Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

INTESTINAL LYMPHATIC TRANSPORT OF DDT AND SAQUINAVIR – THE ROLE OF LIPID BASED FORMULATIONS AND MODULATORS OF P-GLYCOPROTEIN AND CYTOCHROME P450

being a thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICS

at

TRINITY COLLEGE, DUBLIN

by

Brendan T. Griffin, *B.Sc. (Pharm.), M.P.S.I.,*

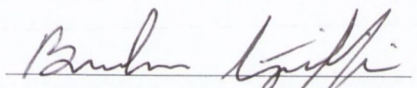
under the direction of

Dr. Caitriona M. O'Driscoll, *B.Sc. (Pharm.), M.A., Ph.D., M.P.S.I.*

Declaration

This thesis is submitted by the undersigned to the University of Dublin, Trinity College, for examination for the degree of Doctor of Philosophy. It has not been submitted as an exercise for a degree at any other University. I myself carried out all the experimental work described, except where duly acknowledged. This manuscript was completely written by me with the aid of editorial advice from Dr. Caitriona O'Driscoll.

I give permission for the Library to lend or copy this thesis upon request.



Brendan T. Griffin, October 2001

PUBLICATIONS AND POSTERS ASSOCIATED WITH THIS THESIS

B.T. Griffin and C.M. O'Driscoll (1999). The effect of bile salt micelles on the lymphatic transport of DDT in the mesenteric lymph duct-cannulated anaesthetised rat.
Presented at Trinity College Dublin/Queens University Belfast Joint Research Pharmacy Seminar.

B.T. Griffin and C.M. O'Driscoll (1999). Lymphatic transport of DDT from micellar and lipid self emulsifying systems in the rat. *Pharm Sci. 1 S4224 (Abstract)*
Presented at the Annual Meeting for the American Association of Pharmaceutical Scientists, Nov. 14-18 in New Orleans, LA.

B.T. Griffin and C.M. O'Driscoll (2001). Targeting of saquinavir to the intestinal lymphatics in the rat - the influence of P-glycoprotein and cytochrome P450 modulators. *Pharm Sci. 1 S3175 (Abstract)*
Presented at the Annual Meeting for the American Association of Pharmaceutical Scientists, Oct 21-25 in Denver, CO.

ACKNOWLEDGEMENTS

I wish to express my gratitude to my supervisor, Dr. Caitriona O'Driscoll for her advice, support and understanding, throughout my postgraduate years. I acknowledge and appreciate most of all, her guidance and insightful discussions during the preparation of this thesis.

I would like to thank the staff of the Bioresources Unit for their help in relation to animal studies. I am particularly indebted to Ann Brayley, for assistance in animal handling and for insisting that a 12-hour experiment must start before 8.15am. I would like to thank all the staff of the School of Pharmacy for their willingness to be of assistance whenever the need arose. I would like to mention Prof. Owen Corrigan, Prof. Pat Deasy, Dr. Ann Marie Healy, Dr Majella Lane, Nuala Collins, Brian Canning, Pat Quinlan and Liesa Eckhardt, who have all assisted me at various times. My thanks are also due to Dr. Richard Maguire, Dr. John Gilmer and Dr. John Walsh for expert advice on analytical chemistry, and Dr. John Clancy for helpful discussions of validation and also for the gift of the cyclosporin sample. I wish to thank Dr. Hugh Wiltshire and Roche for the sample of saquinavir and Dr John Tjia in Liverpool for his help establishing the saquinavir assay.

I would like to thank all my friends and postgraduate colleagues, too numerous to mention, who helped make my college years unforgettable. I wish to thank especially Stephen, Richie, Len, Anthony, Karl, Denis, Mark, Sally-Ann, Colleen, Fergal, Karen, Bronagh, Nicole, Emma, Deirdre, Fiona, Helena, Sandra, and the 'chemistry, 'cognosy and 'cology crew. Cheers to the lads, Jason, Johnny Eggs, Fergie, Mike, Wheelie and Dessie. And no, Docco, I have not got a cure for AIDS, just yet!

It would not have been possible to do this without the love and support of my mother, Mary and father, Joe, who always encouraged me and reminded me not to lose sight of the final goal. I am very grateful for the opportunities they have presented me with, and proud of the way they brought me up, and have promised them to never lose sight of that. To my brother and sisters, Joseph, Ann Marie and Bernie, thanks for the being great friends as well.

Finally, my special thanks to Sinead, for the love and encouragement when my morale was low, for the patience and kindness when the pressures got too high and for simply making all this worthwhile.

SUMMARY

The experiments in this thesis represent biopharmaceutical investigations, performed in an attempt to evaluate potential dosage form strategies designed to promote the intestinal lymphatic transport of lipophilic compounds. DDT is a highly lipophilic model compound, which is routinely used in mechanistic lymphatic transport studies. Saquinavir is a recently approved, highly lipophilic, potent HIV protease inhibitor, which exhibits poor bioavailability due to poor dissolution and absorption characteristics and extensive first pass metabolism.

Initially, the solubilization capacities of a series of lipid micellar systems for both lipophilic compounds were examined. The lipid vehicles investigated included simple and mixed micelles, of both naturally occurring (e.g. bile salts) and synthetic (e.g. cremophor EL and TPGS) surfactants. The saturation solubility of both DDT and saquinavir in the presence of these micellar systems were all significantly improved, relative to aqueous solubility. The solubility of both lipophilic compounds in mixed micelles, containing the long chain, oleic acid, increased with increasing fatty acid content, relative to the simple micelles. The micellar systems produced from the synthetic surfactants, TPGS and Cremophor EL, generally displayed a greater solubilization potential for both lipophilic compounds, compared to the bile salt micelles, although some differing solubilization mechanisms between naturally occurring and synthetic micelles were observed.

The effects of a range of lipid-based formulations on the intestinal lymphatic transport of DDT and saquinavir were investigated *in situ* in the anaesthetised rat. Formulation in a mixed micellar state was found to enhance lymphatic transport, relative to simple micelles. The incorporation of the long chain fatty acid appears to be critical; promoting intestinal triglyceride turnover, and resultant lipoprotein synthesis. The proposed mechanisms whereby the micellar systems improved lymphatic transport were an improved drug transfer across the enterocytes, coupled with a stimulation of triglyceride turnover.

Having identified the potential advantages of oleic acid at promoting lymphatic transport, research was centred on the design of a lymphotropic self-emulsifying lipid based formulation. SEDDS facilitate the formation of solubilized phases from which absorption may occur. From a formulation standpoint, SEDDS may be preferable to micellar vehicles because of an ability to solubilise high concentrations of the drug while providing a suitable vehicle for delivery in a soft gel capsule. Using a pseudo ternary phase diagram to

guide formulations efforts, the conditions required for the formation of a SEDDS that maintains a microemulsion structure on infinite dilution were assessed using oleic acid as the oil phase. The extent of transport of DDT and saquinavir was similar to that obtained with the mixed micellar systems. The maximal rate of transfer is delayed compared to the mixed micellar systems, possibly reflecting the delay involved in converting from a microemulsion phase to a pre-absorptive phase, represented by the bile salt micelles. By comparison the lymphatic transport from a crude oleic acid dispersion is poor, more prolonged, and incomplete after 8 hours. Dispersion of lipid droplets into a finely emulsified state appears to be an essential step for intestinal absorption of lipophilic compounds and lipids. The kinetics of lipid turnover induced by the lipid formulation appear to play a role in efficient drug transfer to intestinal lymph, with the more efficient lipid processing from the SEDDS formulation, compared to the oleic acid dispersion, and the NaC:OA mixed micelles, compared to the NaC: phospholipid mixed micelles, resulting in increased apparent loadings of DDT in intestinal lymph triglyceride.

For all the lipid formulations, there was a strong positive correlation between the lymphatic transport rate of both DDT and saquinavir, and the triglyceride turnover in intestinal lymph, confirming that intestinal lymphatic transport occurs in association with re-synthesized long chain lipids (i.e. intestinal lipoproteins). For the study with DDT, a strong positive correlation was observed between the cumulative extent of DDT transport after 6 hours and the total amount of triglyceride recovered in lymph after 6 hours. This suggests that the two crucial determinants of intestinal lymphatic transport are (a) an ability of the lipid formulation to stimulate a maximal extent of lymphatic C_{18} lipid turnover and (b) the solubility of the drug candidate in the re-synthesized C_{18} triglyceride. The limited solubility of saquinavir in triolein, compared to DDT, was one of the contributing factors for the low extent of intestinal lymphatic transport observed.

The experimental results obtained following co-administration of P-glycoprotein (P-gp) and cytochrome P450 (CYP) modulators confirm the potential for these efflux and/or elimination processes to significantly limit the intestinal lymphatic transport of saquinavir and also the absorption of saquinavir into the systemic circulation. These findings provide a rationale for attempts to improve the lymphatic targeting of saquinavir by concomitant administration of P-gp and or CYP modulators, which may significantly increase its therapeutic value for treatment of HIV infection in the lymphatics.

CONTENTS

MATERIALS AND EXPERIMENTAL METHODS

RESULTS AND DISCUSION

GLOSSARY OF TERMS

μg	microgram
μl	microlitre(s)
^{0}C	degrees Celsius
AIDS	Acquired immunodeficiency syndrome
AUC	Area under the curve
clog P	Theoretical Log P calculated using clog P software
CSF	cerebral-spinal fluid
Css	Saturation solubility
CMC	The critical micelle concentration
CYP	Cytochrome P450 3A
D/S	Dose to solubility ratio
FA	Fatty acid
g	gram(s)
HIV	Human immunodeficiency virus
HLB	Hydrophile/Lipophile balance
HGC	Hard gelatin capsule
HPLC	High Performance Liquid Chromatography
i.v.	intravenous
i.m.	intramuscular
l	litre(s)
LOD	limit of detection
mg	milligram
min	minute(s)
ml	millilitre
mM	millimolar
MSC	Model Selection Criteria
Mw	Molecular weight
NaC	Sodium Cholate
nm	nanometer
OA	Oleic acid
P	The partition coefficient of a drug between two phases usually expressed as a log value
PCS	Photon Correlation Spectroscopy
PEG	Polyethylene glycol

P-gp	P-glycoprotein
pH	$-\log_{10}[H^+]$
PKa	$-\log_{10}$ [Ka] (the negative log of the dissociation constant of a weak acid)
rpm	revolutions per minute
s.c.	subcutaneous
S.D.	Standard Deviation
S.E.	Standard Error
S/So	Solubility enhancement expressed as the ratio of solubility in micellar solution (S) and solubility in buffer solution (So)
S:CoS	Surfactant to co-surfactant ratio
SEDDS	Self emulsifying drug delivery systems
SQV	Saquinavir
SGC	Soft gelatin capsule
t	Time
$t_{1/2}$	half-life
TG	Triglyceride
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
u.v.	Ultraviolet
Vd	Volume of distribution
VLDL	Very low-density lipoproteins

ORIGIN AND SCOPE

It is now well established that many lipophilic compounds are absorbed, to a certain extent, via the lymphatic route following oral administration. The mechanisms whereby lipophilic molecules are absorbed from the gastrointestinal tract and transported to the general circulation are complex and poorly understood (Porter & Charman, 1997). There are a number of biopharmaceutical advantages associated with significant drug transport via the intestinal lymphatics. These include the potential to increase drug absorption, the avoidance of hepatic first pass metabolism (because the lymphatics drain directly into the internal jugular vein), to prolonged delivery to the systemic circulation, and the potential to selectively target compounds to particular regions of the lymphatic circulation.

The intestinal lymphatics are responsible for the transport of dietary lipid from the absorptive cells of the intestine to the systemic circulation (O' Driscoll, 1992). For a lipophilic drug to be preferentially absorbed by the intestinal lymphatics, the drug is best formulated with a lipid vehicle; the co-administered lipid will serve in large part as the required substrate and constituent for lipoprotein synthesis by the intestinal enterocytes. Triglycerides undergo initial lipolysis in the intestinal lumen to the corresponding fatty acids and monoglyceride and these entities are absorbed into the epithelial cells of the gut. The triglyceride is then re-synthesised in the epithelial cells and is ultimately transported as the major component of chylomicrons (a class of lipoprotein), which are the major lipoidal transport system of the intestinal lymphatics (Tso et al., 1987). It is presumed the lipophilic drugs are transported in the triglyceride core of the chylomicrons (Charman & Stella, 1986a). Long chain fatty acids promote chylomicron formation to a greater extent than small and medium chain fatty acids which may be absorbed directly by the portal blood system (Caliph et al., 2000).

In order to define some of the parameters affecting the lymphatic uptake and transport of lipophilic drugs, in this thesis, the effects of a series of lipid vehicles on the intestinal lymphatic transport of lipophilic compounds in a mesenteric lymph duct cannulated rat model were investigated. DDT was chosen initially as a model compound because it is highly lipophilic, relatively metabolically stable and well transported by the intestinal lymphatic system of the rat (Palin et al., 1982).

Previous studies from this laboratory (Obodozie, 1997) examined the absorption of DDT from bile salt micellar systems in an *in situ* single pass perfusion model (Komiya et al., 1980). DDT appearance in the blood was monitored simultaneously with the

disappearance from the gut, during perfusion, in order to check the correlation between kinetic parameters of these two processes, as well as monitor the mass balance of quantities transported across the mucosal membrane (Figure 1.1). The theoretical line AB represents the situation where disappearance from the gut equals appearance in the general circulation. Although DDT transport across the intestine was low for both simple and mixed micelle formulations, it was lower in the mixed micellar systems than in the simple micellar systems. The rate of disappearance from the lumen was greater than the rate of appearance in the blood. Incorporation of lipids to the simple micelles to form mixed micelles, further decreased the appearance rate of DDT in the general circulation approximately 3.6-fold, relative to the simple micelles. It was proposed that the absence of a mass balance suggested that DDT was being diverted to the lymph, with the mixed micellar systems promoting this effect to a higher degree than the simple micellar systems. These results highlighted the potential of micellar systems to target the lymphatics, and bile salt/fatty acid micelles in particular. In order to test these hypotheses it was necessary to establish a mesenteric duct cannulated rat model to study the lymphatic transport of DDT from micellar systems.

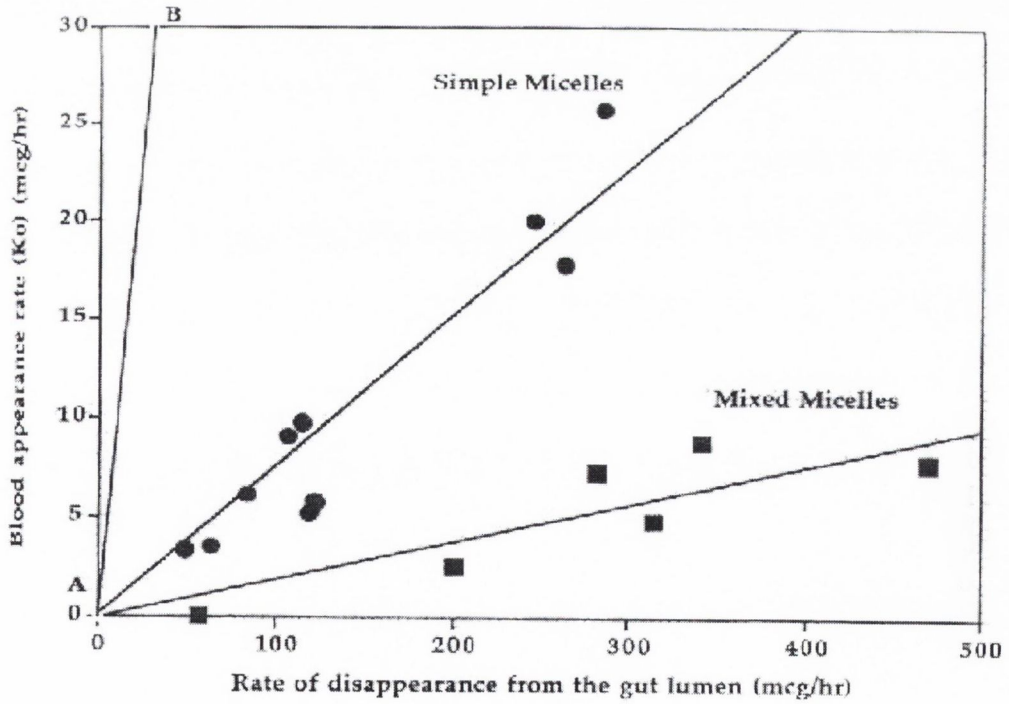


Figure 1.1 Blood input rate vs. disappearance rate of DDT from the gut lumen, in the simple and mixed bile salt micellar systems (Obodozie, 1997).

The potential of lipidic self-emulsifying drug delivery systems (SEDDS) for improving the extent and reproducibility of absorption for lipophilic drugs has received much attention recently, particularly with the arrival of the soft gelatin capsule formulation of cyclosporin A, Neoral® from Sandoz (Mueller et al., 1994). The principal characteristic of a SEDDS is their ability to form fine oil-in-water emulsions or microemulsions upon mild agitation following dilution by aqueous phases. Ideally, SEDDS formulations of lipophilic drugs facilitates their subsequent assimilation with the relevant bile salt micellar structures, therein preventing drug precipitation, which would be expected to improve the extent and consistency of lipophilic drug absorption (Pouton, 1997). In published literature, both long-chain and medium-chain triglyceride oils with different degrees of saturation have been used as the lipophilic phase for the design of SEDDS (Charman et al., 1992, Shah et al., 1994, Craig et al., 1995). For this study it was deemed desirable to form a lipophilic phase with the long-chain free fatty acid, oleic acid. Charman and Stella (1986a) reported that the long chain free fatty acid form offered advantages over the triglyceride equivalent in terms of a two-fold higher extent of lymphatic transport of DDT, with a shorter lag time. Formulating a long chain fatty acid as a SEDDS was therefore desirable with a view to forming a readily dispersed lymphotropic microemulsion, which provides a lipidic substrate for efficient chylomicron synthesis and subsequent lymphatic drug transport.

Saquinavir is a highly lipophilic, HIV protease inhibitor approved for use in the treatment of acquired immuno-deficiency syndrome (AIDS). However, the bioavailability of saquinavir is relatively low i.e. 4-12% depending on the formulation (Vella & Floridia, 1998). The bioavailability is increased by food. The poor bioavailability may be due to a combination of factors including poor aqueous solubility, resulting in poor dissolution characteristics and extensive first pass metabolism. Saquinavir is a substrate for efflux by P-glycoprotein and metabolism by CYP 3A4 however the contribution of these physiological processes to the oral bioavailability of saquinavir has yet to be established.

Despite low bioavailability and high plasma protein binding, saquinavir has exhibited substantial and durable *in vivo* antiviral activity both as a single agent and in particular in combination with zidovudine and zalcitabine (Moyle & Gazzard, 1996). HIV virus appears to proliferate within the lymphatic system and it has been found that viral load in certain type of cells, such as lymphocytes and macrophages, is much higher than in other cells (Pantaleo et al., 1993; Embretson et al., 1993; Li & Chan, 1999). These high viral

load cells can serve as a reservoir for HIV. Therefore it is obvious that targeting delivery of anti HIV agents specifically to these cells would increase the efficacy of therapy.

Saquinavir is an ideal candidate for intestinal lymphatic targeting for the following reasons: (i) high lipophilicity, (ii) extensive first pass metabolism, (iii) increased bioavailability following co-administration of fatty meal and (iv) the therapeutic benefit of targeting this potent HIV protease inhibitor to the lymphatic system. To-date one study on intestinal lymphatic transport of a HIV protease inhibitor has been reported in published literature; Lin et al., (1996) examined the lymphatic transfer of ^{14}C-indinavir after oral administration in a citric acid solution to rats (n=2). The kinetics of intestinal lymphatic transport were not reported. Peak concentrations were found in lymph 0.5hr after dosing. The authors concluded that indinavir was distributed quickly into and out of the lymphatic system and noted also that the rapid lymph transfer is of clinical relevance, because a primary clinical hallmark of AIDS is the depletion of CD4 lymphocytes and pathology of associated lymphoid tissues.

Since the effect of saquinavir on viral replication is dose dependent (Kitchen et al., 1995; Shapiro et al., 1996), and therapy is expensive, in this study a number of approaches were examined, in an attempt to solve some of the limiting factors relating to oral delivery of saquinavir and to explore the potential to enhance intestinal lymphatic transport. Formulation in lipid micellar and self-emulsifying formulations were performed to increase the solubilization within the intestine and improve absorption from the gastrointestinal tract. The potential for these lipid formulations to promote intestinal lymphatic transport and thereby avoid hepatic first pass metabolism was examined. Finally, the effects of co-administration of modulators of the P-glycoprotein efflux mechanism and the cytochrome P450 metabolic enzymes on the intestinal lymphatic transport were assessed.

GENERAL INTRODUCTION

2. Drug delivery to the intestinal lymphatics

2.1. Introduction

The lymphatic system is an elaborate network of specialised vessels distributed throughout the vascular regions of the body. The primary and well-recognised function of the lymphatics is to drain the capillary beds and to return extracellular fluid to the systemic circulation, thus maintaining the body's water balance (O' Driscoll, 1992). However, the structure and function of the lymphatics throughout the body are not uniform, and in specific areas, the lymphatics perform a specialised role. For example, the intestinal lymphatics are responsible for the transport of dietary fat and lipid soluble vitamins to the systemic circulation.

Orally administered drugs gain access to the systemic circulation via two separate and functionally distinct absorption pathways – the portal blood and the intestinal lymphatics. The physiochemical and metabolic features of the administered drug and the characteristics of the administered formulation, largely dictate the relative proportion of drug absorbed via these two pathways.

The portal blood represents the major absorption pathway for the vast majority of orally administered drugs as it has a high capacity to transport both water soluble and poorly water soluble compounds. For drugs that act either at the level of a well perfused organ/target cell, or for drugs designed to treat disease states of highly vascularized organs, the systemic concentrations of drug resulting from absorption via the portal blood are generally a good indicator of potential clinical efficacy. However, a significant drawback associated with absorption via the portal blood is the potential for first pass metabolism.

The intestinal lymphatics are a specialised absorption and transport pathway for highly lipophilic drugs, lipids and lipidic derivatives, and lipophilic xenobiotics (Porter & Charman, 1997). Once a drug has gained access to the intestinal lymphatics (generally in association with specific lipoproteins formed in the enterocyte), it will drain from the mesenteric lymphatics into the cysterna chyli. The cysterna chyli, which also collects hepatic and regional lumbar lymph, then drains into the thoracic lymph duct, which empties into the systemic circulation at the junction of the left internal jugular and left subclavian veins. Therefore, the consequential drug delivery opportunities associated with

intestinal lymphatic transport are the potential to avoid hepatic first pass metabolism after oral dosing.

B- and T- lymphocytes, which play a major role in maintaining the immune system, also circulate through the lymphatics in relatively high concentrations compared with the systemic blood. Consequently there is considerable interest in the specific delivery of anti-infective or anti-viral agents to the lymph to combat lymphocyte destruction by, for example, human immunodeficiency virus. The lymph also serves as a primary conduit for the dissemation of tumour metastases and has been widely explored as a target for immunogenic and cytotoxic agents designed to combat the spread of metastases from solid tumours (Hirnle, 1997). The benefits of targeted delivery of drugs to regions of the lymphatics that are directly supplied by lymph from the mesenteric lymphatics are thus readily apparent. These regions are often poorly perfused by the systemic circulation making it difficult to attain adequate concentrations at the target organ/cell after the compound has been absorbed via the portal blood.

The process of intestinal lymphatic drug transport often continues over time periods longer than typically observed for drug absorption via the portal vein. Consequently, drug transport via the intestinal lymphatics may be utilised to prolong the time course of drug delivery to the systemic circulation (Porter & Charman, 1997).

2.2. General structure of the lymphatic system

The lymphatic system consists of a network of thin walled vessels that drain fluid (lymph) from the interstitial spaces. Like the vascular system, the lymph is responsible for the transport of fluids and cellular elements around the body; however, unlike blood vessels the lymphatics do not form a circular system. Rather, the flow of lymph is unidirectional, recovering fluid from the periphery and returning it the vasculature (Yoffey & Courtice, 1970).

The lymphatic capillaries (also referred to as initial or terminal lymphatics) collect lymph from the periphery and unite to form prenodal collecting vessels that transport lymph to the regional lymph nodes. Postnodal lymph vessels carry lymph between successive sets of lymph nodes or to larger lymphatic collecting vessels. Eventually, the larger lymph-collecting vessels drain lymph from the final set of lymph nodes into the lymph ducts.

Lymph from the intestinal, hepatic and lumbar regions drains into the cisterna chyli. The cisterna chyli acts as a collecting reservoir at the distal end of the major lymphatic vessel (the thoracic lymph duct). The thoracic lymph duct ascends upwards from the cisterna chyli, receiving lymph from the mediastinum and eventually from all parts of the body except the upper quadrant. The thoracic duct empties directly into the venous blood at the junction of the left internal jugular vein and the left subclavian vein. Lymph from the upper right quadrant of the body (the right side of the head and neck, the right arm, and the right side of the thorax) may drain into a right thoracic lymph duct that empties directly into the venous system at the junction of the right internal jugular vein and the right subclavian vein. If the right thoracic duct is absent, lymph from the lymphatic trunks draining the upper right side of the body drains into the (left) thoracic duct and to the left jugular vein as before.

The hydrostatic and oncotic mechanisms controlling the capilliary filtration of plasma and solutes and the production of lymph have been reviewed by O' Driscoll (1992). Briefly, most of the constituents of plasma move freely through capilliary walls to form interstitial fluid; however, more fluid leaves the blood capillaries than is returned by absorption and the excess fluid (~3Litre/day) drains into the lymphatics and becomes lymph. Importantly, the lymph is the primary route of return of filtered (interstitial) plasma proteins to the circulation, and is responsible for the maintenance of plasma protein balance.

Lymph contains most of the components of plasma, although generally at lower concentrations. For example, the protein concentration in lymph is about two-thirds of that found in serum (Bergstrom & Werner, 1966) and the concentrations of fibrinogen and prothrombin in lymph are less than in plasma (Yoffey & Courtice, 1970). The levels of electrolytes, nonelectrolytes such as urea and creatinine, and iron and transferrin are not statistically different in lymph than in plasma (Yoffey & Courtice, 1970)).

A specific function of the lymphatic system is the transport of dietary fats from the gastrointestinal tract via the intestinal lymphatics. The concentration of lipid in the thoracic and intestinal lymph is therefore elevated postprandially; however, the concentration of lipid in the peripheral lymph is generally lower than in plasma. The rates of lymph production as well as the content of lymph draining the small intestine are largely dependent on the absorptive state of the intestine and the constituents of chyme. Studies on the formation and transport of gastrointestinal lymph indicate that these are dynamic

processes that are regulated by both local (intrinsic) and remote (extrinsic) factors (Benoit & Zawiega, 1994).

The direction of lymph flow dictates that interstitial fluid is absorbed form the periphery and transported to the vasculature via the lymph nodes. In this regard the lymph nodes fulfil a defence function, filtering lymph and trapping foreign substances and antigen in reticular fibres where macrophages and lymphocytes remove foreign objects by phagocytosis or mount cellular or humoral immune responses to the antigen. All lymph passes through at least one set of lymph nodes on its passage from the periphery or intestinal tract back to the systemic circulation ((O' Driscoll, 1992)). Lymph enters the lymph node via one, or many, afferent lymph ducts and flows through medullary sinuses lined with large numbers of macrophages, which are responsible for the phagocytosis of cellular and particulate material. The lymph exits the node through the hilus into (usually) a single efferent lymphatic. The exchange of various materials between the blood and lymph occurs within the lymph node, however, the mechanisms of this exchange are poorly understood. The lymph nodes are also a centre for lymphocyte proliferation with B-, T- lymphocytes, and B-lymphocyte derived antibodies entering the lymph via the lymph nodes.

Lymphatic capillaries are closed at their peripheral ends and consist of a single layer of nonfenestrated endothelial cells with an incomplete basal lamina. The endothelial layer is made up of overlapping endothelial cells that have more 'open' intercellular junctions than those in blood capillaries. Estimates of intercellular junctional distances range from several microns (Casley-Smith, 1980; Leak, 1980) to 15-20nm (O' Morchoe et al., 1982). The large intercellular junctions facilitate the preferential transport or drainage of macromolecules from the interstitial space into the lymphatics. The rate of filtration and re-absorption of fluid across the vascular capillaries is large (20L/day-40L/day) in comparison to the small quantities of interstitial fluid drained by lymph (2L/day-4L/day). Consequently small molecules (<2000 Mw) with approximately equal affinity for the lymph and the blood are predominantly cleared by the blood vessels. Particulates and molecules of increasing molecular size increasingly favour drainage into the more open capillaries of the lymph.

2.3. Drug access to the lymphatics after parenteral administration

Drug molecules entering the peripheral lymph are similarly channelled via the lymph nodes to the systemic circulation, facilitating drug delivery to the lymph, the lymph nodes, and eventually the systemic circulation. To gain access to the lymph, prospective drug molecules must be either introduced directly into the lymphatic capillaries (by injection) or drain into the lymph from the interstitial space. Drugs may access the interstitium via parenteral delivery (e.g. s.c. or i.m. injection). Lymphatic selectivity is most effectively provided by direct injection of drugs into the lymphatic vessels. Although this approach has been reported for some chemotherapeutic compounds, (Order et al., 1975 & 1977), the technical difficulties involved in intra-lymphatic injection have limited its practical employment, and drug access to the lymph is generally achieved after administration to regions that are well drained by lymphatic capillaries i.e. the interstitial spaces.

After intravenous administration, most drugs extravasate and access the interstitial space. Indeed for the majority of medicinal products this is a prerequisite for activity, since most sites of action are extravascular. Although the majority of the mass of these compounds is subsequently reabsorbed into the blood capillaries, a small proportion may drain into the lymph. For most small molecules the concentrations of drug in the blood, the extracellular (interstitial) fluid, and the lymph equilibrate across the capilliary beds and the ratio of drug concentration in the lymph (C_L) to drug concentration in the blood (C_B) approaches unity. In the case of larger molecules, decreased capilliary permeability may limit extravasation and impede access to the interstitial space and the lymph. After i.v. administration, high molecular weight dextrans and dextran copolymer have been shown to accumulate in the lymph nodes (up to 30-40%/g) (Takakura et al., 1990; Papisov et al., 1994). However, the concentrations of dextran in peripheral or thoracic lymph were not determined in these investigations, and the mechanism of accumulation could represent extraction into the lymph nodes from either the systemic circulation or lymph. Liposomes appear to have little, if any, specific affinity for lymph or lymph nodes after i.v. administration (Allen et al., 1993; Kim et al., 1993). A recent report has described enhanced concentrations of 2'3' dideoxyinosine (ddi) in the lymph nodes 3 hours after i.v. administration of a liposome formulation (particle size 112nm); however, the authors also reported a significant increase in the plasma half-life of ddi from the liposomal formulation, and the enhanced drug concentrations in the lymph nodes most likely reflect equilibration of ddi into the lymph from the high concentrations of drug obtained in plasma (Harvie et al., 1995).

Direct administration to the interstitium, by s.c., i.m., or i.p. administration, circumvents many of the limiting problems of extravasation after i.v. administration, and provides for selective delivery of drug molecules to those areas best drained by the lymphatic capillaries. Two general approaches have been developed to promote lymphatic transport after interstitial administration, both of which rely on the differential permeability of the lymphatic capillaries (in comparison to blood capillaries) to molecules of increasing size; first, the use of macromolecules or drug conjugates; second, the formation of particulate or colloidal delivery systems that may be used to generically promote lymphatic delivery of drug molecules with a wide range of physiochemical properties. Porter (1997) has recently reviewed lymphatic drug transport after parenteral administration.

2.4. Drug access to the lymphatics after enteral administration

The lymphatic capillaries of the small intestine originate as single, closed vessels (lacteals) located in the centre of each intestinal villi. The lacteals lie within the interstitium, approximately 50μm below the intestinal epithelium, and are approximately 20μm in diameter. Like the peripheral interstitium, the intestinal interstitium consists of reticular and collagen fibres supporting a gel-like mass of glycoproteins, including hyaluronic acid.

The lymphatic capillaries of the large intestine are fewer in number and smaller in size than the lacteals of the small intestine and originate much deeper in the intestinal mucosa, up to 300-400μm from the epithelial surface. Lymphatic capillaries from both the small and large intestines form a network of lymphatic capillaries in the mucosa and submucosa and eventually form larger collecting lymphatics. The collecting lymphatics from the small intestine and the ascending and traverse colon join to form the superior mesenteric lymph duct. The collecting capillaries from the descending and sigmoid colon drain into the inferior mesenteric lymph duct. The mesenteric lymph runs into the cisterna chyli and via the thoracic lymph to the systemic circulation.

The permeability characteristics of the intestinal lymphatic capillaries mirror those of the peripheral capillaries and are significantly more permeable to large molecules than the blood capillaries. Unlike the peripheral interstitium, however, the resistance to the intestinal interstitium varies depending on the status of fluid absorption by the intestine. During fluid absorption the interstitial volume increases, porosity is increased, and lymph flow may increase up to 20-fold (Barrowman, 1978; Granger et al., 1980; Lee, 1984).

Following absorption from the gastrointestinal tract, blood and lymph capillaries compete for the transport of drug molecules to the systemic circulation. The majority of enterally administered compounds are absorbed into the portal blood, since the rate of fluid flow through the portal blood is some 500 times greater than that through the lymph (Reininger & Saperstein, 1957). However, the relatively 'open' structure of the lymphatic capillaries may select for lymphatic transport of high molecular weight (macro) molecules or colloids.

Specifically, access to the intestinal lymph (as opposed to the portal blood) is provided by the differences in endothelial architecture between lymph and blood vessels. Whereas the vascular endothelial barrier features tight inter-endothelial junctions and a continuous basal lamina, the lymphatic vessels are characterised by either discontinuous or absent basal lamina and relatively wide inter-endothelial junction distances (Schmid-Schonbein, 1990). The transcellular access of small, lipophilic molecules into either lymph or the blood is therefore relatively unimpeded and under these circumstances absorption into the blood predominates, due to the significantly higher blood flow compared to lymph flow, aiding the mass transport process. However, for large (>10000Da) colloidal structures, e.g. lipoproteins, transcellular transport into the blood is significantly impeded and paracellular access across the more 'leaky' lymphatic endothelium is favoured.

The gastrointestinal tract presents a formidable barrier to the absorption of large molecules and particulates and therefore the major challenge in promoting intestinal lymphatic transport is the design of delivery systems that promote the transport of macromolecules or colloids from the intestinal lumen into the interstitium. Three basic mechanisms may be utilized to achieve this goal:

- Lipophilic drugs or prodrugs may gain access to the lymph by intercalation into the lipid digestion pathway. Orally ingested lipids are digested, absorbed, and packaged into colloidal carriers (chylomicrons) in the enterocyte. Chylomicrons are subsequently released into the interstitial space where their size (200nm-800nm) promotes drainage into the intestinal lymph. Extremely lipophilic drugs may associate with chylomicrons after absorption and as a result may gain access to the intestinal lymph.

- Absorption promoters or absorption promoting vehicles may be used to enhance the passage of macromolecules across the intestinal wall. Access to the lymph is

subsequently assured by macromolecular size. The lymphatic transport of small molecules may be similarly enhanced by the synthesis of macromolecular conjugates or prodrugs.

- Stable colloids or particulate systems may be abstracted from the intestinal lumen by gut associated lymphoid tissue (GALT). Absorption via the GALT delivers particulate delivery systems directly to the lymphoid tissue, where passage into the lymph may occur directly or subsequent to phagocytosis by lymphocytes or macrophages (O' Hagan, 1990; Eldridge et al., 1990).

2.5. Lipid digestion pathway

High amounts of dietary lipid, 20-50 gram per meal (Charman, 1998) enter the intestine on a daily basis, yet very little is lost in stools due to highly efficient luminal digestion, absorption and metabolic processing. For some extremely lipophilic drugs and xenobiotics, this pathway represents an opportunity for access to the lymphatic system. Drug transport via the lipid transport pathway requires that the drugs are sufficiently lipophilic to allow *in situ* association with the colloidal products of triglyceride re-synthesis (lipoproteins). It is the size of the lipoproteins, and not the size of the drug, that promotes drainage into lymphatic capillaries. An understanding of lipid digestion is central to rationalizing the design of lipid formulations.

Lingual and gastric lipases are responsible for the initiation of triglyceride hydrolysis, at the sn-3 position, forming the corresponding diglyceride (DG) and fatty acid within the stomach. The liberation of these more amphiphilic lipid digestion products, in combination with the shear produced by antral contraction, facilitates formation of a crude emulsion, which empties into the duodenum. The presence of lipid in the upper small intestine stimulates secretion of bile salts and bilary lipids from the gall bladder and pancreatic fluids form the pancreas. Bilary lipids such as phospholipid (PL) and cholesterol ester (CE) bind to the surface of the emulsified lipid thereby leading to the production of a more stable (in colloidal terms) emulsion, with a smaller particle size (Carey et al., 1983). The process of lipid digestion is completed by the action of pancreatic lipase and co-lipase that quantitatively produces two molecules of FA and the corresponding 2-MG from each TG molecule. The monoglycerides and fatty acids form a liquid crystalline layer on the surface of the emulsion droplet, which may then be

penetrated by bile salts. The reduction in particle size provides a greater surface area available for binding the pancreatic lipase/co-lipase complex, an interfacial enzyme system that preferentially acts at the surface of triglyceride droplets. Since the MG and the partially ionised FA have intrinsic emulsification properties and because FA promotes binding of lipase/co-lipase complex to the emulsion droplet surface the overall process of lipolysis is self-promoting (Borgstrom, 1980; Bernback et al., 1989; Shiau, 1990).

The brush border membrane of the enterocytes lining the small intestine is separated from the bulk aqueous phase of the intestinal contents by a poorly mixed aqueous boundary layer, that constitutes a major barrier to absorption of poorly water-soluble lipid digestion products. Absorption of the products of lipid digestion is facilitated by micellar solubilization that overcomes the barrier to absorption by providing a hydrophilic carrier to promote passage across the aqueous diffusion layer and markedly increases the effective water solubility of the FA and MG. Tso (1994) confirmed the effective aqueous solubility of FA and MG may increase up to 1000-fold in the presence of bile salt micelles. Studies by Hoffman and Borgstrom in the 1960's proposed that the products of lipid digestion were partitioned between an oily phase and a bile salt mixed micellar phase. However current understanding indicates that the products of lipid digestion may be solubilized in a multi-component system containing a number of different physiochemical species. Thus, as lipolysis proceeds digestion products 'pinch off' from the surface of the crude TG/DG emulsion to form liquid crystalline structures, which in the presence of sufficient bile salt concentrations, form multilamellar and unilamellar vesicular structures. Luminal processing of phospholipids by phospholipase A2 produces the corresponding lyso-phospholipid and cholesterol esters are hydrolysed to the free sterol by cholesterol esterase (Borgstrom et al., 1957; Van den Bosch et al., 1965).

The actual absorption mechanisms of the various products of lipid digestion are still unclear, although micelles are not thought to be absorbed intact (Hoffman, 1970; Simmonds, 1972). Lipids are generally believed to be absorbed from a monomolecular intermicellar phase, which is in equilibrium with the solubilized lipid reservoir (Westergaard & Dietschy, 1976). The stimulus for the dissociation of lipid from the mixed micellar phase is unknown, although a number of studies have suggested a role for a microclimate of lower pH associated with the intestinal absorptive site (Lucas et al., 1975; Shiau et al., 1985; Shiau, 1990).

In addition to passive diffusion, evidence is mounting to suggest that active uptake processes may contribute to the absorption of lipid digestion products. Stemmel and co workers (1985, 1988) have described a carrier system termed the micro-villus membrane fatty acid binding protein (MVM-FABP), which may be responsible for the uptake of FA and other lipid substrates into the enterocyte. It is believed however, that passive transport most likely dominates especially under conditions where the luminal lipid concentration is high e.g. post prandially (Poirier et al., 1996; Schoeller et al., 1995).

The metabolic fate of the absorbed lipid is primarily determined by the fatty acid chain length. The traditionally held view is the fatty acids of chain length greater than 12 carbons migrate from the absorptive site of the endoplasmic reticulum where re-esterification and assembly into intestinal lipoproteins (e.g. chylomicrons) occurs prior to secretion into mesenteric lymph. In contrast small and medium chain fatty acids (less than 12 carbons), which make up a small (~10%) but significant proportion of dietary lipid, are primarily absorbed into the portal blood (Kiyasu et al., 1952). Realistically, the dependence of portal and lymphatic transport on the chain length of the absorbed fatty acid is less clear than this general 'rule of thumb' as there are reports of lymphatic transport of medium chain fatty acids and portal blood absorption of long chain fatty acids (McDonald et al., 1980; McDonald & Weidman, 1987; Baugaut & Carlier, 1987).

The mechanisms whereby fatty acids (and potentially drug molecules) are trafficked intracellularly are largely unknown, although evidence points to a role for the intracellular fatty acid binding proteins (FABP), (as distinct from the lumina MYM-FABP) in delivering fatty acids to select cystolic destinations (Davidson, 1994, Thomson et al., 1993). Two cystolic fatty acid binding proteins, I-FABP and L-FABP appear to play an important role in the intracellular transport of absorbed lipids from the absorptive site to the endoplasmic reticulum where fatty acid and monoglyceride are re-esterified to triglyceride (Davidson, 1994; Thomson et al., 1993; Ockner et al., 1992; Storch et al., 2000). Since FABP preferentially binds long chain fatty acids they may also impart a degree of specificity seen in lipid migration to the endoplasmic reticulum and the subsequent re-esterification process. Recent data suggest that I-FABP (but nor L-FABP) can directly extract fatty acids from membranes (Thumser & Storch, 2000) and binds fatty acids presented to the apical side of the enterocyte more avidly than those presented basolaterally (Alpers et al., 2000) supporting the notion that I-FABP may be more important in the delivery of fatty acids for the brush border membrane to specific

intracellular sites. However more fatty acid appears to be bound to L-FABP in the enterocyte (in comparison to I-FABP) (Alpers et al., 2000) and studies in mice and Caco-2 suggest that I-FABP is not essential for the absorption of dietary fatty acids (Vassileva et al., 2000; Levin et al., 1992). The relative roles of I-FABP and L-FABP in the intracellular processing of fatty acids therefore remain unclear, although evidence describing up-regulation in response to long-chain fatty acid exposure (Poirier et al., 1997) and down-regulation in the presence of epidermal growth factor, EGF (a known inhibitor of triglyceride output) (Darimont et al., 1999), suggest a potential regulatory role in determining triglyceride output.

Absorbed fatty acid may be re-synthesized to the corresponding triglyceride by either of two independent pathways, although both pathways use fatty acyl CoA as a common precursor. The major route of re-esterification is the mono-acylglycerol pathway, which is the most rapid and energy efficient; accounting for approximately 80% of intestinal chylomicron production. The monoacyl glycerol pathway involves the sequential direct acylation of absorbed exogenous 2-monoacylglycerol with activated fatty acids to form the di-, and ultimately triglycerides lipids, and is the major pathway for the formation of TG during exogenous lipid absorption (Tso, 1994; Senior & Isselbacher, 1962). The minor route of triglycerides formation, termed the phosphatidic acid pathway (also known as the glycerol-3-phophate or α-glycerophosphate pathway), is a more complicated pathway involving the sequential acylation of endogenous glycerol-3-phosphate with 3 molecules of activated fatty acid in a series of biochemical steps (Johnson, 1976). Interestingly the intermediate phosphatidic acid product acts as a substrate for both the eventual formation of triglycerides and the corresponding phospholipid. Since the phosphatidic acid pathway utilises endogenous glycerol-3-phosphate, it is not dependent on absorbed monoglyceride and appears to be more important in the re-esterification of endogenous fatty acids. Re-esterification of endogenous lipids via the phosphatidic pathway also appears to lead to the production of more dense lymph lipoproteins (very low density lipoproteins, VLDL) as opposed to chylomicrons formed in response to re-esterification of exogenous lipids via the monoacylglycerol pathway (Tso et al., 1987; Tso, 1994; Ockner et al., 1969).

Following re-esterification in the endoplasmic reticulum, triglyceride is processed through a number of intracellular organelles from where it becomes the primary core lipid of chylomicrons. The surface of the intestinal lipoprotein is subsequently stabilised by the sequential addition of phospholipids (which are estimated to cover up to 80% of the

surface) and various apolipoproteins (Zilversmit, 1965). A typical composition of a chylomicron is approximately 93% triglyceride, 1% cholesterol (primarily present as cholesterol esters), 4% phospholipids and 2% protein (composed of B-48, A-I, A-IV and A-II). Chylomicrons ultimately fuse with the basolateral membrane of the intestinal cell facilitating entry into the lamina propria and mesenteric lymphatics. Following release into the interstitial space, chylomicrons are preferentially absorbed by the open capillaries of the lymphatic system and transported via the intestinal lymphatic ducts to the thoracic duct and the general circulation.

The processing of dietary triglyceride lipid by the gastrointestinal tract is an efficient and high capacity process. The average daily Western diet consists of ~100-150g of triglyceride (Wang, 1987) (as much as ~30% of total caloric intake), and under normal physiological conditions >95% of the ingested lipid is processed and absorbed. Using sequential perfusion of the rat intestine with lipid and Pluronic L-81, which blocks lymphatic TG transport, Tso et al., (1982) estimated that a lymphatic TG fatty acid transport rate of between 100-130μmol/h (340-470mg/12h/TG) is the maximal transport capacity of the proximal half of the rat small intestine. This represents approximately a 32-fold increase relative to the estimated endogenous/basal levels of TG turnover, reported by Caliph et al., (2000), of 12.7mg/12hr, and is well above the maximum amount of TG triglyceride achieved in that study following administration of 100mg long chain TG (147mg/12hr). Levels of triglyceride transported in thoracic lymph in dogs have been reported to increase from 0.74g/10hr pre-prandially to 33.66g/10h post-prandially, representing a 45.5 fold increase (Khoo et al., 1999a). These results confirm the high capacity of the intestine to rapidly produce lipoproteins.

2.6. Lymphatic drug transport of lipophilic drugs via the lipid digestion/absorption pathway

Transport via the intestinal lymphatics has been shown to contribute to the absorption of a number of highly lipophilic drugs and xenobiotics - for example, cyclosporin (Ueda et al., 1984), naftifine (Grimus & Schuster, 1984), probucol (Palin & Wilson, 1984), mepitiostane (Ichihashi et al., 1991) lipophilic vitamins and vitamin derivatives (Kuksis, 1987), halofantrine (Porter et al., 1996), DDT (O' Driscoll et al., 1991) and related analogues (Sieber, 1974), benzo(a)pyrene (Laher et al., 1984) and polychlorinated biphenyls (Busbee et al., 1985). The exact mechanisms whereby drug molecules access

the intestinal lymph in preference to the portal blood are not well known, although it appears that the critical step is drug association with the intestinal lipoproteins (primarily chylomicrons) assembled in the enterocytes during digestion and absorption of lipids (Myers & Stella, 1992).

Charman & Stella (1986b) theorised that if one assumes a simple partition/partition behaviour, the extent of drug partitioning between the portal blood and intestinal lymph may be estimated from a comparison of the relative rates of mass transfer from each route. The rate of fluid flow in the intestinal lymphatics is approximately 500-fold less than that of portal blood (Bollman et al., 1948; Reininger & Saperstein, 1957), and during peak lipid transport the lipid content of lymph is of the order of 1-2% (w/v). Therefore, the effective mass ratio between lymph lipid and portal blood is of the order of 1:50,000. Consequently the selective lymphatic transport of small molecular weight, water-soluble drugs is unlikely if the route of absorption (portal vs. lymph) is governed by the relative rates of fluid flow. If the route of transport is determined by the relative mass transfer rates of lymph and blood, a candidate molecule requires up to 50,000 times greater affinity for lymph lipid than for portal blood (hence a log P of greater than 4.7)

2.7. Formulation approaches for enhanced lymphatic delivery

As a consequence of the requirement of drugs to associate with lymph lipoproteins in order to enhance the lymphatic transport drug, formulations which contain lipid (and therefore stimulate the turnover of lipoprotein through the enterocyte) have generally been found to enhance the lymphatic transport of highly lipophilic drugs. Many lipid species such as liposomes, micelles and mixed micelles are involved in the various steps of lipid digestion and have been suggested as lymphotropic delivery systems (Takada et al., 1985; Yoshikawa et al 1985; Hirnle, 1997). With few exceptions (Aramaki et al., 1993; Tomizawa et al., 1993), these carriers do not traverse the intestinal epithelium intact, and lipid and drug molecules are absorbed as single molecular entities. Colloidal carriers are therefore unlikely to enhance lymphatic transport of drug molecules per se, and candidate molecules must be sufficiently lipophilic to associate with the endogenous colloidal species (lymph lipoproteins) synthesized within the enterocyte. Notwithstanding this (albeit critical caveat), lipid vehicles may facilitate the lymphatic transport of lipophilic drugs by stimulating the turnover of lymph lipoproteins through the enterocyte, thus

providing an increased lipoprotein-based absorption 'sink' into which lipophilic drugs can partition.

The choice of co-administered lipid is a crucial determinant of the extent of intestinal lymphatic drug transport. Lipids are typically characterized by the (i) lipid class, (ii) lipid chain length, (iii) degree of lipid saturation, and (iv) physical state of the administered lipid.

2.7.1. Effect of lipid class

Charman et al., (1986a & b) examined the effect of lipid class on the intestinal lymphatic transport of DDT in mesenteric lymph duct cannulated anaesthetized rats. Three types of lipid vehicle were utilized in this study, peanut oil (triglycerides), oleic acid (fatty acid) and a 2:1 mixture of oleic acid-mono-olein, which represents the lumenal digestion products of triglycerides. The dose volume of lipid in all studies was 200μl, containing 2mg DDT and infused intraduodenally over 2 hours. Cumulative intestinal lymphatic transport of DDT was two-fold greater in either oleic acid or the 2:1 mixture of oleic acid:monolein compared with the equivalent triglyceride based lipid vehicle. A shorter lag time was also associated with administration in the fatty acid vehicle when compared with the triglyceride vehicle. The authors suggested that lipid formulations based on fatty acids are preferred, in terms of maximal lymph transport, than triglyceride-rich lipid formulations, that require additional pre-absorptive digestion.

2.7.2. Effect of lipid chain length

Vitamin D3 was more efficiently absorbed (approximately three times higher) after administration to fasted human volunteers in peanut oil (a long chain triglyceride containing primarily oleic acid) than in Miglyol 812 (a medium chain triglyceride containing primarily C_8-C_{10} fatty acids (Holmberg et al., 1990). Since vitamin D3 is almost exclusively absorbed by the lymphatic route, the low absorption observed when administered with the medium chain triglyceride likely represents a reduction in lymphatic transport, secondary to insufficient chylomicron formation. Palin and co-workers reported similar effects on the absorption of two highly lipophilic, lymphatically transported

compounds DDT and probucol (Palin et al., 1982; Palin & Wilson, 1984). Highest plasma concentrations were observed after administration of either compounds in peanut oil (primarily C_{18} lipids) and the lowest plasma levels were evident following administration of liquid paraffin (a poorly absorbed, non-digested mineral oil). Intermediate plasma levels were observed after administration of Miglyol 812. It was postulated that digestion of peanut oil within the small intestine resulted in the liberation of long-chain fatty acids, which effectively promoted chylomicron formation and consequently the lymphatic transport of DDT or probucol, whereas the digestion of Migylol 812 led to the production of medium chain fatty acids which are absorbed to a greater extent into the portal blood and were therefore incapable of supporting significant lymphatic drug transport. The low plasma levels of DDT and probucol observed after administration in the liquid paraffin vehicle were consistent with an inability to form chylomicrons, secondary to poor digestion and absorption of the vehicle.

Nankervis et al., (1996) evaluated the impact of the choice of formulation lipid (long-chain TG, long-chain FA, or a medium-chain TG) on the lymphatic transport of three retinoids. After oral administration, the lymphatic transport of the retinoids was low (<1% of the dose), but again the total oral bioavailability was low and the contribution to oral bioavailability conveyed by the lymphatics appeared to range from less than 1% to as much as 75% (figures broadly estimated from drug transport rate data). The lymphatic uptake of the three retinoids also reflected their lipophilicity, the rank order of increasing lymphatic uptake from each oil was temarotene (log P ~8.7) > etretinate (log P ~ 7.8) > isotretinoin (log P ~ 6.8). The impact of formulation components on the route of drug transport was significant. For example, the combined rate of uptake of temarotene into both plasma and lymph after oral administration in Miglyol 812 (a medium-chain TG) was 9.5μg/hr/kg, of which approximately 13% was delivered by intestinal lymph. By changing the oil from Miglyol 812 to cotton-seed oil (a long chain TG) the total rate of uptake into the lymph plus plasma was reduced marginally to 8.9 μg/hr/kg, but the fraction absorbed by the lymph was enhanced four-fold to approximately 45%. While the total amounts absorbed by each route were not stated (only the rate), it is clear that long-chain lipid vehicles (especially cotton seed oil and linoleic acid) proved to be more effective in promoting the lymphatic transport of retinods than a medium chain triglyceride oil (Miglyol 812). The authors suggested that significant alteration in the proportion of the dose absorbed by the intestinal lymph may be achieved by changing the vehicle of delivery.

Caliph et al., (2000) reported that the lymphatic transport of halofantrine (Hf) was highly dependent on the chain length of the co-administered triglyceride lipid, and increased with increasing chain length (peanut oil, long-chain C_{18} TG, 15.8% of dose > Captex 355, medium-chain (C_{8-10}) triglyceride, 5.5% of dose > tributyrin , short-chain (C_4) triglyceride, 2.22% > lipid free vehicle , 0.34%). Interestingly, similar trends were also observed in the intestinal lymphatic transport of triglyceride lipid, confirming that the extent of lymphatic lipid transport was dependent on lipid chain length, and was negligible for short chain triglyceride, marginal for medium chain triglyceride, and optimal for long chain triglyceride. The co-administration of formulation lipids also enhanced the total systemic availability, which was defined as the mass transported directly into the lymph plus the proportion of dose absorbed via the blood into the systemic circulation, relative to an i.v. control (C_{18}, 22.2% of dose>C_{8-10} 19.2% > C_4, 15.5% > lipid free vehicle, 6.4%). In animals dosed with the long chain triglyceride vehicle, lymphatic transport accounted for 70% of the total available drug, whereas administration of the medium or short chain triglyceride or lipid free formulations, the majority of the drug was absorbed via the non-lymphatic route (i.e. portal route).

2.7.3. Effect of degree of unsaturation

The differences between saturated, monounsaturated and polyunsaturated lipids on intestinal lymphatic transport is less clear-cut. Dietary intake of fats has received considerable attention in the last few decades since diets high in fat, and in particular saturated fats, have been linked with high cholesterol blood levels and coronary heart disease. Noguchi et al., (1985a) reported a doubling of lymphatic transport of testosterone undecanoate after administration in a monounsaturated oleic acid lipid vehicle when compared with a polyunsaturated (MaxEPA) lipid vehicle. Similarly McDonald et al., (1980) reported a decreased lymph transport/portal blood transport ratio for C_{18} fatty acids of increasing degrees of unsaturation.

A decrease in the lymphatic transport of lipid soluble vitamins, A and D, was reported after administration of lipid vehicles with increasing polyunsaturated fatty acid content (Kuksis, 1987). Clarke et al., (2000) have recently documented the increased lymphatic transport of two carotenoids (lycophene and asataxathin) after intraduodenal infusion in a mixed micellar solution containing a monounsaturated lipid source, olive oil (65.3% $C_{18:1}$, 14.5%

$C_{18:2}$), when compared to an identical micellar solution containing the more unsaturated corn oil (26.3% $C_{18:1}$, 58.2% $C_{18:2}$). Porsgaard & Hoy (2000) have also shown that the extent of lymphatic transport of α-tocopherol from rapeseed (61.4% $C_{18:1}$, 22.7% $C_{18:2}$), soybean (23.4% $C_{18:1}$, 50.6% $C_{18:2}$) and sunflower (24.6% $C_{18:1}$, 60.6%$C_{18:2}$) oils is proportionally highest (78.8% of dose) after administration as soybean oil (principally $C_{18:2}$), followed by rapeseed ($C_{18:1}$) oil (45.7% of dose) and sunflower ($C_{18:2}$) oil (21.4% of dose), showing no clear dependence of the efficiency of lymphatic transport on the degree of unsaturation of the lipids.

Recent evidence gained using Caco-2 cells has shown that the stimulation of lipoprotein secretion and triglyceride output in Caco-2 cells is maximal after incubation with $C_{18:1}$ fatty acids followed by $C_{18:2}$ and $C_{18:3}$, and that lipoprotein and triglyceride output after incubation with fully saturated $C_{16:0}$ and $C_{18:0}$ fatty acids is significantly lower (Van Greevenbroek et al., 1996 & 2000 &2001; Field et al., 1995 & 1988). Incubation with $C_{18:1}$ and $C_{18:2}$ fatty acids also resulted in secretion of lipoproteins of a density similar to chylomicrons/VLDL, whereas, $C_{16:0}$ lipids resulted in lower triglyceride output, higher phospholipid output, and the secretion of intermediate density lipoproteins (IDL)/low density lipoproteins (LDL) (van Greevenbroek et al., 1995 & 1996). Subsequent studies revealed that the chylomicron/VLDL like lipoproteins secreted in response to incubation with unsaturated lipids contained a much larger proportion of exogenous lipid than the IDL/LDL lipoproteins secreted in response to incubation with unsaturated lipids (Van Greevenbroek et al., 2000). Interestingly, these differences were obscured when mixtures of fatty acids representative of the mixtures of fatty acid present in common dietary oils (olive oil, corn oil and cream fat) were incubated in Caco-2 cells and subsequent studies showed that the small proportion of unsaturated lipid present in these blends (and presumably in various animal fats and vegetable oils) was sufficient to stimulate secretion of VLDL/chylomicron like lipoproteins. These trends are also consistent with a previous *in vivo* examination of the lymphatic transport of palmitic acid, oleic acid and the trans-isomer of oleic acid, elaidic acid, where the authors suggested that administration of oleic acid led to re-esterification via the monoglyceride pathway and secretion of chylomicrons, whereas the fully saturated palmitic acid and elaidic acid, resulted in higher phospholipid output, in the form of smaller lipoproteins, possibly via stimulation of the α-glycerophosphate pathway (Bernard et al., 1987).

2.7.4. Effect of dispersed state

Porter et al., (1996a & b) examined the lymphatic transport of the lipophilic antimalarial, halofantrine (Hf), in both conscious and anaesthetized rat models. The calculated log P of the clinically available form, halofantrine hydrochloride (Hf.HCL) is high with a value of 8.5; the extent of lymphatic transport of Hf.HCL was low, due to the low triglyceride solubility of approximately 1mg/ml. In an attempt to increase the triglyceride solubility, the amorphous freebase of halofantrine was examined and was found to be soluble in peanut oil in excess of 50mg/ml. In the anaesthetized rat model, lymphatic transport was found to be a major contributor to the bioavailability of Hf, as demonstrated by the recovery of up to 20% of the administered dose in the intestinal lymph. The rank order effect of the vehicles for the promotion of lymphatic transport was micellar > emulsion > lipid solution supporting the contention that formulation of lipids as increasingly disperse systems may enhance lymphatic transport.

In contrast, the lymphatic transport of Hf following administration of these lipid formulations in the conscious rat model was independent of both the class of administered lipid (triglyceride or fatty acid) and the extent of formulation dispersion (micellar lipid or lipid solution). In particular, formulation of Hf in a non-dispersed lipid solution did not limit the extent of lymphatic drug transport after oral dosing to conscious rats. The authors suggested that the lower extent of lymphatic transport of Hf from the lipid solution formulation in the anaesthetized animal model may be a function of the absence of gastric processing in the anaesthetized model (drug administered intraduodenally). Supporting this contention is the similar extent of lymphatic transport of Hf from the dispersed micellar formulation in both the anaesthetized and conscious rat models. The decreased lymphatic transport of Hf from the non-dispersed formulations in the anaesthetized model may reflect a decrease in the ease and extent of processing of the lipid formulations compared with the crude emulsion that would form *in situ* after initial gastric processing by preduodenal lipases and the shear action of the stomach. Crucially, it appears that attainment of a micellar state in the duodenum, either by direct administration of micellar lipid, or via formulation in a digestible lipid formulation appears to facilitate lymphatic drug transport.

Liu et al., (1991), reported the lymphatic absorption of vitamin D3 in a Milk Fat Globule Membrane (MFGM) emulsion dosage form was 1.4 fold higher than that in the tween 80

emulsion. It was found that the MGFM emulsion was much more strongly subject to lipolysis by pancreatic lipase and that the membrane fluidity of the MFGM micro-dispersion was greater than that of the Tween 80 micro-dispersion in the presence of both taurocholate and pancreatic lipase. The results suggest that the difference between the promotion effects of lymphatic absorption of Vitamin D3 by MGFM and Tween 80 may be due to the formation of MFGM-bile salt micelles, which have a higher membrane fluidity. The results emphasise that the formation of MFGM-bile salt mixed micelles in the intestine is crucial for the promotion of lymphatic drug transport.

2.8. Other examples of targeted lymphatic drug transport from lipid based formulations

Recent reports have described a delivery system which claims to redirect appreciable quantities of drugs with little inherent affinity for the lymphatics (as estimated from a moderate lipophilicity), through the lymphatics (White et al., 1991; Barnwell et al., 1992; Barnwell & Attwood, 1996). The specific aims of these studies was to design a formulation capable of redirecting the absorption of propranolol via the intestinal lymphatics thereby decreasing first pass metabolism and increasing oral bioavailability. The initial report by White and co-workers examined the lymphatic absorption of propranolol in a pig model after oral administration. In this study, a lymph vessel near the mesenteric root was isolated and ligated in each pig thereby providing a means for determining lymph concentrations of propranolol rather than the extent of transport. A commercial immediate release propranolol formulation (Inderal®) and two test formulations containing mixtures of propranolol and bile salts (subsequent reports suggest the proprietary formulation also contains oleic acid) were compared in a parallel study in three separate pugs. Higher lymph concentrations of propranolol were detected in the first 30 minutes after administration of the test formulations compared with Inderal control and an increase in the systemic concentrations of propranolol were noted over the same time period relative to portal blood concentrations. This finding led to the conclusion that the test formulation had redirected propranolol to the systemic circulation via the intestinal lymphatics. However, as the total quantity of lymph draining from the intestine was not collected, it is not possible to unequivocally assign lymphatic transport as the basis for any enhanced oral bioavailability. The log P octanol/water of propranolol base is 3.26, which is low in comparison with other compounds that are transported to significant extents via the intestinal lymphatics.

In subsequent studies the authors have ascribed lymphatic transport of propranolol as being due to its lipophilicity, formation of an ion-pair between oleic acid and propranolol (Green & Hadgraft, 1987), and the reported hormone like effects of oleic acid in stimulating secretion of VLDL and chylomicrons (Davidson et al., 1988; Pullinger et al., 1989; Homan et al., 1991; Moberly et al., 1990). A further study conducted in 10 healthy male subjects which compared a sustained release, once a day formulation containing oleic acid and propranolol (Hepatic Avoidance using Lymphatic Output, HALO®) with the commercial sustained release propranolol formulation (Half-Inderal®) revealed a statistically significant doubling in the mean plasma AUC values after administration of this bile salt/oleic acid based formulation (Barnwell).

Hauss and co-workers investigated the intestinal lymphatic transport of CI-976, a lipophilic lipid regulator drug with poor water solubility and low bioavailability (log P 5.83) (Hauss et al., 1994). In control animals (no lymph collection), the mean plasma $AUC^{0\rightarrow 14h}$ values were 19.5μg.h/ml after administration of the emulsion formulation and 46.9μg.h/ml after administration of the suspension formulation. In lymph cannulated rats, plasma $AUC^{0\rightarrow 14h}$ values after administration of the emulsion and suspension formulation were 11.24 μg.h/ml and 19.98 μg.h/ml respectively. By comparing the plasma AUC values for control animals with animals with lymph fistulas (i.e. when the drug transported by the lymph was collected and could not contribute to overall bioavailability), the proportion of the plasma AUC value contributed by lymphatic transport was found to be significant and similar for both delivery systems (43% for the emulsion formulation and 57% for the suspension formulation). Whilst the absolute amount of drug transported through the lymph was low (<1% of dose), the percent of the administered dose recovered directly from the lymph was 7-fold greater for the emulsion formulation. Distribution studies showed that administration of the emulsion formulation resulted in a 43% greater accumulation in perianal fat as compared with administration of the suspension formulation. The authors suggested that formulation as an emulsion enhanced the lymphatic transport of CI-976, leading to entry of the drug into the plasma encapsulated in chylomicrons. Rapid redistribution of chylomicrons from the plasma to the fat subsequently explained the altered drug distribution profile and the lack of a proportionally greater contribution to the plasma AUC from the lymphatically transported CI-976 after dosing as an emulsion. These results demonstrate, particularly for compounds with low bioavailability, that small amounts of drug absorption via the lymph may have a significant impact on the total plasma AUC value, and that the proportion of the dose transported via

the lymph is formulation dependent. The authors concluded that plasma concentration-time data alone might not fully reflect relative bioavailability and pharmaco-dynamic potential of lipophilic drugs dosed in lipid vehicles.

Hauss et al., (1998) have also studied the lymphatic transport of a lipophilic, poorly water soluble, anti-inflammatory agent and potent inhibitor of leukotriene (LTB4) synthesis, ontazolast. The total amount of lymphatic transport over the 24h post dosing period was found to be 20-25 fold greater for a Peceol SEDDS formulation and 50-fold greater for a soybean emulsion formulation, when compared with an aqueous based suspension formulation. The authors reported a concurrent increase in the lymphatic transport of triglyceride lipid that mirrored the trend in lymphatic drug transport.

Myers and Stella (1992) examined the factors affecting the lymphatic transport of penclomidine, a lipophilic cytotoxic agent. The solubility in triolein was determined to be 175mg/ml and the log P calculated as 5.36. The extent of transport was lower than expected, given these physical characteristics, with 1.5% of the administered dose being recovered in intestinal lymph after 12h. The authors suggested that the high affinity of penclomidine for red blood cells may be important during 'trafficking' of the penclomidine between the enterocytes, through the interstitial fluid and eventually to the lymph versus portal blood. Although penclomidine displays highly lipophilic characteristics, and should readily associate with lymph triglyceride, the red blood cell affinity may cause a skewing or shift in the pseudo-equilibrium between chylomicron bound and unbound drug, by providing a sink towards which the drug is drained (i.e. red blood cells).

In the literature the degree of lymphatic transport is generally expressed as a % of dose administered. From a lymph targeting perspective, a relatively small extent of absolute lymphatic transport (when assessed as a fraction of absolute bioavailability) may be adequate to provide the desired pharmacological endpoint, such as site specific delivery to B- and T- lymphocytes (e.g. immunogenics, anti HIV agents). In these situations it is the concentrations achieved in the lymph, and more specifically the lymph/plasma ratio that may be a more relevant parameter. The selective delivery of the immunosuppressant, cyclosporin A, illustrates this. Takada and co-workers noted that as lymphocytes circulate mainly in the lymphatic systems in the body, the immunosuppressive activity of CsA might be related to the CsA concentration in the lymphatic system (Takada et al., 1988). A polyoxy 60 castor oil (HCO-60) micellar formulation increased the lymphatic

concentration of CsA by approximately 20 times compared to the conventional olive oil preparation (Takada et al., 1985 & 1986). Moreover, a basic pharmacological study using the rat heart transplantation study showed that the mean survival time (in day) of the transplanted rat heart was significantly longer for the new formulation with the proposed selective lymphatic transporting ability, than with the olive oil formulation (Yasumura et al., 1986; Takada et al., 1987). In order to transfer CsA selectively into thoracic lymphatic system, Yanagawa et al., (1989) administered CsA to rats at a dose of 10mg/kg orally in lipid microspheres composed of olive oil and soybean oil, egg phosphatidyl choline and glycerol. Two hours after dosing, CsA thoracic lymph concentration, when administered as lipid microspheres, was approximately 46 times greater after dosing in comparison with a conventional olive oil preparation. In a similar study, Takada et al., (1989) administered solid dispersions to rats containing CsA, (7mg/kg), surfactant (HCO-60) with 'selective lymphatic targeting capability', and an enteric coating material. The highest achieved lymph levels (7680ng/ml) occurred after administration of a solid dispersion of CsA in hydroxy-propyl methylcellulose phthalate (HP-55®). The percentage transferred into the lymphatics after 6h was approximately 2% of the administered dose.

2.9. Lipophilic prodrug approaches for enhanced lymphatic delivery

The molecular and physiochemical features of candidate compounds for lymphatic transport are restrictive due to the requirement for high lipophilicity. Therefore, the design of lipophilic prodrugs is a logical approach for the enhancement of lymphatic transport. Early attempts at increasing the lipophilicity of candidate compounds concentrated on the synthesis of simple esters by condensation with long chain fatty acids. However, the *in vivo* performances of such simple ester prodrugs generally met with mixed success and more sophisticated approaches have subsequently been examined. These 'more sophisticated' prodrugs have typically been designed to mimic key structures in lipid digestion and re-esterification processes, thereby facilitating the incorporation of the prodrug into such pathways.

A number of studies have examined ester and ether prodrugs of testosterone to enhance its lymphatic transport and bioavailability (Tauber et al., 1986; Geelen et al., 1977; Horst et al., 1976; Noguchi et al., 1985a). The steroid is widely used in the treatment of male hypogonadism and with potential utility in the treatment of postmenopausal breast carcinoma and osteoporosis. Oral testosterone therapy in humans is limited by extensive

pre-systemic metabolism, as the absolute bioavailability is approximately 4%. The extent of intestinal lymphatic transport of testosterone is also low (0.001% of dose). However the oral administration of a lipophilic ester prodrug (testosterone undecanoate) increased the extent of lymphatic transport 1000-fold and improves absolute oral bioavailability of testosterone to approximately 7% (Tauber et al., 1986). Exclusive transport of testosterone undecanoate via the mesenteric lymph was demonstrated (Horst et al., 1976). Noguchi and co-workers (1985) have shown that the extent of lymphatic transport of a series of testosterone esters in the rat was proportional to the lipophilicity of the administered prodrug, the palmitate prodrug being the most efficient in terms of enhanced lymphatic transport of total testosterone (prodrug plus free testosterone). Approximately 1.25% of the administered dose of the palmitate prodrug was transported via the mesenteric lymph over an 8h period post dose. Assuming a lymph flow of approximately 1ml/hr in the anaesthetized rat, this represents an average lymph concentration of 100-fold higher than the peak systemic blood concentration. Viewed in the context of site specific delivery, a 100-fold improvement in the concentration of drug at a target site (e.g. lymph) relative to systemic blood is highly significant (it should be noted that in this case the lymph/blood concentration ratio is helped by the extensive presystemic metabolism of testosterone), and while the majority of testosterone recovered from the lymph was in the form of the prodrug, these data demonstrate the utility of ester prodrugs to enhance the lymph/blood therapeutic ratio of prospective drug candidates, particularly if both prodrug and parent have biological activity.

The fat-soluble vitamins (A, D, E and K) are poorly water-soluble and rely almost exclusively on transport via the intestinal lymphatics for absorption (Kuksis, 1987). The major problems associated with the formulations of these vitamins are both low absorption and chemical instability. Aliphatic esters have been synthesized in an effort to both improve stability and to enhance absorption and lymphatic transport (Fernandez & Borgstrom, 1990; Nakamura et al., 1975; Gallo Torres, 1970a & b). Many of these approaches have led to enhanced vitamin concentrations in intestinal lymph after oral prodrug administration. However this is generally not a function of the prodrug directly enhancing lymphatic transport since the prodrug ester is often cleaved prior to absorption. Rather the prodrug appears to enhance drug stability and as a consequence facilitates the absorption of the parent molecules.

An aliphatic ester prodrug of indomethacin, indomethacin farnesil, synthesised to facilitate effective systemic indomethacin delivery with the possibility of reduced gastrointestinal irritation, more directly demonstrates the lymph-directing utility of ester prodrug approaches. After oral administration, the majority of the indomethacin ester was hydrolysed in the intestinal lumen and the parent drug absorbed into the portal blood. However, 12% of the indomethacin ester prodrug was transported through the intestinal lymphatics over an 8 h dose period (Mishima et al., 1990).

A recent example of the utility of ester/ether prodrugs to substantially enhance intestinal lymphatic drug transport involves mepitiostane (MP) a 17 methoxycyclopentane ether derivative of epitiostanol (Ichihashi et al., 1991 & 1992). Epitiostanol (EP) is an anti mammary tumour agent administered intramuscularly as significant first pass metabolism precludes oral administration. MP has been developed as an orally active prodrug, and the enhanced bioavailability observed after oral administration of MP was believed to be a function of decreased first pass metabolism. To probe the mechanism of first pass avoidance, Ichihashi and co-workers (1991) conducted a series of studies examining the lymphatic transport of MP and EP. In the first of these studies ^{14C}-MP dissolved in sesame oil and dispersed in 30 vols of fresh rat bile was instilled directly into the lumen of the rat small intestine ligated at the pylorus and ileocaecal junction. In one group of rats, systemic blood and thoracic lymph were collected and in control rats only systemic blood levels were monitored. In both groups, approximately 80% of the administered radioactivity was absorbed, although plasma levels of total radioactivity and unchanged MP were considerably lower in the lymph fistulated rats (where lymphatic transport could not contribute to systemic levels). Furthermore, 34% of the administered dose of radioactivity was found in the thoracic lymph in the 6 h post-dosing period with 97% of radioactivity due to parent MP. In non-fistulated rats, MP was readily detectable in plasma after oral administration of 0.5 and 5mg/kg doses of MP although plasma levels of EP were negligible. When the oral dose of MP was increased to 20mg/mg, EP was detected in whole blood and various target tissues. These observations suggest significant lymphatic transport of MP, followed by slow conversion to EP, which is subsequently rapidly cleared.

Further studies determined that greater than 90% of the lymphatically transported MP was associated with the chylomicron and VLDL lipoprotein fractions of lymph (Ichihashi et al., 1992). The presence of bile was found to exert a considerable effect on both the total

absorption and the proportion of the absorbed dose transported in the lymph. In the presence of bile, 75% of the dose was absorbed with 55% of the absorbed dose transported in the lymph. In the absence of bile only 3% of the total dose was absorbed with only 5% of this amount being transported in the lymph. These data confirmed the generally held view that lymphatic transport occurs in tandem with efficient in vivo drug solubilization stimulated by bile secretion.

Due to the metabolic lability associated with simple ester-based pro-moieties, other functional approaches have been explored as a means of enhancing lymphatic transport. The most widely used approach in the design of prodrugs has been to target the lipid digestion pathway using monoglyceride mimics. Garzon-Aburbeth et al., (1983 & 1986) assessed the utility of diglyceride prodrugs of L-Dopa and chlorambucil. The oral bioavailability of L-Dopa is low and variable due to first pass metabolism and this led to the suggestion that oral bioavailability would benefit from reduced pre-systemic clearance afforded by significant lymphatic transport. The glyceride pro-drug comprises a glycerol backbone with palmitic acid substituted in the 1- and 3- positions and l-Dopa substituted in the 2-position. The rationale for the prodrug is that the fatty acids in the 1- and 3-positions are cleaved during lipid digestion leaving the 2- substituted L Dopa derivative, as a 2- monoglyceride mimic, which is absorbed and incorporated in to the TG re-synthesis pathway. After oral administration of the L-Dopa, only 2% of the administered dose was transported via the intestinal lymphatics (as either L-Dopa or dopamine). In contrast, after oral administration of the l-Dopa diglyceride prodrug, 8.3% of the administered dose was present in the lymph as the diglyceride prodrug with a further 14.9% of the administered dose present as related glycerides. Although the pharmacokinetic data reported for the l-Dopa diglyceride prodrug were limited, the increase in lymphatic transport translated to an approximate doubling of the area under the L-Dopa plasma concentration time profile compared with the oral administration of L- Dopa.

In contrast to L-Dopa, the oral bioavailability of chlorambucil is high. However, its therapeutic utility for the treatment of Hodgkins disease and other lymphomas would likely benefit from specific delivery to the lymphatics and this promoted assessment of a diglyceride prodrug. After oral administration, a significant amount of the 1,3-diglyceride prodrug of chlorambucil (26%) was transported through the lymph whereas only 3.4% of the parent molecule was detected in the lymph after oral dosing of the prodrug. In an efficacy study conducted in mice, the diglyceride prodrug increased the survival times of

mice with a tumour model of disseminated lymphatic leukaemia relative to chlorambucil. As the pharmacokinetic data suggested limited conversion of the prodrug to the parent compound within the lymph, it appears that chlorambucil prodrug may have inherent activity in contrast to the classical prodrug model where activity requires liberation of the parent drug (Garzon-Aburbeh et al., 1983).

The most recent approach to 'functionally derived' prodrugs has targeted the phospholipid re-esterification pathway. Phospholipids are hydrolysed in the intestinal lumen to lyso-phospholipids, which after absorption by the enterocyte, are reacylated prior to their central role in the stabilisation of the lipoprotein surface. The targeted lymphatic delivery of fluorouridine, an anti-tumour agent, has recently been described utilising the dipalmitoylphosphatidylfluorouridine (DPPF), a phospholipid prodrug (Sakai et al., 1993). After oral administration of DPPF, analysis of thoracic lymph revealed the presence of DPPF, the parent fluoururidine and two fatty acid congeners of DPPF. These were subsequently identified as 1-palmitoyl-2-arachidonoylphospatidylfluorouridine (PAPF), and 1-palmitoyl-2-linoleoylphospatidylfluorouridine (PLPF), where the palmitic fatty acid in the 2 position of DPPF has been substituted with arachidonic acid and linoleic acid, respectively. The profile of the fluorouridine-related compounds is consistent with the reacylation of lysophospholipid in the enterocyte. The lymph concentration of the DPPF-related congeners was approx 30-fold greater than the corresponding plasma concentrations, indicative of the specificity of transport into the lymphatics. Unfortunately the bioavailability and the extent of lymphatic transport of DPPF or fluorouridine were not reported.

Prodrugs are a useful means for imparting the necessary molecular characteristics to promote lymphatic transport. Whilst simple ester prodrugs have the capacity to increase partition coefficient and triglyceride solubility, these structures are often metabolically unstable which limits their utility. The use of monoglyceride or triglyceride mimics, and more recently phospholipid mimics, appear to hold promise for selective lymphatic delivery. Unless there is intrinsic biological activity of the prodrug itself, the lability of the prodrug linkage (from either triglyceride, phospholipid or other prodrug strategies) must be sufficient to liberate the active parent molecule at the site of action.

2.10. Models to study intestinal lymphatic transport

A number of animal models have been described for the assessment of intestinal lymphatic drug transport and these have been recently reviewed (Edwards et al., 2001). Lymphatic transport studies are commonly first conducted in the laboratory rat, with larger more complicated models (such as dog, sheep, or pig) subsequently investigated, if warranted. However the utility of lymph fistulation in large animals is limited by considerable logistical and economic constraints. As lymphatic transport can be affected by experimental factors such as the site of cannulation and the period of fasting prior to dosing (Charman et al., 1986a; Porter & Charman, 1997), it is important to standardise procedures when comparing between studies. Ideally sampling strategies for lymphatic transport studies should provide for capacity to estimate both the extent of lymphatic transport, as well as the extent of portal blood absorption, in order to estimate the relative influence of each transport route on systemic bioavailability of the drug/formulation. In this regard, it is reasonable to assume that systemic plasma concentrations of drug in the lymph-fistulated rat reflect absorption of drug via the portal blood (Edwards et al., 2001). Therefore, the contribution of portal blood absorption to bioavailability can be determined by comparison of the relative areas under the concentration-time profiles obtained in lymph-fistulated rats and in a parallel group of sham operated animals with mesenteric lymph intact.

The mesenteric lymph duct cannulated model has been widely used for the assessment of lymphatic transport since validated methodologies in both anaesthetized and conscious animals are available. The mesenteric intestinal lymphatic duct, rather than the traditionally used thoracic lymphatic duct, is the preferred site of cannulation to estimate the ability of the small intestine to lymphatically transport lipophilic molecules (Noguchi et al., 1985a). General anaesthesia precludes oral dosing in the anaesthetized model and consequently drug and lipid formulation are administered intraduodenally. This circumvents the inherent emulsifying action of the stomach, the potential effects of lipids on gastric emptying, but also presents the opportunity to probe some of the mechanistic aspects of lymphatic transport under controlled experimental conditions. However, the conscious rat model best represents the *in vivo* situation in terms of both lack of anaesthetic effects and the ability to orally administer drug formulations. Major drawbacks with the use of restraining cages include the inability of the investigator to evaluate the effect of induced stress on the experimental animal, which is especially crucial when studying the

gastrointestinal absorption profile of a particular drug/formulation, and to easily sample other body fluids and tissues. Anaesthesia may well have an effect on the lymphatic transport of the model compounds; however it would at least be expected that the same trends exhibited in data collected from the cannulated anaesthetised animal would apply to the conscious animal (Porter & Charman, 1997).

The advantages of lymphatic transport studies performed in rats are that they are robust, inexpensive and straightforward in regard to drug administration and sampling. The average success rate is approximately 70% (Edwards et al., 2001) when a trained operator is employed. The limitations of lymphatic transport data from rats however should not be overlooked. In the rat, bile flow is continuous and independent of food intake, whereas in higher species, food or lipid is required to stimulate the numerous digestion related processes. Therefore, it is difficult to obtain pre- and post-prandial intestinal environments in the rat that are more reflective of the situation in humans. From a drug delivery standpoint, the small size of the rat also precludes the administration of full size, human dose forms and therefore limits the examination of lymphatic transport after dosing of realistic volumes of lipid.

In an attempt to overcome the limitations associated with the rat model and to provide a more accurate assessment of the modes of absorption of lipophilic drugs a number of models have been described using higher animals. A conscious pig model has been described, which allows for sampling, but not collection, of thoracic lymph, and therefore the absolute mass of drug transported in the lymph cannot be calculated (Jensen et al., 1990). A sheep model has also been developed, which samples thoracic lymph (Charman et al., 2000). However given the vast differences in the gastrointestinal profile of ruminants compared to monogastric species, the use of this animal model is likely to be limited to parenteral studies.

Khoo et al., (1999a) have recently described a robust, conscious dog model of intestinal lymphatic transport that allows for administration of prototype human dose forms in representative pre- and postprandial states. The model also allows sampling of both portal and systemic blood. The publication of this model has generated much interest, not only as it was the first of its kind to estimate the contribution of lymphatic transport of drugs in simulated fed and fasted states but by also allowing the potential for enterocyte metabolism to be evaluated by sampling portal blood. After postprandial administration of a 100mg Hf

in a nonlipid dose form, 54% of the administered dose was transported lymphatically over a 5h post dosing period. Previous lymphatic studies conducted in fasted conscious rats demonstrate ~10% of an oral dose of 2mg Hf in 50μl of lipid was transported via the lymphatics over the initial 4h period, with the extent of transport being dependent on the administered lipid and formulation state. This new dog model data indicate that intestinal lymphatic transport was more extensive and rapid than previously observed in rats, and that the lymphatics were the major absorption pathway for Hf after postprandial administration. As expected, lymphatic transport was much lower (1.3% of the dose) after administration of a non-lipid dose of Hf to fasted dogs, presumably because of a lack of adequate lipid source. The preliminary dog data therefore, suggest that intestinal lymph transport may be quantitatively more important absorption route for highly lipophilic drugs than previously assumed based on rat data. Hence a broader set of 'transport criteria' for lipophilic drugs and lipid formulations may need to be adopted regarding likely contribution of lymphatic transport to oral bioavailability.

Many of the principle advances in the understanding of drug delivery processes in recent years have come from a wider appreciation of the role of membrane transporter systems and intracellular drug processing in general. This increased understanding has in large part been driven by the application of cell culture systems and genetically modified animal models, the development of which has allowed isolation and examination of specific transporters, enzymes etc. Many of the biochemical and metabolic features of lipid processing *in-vivo* have been shown to exist in Caco-2 cells. Compared to the small intestine enterocyte Caco-2 cells show uptake of fatty acids, followed by esterification to form triglycerides (Trotter & Storch, 1993; Trotter et al., 1996). They synthesize and secrete similar apoproteins (apo), which are essential for normal assembly, secretion and catabolism of intestinal lipoproteins (Field et al., 1995; Levy et al., 1995). Microsomal triglyceride transfer protein (MTP), which functions in the transfer of lipids from their site of synthesis to interact with apoB during lipoprotein production, has been identified and characterised in Caco2 cells (Mathur et al., 1997; Van Greevenbroek et al., 1998). In the presence of fatty acids, triglyceride rich lipoproteins of similar size and density to those synthesized and secreted in human intestine, are produced by cells (Field et al., 1988; Van Greevenbroek et al., 1996 & 2000). The application of models such as Caco 2 cell line has already significantly increased our understanding of the processes of lipoprotein assembly within the enterocyte (Hussain, 2000), and it is likely that progress in this direction will

lead to a much improved understanding of the effect of different lipid excipients on the absorption of lipophilic drugs (O' Driscoll, 1998).

The potential merits of enhanced intestinal lymphatic transport have thus been summarised. In the past, there are relatively few examples whereby the extent of intestinal lymphatic transport is a major determinant of drug bioavailability. However more examples of extremely lipophilic, highly potent molecules are appearing in the literature and there is an increased awareness that quantification of drug concentrations in the lymph may provide a more accurate indication of drug concentration at the site of action than routine drug concentrations in the plasma. Further progress into understanding the mechanisms involved in and the effect of formulation on intestinal lymphatic transport is needed, with a view to designing more effective lymphotropic delivery systems

3. Lipid based vehicles for oral delivery

3.1. Introduction

Natural and synthetic lipids have generated much academic and commercial interest as a potential formulation strategy for improved oral bioavailability of poorly water-soluble drugs. When administered in traditional solid dose-formulations, these compounds often exhibit low bioavailability as their absorption can be kinetically limited by low rates of dissolution and capacity limited poor solubility. A drug is classified as 'poorly soluble' when its dissolution rate is so slow that dissolution takes longer than the transit time past its absorptive sites, resulting in incomplete bioavailability (Amidon et al., 1995; Horter & Dressman, 1997).

The well-known effect of food for improving the bioavailability of many poorly soluble drugs, where the enhanced absorption is ascribed to the ingested lipid, is ample evidence of the beneficial role that lipids can have on drug absorption. Although the form, content and volume of such dietary lipids is markedly different to what would be included in a pharmaceutical formulation, food effect bioavailability data can be viewed as offering a prospective approach for the design of superior formulations for such drugs.

Lipid formulations can reduce inherent limitations of slow and incomplete dissolution of poorly soluble drugs, and facilitate the formation of solubilized phases from which absorption can occur. The attainment of a solubilized phase will not necessarily arise directly from the administered lipid, but most likely from intra-luminal processing to which lipids are subjected prior to absorption. Therefore an understanding of digestion and the manner in which the endogenous components contribute to drug solubilization is central to rationalizing the design of lipid formulations.

The co-administration of lipids with drugs can also impact on their absorption path. Although most orally administered compounds gain access to the systemic circulation via the portal blood, some highly lipophilic compounds are transported to the systemic circulation via the intestinal lymphatics. Where intestinal lymphatic transport contributes to oral bioavailability, the characteristics of the co-administered lipid significantly influence the extent of drug absorption.

Lipids offer versatility for drug administration as they can be formulated as solutions, suspensions, emulsions, micelles, self-emulsifying systems and microemulsions. From a practical and aesthetic point of view, lipids are ideally prepared as unit dose forms such as sealed hard or soft gelatin capsules. Consequently, much attention has focused on lipid solution and emulsion pre concentrates, which can be prepared physically as stable formulations suitable for encapsulation.

The gastrointestinal tract impacts on the performance of lipid based formulations as triglyceride lipids (and derivatives thereof) are quantitatively and efficiently hydrolysed prior to absorption. In the fed state, the presence of lipid digestion products induces secretion of biliary and pancreatic fluids which dramatically alter the luminal environment of the small intestine. The lipid digestion products are solubilized within the bile salt micelles for their subsequent delivery to the absorptive cells of the gastrointestinal tract. The process of lipid digestion is dealt with in greater detail in Chapter 2.

3.2. Role of bile salt solubilization in the absorption of lipid products

Bile plays a central role in the solubilization of lipid digestion products and poorly water-soluble drugs. Lipid digestion products in the intestine promote a cholescystokinin-mediated contraction of the gald bladder and relaxation of the sphincter of Oddi, leading to the expulsion of bile, with peak flow occurring 30 minutes after ingestion of a meal. The bile salts are categorised into primary salts (sodium and potassium salts of cholic and chenodeoxycholic acid) which are synthesized in the liver, and secondary salts which are the metabolic products of the above two produced by colonic bacteria (deoxycholic and lithocholic acid respectively) and reabsorbed into the liver. The salts exist as glycine and taurine conjugates.

Typical bile concentrations of bile salts in the fasted intestine are 4-6mM compared with postprandial concentrations of 10-20mM (Rautureau et al., 1981). Hofmann and Borgstrom (1962 & 1964), are credited with much of our current understanding of the micellar solubilization and uptake of dietary lipids, who discovered the importance of micellar solubilization in the uptake of lipid digestion products by enterocytes. This concept was furthered by Carey and associates (Carey et al., 1983; Staggers et al., 1990) who discovered the co existence of unilamellar vesicles with bile salt-lipid mixed micelles in the small intestine. They proposed that as lipolysis proceeds, digestion products 'bud

off' from the surface of the crude emulsion that forms after gastric processing, to form large liquid crystalline structures, which in the presence of sufficient bile salt concentrations, form multilamellar and unilamellar structures. The intestinal mixed micellar phase initially described by Hoffman and Borgstrom is now thought to exist in a two-phase equilibrium system with unilamellar lipid vesicles (Tso, 1994).

3.3. Effect of lipids on intestinal permeability

Constituents of the mixed micellar phase may impact on the intestinal permeability of poorly water-soluble compounds via three mechanisms. Firstly, the presence of lipid digestion products and bile salts may alter the intrinsic permeability of the intestinal membrane leading to increased absorption via the paracellular or transcellular. The effect of bile salt mixed micelles on increasing the intestinal permeability of DDT (Obodozie, 1997), or clofazimine (O' Reilly, 1991) in rat intestine and dextromethorphan (Meaney, 1997) in cell culture models has been recently explored. Secondly, solubilization of lipophilic drugs with in the bile salt may facilitate diffusion through the aqueous diffusion layer leading to increased absorption. Thirdly, and conversely, drug solubilization may decrease the intercellular 'free' fraction of drug, which could potentially lead to a decrease in absorption. These divergent effects are the basis for the often confusing and contradictory reports concerning drug absorption after administration as solubilized surfactant based systems.

The inherent permeability enhancing abilities of various bile salts, fatty aids and monoglycerides in the gastrointestinal tract are well known (Anderberg et al., 1993; Aungst, 1993; Swenson et al., 1994). When the profile of many of these naturally occurring 'permeability enhancers' is considered, it is apparent that the intestinal mucosa is frequently subjected to dietary-induced damage and that mechanisms have evolved for rapid repair (Curatolo & Ochoa, 1994). Kvietys et al., (1991) studied the role that lipolytic digestion products play in causing mucosal injury. They concluded that damage to the intestinal epithelium by the products of lipid digestion may be a common occurrence during the normal digestive and absorptive process associated with lipid processing.

3.4. Effect of lipids on physiological membrane function

Recent work has led to the discovery of counter transport efflux proteins known as P-glycoprotein which expel specific drugs back into the lumen of the gastrointestinal tract after absorption has taken place (Benet et al., 1996). Identification of these proteins has led to any investigations to identify drug substances for this route and subsequent studies to inhibit this excretory mechanism (Wu et al., 1995; Thummel et al., 1996). Several studies have examined the effect of lipid derivatives to inhibit these transport mechanisms (Nerurkar et al., 1996 & 1997; Dintaman & Silverman, 1999; Buckingham et al., 1995). Nerurkar et al., (1996) studied the inhibitory properties of commonly used surfactants, Cremophor and Tween 80 to enhance permeability of peptides in Caco-2 cell cultures exhibiting P-gp like activity. Permeability coefficients increased approximately 2.7 fold in the presence of 0.1% cremophor EL indicating that the efflux mechanism can be blocked by the actions of the surfactant. It has also been reported that the plasma concentrations of cremophor EL in patients administered paclitaxel, which is formulated with this surfactant, reach sufficient levels to inhibit P-gp *in vitro* (Webster et al., 1993)

3.5. Lipid formulations and oral bioavailability

Lipid based dose forms encompassing a wide variety of compositional and functional characteristics have been advantageously utilised for the formulation of lipophilic drugs, as reviewed by Humberstone & Charman (1997). In this present review, emphasis will be given to drug absorption from oils, emulsions and microemulsions systems. Particular emphasis will be made of self-emulsifying drug delivery systems (SEDDS). Table 3.1 compares some of the physiochemical characteristics of micelle, microemulsion and emulsion formulations.

Table 3.1 Physiochemical characteristics of micelles, microemulsions and emulsions

Characteristics	Micelles	Microemulsions	Emulsions
Optical aspect	Transparent	Transparent	Milky
Droplet size	2 to 10nm	10 to 200nm	< 5,000nm
Thermodynamic stability	+	++	-
Dilution	-	++	+
Surfactant	Above critical micellar concentration	20-80%	<15%

3.5.1. Drug absorption from oils

The use of oily vehicles as a means by which to administer drugs to the gastrointestinal tract is well recognised. Hargrove et al., (1989) reported a significantly enhanced oral absorption of micronised progesterone when administered as a suspension in a long chain fatty acid vehicle. The formulation increased the peak progesterone plasma concentrations 2.3 fold compared to when administered as a micronised powder without any lipid. Similarly, serum concentrations of phenytoin were approximately 50% greater in rats administered a corn oil suspension than when administered from an aqueous suspension (Chakrabarti & Belpaire, 1978). However, this improvement in bioavailability was lost when the drug was administered in a non-micronised form as a lipid suspension, suggesting that size reduction facilitated the dissolution and solubilization processes. Abrams et al., (1978) investigated the dose dependence of a lipophilic steroid derivative in sesame oil. As the concentration of the drug exceeded its solubility in the oil, the proportional linearity of drug absorbed ceased. These results suggest drug absorption is related to the amount of drug dissolved in the oil phase. A further increase in drug loading above the saturated concentration levels resulted in solid drug particles, which were then subject to the dissolution process.

A number of studies have examined the influence of the nature of the oil on drug uptake from lipid vehicles. For example, Palin et al., (1982) compared plasma concentrations of DDT after oral administration in arachis oil, Miglyol 812, liquid paraffin, and as a suspension in water. The authors reported marked differences in the absorption profile, with the AUC for the arachis oil formulation significantly higher than the other formulations, while also producing the highest extent of lymphatic transport. The authors suggested that the observed effects may be associated with the nature of the oil. Liquid paraffin is composed of non-digestible hydrocarbons; hence drug absorption may be diminished due to entrapment of the DDT within the oil droplets and, as such, remain unabsorbed. Arachis oil is metabolised to primarily long chain fatty acids, which form mixed micelles in the intestine, resulting in increased solubilization capacity. The potential for enhanced permeability and stimulation of lymphatic transport due to liberation of long chain fatty acids was also proposed. Miglyol is digested to the more water soluble medium chain length fatty acids which are primarily absorbed by the portal route, and as such do not stimulate chylomicron production.

3.5.1.1. Role of digestibility of oil

Much debate has centred on role of digestibility of the lipid vehicle. Non-digestible lipids such as mineral oil (liquid paraffin) and sucrose polyesters essentially remain unabsorbed in the intestinal lumen, and can actually limit drug absorption by retaining a proportion of the co-administered drug (Palin & Wilson, 1984; Volpenhein et al., 1980; Rozman et al., 1983). Digestible lipids consist of dietary lipids (glycerides, fatty acids, phospholipids) as well as various synthetic esters. The vehicles are susceptible to the normal digestive processes, as previously described, and the rate and extent of digestion, the colloidal phases formed and the physiological effects of the digestion products formed can all potentially impact on the absorption of co administered drug.

Yamahira et al., (1979) compared the oral absorption of the drug SL-512 from an easily digested medium chain triglyceride and a synthetic, poorly digested lipid, MBLA (N-α-methylbenzyllinoleamide); the drug partition coefficients with respect to the two lipids were similar. The medium chain triglyceride formulation showed a fourfold increase in maximum serum blood concentrations compared to the MLBA, indicating that lipid digestibility was a crucial factor influencing drug uptake. Similar results were obtained when the formulations were given intraduodenally, indicating that alterations in gastric emptying were not responsible for the effect. The authors also showed using a recirculating intestinal perfusion experiment that absorption did not take place directly from the lipid formulation but that lipid digestion was a prerequisite to drug absorption. In support of this, Ritschel et al., (1990 & 1991) demonstrated that bile duct ligation decreased absorption of cyclosporin from long chain fatty acid ester microemulsions, thereby indicating the dependency of cyclosporin absorption on bile secretion and also that lipid digestion and mixed micelle formation are necessary for drug absorption to proceed.

Myers & Stella (1992) investigated the effects of non-digestible and digestible lipid vehicles on the oral bioavailability of penclomidine. The bioavailability was greatest from the medium chain triglyceride vehicle, followed by the long chain triglyceride vehicle and then the mineral oil formulation, with this rank order corresponding to the digestibility of the lipids. The authors also found that the short chain triglyceride formulation gave poor bioavailability results despite an expected rapid digestion of these oils. It was suggested that rapid dispersion and dissolution of the digestion products, resulted in precipitation of drug in the intestine, and as such drug dissolution may be limited suffering a similar fate as

the aqueous suspension formulation. It was also suggested that the lipid digestion products of the medium and long chain triglycerides result in the formation of mixed bile salt micelles with superior solubilization capacity. These studies highlight the importance of both promoting and maintaining drug solubilization when formulating with a digestible lipid formulation.

3.5.2. Drug absorption from emulsions

Emulsification of a lipid vehicle involves a reduction in droplet size usually by the addition of surfactant(s) and an external energy input. An emulsifier will act as a stabilizer of the droplet form of the internal phase. In the absence of an emulsifier, the droplets quickly coalesce and the liquids separate on cessation of the applied agitation. It is logical to assume that as the normal process of digestion converts a lipid mass to a highly disperse emulsified system (utilizing natural surfactants such as bile salts), formulation as an emulsified lipid vehicle may facilitate drug absorption. An increase in surface area as a result of reduction in droplet size will allow a more efficient partitioning of the drug between disperse and continuous phase, and also allow an increase in lipase activity resulting in a rapid formation of mixed micelles. Exogenous surfactants not only serve to promote the emulsification action of endogenous surfactants, but they may also exert influences physiological function in the intestine.

The hydrophobicity of a lipid surfactant can be described by the hydrophile/lipophile balance (HLB) number. This value describes the balance between the hydrophobic and hydrophilic portions of the molecule. A lower HLB number indicates a more lipophilic substance and a higher value a more hydrophilic substance. This parameter is therefore governed by the degree of unsaturated carbon bonds, the fatty acid chain length and the size of the PEG group.

In a continuation of the study by Myers & Stella (1992), the effect of emulsification on blood levels of penclomidine was examined. Emulsified and non-emulsified formulations containing either trictanoin – a digestible oil – or light mineral oil – a nondigestible oil - were compared. For both emulsion formulations, the blood levels of penclomidine were higher compared to non-emulsified formulations. Given the indigestibility of mineral oil, this improvement was ascribed to a particle size effect, while the further improvement in

blood absorption reflects the beneficial effects of both digestibility and reduced droplet size.

Similar results were also observed after administration of griseofulvin in either a solution or emulsion formulation of corn oil to rats (Carrigan & Bates, 1973). Tarr and Yalkowsky (1989) investigated the effect of emulsion droplet size on the absorption characteristics of cyclosporin. Two emulsion were prepared from the commercial Sandimmune® formulation, either by stirring (4μm) or by homogenization (2μm). Cyclosporin blood concentrations were 1.7 times greater for the homogenized emulsion. These results demonstrate the importance of the physical structure of the lipid emulsion.

Reductions in inter and intra-individual variability has also been attributed to a reduction in droplet size (Charman et al., 1993; Kovarik et al., 1994). It may be speculated that an emulsified preparation presents the drug in a more uniform manner (Charman et al 1993). Fed and fasted conditions have also been reported to affect the absorption profile of drugs prone to dissolution rate limited absorption when administered in conventional solid dose forms. Emulsified formulations present the drug in a readily absorbable form and have thus been found to reduce the extent of the food effect (Charman et al., 1993; Mueller et al., 1994), by acting as the lipid load analogous to the fed state.

3.5.3. Drug absorption from microemulsions

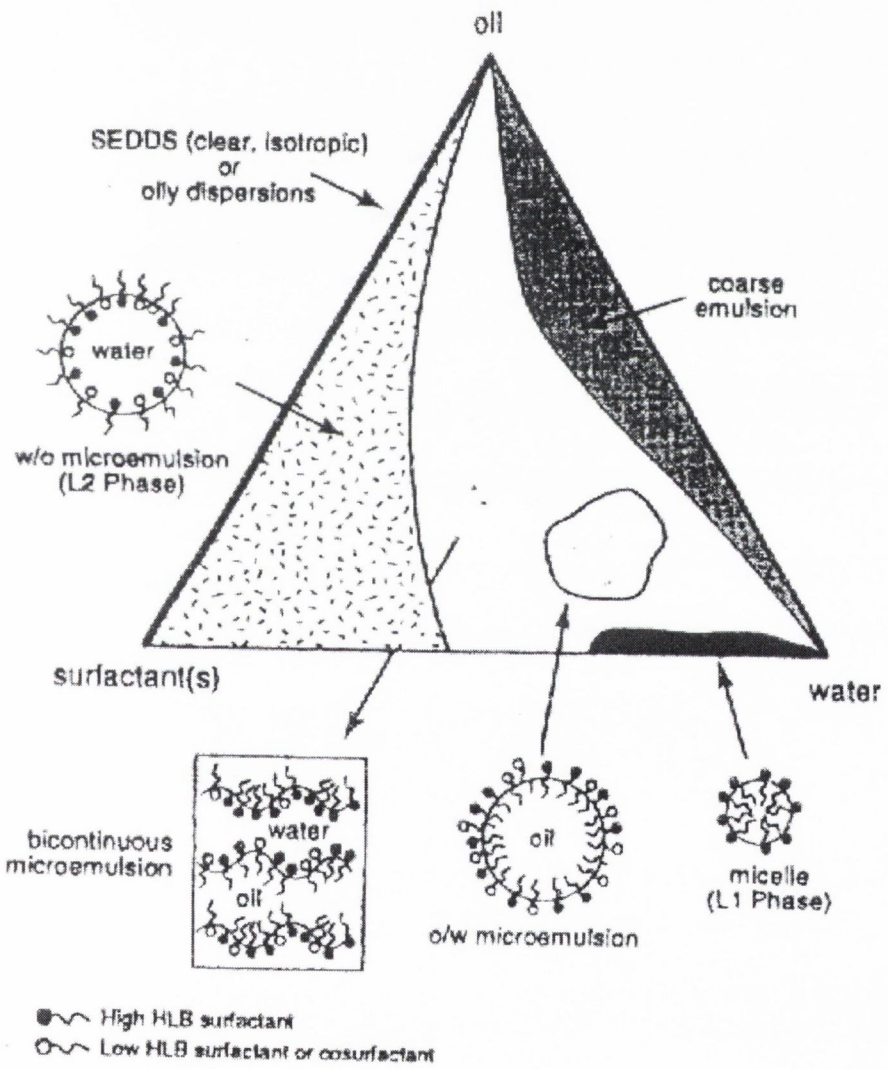
A microemulsion is defined as a quaternary system containing a unique ratio of components including a lipophilic phase, a hydrophilic phase, a surfactant and a co-surfactant (Bhargava, 1987). Main characteristics of this system are low viscosity, isotropicity, thermodynamically stability and organised structures of less than 200nm. Microemulsions appear to be valuable drug delivery systems because of a high solubilising power for some poorly soluble drugs (Lawrence & Rees, 2000).

Microemulsions actually have little in common with classical two-phase emulsions. They are essentially stable, single-phase swollen micellar solutions rather than unstable two-phase dispersions. The dividing line between the size of a swollen micelle (approximately 10-40 nanometers) and a fine emulsion droplet (approximately 100-5,000 nanometers) is not well defined. Another important difference concerns their appearance; emulsions are

cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not. The latter point has obvious implications when considering the relative cost of commercial production of the two types of system.

Microemulsions are usually formulated empirically, though there are some useful guidelines, which have emerged by characterization of the properties of successful formulations (Constantinides, 1995). Their formulation involves a combination of three to five components: an oil phase, an aqueous phase, a primary surfactant and in many cases a secondary surfactant and an electrolyte. These isotropic systems are usually more difficult to formulate than ordinary emulsions because their formation is a highly specific process involving spontaneous interactions among the constituent molecules. Any change in either the components or their relative concentrations in the microemulsion formulation can lead to a change in the nature of the system formed or even in failure of a microemulsion system to form. The selection of components and the concentrations in which these should be present, is determined by the use of a phase diagram. From the phase diagram the extent of the microemulsion region can be identified and its relation to other phases established. The microemulsion region is initially delineated by its isotropic nature and low viscosity. Other regions of the phase diagram can be identified by their characteristic optical structures visible in plane polarized light, or their x-ray diffraction patterns (Swarbrick et al., 1994). Figure 3.1 depicts a hypothetical psuedo-ternary phase diagram.

Figure 3.1 A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phases. Within the phase diagram existence fields are shown where conventional micelles (L1 phase), reverse micelles or w/o microemulsion (L2 phase) and o/w microemulsions are formed, along with bicontinuous microemulsion and coarse emulsion phases. In the absence of water, oil-surfactant blends can be either clear isotropic solutions (SEDDS) or oily dispersions depending on the nature of the oil and surfactant and the oil-to-surfactant ratio. (Adapted from Constantinides 1995).



Microemulsions have generated considerable interest over the years as potential drug delivery systems, with reports of increased absorption, improved clinical potency and potentially decreased toxicity according to literature (Lawrence, 1996; Constantinides, 1995; Attwood, 1994; Sarciaux et al., 1995). The existence of microdomains of different

polarity within a single-phase solution has also led to applications for the delivery of hydrophilic compounds e.g. peptides (Constantinides et al., 1995). Peptide drugs have been successfully formulated into the dispersed aqueous phase of a w/o microemulsion droplets where they are afforded some protection from enzymatic degradation when administered orally (Sarciaux et al., 1995). In addition, the presence of lipid surfactants may serve to increase membrane permeability thereby increasing drug uptake (Constantinides et al., 1996).

Constantinides et al., (1994 & 1995) reported on the formulation and intestinal absorption enhancement of Calcein and an RGD peptide in rats from w/o microemulsions of different composition and particle size. The formulations used in those studies which incorporated non-ionic lipids and surfactants, demonstrated that improved absorption was dependent on the lipid composition of the microemulsion, particularly on the presence of medium chain triglycerides (mono-, di-, and tri-glycerides). In a subsequent study (1996), the evaluated w/o microemulsion, in addition to containing medium chain glycerides, contained ionic lipids in the form of medium chain fatty acids (e.g. caprylic acid). Upon intraduodenal administration in the anaesthetized rat, the absorption of Calcein was improved from 2% in aqueous solution up to ~37% in w/o microemulsions. The absorption enhancement activities of the fatty acids followed the order $C_8 \approx C_{10} > C_{12}$. The results indicate: a) in pseudo-ternary phase diagrams the microemulsion existence field was significantly modified by the presence of ionic lipids; b) there was a significant contribution to the observed absorption enhancement of Calcein by medium chain fatty acids with the C_8/C_{10} fatty acids being most effective; and c) absorption enhancement of Calcein was significantly reduced in the absence of low levels of C_8/C_{10} mono-/di-glycerides. Interestingly a w/o microemulsion which could be converted to an o/w microemulsion upon dilution with excess water (≥ 20-fold), having an effective droplet size of 73nm and a polydispersity of 0.364, was identified. A series of other w/o microemulsions upon dilution of excess water produced conventional o/w emulsions based on visual observation. Whether or not upon dilution with excess water the w/o microemulsion is inverted to an o/w microemulsion or a conventional emulsion did not appear to affect the *in vivo* performance as there were no observed differences in regard the extent of bioavailability enhancement. It must be remembered however that in this study the aim was to improve the absorption of water-soluble compounds.

Microemulsions therefore confer advantages on oral drug absorption on two fronts: firstly, the lipids present in the formulation may enhance drug absorption and solubility in a similar fashion as typical oily vehicles, the mechanisms of which are outlined above. Secondly, the higher concentrations of surfactants may exert an effect on permeability, by disrupting the cell membrane (Swenson & Curtalo, 1992). Surfactant monomers are capable of partitioning into the cell membrane where they form polar defects in lipid bilayer. At high surfactant concentrations in the cell membrane, surfactant-surfactant contact occurs, and the membrane can be dissolved into surfactant-membrane mixed micelles. In general, surfactants which are too hydrophobic to be water soluble, are poor enhancers, whereas surfactants that are very hydrophilic cannot partition into the hydrophobic domains of the lipid bilayer. In agreement with these observations, (Crison & Amidon, 1999) have reported a trend in the improvement of the bioavailability of nifedipine in dogs using a high surfactant (Labrasol HLB 14) over that of a surfactant with a low HLB (lauroglycol HLB4), even though both formulations appeared to solubilise the drug to the same extent. In this study a five-fold increase in bioavailability with Labrasol and a three-fold increase with lauroglycol were observed compared to a powder formulation. However, no general correlation exists between the extent of absorption enhancement and the HLB value of the surfactant (Miyamoto, 1983)

3.6. Self-emulsifying drug delivery systems (SEDDS)

The dispersibility of the administered lipid and drug impacts on the extent of drug absorption from lipid vehicles. However, the use of conventional oil, emulsions and microemulsions, as described above, is limited due to inherent physical stability, large volume of the formulation and poor precision of dose. An approach for avoiding these problems is the use of Self-emulsifying drug delivery systems (SEDDS). Self-emulsifying drug delivery systems (SEDDS) are mixtures of oils and surfactants, ideally isotropic, sometimes including cosolvents, which self-emulsify on contact with aqueous phases, under conditions of gentle agitation, similar to those which would be encountered in the gastrointestinal tract. Hydrophobic drugs can often be dissolved in SEDDS allowing them to be encapsulated as unit dosage forms for peroral administration. These formulations can be formulated into soft gelatin capsules allowing precise and convenient unit dose forms. When such a formulation is released into the lumen of the gut it disperses to form a fine emulsion, so that the drug remains in solution in the gut, avoiding the dissolution step which frequently limits the rate of absorption of hydrophobic drugs from the crystalline

state. Generally this can lead to improved bioavailability, and/or a more consistent temporal profile of absorption from the gut (Pouton, 1997).

The flagship product Sandimmune®, which generated much of the interest in SEDDS, is composed of oil, ethanol and labrafil in a ratio of 40:18:42 with 100mg/ml cyclosporin, and forms a crude emulsion on gentle mixing with water (Vonderscher & Meinzer, 1994). A new lipid based formulation of cyclosporin, Neoral®, which contains corn oil, gelatin, glycerol, dehydrated ethanol and Labrafil M21125CS, has been described either as a pre-microemulsion concentrate or self-microemulsifying drug delivery system (SMEDDS). In this case the lipid blend forms a fine o/w microemulsion on dilution. In a study by Meinzer et al., (1995) the droplet size distribution of Sandimmune® and Sandimmune Neoral® was determined by means of dynamic laser light scattering. The range for Sandimmune was determined as 1,000-5,000 nanometers with a mean of 1,243.1 and a polydispersity value of 0.561. Neoral had a particle size range of 20-50 nanometers with a mean of 25.8 and a polydispersity value of 0.038. The Neoral® microemulsion pre concentrate was shown to increase the oral bioavailability of cyclosporin approximately 2 fold, compared with the Sandimmune® capsule, while also reducing inter and intra subject variability (Kovarik et al., 1994). It is very likely that the difference between these two soft gelatin oral formulations may be due at least to some extent to the fact that the Neoral composition leads to microemulsion formation. This should allow better solubilization and hence dissolution and absorption of the cyclosporin. Furthermore, an increased post prandial bioavailability of cyclosporin when administered as Sandimmune® is reported, suggesting that the drug absorption from the crude emulsion was dependent on both on *in vivo* digestion and dispersion in the intestine. In contrast, the relative lack of effect of a fatty meal on absorption of Neoral® indicates that this formulation may act independently of intestinal digestion (Mueller et al., 1994). The absorption of cyclosporin was also less dependent on bile levels when administered in the Neoral formulation (Trull et al., 1995).

There have been numerous reports outlining improvements in bioavailability when drugs are administered in SEDDS compared to conventional dosage forms (Table 3.2). Kommuru et al., (2001) reported a two-fold increase in bioavailability of coenzyme Q10 following administration in two SEDDS formulations compared to a powder formulation. Interestingly, there were no significant differences in bioavailability between a solid dose form and a simple oil-based formulation suggesting that the surfactants present in the SEDDS blend facilitate drug absorption either by promoting drug solubilization and

penetration through the aqueous boundary layer, or increasing membrane permeability (Kommuru et al., 1999). In multiple dosage studies carried out in HIV infected patients, the administration of a SEDDS formulation resulted in a larger AUC and higher peak and through concentrations of the HIV protease inhibitor SC-52151 than a corresponding elixir formulation (Fischl et al., 1997). Shah et al., (1994) examined the bioavailability of Ro-150778, a highly lipophilic naphthalene derivative, demonstrating a four-fold increase in bioavailability for the SEDDS compared to that of a PEG 400 solution and a 20-fold increase in bioavailability compared to a standard tablet of micronised drug after administration to fasted dogs. It was proposed that the reduced bioavailability of the drug in the PEG 400 formulation was due to precipitation within the intestine, resulting in dissolution rate limited absorption. The SEDDS formulations may act both to maintain the drug in solution, but also facilitate dispersion, resulting in a faster dissolution rate of the drug.

Lin et al., (1991) examined the oral absorption of a potent CCKb receptor antagonist, L-365260. Maximum plasma concentrations Cmax and bioavailability values were 7-8 -fold higher for SEDDS and PEG 600 formulations than tablet and methylcellulose suspensions (Lin et al., 1991 &1996). No significant difference was reported between the SEDDS and PEG 600 preparation, suggesting that maintaining the drug in solution, albeit by different mechanisms, within the intestine was a crucial factor for the improved drug absorption. Charman et al., (1992) reported similar extents of bioavailability following administration of the poorly soluble antiviral compound, WIN 54954, in PEG 600 and a SEDDS to fasted dogs. The plasma profiles obtained after administration of the SEDDS were more reproducible than those observed after administration of the PEG solution, and previous studies where WIN 54954 was administered as a soybean oil solution had produced erratic plasma profiles. These combined observations indicate that a more consistent absorption of the SEDDS formulation was a function of the inherent solubilization and dispersion produced by the SEDDS, and not merely a function of the lipid component or solution nature of the vehicle.

Table 3.2 Commercial lipid based formulations exhibiting enhanced bioavailability

Product	***Formulation type***	***Strength***	***Dose***	***Excipients***	***Bioavailability enhancement***
Neoral® *(cyclosporin)*	Liquid filled soft gelatin capsule	25 and 100mg	Up to 1g/day	Mono-di-triglycerides, polyoxyl 40 hydrogenated castor oil, α-tocopherol and PEG	20-50% compared to Sandimmune
Fortovase® *(saquinavir)*	Liquid filled soft gelatin capsule	200mg	18 capsules daily	Medium chain mono and diglycerides, povidone and α-tocopherol	AUC increase 3.3-fold compared to Invirase®
Agenerase® *(amprenavir)*	Liquid filled soft gelatin capsule	150mg	16 capsules daily	TPGS, PEG 400 and PEG	Conventional oral formulation gave no detectable blood levels
Norvir® *(ritonavir)*	Liquid filled soft gelatin capsule	100mg	12 capsules daily	Oleic acid, ethanol, polyoxyl 35 castor oil	Similar to an oral solution

3.6.1. Mechanisms of self emulsification

The process by which self-emulsification takes place is not yet clearly understood. It is generally accepted that self-emulsification occurs when the entropy change that favours dispersion is greater than the energy required to increase the surface area of the dispersion (Reiss, 1975). Formation of an emulsion involves the generation of a larger interface between the two liquids than is found in the unmixed state, hence it is necessary to input energy into the system according to the equation 3.1.

$$\Delta G = \sum_i N_i \pi r_i^2 \sigma \qquad \textbf{Equation 3.1}$$

where ΔG is the free energy associated with the process (ignoring the free energy of mixing), N is the number of droplets of radius r and σ represents the interfacial energy. With time, the two phases of the emulsion will tend to separate, in order to reduce the

interfacial area, and subsequently the free energy of the system. Therefore, the emulsions resulting from aqueous dilution are stabilised by surfactants, which form a monolayer around the emulsion droplets and hence reduce the interfacial free energy, as well as providing a barrier to coalescence. In the case of self-emulsifying systems, the free energy required to form the emulsion is either very low and positive, or negative, then, the emulsification process occurs spontaneously (Craig et al., 1995). The involvement of liquid crystalline formation liquid crystal (LC) formation in the self-emulsification process has been extensively studied, and in particular the tendency for water to penetrate the LC phase (Groves et al., 1974; Pouton, 1985; Wakerly et al., 1987). Craig et al., (1995) used the combination of particle size analysis and low frequency dielectric spectroscopy to examine the self-emulsification of a series of Imwitor 742/Tween 80 systems. The dielectric studies provided evidence that the formation of the emulsions may be associated with LC formation, although the relationship was clearly complex. The above technique also showed that the presence of drug may alter the emulsion characteristics possibly by interacting with the LC phase (Craig et al., 1993). In practice, disruption of the oil-water interface is caused by penetration of water into the formulation or diffusion of cosolvents away from the formulation (Pouton, 1997).

3.6.2. Biopharmaceutical aspects on SEDDS

The *in vivo* performance of SEDDS formulations can be related to various factors:

- The rate of emulsification i.e. the mixtures ability to form emulsions, microemulsions, micellar solutions or fine emulsions spontaneously. Pouton (1985) proposed estimating the efficiency of self-emulsification by evaluating the rate of emulsification. Turbidity measurements were used to identify efficient self emulsifying systems by establishing whether the dispersion reached equilibrium rapidly and in a reproducible time

- The mixtures ability to self-emulsify in a large field of dilution with water. The ability of a microemulsion to be diluted is essential for its use as a drug delivery vehicle since, after administration, it will almost certainly be diluted by body fluids. The use of hydrophilic co-surfactants can lead to the destruction of the microemulsion upon *in*

vivo dilution due to the partitioning of the co surfactant out of the interfacial region into the continuous phase (Lawrence & Rees, 2000).

- The droplet polarity, size and size distribution formed on dilution. Larger droplets are less stable than smaller droplets due to their larger area to volume ratio, and so will tend to grow at the expense of the smaller droplets (Shaw, 1980). Also the diffusion path for the drug will decrease with the reduction of the radius of the droplets. Droplet size may also affect the rate and extent of drug release (Tarr & Yalkowsky, 1989). An appropriate polarity in combination with small droplet size will permit an acceptable rate of release of the drug (Shah et al., 1994). The polarity of the oil droplets is governed by the proportion of oil and surfactants, the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular weight of the hydrophilic portion and the concentration of the emulsifier. Craig et al., (1996) found that the self-emulsifying behaviour was shown to be dependent on the polarity of the oils, with polar hydrophilic oils (i.e. high HLB) displaying the clearest tendency to self emulsify forming o/w emulsions.

- Significance of digestibility. Mac Gregor et al., (1997) reported that formulation of oils with surfactants may affect digestibility as hydrophilic surfactants, such as Cremophor RH40, may inhibit lipolysis, and as a result one of the mechanisms facilitating *in vivo* drug uptake may be compromised. Assessment in an *in vitro* lipolysis model is recommended. Patel et al., (1999) reported that despite superior self-emulsification and drug solubilization properties, hydrophilic oils displayed unfavourable lipid digestion. It was suggested that the polyethylene glycol side chain of some polyglycolysed glycerides, such as Labrasol and Labrafac CM10, may hinder the binding of the lipase/co-lipase complex to the surface of the o/w droplet.

Pouton has recently proposed a classification system for SEDDS based on similar performance characteristics (Pouton, 2000)

Lipid vehicles, and SEDDS in particular, offer a potentially useful means of enhancing oral absorption of a number of drugs. There has been a traditional reluctance to develop lipid based dose forms due to potential problems of chemical and physical instability, and a traditional preference for solid dosage forms. However, there is a current resurgence of interest in lipid based dose forms due to the potential commercial and pharmaceutical

benefits, and an industry trend towards discovery/development of increasingly hydrophobic (and potent) new chemical entities e.g. approximately 40% of new drug candidates have poor water solubility (Robinson, 1996). The emergence of a few commercial examples, most notably cyclosporin, ritonavir, saquinavir and amprenavir has furthered interest in SEDDS formulations (Table 3.2). Clearly there are several areas associated with lipid based oral dosage forms in which further knowledge would be desirable. It is my view that paramount among this topics is the issue of digestibility. The formulation strategies towards increased sophistication of lipid-based vehicles has tended to 'by-pass' the natural processes of lipid digestion, dispersion and absorption; a strategy that has resulted in the Neoral® formulation acting apparently independently of bile and co-administered food. However the choice between digestible versus non-digestible formulations will be drug specific, and understanding the potential for the lipids administered, whether digested *in vivo* or administered pre-digested, to interact with physiological processes, pose some interesting challenges e.g. bile salt-lipid mixed micelle solubilization capacity, P-gp and P-gp like interaction, inhibition of lipolysis and promotion of intestinal lymphatic transport.

4. Saquinavir

4.1. Introduction

The AIDS epidemic has reached catastrophic proportions, affecting people from all socio-economic groups around the world. According to the World Health Organisation, 15,000 people worldwide become infected daily with the AIDS virus, the human immunodeficiency virus (HIV), joining the 30-50 million who already have it or have died from it. Over $3 billion is spent annually on drug therapy development worldwide (W.H.O.estimates).

Antiretroviral therapy is associated with delayed disease progression and prolonged survival in patients with advanced HIV infections, but the duration of both clinical benefit and viral suppression is limited, and viral strains with decreased susceptibility emerge (Fischl, 1989; Vella et al., 1992; Collier et al., 1996). Current antiretroviral therapies include the reverse transcriptase inhibitors, which have limited activity and significant toxicity (Hirsch & Daquila, 1993) and the recently developed HIV protease inhibitors. However, the disease eventually progresses to the acquired immunodeficiency syndrome (AIDS) despite the use of these agents.

Peptidic inhibitors of the HIV-encoded protease have had a major impact on the AIDS epidemic, by increasing patient survival and decreasing disease progression (Flexner, 1998). Unfortunately these agents must be given in combination with other active antiretroviral drugs, and regimens associated with clinical benefit have a number of disadvantages and much room for improvement.

4.2. Rational drug design of HIV protease inhibitors

A brief history of the development of these compounds is instructive. HIV protease, an aspartic endopeptidase, plays an important role in the HIV replication cycle. The protease gene was recognised within the sequence of the first HIV-1 genome, published in *Nature* in 1985 (Ratner et al., 1985), prior to identification of a functional protein. The HIV protease enzyme targets HIV-specific amino acid sequences in the gag and gag/pol polyproteins whose cleavage is essential for the maturation of the nascent virion (Kohl et al., 1988). Inhibition of this enzyme leads to the production of immature non-infectious progeny (Darke et al., 1994). Although mammalian cells contain a number of aspartyl

proteases, none appear to efficiently cleave gag polyprotein, and conversely the HIV–encoded protease is not known to cleave any host cell-encoded proteins (Flexner et al., 1988). The first reports of inhibitors of this enzyme appeared in 1988 (Billich et al., 1988). These initial peptidic inhibitors were of low potency, and most suffered poor pharmacokinetic characteristics due to their very high lipophilicity, high molecular weight and poor water solubility. The first selective and potent inhibitors appeared within a few months (Roberts et al., 1990). One of these compounds, saquinavir, became the first protease inhibitor approved for prescription use in the U.S. Saquinavir entered phase I trials in 1992, and just 3 years later, in December 1995, this drug received accelerated approval from the FDA for use in combination with antiretroviral nucleosides. This was one of the most rapidly developed and approved drugs in modern times. However, an even shorter timeline was completed just a few months later, in March 1996, when ritonavir received full approval for the treatment of patients with advanced AIDS, indinavir, whose clinical development paralleled that of ritonavir, received FDA approval a few weeks later. A fourth peptidic protease inhibitor, nelfinavir, was approved in 1997, and a fifth, amprenavir, was approved in 1999. All approved protease inhibitors cause a rapid and profound decline in plasma HIV viral loads in patients, as measured by quantitative polymerase chain reaction or branched DNA assays of HIV RNA copies per ml of plasma, which usually results in a sustained increase in CD4 cell count.

Although combination chemotherapy with protease inhibitors has had a major impact on the morbidity and mortality of HIV infection, these new drug regimens are associated with a number of problems. First, many patients cannot manage the large number of tablets/capsules and the strict dietary requirements and have difficulty adhering to the prescribed regimen. For this reason, in some settings, treatment failure with initial combination regimens may be as high as 50% (Volberding, 1998). Inter-individual variability in pharmacokinetics is large, as indicated by a coefficient of variation for all mean AUC of > 30% for all five approved protease inhibitors. Several factors contribute to pharmacokinetic variability, including the effects of first pass metabolism and food. A high fat meal substantially increases the bioavailability of saquinavir and nelfinavir, but reduces the bioavailability of indinavir and amprenavir. The same high fat meal increases the bioavailability of ritonavir capsules but decreases the bioavailability of ritonavir liquid formulation. It is currently recommended that nelfinavir and saquinavir be given with a moderate-fat meal, and that indinavir be given in the fasted state or with a light, low-fat snack. Amprenavir and ritonavir may be taken with or without food, but amprenavir

should not be given with a high fat meal. Therefore, improving the convenience, tolerability and cost of available regimens and promoting the long-term adherence to effective regimens are priorities for clinical and preclincal drug development. Unlike that of nucleoside analogues, which undergo phosphorylation resulting in sustained intracellular concentrations of their active triphosphate derivatives, the activity of protease inhibitors is dependent on the continuous maintenance of circulating concentrations that suppress viral mutation. Consequently maximal viral suppression is achieved with frequent, high doses of protease inhibitors in combination with nucleoside analogues.

4.3. Saquinavir

Saquinavir is an HIV protease inhibitor which, formulated as a hard-gel capsule (HGC), was the first drug of its class to become available for the treatment of patients with HIV infection. Despite the beneficial effects that saquinavir HGC-containing combination regimens have shown in the treatment of patients with HIV infection, the HGC formulations has limited oral bioavailability and has shown only modest antiviral activity *in vivo*. To overcome this limitation (with the aim of improving antiviral efficacy), a soft gel capsule (SGC) formulation of the drug has been developed, which provides greater systemic exposure to the drug. At the recommended dosage of 1200mg t.i.d, the SGC formulation of saquinavir achieves plasma concentrations > 8 times higher than those in patients receiving saquinavir HGC 600mg t.i.d.

4.3.1. Overview of pharmacodynamic properties

Saquinavir is a highly selective and potent inhibitor of HIV protease enzyme (Roberts et al., 1990). Saquinavir has been developed using computer-assisted design techniques and mimics the transition state of the phenylalanine-proline cleavage site in HIV polypeptides (Vella, 1995; Perry & Noble, 1998). Notably, whereas the nucleoside reverse transcriptase inhibitors (NRTIs) show anti-HIV-1 activity in acutely infected cells only, saquinavir, like other protease inhibitors, is active in cells either acutely or chronically infected with the virus (Moyle & Gazzard, 1996).

In vitro, saquinavir shows activity against HIV-1, including zidovudine-resistant strains; concentrations required to produce 50% inhibition of various strains of HIV-1 ranged from 1 to 30nmol/L (≈0.77 to 23.1ng/ml). Additive or synergistic *in vitro* antiviral activity

occurs with saquinavir in combination with most NRTIs and/or drugs with anti-HIV activity. However, because saquinavir binds extensively to plasma proteins *in vivo*, in vitro IC50 values are useful only as a guide to required therapeutic plasma concentrations. In fact, therapeutic plasma concentrations of saquinavir should be at least twice those of effective *in vitro* antiviral concentrations (Moyle & Gazzard , 1996).

4.3.2. Overview of pharmacokinetic properties

4.3.2.1. Absorption

The mean absolute bioavailability in healthy volunteers of saquinavir after a single oral 600mg dose of the HGC formulation (taken with food to optimise absorption) is 4% (CV=17%, 1-9% range), eighteen times more than the bioavailability in the fasted state. This is the result of both limited absorption from the HGC and extensive first pass metabolism; however steady state exposure appears to be greater in HIV-infected people than in healthy volunteers (Noble & Faulds, 1996). Although the mean absolute bioavailability of saquinavir SGC has not been determined, a single 600mg dose of saquinavir was estimated to have a relative bioavailability of 331% compared with a single dose of saquinavir HGC 600mg (Perry & Noble, 1998). The pharmacokinetic characteristic dictates three-times-daily dosing in humans.

Mean maximum plasma concentrations (C_{max}) values of saquinavir ranged from 301.2 to 2181 ng/ml in patients with HIV infection after doses of saquinavir SGC 400, 800 or 1200mg t.i.d. A lower saquinavir C_{max} (197.5ng/ml) occurred in HIV-infected patients who received saquinavir HGC 600mg t.i.d. C_{max} occurs 3.3 hours post administration in the fed state and after less than 1 hour in the fasted state. The terminal half-life of saquinavir after i.v. administration was 13.2 hours. Saquinavir showed a greater than dose-proportional increase in mean $AUC_{0\rightarrow 8h}$ over the 400 to 1200mg dose range given 3 times daily. The pharmacokinetics appear to be non-linear, and as such it is not possible therefore to predict steady state profiles from pharmacokinetic measurements made after a single dose.

Absorption of saquinavir is markedly enhanced by the presence of food, especially if its fat content is high. In 12 healthy volunteers who received a single 800mg dose of saquinavir SGC with a 1000kcal breakfast, the mean AUC over 12 hours (1120ng/ml.h) was almost 7 times higher than in fasted volunteers (167ng/ml.h) who received the same dose (Noble &

Faulds, 1996). In addition, absorption of saquinavir was increased 2 fold when a single 600mg dose was administered after a 'heavy' breakfast in comparison with a 'light breakfast'. Secondary peaks (several hours after dosing) in the plasma concentration/time profile frequently occur shortly after ingestion of food, after oral and not i.v. administration, and it has been postulated that the gastrocolonic response is responsible for this observed increase in absorption following ingestion of food (Kenyon et al., 1998).

4.3.2.2. Distribution

Saquinavir has a large volume of distribution (>700L) and is highly plasma protein bound (>97%) (predominantly to α_1-acid glycoprotein). Since saquinavir is bound extensively to plasma proteins, the large Vd, approximately fifteen times that of body water, is indicative of strong tissue binding of this drug and may predispose to plasma protein displacement interactions. As only the unbound concentration of the protease inhibitor to which the virus is exposed counts, plasma protein binding of HIV protease inhibitors has to be taken into account in assessing their therapeutic efficacy. Saquinavir is a high affinity substrate for the drug-transporting protein P-glycoprotein; binding to this protein is believed to contribute to the limited oral bioavailability of the drug.

Human immunodeficiency virus appears to reside in the brain, as evidenced by the presence of large quantities of un-integrated viral DNA in the brains of HIV-infected individuals (Pang et al., 1990). The mechanism by which HIV enters the brain is not well understood; however the resulting infection leads to a number of central nervous disorders such as AIDS dementia complex and HIV encephalopathy (Spencer & Price, 1992; Lipton & Gendelman, 1995). Furthermore, the blood brain barrier may inhibit the transport of anti-retroviral agents into the brain, which might serve as an occult reservoir for viral replication. Similar so called 'sanctuary sites' include the testes and the lymphoid tissue. To combat infection and inhibit viral replication of HIV in the brain and the CNS, antiretroviral agents must cross the blood brain barrier. Glynn & Yazdanian (1998), studied the permeability of a series of protease inhibitors in an in vitro blood brain barrier model and found that saquinavir had the lowest permeability. Despite the large volume of distribution, the concentrations of saquinavir in the CSF appear to be negligible. Five hours after oral administration of saquinavir HGC (dose not reported) as part of saquinavir combination therapy, the drug was undetectable in CSF of 6 HIV infected patients treated for $\geq$ 2 weeks (Moyle et al., 1999).

4.3.2.3. Metabolism and elimination

After oral administration, saquinavir undergoes rapid and extensive first pass metabolism, predominantly by cytochrome p4503A4 isoenzyme. Several mono- and di- hydroxylated metabolites are produced which have negligible activity. Elimination of saquinavir is predominantly non-renal, with about 1% of the drug excreted unchanged in the urine and 88% eliminated in the faeces as unchanged drug and metabolites, which suggests that bilary excretion is a major route of elimination (Noble & Faulds, 1996). ^{14}C-saquinavir studies revealed that 13% of circulating radioactivity was attributed to unchanged drug after oral administration of 600mg saquinavir, compared to 66% of circulating radioactivity following i.v. administration. Other reports estimate that 30% of the dose is absorbed (Vella & Floridia, 1998). This data reflect the high first pass metabolism of saquinavir.

The systemic plasma clearance of saquinavir (CL) after intravenous administration in a postprandial state has been estimated to be 1.14L/hr/kg (Hsu et al., 1998), which is higher than hepatic plasma flow ($Q_H \approx$ 50L/hr or 0.7L/hr/kg). Because hepatic blood flow may increase transiently during non-fasted conditions, particularly after a protein-rich meal (Orrego, 1965), it is possible that the higher than expected systemic clearance of saquinavir was partly caused by postprandial conditions. Another possibility is that intravenously administered saquinavir is partly eliminated trans-intestinally (e.g. through P glycoprotein) or through intestinal CYP3A.

Saquinavir exhibits substantial inter-individual variability (Barry et al., 1998). Point estimates for inter-individual variance terms associated with saquinavir disposition range between ~0.3 and ~0.6. These values give rise to approximate coefficients of variation between 58 and 85%, and correspond to roughly 9-fold and 21-fold differences between upper and lower limits of approximate 95% predictive ranges in humans (Lunn & Aarons, 1998).

4.3.3. Therapeutic efficacy

The efficacy of antiviral drug therapy in the treatment of patients with HIV infection is best established by its effects on clinical end-points, such as disease progression and/or mortality over time. However, because of the slow rate of progression of HIV disease, the use of clinical end-points is not always possible; thus measurements of surrogate markers

for the disease progression (primarily plasma HIV RNA levels and CD4+ cell counts) are used initially to determine the efficacy of antiretroviral drug treatment in many clinical trials.

Over recent years, it has become established that combination regimens of antiretroviral drugs produce greater and more durable suppression of viral replication than antiretroviral monotherapy in patients with HIV infection. Combination antiretroviral therapy drug regimes may also prevent or delay the emergence of drug resistant strains of HIV-1 in vivo. Based on these results, recent research has focused on identifying combinations of antiretroviral drugs with additive and/or synergistic antiviral effects.

Progression to acquired immunodeficiency syndrome event or death decreased by 49% among patients treated with saquinavir in combination with zalcitabine and zidovudine, compared with patients treated with zalcitabine and zidovudine alone. Mortality rate as a single end point decreased by 68%. Patients took treatment for a median of about 1 year and participated in follow-up study for as additional 1.5 years. (Saquinavir product info., 1998)

4.4. Potential place of saquinavir in the management of HIV infection

Optimism that HIV disease may be manageable over the long term has been prompted by the encouraging results of trials of various protease inhibitors in combination with NRTIs (Deeks et al., 1997). The clinical evidence to date indicates that treatment of HIV-infected patients with these combination regimens leads to marked reduction in plasma HIV RNA levels, with sub-quantifiable plasma RNA levels achieve and maintained in many patients. In view of these findings, updated guidelines from the International AIDS Society US panel now recommend that initial therapy should comprise a protease inhibitor plus two NRTIs (Carpenter et al., 1997). The recent guidelines also reinforce that patients with symptomatic infection and/or patients with CD4+ cell counts < 500 cells/μl should receive combination antiretroviral drug therapy. In comparative combination therapy trials, saquinavir SGC in combination with 2 NRTIs was as effective as indinavir plus 2 NRTI's in anti-retroviral -naïve or -experienced patients.

Although combinations of a single protease inhibitor and 2 NRTIs are widely considered to be the 'gold standard' treatment regimen for patients with HIV infection, 4-drug

combinations of 2 protease inhibitors and 2 NRTIs may be a useful option (Perry & Noble, 1998). Issues of compliance and tolerability, however, need to be addressed before this therapeutic approach can be recommended for the routine management with HIV disease. Notwithstanding the documented efficacy of the protease inhibitors in the treatment of patients with HIV infection, these agents have several limitations and from a pharmaceutical scientist point of view represent considerable opportunities for improvement. For example, protease inhibitor compliance may be less than optimal because of the large 'pill burden' that must be adhered to to achieve therapeutic concentrations. Combination protease inhibitor, such as saquinavir plus ritonavir, may be beneficial in terms of improving antiviral activity, by allowing a reduction in dosage or frequency and possibly improving compliance. However as the possibility of enhanced toxicity needs to be considered, the relative pharmacokinetic and clinical benefits of protease combinations needs to be clearly established.

4.5. Therapeutic goals of combination therapy

The key to successful therapy is to reduce disease progression and to prolong survival. This is directly related to maximal drug concentrations, which attain the dual goal of reduced viral replication and reduced risk of resistance development.

4.5.1.1. Reduced drug resistance

Drug resistance is a major obstacle to successful long-term suppression of HIV with protease inhibitor containing regimens. As a result of high viral turnover and because of the frequency of mutations appearing during each replication cycle of HIV, drug resistant viral mutants are generated or selected under the selective pressure of antiretroviral drugs (Moyle & Gazzard, 1996). Resistance is associated with specific, well-characterized mutations in the HIV protease gene (Boden & Markowitz, 1998). Resistance is a staged process wherein the virus acquires a single primary amino acid change that produces only a slight (generally less than five fold) change in drug sensitivity. Thereafter, additional secondary mutations accumulate that confer ever-increasing resistance. Amino acid changes associated with primary resistance generally reside in the enzyme's catalytic site; whereas secondary mutations may be distant from the catalytic site. It is thought that many secondary mutations are compensatory; allowing improved proteolytic activity in the presence of primary active site mutations (Boden & Markowitz, 1998). Cross-resistance among peptidic protease inhibitors is substantial; especially once a virus acquires a number

of secondary resistance mutations. Exposure to one protease inhibitor may select virus that is resistant to all other drugs in the class, even those the patient has not yet received.

The risk of developing resistance is related quantitatively to plasma drug concentrations. Higher doses of drug produce higher plasma concentrations and are associated with greater duration of antiviral response and a decreased risk of genotypic or phenotypic resistance (Flexner, 1998). Data from a study of ritonavir monotherapy suggest that the rate of accumulation of resistance mutations is inversely proportional to trough concentration of drug (C_{min}) during an average dosing interval (Molla et al., 1996). Dosing regimens maintaining plasma drug concentrations above some resistance threshold might therefore suppress the emergence of resistant strains. Non-compliance appears to play an important role in the development of drug resistance. Compliance monitoring in patients taking high dose saquinavir suggests that an increased frequency of genotypic resistance is associated with sporadic drug taking behaviour (Vanhove et al., 1996).

The initial pattern of resistance to saquinavir appears to be different from those that develop during therapy with the other protease inhibitors. In contrast the resistance profiles of indinavir and ritonavir are similar, thus indicating the increasing risk of cross-resistance between these two drugs.

4.5.1.2. Increased plasma concentrations

Dual protease inhibitor regimens take advantage of inherent pharmacokinetic interactions between the protease inhibitors to produce significant increase in plasma concentrations. The most significant of these is the saquinavir- ritonavir interaction, which will be looked at in greater detail.

4.6. Ritonavir-saquinavir interaction - a unique synergy

Ritonavir increases saquinavir concentrations at steady state by up to 30-60 fold, allowing reduction of saquinavir dose and dosing frequency (Merry et al., 1998; Flexner, 2000). These benefits reflect inhibition of pre-systemic clearance and first pass metabolism, as well as inhibition of systemic clearance mediated by hepatic and intestinal cytochrome P450 3A4. A complete list of possible benefits from dual protease inhibitor regimens is provided in Table 4.1.

Table 4.1 Potential clinical advantages of dual protease inhibitor therapy (adapted Flexner 2000)

Pharmacokinetic effects	Clinical consequences	Other potential benefits
Increased bioavailability	Reduced dose	Decreased pill burden/ improve compliance
Decreased systemic clearance	Reduced cost of therapy	Decrease cost of therapy
Increase AUC	Increased antiretroviral activity	Improved Convenience
Increased trough Cmin	Less likelihood of resistance	Dual agents lacking cross resistance
Reduced pharmacokinetic variability	More predictable drug concentrations	
Increase formation of active metabolites		
Decreased clearance of active metabolites		

In a single dose crossover study ritonavir increased the saquinavir AUC 50- to 132- fold and increased the saquinavir C_{max} by 23- to 35- fold (Hsu et al., 1998). For a fixed dose of ritonavir, the pharmacokinetics of saquinavir were relatively proportional to saquinavir dose. Saquinavir had a small but statistically significant effect on the ritonavir AUC (6.4% mean increase in this study). These results suggest that the poor oral bioavailability, and non-linear absorption kinetics of saquinavir reflects extensive first-pass metabolism rather than poor absorption. The increase in saquinavir concentrations with ritonavir is the result of improved bioavailability, perhaps as much as 100%, with little effect on post-absorptive systemic clearance.

Hence, the interactions between ritonavir, converts saquinavir from a drug with highly variable and extensive first pass metabolism to an agent in which first pass loss is quite low. Hsu et al., (1998), estimated through a series of pharmacokinetic calculations, that the 50 to 400 fold increase in saquinavir AUC, with and without ritonavir, was primarily due an almost complete inhibition of first pass metabolism, either trans-intestinally or in the liver. The post absorptive contribution of ritonavir was estimated to be much lower, possibly only contributing to a four- to five-fold increase in AUC.

In contrast to ritonavir, other known inhibitors of cytochrome P450 3A4 increase the steady state AUC of saquinavir by no more than fivefold, and inhibitors of intestinal cytochrome p450, such as grapefruit juice, increase the saquinavir AUC by no more than three fold (Hsu et al., 1998; Flexner, 1998). Ritonavir does not affect the

pharmacokinetics of other p450 substrates to nearly the same extent as it affects saquinavir, even those with extensive first pass metabolism - increasing AUC by no more than fivefold (Flexner, 1998). This suggests a unique chemical specificity for the interaction between ritonavir and saquinavir. It is likely that ritonavir inhibits intestinal CYP450 3A4, and recent data suggest that ritonavir may also be a potent inhibitor of P-glycoprotein (Drewe et al., 1999; Bommhardt et al., 1994). Selective interaction with one or both of these pathways may account for the surprising magnitude of the ritonavir effect on saquinavir oral bioavailability.

Dual protease inhibitor regimens are unique in that both drugs are active for the disease being treated and both attack the same pharmacological target. The magnitude of the pharmacokinetic interaction between saquinavir and ritonavir is one of the largest ever-described in human subjects. An added pharmacokinetic benefit of combining ritonavir with saquinavir is a reduction in inter-subject variance. Ritonavir reduced the percent coefficient of variability for saquinavir pharmacokinetic parameters from about 70% to about 30% (Hsu et al., 1998). An additional rationale for the combination of ritonavir and saquinavir is the divergence in initial mutations in HIV protease selected by each agent during monotherapy. Further evidence of the increasing acceptability of dual protease therapy, is that the clinical trials of ABT-378 (lopinavir), an investigational peptidic HIV protease inhibitor, have principally involved co administration of ritonavir, taking advantage of similar interaction to develop a novel antiretroviral regimen (Hurst & Faulds, 2000; Koulson, 1998). A soft gelatin capsule formulation containing the two-protease inhibitors (Kaletra®) has been recently marketed in the US; the antiviral activity being wholly attributable to lopinavir (Abbott product info.).

4.7. Targeting of drug concentrations to 'sanctuary sites'

The concept of assessing the therapeutic efficacy of an antiviral regime based on the immunological and virological status in lymphoid tissue, as opposed to the traditional immunological and virological markers of the disease in plasma (e.g. plasma HIIV RNA levels) has been discussed extensively in mediacl literature (Pantaleo et al., 1993; Pantaleo et al., 1998; Cavert W et al., 1997; Finzi et al., 1997; Schrager & D'souza, 1998; Dantzig et al., 1999). The measurement of viral load in plasma is a useful guide to prognosis and to the efficacy of antiretroviral therapy (Mellors et al., 1996). Ultimately however the impact of treatment can only be assessed completely in the lymphoid tissue (LT) reservoirs, where

most of the virus is produced in CD4+ T lymphocytes, macrophages and other lymphoid mononuclear cells (MNCs) and is stored in immune complexes on the surfaces of follicular dendritic cells (FDCs). In the asymptomatic stage of the infection, both lymphoid tissue viral compartments (i.e. MNCs and FDCs) exceed by orders of magnitude the quantity of free and cell-associated virus circulating in the blood stream (Haase et al., 1996). These high viral load cells can serve as a reservoir for HIV. For example, combination therapy can reduce plasma virus to undetectable levels, indicating that prolonged treatment might eradicate the infection. However, HIV-1 can persist in a latent form in resting CD4+ cells, and in patients receiving effective therapy, it was estimated to take up to 60 years to eradicate latent virus in this reservoir, providing a mechanism for life-long persistence of HIV-1 (Finzi et al., 1999, Zhang et al., 1999). Gunthard et al., (2001) suggested that in patients receiving potent antiretroviral therapy for 2 years resulting in sub-quantifiable viral load in plasma, the greatest viral burden may continue to be in lymphoid tissue rather than the CNS or genitourinary compartments.

Therefore it is obvious that targeting delivery of anti HIV agents specifically to these cells would increase efficacy; a strategy that would potently control viral replication, delay the emergence of resistance and ultimately improve the clinical outcome. To enhance the localization and intracellular delivery of anti-HIV drugs, nucleoside analogues have been encapsulated in liposomes (Desormeaux et al., 1994; Harvie et al., 1995; Dipali et al., 1997) or antibody bearing liposome (Betageri et al., 1993), covalently bond to neoglycoprotein (Molema et al., 1990)) and synthesized in prodrug form (Bibby et al., 1996). Cellular uptake and localisation of anti-HIV drugs have been enhanced in the lymphoid tissue, lymphocytes and monocytes/macrophages using these approaches. Nano-particles prepared from polyexylcyanoacrylate or human serum albumin have been used as carriers for the delivery of AZT, saquinavir and ddC to macrophages/monocytes (Bender et al., 1994; Bender et al., 1996)

Protease inhibitors, such as saquinavir, represent an essential tool to obtain maximal viral suppression of the HIV replication. Current evidence strongly indicates that optimal antiretroviral treatment must be based on combinations of drugs able to induce complete and sustained inhibition of viral replication and that viral 'rebounds' must be controlled by sequential use of regimes designed on the basis of antiretroviral potency, tolerability and resistance profiles (Vella & Floridia, 1998). Highly active antiviral therapy involving HIV protease inhibitors has dramatically improved the clinical management of HIV-1 infection,

however, significant problems remain. Thus despite non-detectable plasma viral RNA levels, low level active replication of the virus is still present in certain sanctuary sites such as the central nervous system, which is often associated with progressive loss of cognitive and motor function characteristics of the AIDS dementia complex (Lipton, 1995, 1998). This may occur because effective levels of antiviral agents are not achieved in the central nervous system within the limits of clinical toxicity (Groothius and Levy, 1997). A similar pharmacological sanctuary site appears to be the testes, contributing to sexual transmission of the infection (Zhang et al., 1998). A common characteristic of the blood-brain and blood-testes barriers is the presence of a membrane efflux transporter, termed P-glycoprotein, in the capillary endothelial cells of these tissues (Thiebaut et al., 1987; Cordon Cardo et al., 1989). Expression of this transporter is polarised to the lumenal surface of the endothelial cell so that uptake of a drug substance is countered by efficient back efflux into the circulating blood, which limits drug entry into the tissue. Recently, Kim and co-workers have shown that, in mdr 1a(-/-) "knockout" mouse, lacking P-gp expression, the brain levels of saquinavir, indinavir and nelfinavir were 7- to 35-fold higher than in syngeneic wild type {mdr 1a(+/+)} animals (Kim et al., 1998).

Choo et al., (2000) examined whether pharmacological modulation of P-gp activity would alter the distribution of HIV protease inhibitors into the brain and testes. Intravenous administration of the novel and potent P-gp inhibitor LY-335979 to wild type mice increased brain and testes concentration of ^{14C}nelfinavir, up to 37-fold and 4-fold, respectively in a dose dependent fashion. Similar effects in brain levels were also observed with ^{14C} saquinavir. Because nelfinavir plasma drug levels were only modestly increased by LY-335979, the increase in brain/plasma and testes/plasma ratios of 14- to 17- and 2- to 5- fold respectively, was due to enhanced tissue penetration. Less potent P-gp inhibitors like valspodar, cyclosporin A, and ketoconazole had modest or little effect on brain/plasma ratios but increased plasma nelfinavir concentrations due to inhibition of CYP3A mediated metabolism. The major finding of this study is that the functional activity of this transporter can be pharmacologically inhibited and that such modulation results in significantly enhanced HIV protease inhibitor concentrations in both brain and testes. Importantly, a highly potent and specific inhibitor of P-gp, such as LY-335979 (Dantzig et al., 1996; Dantzig et al., 1999), can achieve this effect of increasing the distribution of the antiviral drug in these tissues disproportionately to any change in plasma concentration, an observation consistent with a targeted impairment of P-gp mediated efflux in the capillary endothelial cells. Collectively these findings provide "proof of

concept" for increasing HIV protease inhibitor distribution into pharmacological sanctuary sites by targeted inhibition of P-gp using selective and potent agents and suggest a new therapeutic strategy to reduce HIV-1 viral replication.

5. Role of intestinal cytochrome p450 and P-glycoprotein in the first-pass extraction of saquinavir

5.1. Introduction

It has long been recognized that some drug molecules are much less effective when administered by mouth than when given parentally. Classical teachings of the factors that influence oral bioavailability have included drug solubility, membrane permeability and hepatic first pass metabolism. Discussions on the contribution of the chemical properties of the drug and formulation issues as they relate to these factors were usually included. In addition, hepatic blood flow, protein binding and hepatic intrinsic clearance were generally addressed. While factors that limit drug release in the gastrointestinal lumen can be overcome with proper drug formulation, first pass metabolism is an unavoidable obstacle to the achievement of optimal bioavailability. Indeed for some drugs e.g. lidocaine, paclitaxel, it effectively precludes oral drug therapy. For others such as the new HIV protease inhibitor, saquinavir, and the immune suppressant, tacrolimus, a mean oral bioavailability of <20% was accepted by regulatory approval bodies in the absence of better alternatives. Unfortunately, a metabolic barrier that limits oral bioavailability often brings with it the problem of significant inter-individual variability in systemic blood concentrations of drug as a consequence of variability in metabolic enzyme expression. Understanding the biochemical and physiological mechanisms for the variability in the extent of first pass drug metabolism is an essential step in optimising oral drug therapy.

Two recent discoveries have opened new views on the barriers for oral absorption of drugs. First, that cytochrome P450 3A4 (CYP) and CYP3A5 are expressed at high concentrations in upper intestinal enterocytes, in addition to hepatocytes (Watkins et al., 1987). Secondly that P-glycoprotein is expressed at the lumenal surface of the intestinal epithelium (Thiebaut et al., 1987). Both can potentially limit the systemic availability of certain drugs (Wacher et al., 1995). As saquinavir acts as a substrate for both P-gp and CYP3A4 in may prove instructive to examine these mechanisms and in particular how they affect the mechanisms of absorption of substrates.

5.2. Contribution and relevance of first pass metabolism.

Drug metabolising enzymes in the liver and gut mucosa are well situated to limit the systemic exposure to foreign molecules that have been absorbed from the gastrointestinal

lumen. It has been postulated that this evolutionary development provides an advantage to herbivorous or omnivorous animal species since numerous molecules, such as the alkaloids, with profound and sometimes adverse pharmacological effects, are found in plant food sources. Any foreign molecule that is absorbed into capilliary beds of the small and large intestine must pass through the liver via the hepatic portal vein before access to the rest of the body. Only those that are absorbed into the lymphatic system or distal rectum effectively by pass the liver.

The hepatic portal vein branches within the liver to form the hepatic acinus, a complex capilliary bed intimately associated with cells of the liver, including parenchymal cells, the primary site of drug metabolism. From the perspective of first-pass drug delivery, a hepatic portal-parachymal cell concentration gradient drives the diffusion of drug across the vascular endothelium, the Space of Disse, and finally, the sinusoidal plasma membrane of the hepatocyte. Diffusion of drug into the hepatocyte competes with bulk flow of blood that removes drug from the sinusoidal space into venules that ultimately form the hepatic vein. The uptake of drug into parachymal cells is not obligatory during first-pass through the liver. However, drug that enters the parachymal cell is subject to a wide variety of metabolic and excretory processes. Under conditions when metabolism is rapid and diffusion from blood to the intracellular enzyme active site is not rate-limiting, metabolism acts to maintain the concentration gradient and promote the extraction of drug from the vascular compartment.

5.2.1. Cytochrome P450 enzymes

From the standpoint of first pass metabolism, the family of Cytochrome P450 enzymes represents the most important of the hepatic enzymes. Although a spectrum of drug biotransformations can occur during first pass, the most common are oxidations catalysed by cytochromes P450. It is the isoenzymes CYP2D6, CYP 3A4, CYP 1A2, CYP2C9 and CYP2C19 that are most often implicated in first pass elimination. For any given substrate, enzyme specificity, enzyme content, substrate binding affinity and sensitivity to irreversible catalytic events all play a role in determining the overall efficiency, or intrinsic clearance, of elimination. CYP3A4 (CYP) is the most frequently listed enzyme.

5.3. Intestinal versus hepatic CYP metabolism

Traditional teaching of clinical drug metabolism has been that hepatic metabolism is of primary importance. The influence of intestinal biotransformations has, up to recently, been generally disregarded, primarily because it was believed that the liver to intestinal ratio of cytochrome P450 has been reported to be approximately 20 (Tam, 1993). However since Watkins and co-workers reported that a major Cytochrome P450 enzyme, CYP3A4, is relatively abundant in the intestinal mucosa (Watkins et al., 1987; Kolars et al., 1992), the potential of the intestinal enzyme system to significantly reduce oral bioavailability has been a major topic for discussion. The fact that the possibility of intestinal metabolism contributing to drug metabolism has traditionally not been addressed has lead to the potentially false assumption that hepatic first pass metabolism or that poor absorption is entirely responsible for low bioavailability. Watkins (1992) proposed that despite the relatively small amount of P450 in the intestinal mucosa relative to the liver (i.e. mean microsomal CYP3A4 contents are reported to be ~50% of the levels found in human liver) the intestine plays a major role in drug metabolism. The location of the P450, just below the microvillus border, maximises drug metabolism as it crosses the intestinal wall. In addition, Watkins (1992) suggests that the exposure of enterocytes to high drug concentrations increase the relative importance of intestinal metabolism, since CYP3A4 is a low affinity, high capacity enzyme. The high level of specific CYP P450 becomes of even greater importance when it is recognised that more than 50% of drugs, for human administration, may be substrates for this enzyme (Benet et al., 1996).

CYP dependent metabolic activity of gut mucosa compares favourably to liver. In studies that compared both hepatic and intestinal (duodenum or jejunum) microsomes, mean maximal metabolic conversion of erythromycin (Watkins et al., 1987), midazolam (Thummel et al., 1996), and tacrolimus (Lampen et al., 1995) for the intestine were approximately 45-118% of the corresponding mean maximal rate for human liver when activities were normalised for protein concentration. When normalised for total nmol of P450, intestinal microsomes proved metabolically superior, reflecting the greater contribution of the CYP3A fraction to total p450 content (Thummel et al., 1997). In a study of human hepatic and colonic mucosal tissue slices, Vickers et al., (1992) reported an approximately two-fold greater rate of cyclosporin metabolism, per gram tissue, for intestine than for liver.

The oral bioavailability of CYP3A4 substrates can be profoundly altered by modulators of enzyme catalytic activity (Thummel & Wilkinson, 1998). Results from *in vivo* interaction studies suggest that both hepatic and intestinal metabolic extraction is sensitive to the effect of known CYP3A4 inducers, such as rifampicin and phenytoin (Hebert et al., 1992; Fromm et al., 1996) and CYP3A4 inhibitor, ketoconazole (Gomez et al., 1995). However the effect of a co-administered modulator of CYP3A4 function might be expected to be more pronounced at the level of intestine, compared to the liver, based on presumed local concentration differences during the period of modulator absorption (Hebert, 1997; Wu et al., 1995). In some cases, it is even conceivable that only the small intestine would be subject to the enzyme altering effects of the xenobiotic. For example, ingestion of grapefruit juice causes an increase in midazolam and cyclosporin AUC's presumably due to a reduction in intestinal first pass metabolism, after oral administration of the drugs, but not after i.v. administration (Kupferschmidt et al., 1995).

Cyclosporin and midazolam are two recent examples of drugs that exhibit substantial intestinal metabolism. In a series of studies in rats and humans, Watkins and co-workers found that intestinal CYP3A exhibited significant *in vivo* metabolic activity towards cyclosporin (Kolars et al., 1991; Kolars et al., 1992). By administering cyclosporin directly into the duodenum of a patient during the anhepatic phase of a liver transplant operation, the investigators unambiguously demonstrated that the primary cyclosporin metabolites measured in hepatic portal vein and systemic blood were of intestinal mucosal origin. Pharmacokinetic data from healthy volunteers also supports a role for intestinal CYP3A in first pass cyclosporin metabolism. From an analysis of cyclosporin AUC following i.v. administration, in absence and presence of CYP3A induction by rifampin and phenytoin, CYP inhibition by ketoconazole (Hebert et al., 1992; Wu et al., 1995; Gomez et al., 1995) it was concluded that the intestine and not the liver was responsible for most of the first pass cyclosporin elimination after oral administration (up to 60%). In addition the data with cyclosporin indicate that the CYP3A modulators exerted greater inductive or inhibitory effects on intestinal extraction than on hepatic extraction.

Thummel et al., (1996 & 1997), conducted a series of studies in humans indicating that Midazolam undergoes extensive intestinal first pass metabolism. Midazolam is eliminated entirely (>97%) by oxidative biotransformation reactions catalysed almost exclusively by CYP3A subfamily (Fabre et al., 1988; Gorski et al., 1994). A study of i.v. and oral midazolam pharmacokinetics in twenty healthy volunteers showed indirect estimates of

intestinal and hepatic first pass extraction fractions that were 44 ± 14% and 43 ± 24%, respectively, with an overall bioavailability of 30%. To verify directly the significance of the small intestine as a site of first-pass metabolic extraction in humans and to assess inter-individual variability associated with that process, the extraction of midazolam in a small group of liver transplant patients was examined (Paine et al., 1996). They were studied during the anhepatic phase of their transplant operation, a time when the metabolic activity of extrahepatic organs could be directly assessed *in vivo* without the confounding influence of the liver. The mean intestinal first pass extraction was estimated to be 43%. The predicted variability in intestinal first pass extraction fraction for healthy volunteers was high (0-77%), similar to that observed in the five anhepatic subjects (14-59%).

Recently, Fromm et al., (1996) have reported on the intestinal and hepatic first-pass metabolism of another low bioavailability CYP3A substrate, verapamil (18.2 and 5.3% for R- and D- isomers, respectively). Utilizing steady-state AUC data following oral administration of racemic verapamil and a pulsed i.v. tracer dose of stable isotope labelled racemic verapamil, the investigators concluded that first-pass intestinal extraction of S- and R-verapamil in healthy volunteers was substantial. The mean calculated intestinal and hepatic extraction of S-verapamil was ~58% and 62%, respectively, for an overall oral bioavailability of 16%. Extraction ratios for R verapamil were similar. Treatment with rifampin induced both hepatic and intestinal metabolism, but like findings with cyclosporin, the increase for the intestine (up to 92%) was greater than the hepatic increase (up to 82%).

CYP3A, the major isoform in the metabolism of saquinavir, is present in intestinal tissue (Kolars et al., 1994). Although the concentration of CYP3A is much lower than that in the liver, the intestinal epithelial cells are subject to very high unbound drug concentrations during the absorption process. Thus it is likely that saquinavir undergoes partial first metabolism by these intestinal enzymes. Co-administration of commercial HGC saquinavir with a single strength grapefruit juice increased the bioavailability of saquinavir 50%; double strength grapefruit juice increased the AUC 100% (Hsu et al., 1998; Kupferschmidt et al., 1998). In addition to inhibition of CYP3A metabolism, it has been shown that grapefruit juice down regulates the enterocytes CYP3A content but appears to have no effect on the activity of hepatic CYP3A (Lown et al., 1997). Fitzsimmons & Collins, (1997) examined the potential of small intestinal microsomes to metabolise saquinavir. A high rate of intestinal oxidation was observed *in vitro* which suggested that

the small intestine may play a crucial role in the extensive first pass metabolism of saquinavir, and therefore in its low relative bioavailability. However the overall contribution of the intestinal tract to the first pass metabolism and to the low bioavailability remains to be determined.

Thummel et al., (1997) attempted to predict the first pass extraction of a series of substrates utilizing a pharmacokinetic model to predict the first order intrinsic metabolic rate, which takes into account the published V_{max} and K_m values for liver microsomal CYP3A specific reactions and estimations for the total amount of CYP3A in the small intestine. The highest clearance value from a series of twelve common CYP3A substrates was saquinavir with an intrinsic clearance of 34926ml/min. These figures were then used to approximate a first pass extraction ratio for an oral dose. A value of 99.6% for saquinavir was obtained. This agrees well with the reported low extent of bioavailability (4%) of an oral dose. Interestingly, the dose of some of the CYP3A substrates used in this study greatly exceeds the cumulative maximum metabolic capacity of the entire small intestine. Hence intrinsic intestinal clearance alone is not the only mechanism involved in the reported high first extraction for these compounds and the authors suggest potential reasons include the effect of apical P-gp or hepatic first pass metabolism.

Because of the large number of substrates that CYP3A metabolise, the contribution of intestinal enzymes to first pass drug metabolism has become the focus of a great deal of research. Not only might intestinal CYP3A reduce the oral bioavailability of a compound, but it might also be a major source of inter-individual variability in blood levels and drug response as a consequence of variable constitutive enzyme expression and drug-drug interactions. CYP3A accounts for approximately 20% of the total P450 in the liver and 70% in the jejunal mucosa (Watkins et al., 1987). Kolars et al., (1992) found CYP3A4 to be the predominant CYP3A expressed in human enterocytes with a ten fold variability in CYP3A4 concentration in five human subjects. Lown et al., (1994) found a six-fold variability in CYP3A activity, as measured by midazolam hydroxylation, and an eleven-fold variability in CYP3A4 protein content in human intestinal biopsies.

However many other CYP3A substrates do not appear susceptible to a gut wall first pass, possibly because of enzyme saturation during first pass or a limited intrinsic metabolic rate. Both direct biochemical and indirect *in vivo* clearance data suggest significant inter-

individual variability in gut wall CYP3A dependent metabolism. The source of this constitutive variability is largely unknown.

5.4. The P-glycoprotein efflux transporter

Clinical resistance to chemotherapeutic drugs is a major problem in the treatment of cancer. One form of drug resistance, termed multi-drug resistance (MDR), is defined as the ability of cells exposed to a single drug to develop resistance to a broad range of functionally unrelated drugs due to enhanced output transport (efflux) of drugs, mediated by a membrane glycoprotein "drug transport pump" (Gottesman & Pastan, 1993). The most consistent alteration found in MDR cell lines is an increased expression of a high molecular weight cell surface glycoprotein (P-glycoprotein), with a concomitant decrease in the accumulation and retention of cytotoxic drugs (Kartner et al., 1983). The human MDR1 gene, which encodes P-gp, was cloned and sequenced in 1986 (Chen et al., 1986). P-gp is recognised as a member of the ATP-binding cassette (ABC) super-family of membrane transport proteins, with overall homology with a number of bacterial, yeast, insect and other mammalian transport systems (Higgins, 1992). A model of MDR in which P-gp functions as an energy dependent drug efflux pump of broad specificity, reducing intracellular drug accumulation is widely accepted (Hunter & Hirst, 1997). The current model of the mechanism of action of P-gp is that drugs can be detected and expelled as they enter the plasma membrane in the manner of a "hydrophobic vacuum cleaner" (Raviv et al., 1990). Virtually every study of a series of structurally related drugs differing in their affinity for P-gp demonstrates that the most important determinant is their relative hydrophobicity and that substrates for the transporter have a partition coefficient (octanol/water) of approximately 2 or greater (Ford et al., 1990; Zamora et al., 1988; Germann et al., 1993). However very hydrophobic agents, such as campotothecin, which are sparingly soluble in water, are not substrates, indicating that some water solubility is required for recognition of P-gp. One proposed mechanism of action for this ATP-dependent transporter is that P-gp acts essentially as 'flippase' that detects drug within the inner leaflet of the membrane and 'flips' it into the outer leaflet (from which it can diffuse away from the cell) or directly into the extracellular space (Higgins & Gottesman, 1992; Vanhelvoort et al., 1996).

After the description of P-gp in MDR cell lines, expression in normal human tissues was documented. The pattern of epithelial cell expression has been extensively studied, using

in situ hybridisation techniques, and found to be primarily associated at the villus epithelium of the small intestine (Trezise et al., 1992) and displays complementary expression with CFTR (cystic fibrosis transmembrane regulator protein, another member of the ABC gene family). Thus CFTR expression is observed in the crypts, but switches to P-gp expression as enterocytes differentiate and move up the crypt-villus axis, to be maximal at the villus tips. This pattern of epithelial cell expression has led to the suggestion that P-gp functions in tissues such as kidney, bowel and the biliary tree to facilitate the excretion and/or minimise the absorption of toxic natural products that are ubiquitous in our diet and environment. The expression of P-gp in capillaries of the brain, testis and papillary dermis of the skin, but not in mid-sized and large blood vessels, is associated with the continuous non-fenestrated arrangement of endothelial cells at these sites and the recognition of these anatomical locations as blood tissue barriers (Cordon Cardo et al., 1989 & 1990). This may limit the penetration of cytotoxic agents into these tissues resulting in the creation of pharmacological sanctuary sites. The specific location of P-gp expression, therefore, indicates that it could be a factor that limits intestinal absorption and diffusion, for instance, across the blood brain barrier, of xenobiotics, as well as feature that participates in the biliary, renal and intestinal clearance of drugs.

P-gp has attracted much attention as a possible physiological determinant of the disposition of drugs. The functional significance of P-gp as a drug efflux pump in certain animal tissues was clearly demonstrated by generating a 'knock out mouse' that was disrupted with P-gp encoded by mdr 1a gene (Schinkel et al., 1994). In mice lacking an mdr 1a gene product, distribution of an anti-cancer drug, vincristine, and an insecticide, ivermectin, is enhanced in many tissues especially the brain. Intestinal distribution of these agents was also increased in those mice, which is consistent with previous findings that P-gp is localized on the lumenal membrane of the intestinal epithelial cells and transports certain drugs, including peptides, out of the cells into the lumen (Hunter et al., 1991; Hsing et al., 1992). Sparreboom et al., (1997) reported using mdr1a (-/-) mice to study the effect of gut p glycoprotein of the pharmacokinetic of paclitaxel. The area under the plasma concentration- time curves was 2- and 6- fold higher in mdr1a (-/-) mice than in wild type mice after i.v. and oral drug administration, respectively. This represents an increase in oral bioavailability from 11% in wild type mice to 35% in mdr1a (-/-) mice. Also, after i.v. administration, these mice excreted significantly less drug into the intestinal lumen than wild type mice, 3% vs. 11%. These data confirm that P-gp may limit the oral

bioavailability, enhancing excretion, and lowering the amount of drug that crosses the intestinal epithelium.

Studies such as Kim et al., (1998), provide direct evidence in support of the hypothesis that the drug efflux mechanism at the apical surface of intestinal epithelium sustains a secretory detoxification function and renders the epithelium relatively impermeable to substrate. The P-gp mediated transport of saquinavir was confirmed by measurement of the specific and directional flux in a HCT-8 epithelial cell monolayer system. The transport was inhibited by established P-gp reversal agents. Thus in the simplest model, P-gp is an additional component influencing the apical drug permeability (Hunter & Hirst, 1997). The absorption of P-gp substrates is limited to an extent that is dependent upon their passive permeability balanced against their affinity for P-gp.

Interestingly, HIV infection of either T cell or monocytic cells resulted in increased P-gp expression and decreased levels of accumulation of AZT (Gollapudi & Gupta, 1990). Additional investigations demonstrated that MDR-expressing cells are also resistant to AZT and DDC (Yusa et al., 1990). Freshly isolated CD4+ and CD8+ cells express low levels of P-gp; however, activation of these cells significantly increases the levels of expression (Gupta et al., 1992). Hence one effect of the action of P-gp may be to reduce the intracellular level of anti-viral drugs (i.e. conferring resistance) in these pharmacological target cells.

It must be remembered that no single efflux explains the diversity of drug secretion displayed by the intestine. Identification of the role of other secretory efflux systems and their molecular characterisation will be required before the importance of intestinal secretory systems in drug absorption and elimination can be fully described.

5.5. P-gp/CYP counter transport mechanism

Poor oral bioavailability for many drugs is generally attributed to poor solubility in the gastrointestinal fluids, poor membrane permeability and/or extensive hepatic first pass elimination, all of which are inherently true of saquinavir. Formulation strategies have thus centred on challenging all three limitations. Recently however, the recognition of both CYP 3A mediated metabolism in the small intestinal enterocytes, and P-gp counter transport processes has led to a new understanding of the poor oral drug bioavailability.

This new awareness has helped explain the high inter-patient and possibly intra-patient variability in the pharmacokinetics of CYP/P-gp susceptible compounds, such as saquinavir. For example, cyclosporin, a highly lipid soluble, large molecular weight compound does not appear to have absorption problems, with approximately 86% of the commercial formulation being absorbed intact in healthy volunteers. Rather the low bioavailability of cyclosporin results from extensive metabolic extraction in the gut which approaches 60% (Benet et al., 1996). Benet and co-workers have suggested that the striking overlap in structural specificity between substrates for P-gp and CYP potentially reveals a complementary metabolic/anti-transport process to limit oral bioavailability of a large number of drug substances. Recognition of this potential for metabolism and counter transport process in the intestine leads to a new perspective on improving drug bioavailability that differs from the traditional physio-chemical approach.

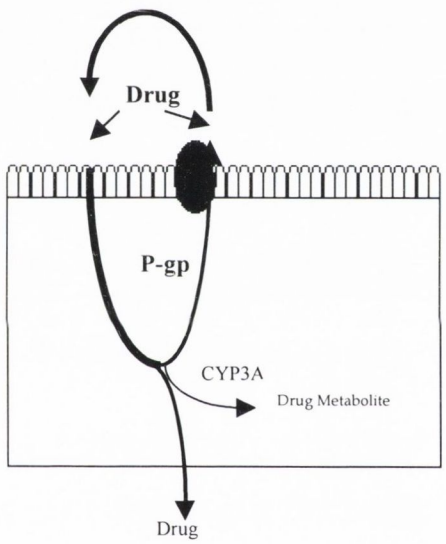


Figure 5.1 Model of drug absorption in the presence of both P-glycoprotein and Cytochrome P4503A. Drug entering the epithelium may either be absorbed across the epithelium, effluxed via P-gp and/or metabolised by CYP3A. The recirculation of the drug, via P-gp increases the likelihood of metabolism (adapted from Hunter and Hirst, 1997).

Benet et al., (1996) have proposed a synergistic role for P-gp and CYP in the intestinal elimination/excretion of drugs (Figure 5.1). It has been suggested that the extensive gut metabolism is enhanced by P-gp which actively transports the compound back into the lumen, thereby increasing its residence time in intestinal tissue, and hence the chance for metabolic transformation. Recognition of interactions between CYP and P-gp has led to additional concepts on the mode of drug absorption/elimination as proposed in the diagram

above. Interestingly, Lown et al., (1997) found no correlation with enterocyte P-gp content and either intestinal CYP3A4 content of hepatic CYP3A4 activity (as measured by the erythromycin breath test). Although P-gp and CYP3A4 may work together to minimize the exposure of the body to environmental toxins, they appear to be regulated separately.

In support of this hypothesis is the observation that the quantities of intestinal enzymes, although highly variable, do not appear to be the key to explaining the variability of oral cyclosporin pharmacokinetics. Of more importance is the quantity of intestinal P-gp, which accounts for approximately 17% of the variability in oral cyclosporin pharmacokinetics. In this study, Lown et al., (1997) demonstrated the clinical importance of P-gp in the pharmacokinetics of oral cyclosporin. Measurements of hepatic CYP3A4 activity and small intestinal biopsies, to measure CYP3A4 and P-gp in the gut, were taken in nineteen kidney transplant patients. Steady state oral cyclosporin pharmacokinetics was also performed in all subjects. It was found the 56% of the variability in apparent oral cyclosporin clearance could be explained by the variability in liver CYP3A4 activity. An additional 17% of the variability was explained by intestinal P-gp levels, which varied eight fold among the patients. The intestinal CYP3A4 levels varied ten fold, but did not appear to influence the variability of oral cyclosporin pharmacokinetics. These results suggest that, although intestinal metabolism was important, for the individuals in the study, it appears as though they have a sufficient quantity of intestinal CYP3A4 to metabolise cyclosporin. However, it was the quantity of P-gp, which enhanced drug exposure to, or the catalytic efficiency of, CYP3A4 that was important in the variability of oral cyclosporin delivery.

There is some concordance of P-gp and CYP3A substrates and modulators including verapamil, cyclosporin and taxol, although the complementarity of substrate/modulators is incomplete (Wacher et al., 1995). Co-administered modulator may act at the level of P-gp, CYP3a or both. Thus, substances which either modulate metabolism and/or secretion are an attractive avenue to explore in the optimisation of oral drug bioavailability. The bioavailability could be maximised by increasing net drug absorption and reducing biotransformations in the gut, by using either cytochrome P450 drug metabolism inhibitors, or P-gp drug transport inhibitors. Several of these inhibitors show overlap in the inhibition profiles. However, toxic effects of these modulators and increased toxicity of the co-administered drug in locations other than in the gut would have to be considered. The potential use of modulators of these systems has yet to be investigated, as has their

usefulness in the clinical setting to be determined. In some cases, however this mode of increasing bioavailability may already be, in a less planned manner, taking place, as is the case for the ritonavir–saquinavir regimen. Understanding the fundamental mechanisms underlying drug secretion and metabolism limiting drug absorption will allow development of optimal oral dosing protocols, including the use of agents to specifically enhance absorption by reducing specific secretory efflux systems.

Recognition of the relative contribution of P-gp and cytochrome P450 to oral drug delivery sheds new light on the absorption mechanisms of saquinavir and also the likely effects of co-administered P-gp/CYP modulators. For example the absorption pharmacokinetics of saquinavir are non-linear; absorption increases supra-proportionally to dose (Noble & Faulds, 1996). Similar observations have been reported for cyclosporin (Ueda et al., 1984). This most likely reflects saturation of either the active transport or metabolic processes. Thus there seems to be a threshold above which transport/metabolic systems are saturated and passive diffusion across the gut wall becomes the rate-limiting step. The threshold for a given compound will be difficult to predict since in addition to its affinity for efflux mechanism and/or metabolic enzymes, it will depend on a number of other factors such as solubility and dissolution rate or the degree of counter expression in the individual.

5.6. P-gp/CYP modulators

Recently medicinal chemists have become interested in the design and synthesis of potential inhibitors of P-glycoprotein as therapeutic agents to increase the efficacy of anti tumour agents (i.e. to prevent the efflux of these antitumour agents in multidrug resistant tumour cells (Zamora et al., 1988; Ford et al., 1990). However such inhibitors could also potentially be used to enhance the intestinal absorption of drugs whose permeabilities are limited by an apically polarised efflux system. It is also possible that some excipients (e.g. surfactants), which are commonly added as solubilising agents for orally administered pharmaceuticals, may function to enhance intestinal permeability of drugs by inhibiting the efflux mechanism in the intestinal mucosa, in addition to favourable absorption rates for physiochemical reasons (Nerurkar et al., 1996 & 1997). To date however, these potential effects of nonionic surfactants have only been demonstrated *in vitro* in cell culture lines, where it is believed that the monomer was the active species, partitioning into the plasma membrane, and inhibiting P-gp through a membrane fluidising mechanism. For example,

it is well recognized that several surfactants (e.g. Tween 80, Cremophor EL), are inhibitors of P-gp in cancer cells and thus enhance the uptake of antitumours agents (Woodcock et al., 1990). Nerurkar et al., (1996) have shown that cremophor EL and Tween 80 enhance the permeability of model peptides across Caco2 cells by inhibition of the apically polarised efflux system. The authors postulated as to the mechanism by which these surfactants inhibit this efflux system. Cremophor EL and Tween 80 are shown to integrate within the cell membranes (Zordon-Nudo et al., 1993) and thus change their microviscosity (Friche et al., 1990). This results in a loosening of the phospholipid bilayers of cells, resulting in the loss of secondary and/or tertiary structures of the membrane proteins, thus altering their biological activity. However, it has also been reported that the zwitterionic surfactant CHAPS, which can integrate within the phospholipid bilayers, does not show an inhibition of P-go even at high concentrations (Woodcock et al., 1992; Zordon-Nudo, 1993). Hence the observed differences between non-ionic and zwitterionic surfactants in the inhibition of P-gp may be due to the differences in the binding affinities of these surfactants to the hydrophobic portion of these efflux systems (Zordon-Nudo, 1993). This in turn means that the 'membrane fluidising' induced inhibition of this efflux system by non-ionic surfactants such as Tween 80 and Cremophor EL may have a component of specific binding to the hydrophobic domain of the protein along with the non-specific changes in its secondary and/or tertiary structure of the protein.

Charman and co-workers (Khoo et al., 1998) have recently described the use of a CYP3A4 inhibitor, ketoconazole, in a dog model to assess the involvement of CYP3A4 mediated metabolism in the *in vivo* metabolism of halofantrine (Hf). Plasma profiles of Hf and Hfm (the CYP-metabolite of Hf) were compared. The plasma Hfm/Hf AUC ratio after fasted administration of Hf without ketoconazole was 0.56; whereas the ratio after fasted oral administration with ketoconazole was less than 0.05. Interestingly, it was observed that the effect of enzyme inhibition was similar to that observed when the dog was dosed with Hf with a high fat meal. The postprandial administration of Hf produced a similar Hfm/Hf plasma AUC profile as did the co-administration of ketoconazole in the fasted state. Potential mechanisms by which the pre-systemic metabolism of post-prandially administered Hf may be decreased include (a) an inhibition of CYP/P-gp by lipid components, (b) a lipid based induced recruitment of intestinal lymphatic transport and (c) the 'trafficking' of Hf through the enterocyte such that metabolism is avoided.

Verapamil appears to be metabolised at least in part by CYP3A4 (Kroemer et al., 1993). It has been shown to reverse multidrug resistance in chinese hamster ovary cells (Dodic et al., 1995), competitively inhibit vinblastine transport by P-gp in multidrug resistant human cancer cells (Horio et al., 1991) and reverse multidrug resistance *in vitro* in mouse leukemic cells that express high levels of P-gp (Barancik et al., 1994). Saitoh & Aungst (1995) demonstrated that verapamil is efficiently secreted by P-gp in rat small intestinal segment, and demonstrated the existence of other P-gp-like efflux mechanism with differing substrate specificities, depending on the intestinal site. Verapamil has also been shown to decrease P-gp expression three fold in a multidrug resistant human leukaemia cell line after 72 hr of exposure as well as to decrease P-gp mRNA two fold within 24 h. The effects on P-gp expression were reversed within 24h of discontinuation of exposure to verapamil (Muller et al., 1994). Hence the effects of verapamil in vitro may be summarised as follows: verapamil may compete with other CYP3A4 substrates for the metabolism; inhibit P-gp transport and decrease P-gp expression.

Ketoconazole has been shown to inhibit CYP3A4 *in vitro* (Labroo et al., 1995) as well as to inhibit P-gp drug efflux activity (Siegsmund et al., 1994). Interestingly, there was no differences between transepithelial transport from the basal to apical and from the apical to basal transport of ketoconazole in P-gp expressing Caco2 cells (Takano et al., 1998). In addition, there was no effect of verapamil on the transport of ketoconazole. This suggests that ketoconazole could inhibit P-gp non-competitively, but that the drug itself may not be transported by P-gp. Kim et al., (1999) reported that ketoconazole's disposition in mdr1a (-/-) mice, was not statistically significant from wild type mice. The authors suggested that ketoconazole is unlikely to be a substrate for P-gp and also suggests the likelihood of other efflux transporters being involved in ketoconazole cellular transport. Ketoconazole appears to be a competitive inhibitor of cyclosporin metabolism in human hepatocytes and liver microsomes (Maurice et al., 1992; Pichard et al., 1990). Jurima-Romet et al., (1994) found ketoconazole was a competitive, reversible inhibitor of CYP3A metabolism (using terfenadine metabolism as a marker) in human liver microsomes (Jurimaromet et al., 1994). However, Wrighton and Ring (1994) using 1'-hydroxy midazolam formation as a marker, found ketoconazole to be a high affinity, non-competitive inhibitor of CYP3A catalytic activity in human liver microsomes. *In vitro* methodological differences may account for the mechanistic discrepancy (Wrighton & Ring, 1994).

In acting as a substrate to both P-gp and CYP3A, cyclosporin is implicated in a wide number of interactions of this type. Cyclosporin is one of the most potent MDR reversing agents and clinically it is a modulator that raises most interest (Ford & Hait, 1990). In one study where 10 patients whose cyclosporin plasma concentration exceeded 2μg/ml, systemic exposure of etoposide (AUC) increased by 80%, with a 36% decrease in the total clearance and a 108% increase in plasma half-life (Lum et al., 1992). The authors of this study concluded that this pharmacokinetic interaction was consistent with alterations of P-gp activity in normal tissues. The addition of cyclosporin resulted in a 55% increase in doxorubicin AUC, and a 350% increase of the doxorubicinol metabolite (Erlichman et al., 1993). Adverse neurological symptoms have been observed which, upon investigation in the rat were postulated to be indicative of P-gp modulation at the blood brain barrier (Barbui et al., 1992). When cyclosporin A was recognised as the most potent of the first generation of MDR modulators, numerous cyclosporin analogues were screened for their ability to inhibit P-gp efflux. PSC833 emerged as the leading pre-clinical candidate, with greater potency as a modulator than cyclosporin A and no immunosuppressive activity or nephrotoxicity. A number of phase I/II trials using co-administration of PSC 833 as a strategy to improve therapeutic efficacy are currently under way (Boote et al., 1996; Lum et al., 1995).

For the majority of clinical drug interactions the exact mechanisms are not completely understood. Many of the studies using Pgp/CYP3A modulators are done *in vitro* and the subsequent interpretation to the effect *in vivo* may be limited. For example, D-verapamil in combination with paclitaxel i.v. resulted in a 2-fold increase in AUC and a 50% decrease in paclitaxel clearance (Berg et al., 1995) (dex- (D-) isomer, is less cardiotoxic (Ferry, 1985) but equally potent as a P-gP modulator (Hollt et al., 1992). Similarly, the unique interaction between saquinavir and ritonavir confers significant bioavailability increases. However, it must be remembered that these studies do not allow differentiation of the relative contribution of P-gp and/or CYP3A modulation at the various sites in the body. The modulation may thus be in part a reflection of inhibition of either hepatic, biliary, or intestinal P-gp function. Therefore, while *in vitro* mechanistic studies in cell culture models prove useful indicators of potential contribution of intestinal anti-transport processes, further detailed mechanistic and metabolic studies *in situ* and *in vivo*, similar to those described for midazolam and cyclosporin, are warranted.

The Loc-I-Gut® perfusion studies, reported by Lennernas and co-workers may prove invaluable in understanding drug-drug interactions of this kind (Lennernas et al., 1997). The procedure involves a regional single pass perfusion of the jejenum in human volunteers and facilitates the investigation of drug-drug interactions in the pre-systemic gut wall *in vivo*. Sandstrom et al., (1999) used this technique to examine the *in vivo* effects of ketoconazole on the intestinal permeability and the presystemic metabolism of each enantiomer of (R/S) verapamil. Ketoconazole did not affect the jejunal Peff of (R/S)-verapamil but did increase the overall transport into the systemic circulation (bioavailability) most likely by inhibition of gut wall metabolism. This suggests that ketoconazole is a significantly more potent inhibitor of CYP3A4 than of P-gp *in vivo* in human intestine.

5.7. Specific interactions of saquinavir

Alsenz et al., (1998) found that for saquinavir, the basolateral to apical secretion exceeded apical (AP) to basolateral (BL) absorption by factors of 50-70 fold. Active efflux was inhibited by verapamil and cyclosporin A and in a similar study ketoconazole reduced BL-AP efflux and increased AP-BL transport of saquinavir (Profit et al., 1999). While a number of similar *in vitro* studies have been performed, demonstrating P-gp mediated and CYP3A4 mediated effects in cell culture lines, the relative contribution to the *in vivo* situation is poorly defined. The most widely documented pharmacokinetic interaction involves the saquinavir-ritonavir therapy, as discussed. Pharmacokinetic interactions in human volunteers involving concomitant administration of ketoconazole (200mg q.i.d) and saquinavir 600mg t.i.d.) resulted in 3-fold increases in saquinavir AUC and Cmax, but had little effect on the absorption kinetics of ketoconazole (Invirase® Product info). The bioavailability of saquinavir in humans has been shown to be doubled when grapefruit juice is co administered. 6', 7' – Dihydroxybergamottin, bergamotin and other furansocoumarin derivatives are constituents in the grapefruit juice that are the most likely candidates responsible for the interaction. The mechanism involved here is believed to be mediated by suicide inactivation of intestinal CYP3A4, followed by CYP3A4 protein degradation, which requires regeneration of active CYP3A4 to resume normal absorption (Lown et al., 1997; Schmiedlin-Ren et al., 1997). Interestingly, red wine has been shown to inactivate CYP3A4 in vitro and potentially cause drug interactions by the same mechanisms (Chan et al., 1998).

Cuvelier et al., (1998) examined the absorption mechanisms of saquinavir in rat intestinal loops. Intestinal loops were filled with a solution containing saquinavir alone (0.2mg/ml) or associated with a series of active transport substrates (e.g. ritonavir (1mg/ml), ketoconazole (1.88mM)). The influence of cyclosporin (10mg/kg) by IV route was also studied. The $AUC_{0\rightarrow 1h}$ was determined over 60 minutes, by sampling portal serum concentrations. The percentage of saquinavir absorbed after 1 hour was determined by determining the quantity of drug remaining in the loops. The extent of absorption of saquinavir was high (>80%), but undetectable portal blood concentrations suggesting extensive gut wall metabolism. Saquinavir concentrations in portal blood were only detectable following co-administration of ritonavir, ketoconazole and cyclosporin (LOD for assay 10ng/ml). The $AUC_{0\rightarrow 1h}$ determined from portal plasma sampling following co-administration of cyclosporin, ketoconazole and ritonavir, were 4.3, 3.9 and 7.1-fold higher, respectively, than with saquinavir alone.

MATERIALS AND EXPERIMENTAL METHODS

6. Materials and experimental methods

6.1. Materials

DDT (1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane)	Sigma
Mithotane (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane	Aldrich
Saquinavir Ro-31-8959/000	Roche
Saquinavir Mesylate Ro 31-8959/003	Roche
Ro 31-9564	Roche
Verapamil	Sigma
Cyclosporin	Sandoz
Ketoconazole	Tocris
Lecithin (Lipoid EPC; 98% Phosphatidyl choline from egg lecithin)	Lipoid
Cholic acid, sodium salt from sheep or ox bile	Sigma
Oleic acid (cis-9-Octadecenoic acid)	Sigma
Labrasol (Saturated polyglycolysed C_8-C_{10} glycerides, 50-80%C_8; 20-50%C_{10})	Gattefosse
Plurol Oleique (Polyglyceryl oleate)	Gattefosse
Cremophor EL (Polyoxyl 35 castor oil)	BASF
TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate)	Eastman
Capmul MCM (Mono-, di-, and triglycerides of C_8 and C_{10} fatty acids)	Abitec
Tween 80	Sigma
Sodium Acid Phosphate ($NaH_2PO_4.2H_2O$)	Riedel-de-Haen.
Sodium Phosphate ($Na_2HPO_4.12H_20$)	Riedel-de-Haen.
Sodium Chloride (NaCl)	Riedel-de-Haen
Acetonitrile (HPLC grade)	Rathburn
Acetone	Labscan
Methanol	Rathburn
Diethyl ether (HPLC) grade	Rathburn
Millipore filters (0.2μm)	Gelman
25Gx5/8" and 23Gx1" sterile needles	B. Braun
Quartz-glass suprasill light scattering cells (10mm)	Optiglass

Nitrogen	BOC gases

All chemicals were used as supplied by manufacturers, with their alleged purities

6.2. Instrumentation.

Temperature regulated water bath, manufactured by Gallenkamp.

Temperature regulated magnetic hot plate with stirrer.

Sigma 203 centrifuge

Balance, Mettler AE 240

Sonic bath.

pH meter

Perfusor V and Perfusor secura FT manufactured by Braun.

Autosizer Lo-C, manufactured by Malvern, UK.

Gilson transfer pippetes, P20, P200, P1000

Sorval ultracentrifuge

Hetosic cooling condenser model CD52, Heto Lab equipment

Hetofrig ice bath Model CB2, Heto Lab equipment

Triglyceride Enzymatique PAP 150 assay (bioMeirieux).

Waters HPLC (LC Module 1®), equipped with 486 detector and autosampler

6.3. Assay of biological matrices

Apparatus. High Performance Liquid Chromatography (HPLC) was carried out using a Waters HPLC pump model equipped with ultraviolet detector with variable wavelength, equipped with an auto-sampler. Daily standard curves were constructed using the ratios of the observed peak areas of drug and the internal standard. The unknown concentrations were computed from the unweighted liner regression equation of the peak area ratio against concentration for the calibration curve (r^2 >0.99).

6.3.1. Assay of DDT in lymph and blood samples

An HPLC procedure, similar to that previously described (O' Driscoll, 1991) was used to analyze DDT in blood and lymph. DDT detection was performed at λ=238nm. The stationary phase was a reversed phase MOS Hypersil C8 column [15cm x 4.6cm id.; particle size 5μm], equipped with a guard column of the same material. The mobile phase, 75% Acetonitrile in HPLC grade water was always freshly prepared, filtered (0.2μm), and degassed. The flow rate was 2ml/min. Peak area measurements were analyzed with Millenium[32] software (Version 3.05.01).

Extraction procedure of DDT from lymph and plasma

From the blood or lymph samples, a 150μl aliquot of lymph or a 200μl of blood was taken and placed into a 15ml glass centrifuge tube. 20μl of a 100μg/ml solution of internal standard, Mitothane, in acetonitrile: water (75:25) was added to each sample. For the standard curve, a spike of DDT dissolved in acetonitrile: water (75:25) was added at this point. 1ml of normal saline and 5ml of distilled diethyl ether was added. The tubes were vortexed for one minute. To separate the aqueous and organic phases, the tubes were centrifuged at 5000rpm for 3 minutes. The tubes were then placed into a dry ice/acetone bath to freeze the aqueous layer. After a 30 second centrifugation, the ether layer was decanted into a clean 5ml centrifuge tube and evaporated to dryness under a stream of nitrogen. The samples were reconstituted in 120μl of mobile phase, vortexed for 1 minute and analyzed by HPLC. A 40μl aliquot of the reconstituted sample was injected onto the HPLC system previously described.

6.3.2. Assay of saquinavir in plasma and lymph

The HPLC assay for saquinavir in lymph and plasma was similar to that previously described by (Wiltshire et al., 2000). Saquinavir detection was performed at λ=238nm. The stationary phase was a 5-μm Megellen C8 column (250 x 4.6mm, Phenomenex), equipped with a guard column of the same material. The mobile phase consisted of a 63:37 mix of acetonitrile: ammonium acetate (10mM) and the flow rate was 1ml/min. Retention times for saquinavir and internal standard (Ro 31-9564) were 9 and 21 min respectively.

Extraction procedure of saquinavir from lymph and plasma

20μl of a 5μg/ml solution of internal standard, Ro 31-9564 in mobile phase was added to either 200μl of plasma or lymph sample in a 15ml glass centrifuge tube. The lymph/plasma samples were then made basic by the addition of 50μl of 10M K_2CO_3 and extracted with diethyl ether (4mL) for 10 minutes in a head to tail mixer. Following centrifugation of the mixture for 5 min at 3000rpm, the tubes were placed into a dry ice/acetone bath to freeze the aqueous layer. The organic phase was decanted and evaporated to dryness under a stream of nitrogen. The residue was dissolved in HPLC mobile phase (0.15ml), vortexed for 1 minute, and 50μl was injected onto the column.

6.3.3. Validation of HPLC assays

Quantitation of DDT in Biological matrices

The validity of the HPLC assay for DDT was established through a careful study of the linearity of response, reproducibility of standard curve and extraction recovery. Figure 6.1 is a representative chromatogram of plasma standard extract with the identified peaks at 3.5 and 2.6 min being DDT and IS respectively. Recovery of DDT was calculated by comparison of the peak heights of the DDT and IS peaks recovered from spiked plasma samples with the peak heights of the injected standard solvent solutions and were in excess of 95% for both DDT and internal standard. Within-day precision and the accuracy of the assay were determined by replicate analyses (n=3) of spiked plasma samples at appropriate concentrations. The accuracy of the assay is expressed as ((observed concentration)/expected concentration) x 100). Between-day precision was determined by analysis of spiked plasma samples (n=3) at these concentrations on three different days of analysis. Table 6.2 depicts the

validation data obtained to determine the within– and between-day variability for DDT quantitation.

Table 6.1 The recovery, within- and between-day assay precision and the accuracy of the assay procedure for DDT from spiked lymph samples.

Spiked DDT concentration (μg/ml)	Recovery (%)	Precision		Accuracy (%)
		Within-day	Between-day	
3.34	91.35	2.58	14.96	90
8.35	87.69	1.76	11.94	97
16.7	87.25	1.34	3.51	95
33.4	106.61	1.28	3.58	99
83.5	88.76	1.16	3.22	103
167	88.33	0.25	0.73	99

Table 6.2 The recovery with- and between-day assay precision and the accuracy of the assay procedure for DDT from spiked plasma samples.

Spiked DDT concentration (μg/ml)	Recovery (%)	Precision		Accuracy (%)
		Within-day	Between-day	
0.375	82.56	16.13	20.50	86.2
0.75	84.61	10.88	13.42	98.7
1.875	83.94	5.77	7.23	89.3
3.75	86.49	2.22	6.92	98.77

Precision (CV%) is defined as (standard deviation/mean)%

Accuracy is defined as the percent of the nominal value (calibration standards)

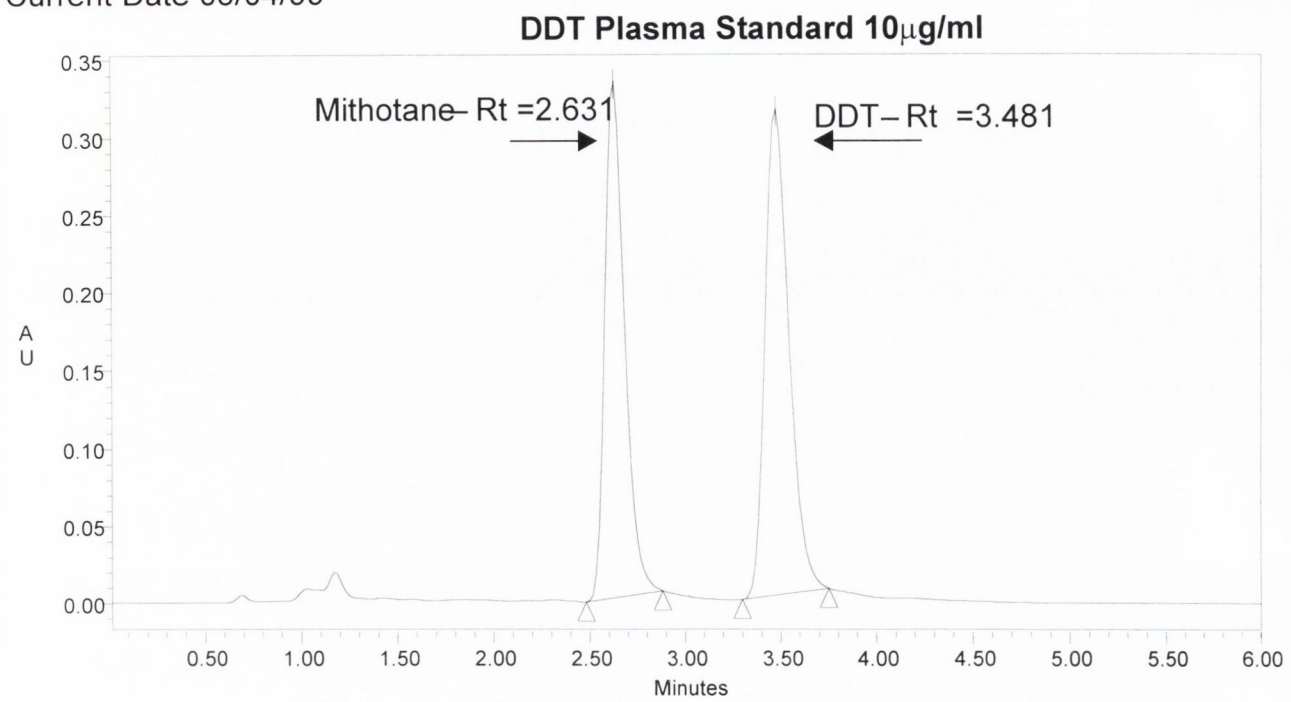


Figure 6.1 Representative HPLC chromatograms of an extracted plasma standard spiked to a concentration of 10µg/ml of DDT (3.48min) and Mithotane (2.63min), according to the methods in section 6.3.1.

The limit of quantitation for DDT in plasma and lymph was estimated to be 0.375µg/ml and 3µg/ml, respectively. The lower limit of quantitaion was defined as the concentration for which the relative standard deviation from the nominal concentration was less than 20%. The higher limit of quantitation for the lymph samples, reflecting a noisier or 'dirtier' base line, was related to the greater amount of interfering compounds in extracted lymph than in plasma.

Quantitation of saquinavir in biological matrices

The validity of the HPLC assay for saquinavir was established through careful examination of the linearity of response, reproducibility of standard curve and extraction recovery. Figure 6.2 is a representative chromatogram of extracted lymph standard with the identified peaks at 8.2 and 20.7min being saquinavir and Ro-315964 respectively.

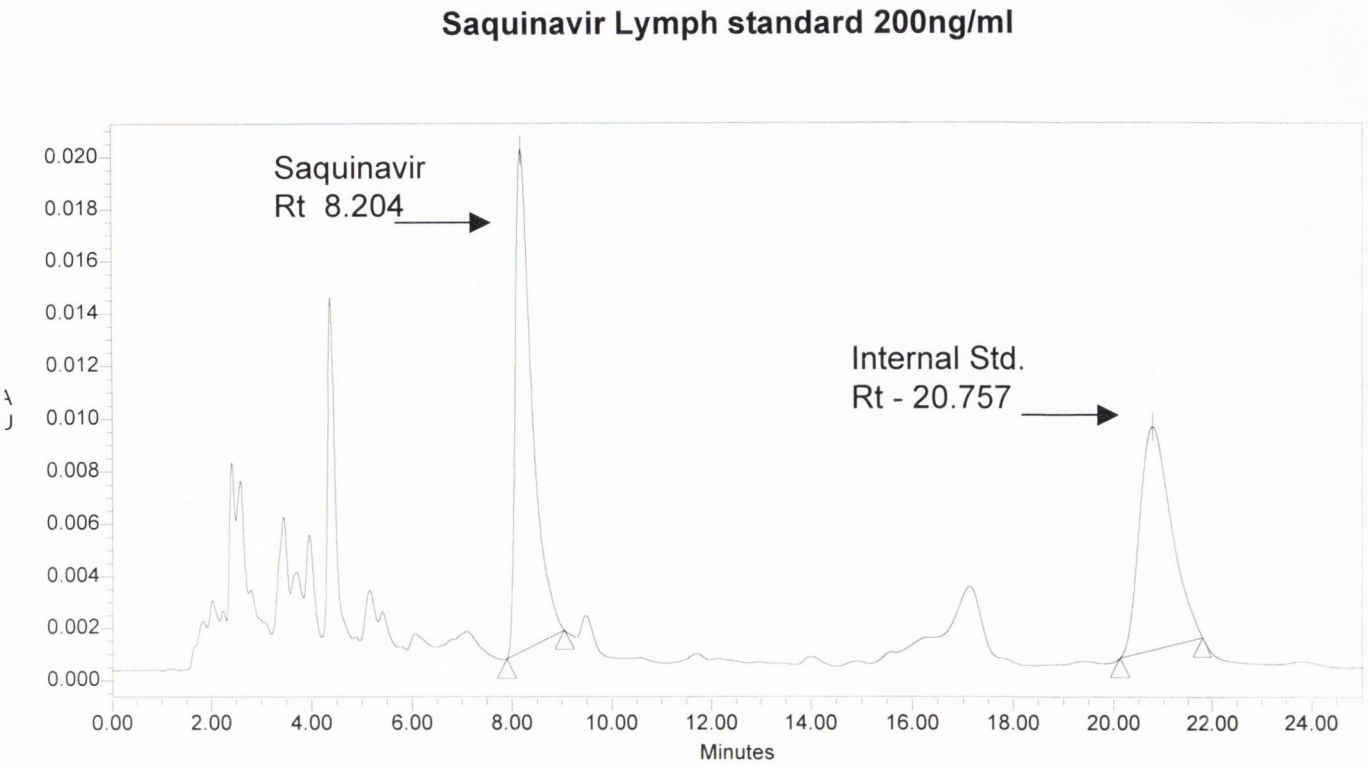


Figure 6.2 Representative chromatograms of an extracted lymph standard spiked to a concentration of 200ng/ml of saquinavir (8.2min) and Ro 31-9564 (20.7min), according to the methods in section 6.3.2.

The analysis of saquinavir in plasma and lymph exhibited excellent linearity ($r^2 \geq 0.99$) over the concentration range in plasma of 25 – 400 ng/ml for plasma and 40 – 800ng/ml for lymph. Regression intercepts for calibration curves were generally very small and were not statistically significant from zero. Table 6.3 and 6.4 summaries the relevant validation parameters for SQV analysis in lymph and plasma. The limit of detection (LOD) in serum and lymph were as low as 5ng/ml. At this concentration the signal to noise ratio was 3. At 25ng/ml in plasma and 40ng/ml in plasma and lymph, respectively the percent deviations (n=3) from the respective nominal concentrations and the R.S.D. were both less than 20%, which were achieved at a signal to noise ratio of 15. The limit of quantitation for saquinavir was therefore defined at 25ng/nl for plasma and 40ng/ml for lymph. The extraction recoveries of saquinavir were all in excess of 93% when compared to the equivalent solvent injection,

within the calibration ranges for both lymph and plasma. The recovery of internal standard was > 97%.

Table 6.3 The recovery, within- and between day assay precision and the accuracy of the assay procedure for saquinavir from spiked plasma samples

Spiked SQV concentration (ng/mL)	Recovery (%)	Precision (CV,%) Within-day	Between-day	Accuracy (%)
27	80.2	10.6	19.9	96.3
54	88.9	6.0	18.2	98.1
108	90.3	2.5	10.9	105.6
216	103.7	5.7	8.7	96.8
432	97.0	1.1	1.6	100.5

Table 6.4 The recovery, within- and between day assay precision and the accuracy of the assay procedure for saquinavir from spiked lymph samples

Spiked SQV concentration (ng/mL)	Recovery (%)	Precision (CV,%) Within-day	Between-day	Accuracy (%)
24.25	80.2	30.46	26.8	51.7
48.5	90.2	19.05	14.2	93.8
97	88.9	4.59	13.2	112
194	90.3	9.55	8.75	101
388	103.7	8.98	6.30	100
776	97.0	4.88	3.69	99.1

6.4. Preparation of lipid formulations

The composition of the phosphate buffered saline solution was $NaH_2PO_4.2H_2O$ and $Na_2HPO_4.12H_2O$ and all experiments were carried out at pH 7.2. The buffer was made isotonic with NaCl. All quantities used were according to the Sorenson's buffer system. HPLC grade water was used and the final solution was checked for pH using a pH meter prior to use.

6.4.1. Preparation of simple micellar systems

Simple micellar systems were prepared by adding appropriate quantities of either Sodium Cholate (NaC), cremophor EL or TPGS in a small quantity of isotonic phosphate buffer (pH7.2) about one-tenth the final volume of micellar solution, at 37°C with constant stirring with a magnetic bar. When the bile salt has dissolved, the solution was made up to final volume, then stirred for a further 30mins, and allowed to equilibrate at room temperature for at least 12 hours.

6.4.2. Preparation of mixed micellar systems

The quantity of Oleic acid (OA) required to make a desired concentration of micellar solution, is weighed out and added drop-wise over 30mins to a simple micellar solution of either NaC, cremophor EL or TPGS dissolved in a volume of buffer equivalent to 30% of the final volume. This is performed at 37°C with continuous stirring. When all the fatty acid is solubilized the solution was made up to the desired volume and stirred for a further 30 minutes. All solutions were viewed in front of a strong light source in order to check that the solution was transparent. All solutions were allowed to equilibrate at room temperature for at least 12hours. For the 2%TPGS: 40mM oleic acid mixed micellar formulation a period of up to 24 hours equilibration was necessary to ensure complete mixed micellar formation. This was believed to be due to the slow dissolution of TPGS aggregates in the buffer solution. In an effort to facilitate the dissolution of TPGS, a 6:1 blend of TPGS: PEG was prepared, which was subsequently formulated with 40mM oleic acid to prepare a mixed micellar solution. TPGS:PEG OA mixed micelle formulations were used in the saquinavir lymphatic transport studies.

6.4.3. Preparation of bile salt/phospholipid mixed micelle systems

Bile salt/phospholipid mixed micelles were prepared according to the method of Mazer et al., (1980). To make a desired concentration of bile salt/phospholipid micellar solution, the appropriate amount of bile salt was weighed into an evaporating dish, and then dissolved in about 5ml of methanol. The appropriate quantity of lecithin was dissolved in 5mls of chloroform, and mixed with the bile salt/methanol mix. The mixture was evaporated under a stream of oxygen free nitrogen gas, until a thick gel-like substance or paste was left and all the methanol/chloroform gone. The residue was freeze-dried to a constant dry weight (which usually took in excess of 24 hours). The dry residue was then re-dissolved gradually in the buffer solution and made up to the desired volume.

6.4.4. Preparation of oleic acid dispersion

The oleic acid dispersion was prepared by adding the appropriate quantity of oleic acid (2%w/v) to a 0.2% Tween 80 aqueous solution and homogenized for 2 x 30second intervals with an Ultra-Turrax. The stability of the resultant dispersion was assessed by taking samples at appropriate time intervals up to 3 hours post homogenization and viewing droplet size and stability under a light microscope. The dispersion was appeared stable over 12 hours with no visible signs of creaming. Photomicrographs of the droplets confirmed that the droplet sizes are similar at 1 hour and 3 hours post-homogenization (i.e. <20μm) confirming adequate stability throughout the course of the experiment. The concentration of the perfused dispersion was samples periodically throughout perfusion, and the concentration of DDT determined, to ensure reproducible dosing.

6.4.5. Preparation of SEDDS

Pseudo-ternary phase diagrams were constructed to examine the formation of w/o and o/w microemulsions using a four-component oil/surfactant/cosurfactant/water system. The four-component system consisted of:

1. The long chain fatty acid, oleic acid.
2. A high HLB surfactant (Labrasol or cremophor EL)
3. A low HLB co-surfactant (Capmul MUM or Plurol Oleique)
4. HPLC grade water

The ternary phase plots were constructed by keeping the ratio of surfactant to cosurfactant fixed (Constantinides, 1995). In preparing each formulation, the following general procedure was employed: appropriate amounts of surfactant/co-surfactant mixture (at fixed ratios of either 1:1, 3:1 and 6:1) and oil were weighed into well-capped glass vials. Samples were shaken for sufficient time to attain equilibrium, maintained at 25^0C; the three component systems were then progressively enriched with water (added drop by drop), while stirring with a magnetic stirrer (1" stirrer bar set at 300r.p.m.). Points of phase separation, turbidity and transparency and changes in viscosity were noted. Regions in which the formulation formed visually stable, isotropically clear solutions were marked on the phase diagram as a microemulsion. The amounts of water added at which transitions occurred were derived by weight measurements. By repeating the experimental procedure for other combinations of surfactant/co-surfactant to oil ratio, the phase boundaries were determined. The microemulsion droplet size of appropriately selected SEDDS blends was determined by photon correlation spectroscopy (Malvern Lo-C model no. MAN 0055). The SEDDS formulations were diluted 1:200 in HPLC grade water to enable accurate measurement, with count rates between 50-200Kcps.

6.5. Solubility studies in lipid solutions

Micellar solutions (5ml) were placed in 10ml glass ampoules to which excess solubilisate (DDT or saquinavir) had been added, sufficient to produce saturation (Hamlin et al 1965). The flame-sealed vials were equilibrated in a thermostated water bath equipped with a shaker at 37^0C for up to 7 days. A vial was sampled from each replicate batch every 24 hours. The samples were filtered though a 0.22μm (Millipore filter), and diluted immediately with an appropriate quantity mobile phase, to prevent precipitation and subsequently analyzed by HPLC. At least three determinations were made per sample, with a minimum of four experiments for each lipid solution.

6.6. Surgical and experimental procedures

In lymphatic transport studies, an anaesthetized rat model has often been employed because it provides a controlled system for evaluating and probing the mechanistic aspects of the lymphatic transport process (Porter & Charman, 1997). The mesenteric intestinal lymph duct, rather than the thoracic lymphatic duct, is the preferred site of cannulation to estimate the

ability of the small intestine to lymphatically transport lipophilic molecules. (Noguchi et al., 1985). A mesenteric lymph-duct cannulated anaesthetized rat model has now been established in our laboratory which allows the estimation of the degree of intestinal lymphatic transport from the small intestine while simultaneously allowing for blood sampling. The triple cannulated rat model, where the mesenteric lymph duct, jugular vein and duodenum are accessed, as detailed by Porter et al., (1996) has been modified in our experiments. Jugular vein cannulation was not attempted as it proved to be a difficult technique to perfect, and increased both the extent of surgical trauma and the duration of surgery. Blood samples were taken by cardiac puncture allowing for the collection of larger volumes of blood samples and reduced risk of blood pooling following repeated venous puncture in the jugular vein.

Surgical procedures were approved by the local animal experimentation ethics committee. Male Wistar rats, weighing 280-320g were fasted for 24 hours with free access to water. The animals were anaesthetized for the duration of the experiment using 50mg/kg sodium pentobarbital given by intraperitoneal injection. Additional injections were given as required (approximately every 2 hours). The animals were shaved from ventral midline to dorsal midline on the animal's right side and on the ventral side of the neck and upper chest. The animals were kept secured on a heating pad to maintain body temperature, and a low watt overhead light was used for this purpose as well, when additional heat was required.

A 7-8 cm lateral incision was made 1cm below the ribs from the right side of the animal to the ventral midline cutting through both skin and muscle layers. The intestines were gently pushed to the animals left side (into the body cavity). A 2 x 2 x 7 cm piece of cotton was placed in the body cavity to hold the relocated intestines in place. A pair of four inch pointed forceps was then placed into the fat pad beneath the right kidney. The forceps, with tips together, were gently pushed through the fat, under the vena cava, raising the peritoneal membrane on the other side of the vena cava. Once the peritoneal membrane had been raised, it was cut to leave the mesenteric artery and the mesenteric lymph duct exposed. While the forceps are under the vena cava the lymphatic cannula can be pulled through. A syringe filled with heparinized saline (1000IU/ml), was used to flush the cannula to prevent lymph clotting. Taking great care, the top of the lymph duct was cut. (The mesenteric lymphatic duct is usually located to the left, anterior, of the mesenteric artery.) The polyethylene end of the cannula was inserted into the lymph duct approximately 3 to 4 mm. At this time, the syringe

can be removed temporarily to check for lymph flow. Any auxiliary lymph ducts to the fight of the artery were cut to ensure all the lymph flow was into the main mesenteric duct. One drop of cyanocrylate glue was placed over the area to hold the cannula and to seal the auxiliary ducts. A 5 x 5mm of muscle tissue which had been cut from the abdominal wall was placed over the super glue area to help secure the cannula and to prevent adhesions of the intestines. At this point the cotton plug was removed and the intestines were gently brought back to the original position. A duodenal infusion of 1.2-1.5ml/h of 0.9% saline was initiated immediately after operation and continued without further interruption unless replaced during a test period by an equal volume of fluid containing lipid.

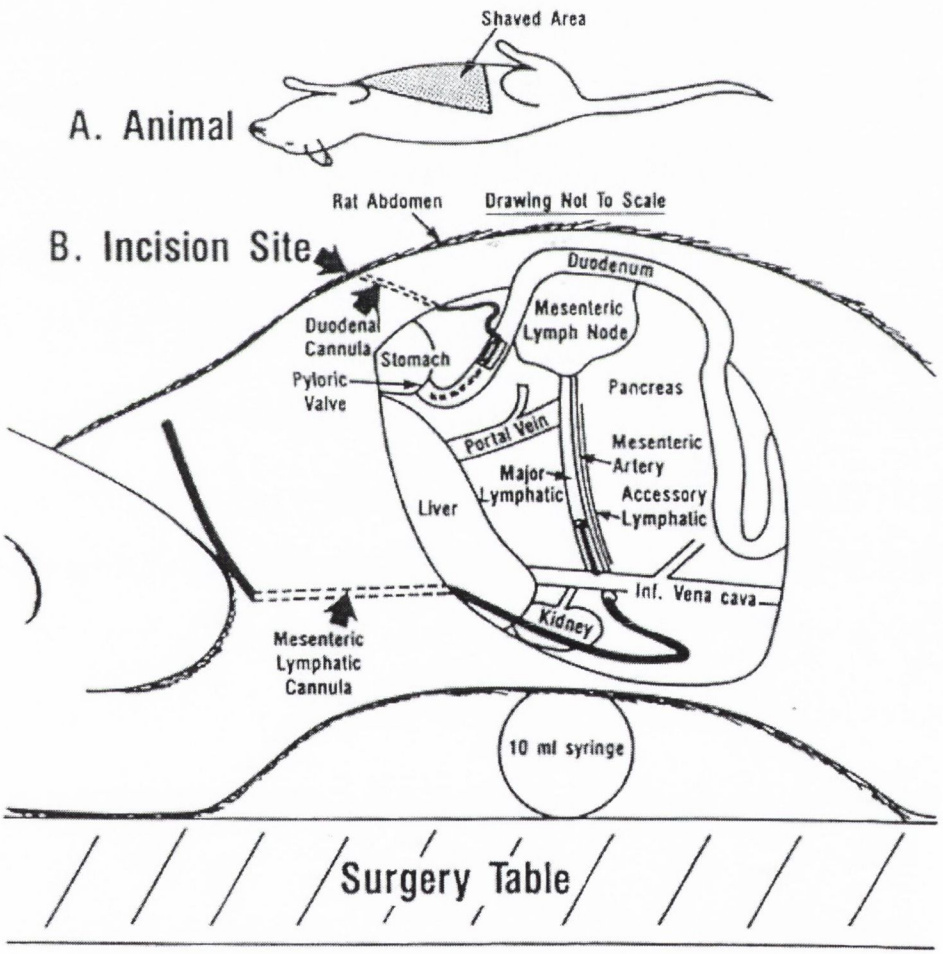


FIGURE 2. Detailed placement of (A) incision, and (B) placement of mesenteric lymphatic and duodenal cannulas. This drawing has been expanded to show detail. Cannulas are drawn in black and the dotted lines show their placement in the tissue.

The duodenal cannula was attached to a trocar and externalized through the abdominal wall. It was then inserted into a small hole made in the duodenum approximately 1cm from the

pylorus. The cannula was secured with one drop of cyanoacrylate glue. The intraperitoneal cannula was inserted between sutures after the closing of the animal's abdominal muscle layers. The application of super glue during the skin layer closure secured the cannula.

Where necessary, a tracheotomy was performed by cutting a 5 x 5 mm hole in the skin above the trachea, to facilitate breathing of anaesthetized rats. The muscle layers were separated with two pairs of four-inch tissue forceps to expose the trachea. A pair of four inch curved tip forceps were inserted under the trachea and a small incision was made on the top of the trachea and the cannula was inserted.

In all cases a period of at least one hour was allowed prior to administration of the drug formulations. This time was allowed to aid the recovery of the animals from the surgical procedures and to let the intestinal motility to return to normal. Since a substantial amount of body fluid is removed during the course of the experiment, the animals were perfused with normal saline at a flow rate of 1.2-1.5ml/hr through the intraduodenal cannula to maintain body hydration and intestinal lymph flow. Drug formulations were administered via the intraduodenal cannula over 3 hours at a flow rate of 1.2ml/hr, for DDT absorption studies, and over 2 hours at a flow rate of 1.5ml//hr for saquinavir absorption studies. Rehydration was stopped while perfusing the test formulations. Blood samples were taken, by cardiac puncture, at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug administration. Mesenteric lymph samples were collected continuously for 1 hour before and 8 hours after dosing in pre weighed cooled glass tubes containing anticoagulant (EDTA or heparin). Lymph collection tubes were changed hourly.

At the conclusion of the experiment, animals were sacrificed by an overdose of i.v. sodium pentobarbital, and the integrity of implanted cannula in each animal was checked. Interval lymph output was determined by weighing the samples collected and any animal not producing consistent lymph flow, and an average, minimum lymph output of 0.3ml/hr was excluded from the study.

Non-lymph cannulated rats - In a separate series of experiments this group were sham operated in terms of the mesenteric lymph duct cannulation and had an intra-duodenal cannula (for rehydration) inserted, as described above.

Intravenous rats – 0.3ml of the i.v. formulation was administered by i.v. bolus injection into the jugular vein over a period of less than 10 seconds. These animals also received a 1.5ml/h intra-duodenal infusion of normal saline throughout the course of the experiment. Serial blood samples (0.2ml) were collected at –5mins, and 5, 10, 15, 20, 30, 45 and 60 minutes, and 0.5-ml blood samples were taken at 3, 4, 6 and 8 hours.

6.6.1. Preparation of drug formulations

Drug concentrations in all lipid formulations were verified on the day of dosing using a validated HPLC assay. The procedure for forming DDT saturated micellar formulations involved sonication for 20 minutes in an ice cooled sonication water bath and stirring for a further 12 hours prior to administration, which allowed sufficient time to reach the DDT saturation solubility in both the bile salt and cremophor micelles. However, for the TPGS micelles, saturation with DDT took considerably longer (i.e.> 72 hours), and as a result the concentration prepared in the simple and mixed TPGS micellar systems was fixed at 200μg/ml and 500μg/ml, representing 74% and 89% of saturation solubility respectively. For the SEDDS and oleic acid dispersion, the weighed quantity of DDT was dissolved in either the SEDDS blend, or oleic acid, which were then dispersed in water or 0.2% Tween 80 respectively. For the saquinavir absorption studies, saquinavir was added to the lipid solutions 2 hours prior to intraduodenal dosing, at a concentration of 1.667mg/ml. Micellar solutions containing 1mM of either verapamil, ketoconazole or cyclosporin, were allowed to equilibrate for 12 hours prior to addition of saquinavir. The i.v. formulation consisted of saquinavir dissolved in a 5% cremophor EL (in phosphate buffered saline pH 7.2) solution, at a concentration of 3.33mg/ml.

6.7. Analysis of lymph triglyceride

Lymph triglyceride (expressed as mg equivalents of C_{18} LCT) was determined using a Triglceride Enzymatique PAP 150 assay (bioMeirieux). The routine triglyceride assay used to quantify triglyceride transport in these studies was based on a commercial enzymatic clinical chemistry assay. This type of analysis relies on hydrolysis of the triglyceride, quantification of the liberated glycerol by reaction with a colourimetric reagent followed by u.v. analysis at 505nm, and recalculation of the mass of triglyceride assuming that the triglyceride present is

the same as it is in the calibrator (which contains C_{18}-based triglycerides present in plasma). Caliph et al., (2000) have shown that the predominant triglyceride in the lymph of rats, regardless of the type of triglyceride dosed, was C_{18} long chain triglyceride. The minimal quantities (or absence) of non-C_{18} based triglyceride therefore justified the use of the enzymatic triglyceride test kit to assess lymphatic lipid transport.

Lymphatic triglyceride transport due to exogenously administered lipid was determined by subtracting the mean baseline or "endogenous" triglyceride transport measured in lymph cannulated rats (n=3) administered saline rehydration solution throughout the experiment, from the total triglyceride transport in animals administered lipid-based formulations.

6.8. Pharmacokinetic data analysis

The pharmacokinetics of DDT and saquinavir were characterized by non-compartmental methods. The parameters are derived from the bolus intravenous equation 1, where C is the plasma or whole blood concentration, C_i is the coefficient, and λ_i is the exponent of the *i*th exponential term.

$$C = \sum_{n=1}^{n} C_i . e^{-\lambda_i t} \qquad \text{where } i = 1 \text{ to n} \qquad \textbf{Equation 6.1}$$

The initial concentration at time zero, C_0, is given by Equation 2.

$$C_0 = \sum_{n=1}^{n} C_i \qquad \textbf{Equation 6.2}$$

The area under the concentration time curve, AUC, is given by Equation 3.

$$AUC = \sum_{n=1}^{n} C_i / \lambda_i \qquad \textbf{Equation 6.3}$$

The volume of the central compartement is given by Equation 4, where D is the dose administered.

$$V_c = D / C_0 \qquad \textbf{Equation 6.4}$$

The clearance, CL, is given by Equation 5.

$$CL = D/AUC \qquad \textbf{Equation 6.5}$$

The elimination half-life, $t_{1/2}$, is given by Equation 6, where λ_i is the smallest exponent and is the apparent elimination rate constant corresponding to the very tail end of the concentration time curve.

$$t_{1/2} = 0.693/\lambda_i \qquad \textbf{Equation 6.6}$$

After intraduodenal administration, the area under the concentration time curve, from time 0 to infinity was calculated as AUC ($t + C_t/\lambda_i$), in which t was the time of last measurable concentration and AUC (t) was calculated with the linear trapezoidal rule.

The bioavailability in sham operated (non lymph cannulated) animals was estimated from the ratio of the doses normalized AUC's after oral and i.v. administration as follows:

$$F = (AUC^{oral}{}_{0\rightarrow\infty h}/D_{oral}) / (AUC^{i.v.}{}_{0\rightarrow\infty h}/D_{i.v.}) \qquad \textbf{Equation 6.7}$$

The percent of the administered dose appearing in the systemic circulation in lymph cannulated animals was calculated in the same way, although in this case, the estimated bioavailability is not a true bioavailability as it only reflects drug absorption directly via the blood and not indirectly via the lymph. The extent of lymphatic transport was calculated using the concentration of drug found in each lymph sample, multiplied by the volume of the lymph produced per hour, and expressed as a cumulative percentage of the dose. Total availability or exposure in lymph cannulated was assessed as the percent dose appearing in the systemic circulation (calculated via the AUC comparison) plus the percent dose collected in the intestinal lymph.

6.9. Photon Correlation Spectroscopy

Photon correlation spectroscopy is a dynamic light scattering technique, which analyses changes in light intensity fluctuations initiated by Brownian motion. This method can be used to determine particle size primarily in the nanometer size range. Brownian motion is the random movement of particles due to bombardment by the solvent molecules that surround them. Larger particles exhibit slower Brownian motion while smaller particles exhibit faster motion. Small changes in light intensity fluctuations are therefore dependent on the particle size. The PCS method rests on the fact that a photon counting detector can track rapid

changes of intensity, if enough photons are present during the sample time in which an individual snapshot of intensity is recorded.

A Malvern Autosizer Lo-C apparatus fitted with a 64 channel 7032 correlator and argon ion external laser, providing the necessary 75mW - 100mW at 488nm with a focused beam, was employed. The scattering cell (Suprasil®) containing the test solution was placed in a thermostated air-cooled (fan assisted) light chamber. The resulting scattered light was detected at an angle of 90^0 by a fixed angle photon detection system which transmitted the signal to the Malvern 7032, 64-channel multibit correlator. The correlation data was analysed by a Malvern application program (LoC version PCS: v1.26), via a Quattro® computer. The method of cumulants was employed in the data analysis. This method is best suited for a unimodal population of particles, given by a monodisperse solution

Scattering cells were acid-washed in chromic acid, rinsed in distilled water and dried in a hot air oven. The test solutions were clarified by a passage through a 0.2μm membrane filter three times. The solution was allowed to equilibrate for 15 minutes in the light chamber prior to starting the sequence program. A minimum of 10 determinations involving five preparations for each sample was done, to obtain average values for the observed particle size. The optimum correlation sample time was determined through monitoring the polydispersity, particle size distribution and scattering intensity obtained by varying the sample times employed in pre-runs of the solution. Solution concentrations producing count rates between 50-200Kcps were desirable. To check for correct operation of equipment, polystyrene latex particles, (diameter 234nm), were used as a standard, well-characterized, monodisperse system. One drop of concentrate was dispersed in 50ml of distilled and filtered water. The merit values describe the relationship between signal to noise ratio. Values between 10-90% are desirable. The Z average mean size corresponds to the mean if the intensity distribution for a narrow distribution. A volume mean can be calculated using Mie theory, however the sample refractive index and absorbance value, denoted as the real and imaginary refractive index, must be known. The polydispersity is an index calculated from fitting a three-parameter polynomial to the log correlation function. It is interpreted as the variance of the supposed log-normal plot.

Because micelles are small particles, the experiments required moderately high laser power, medium sized photomultiplier aperture and short sample times (so as to avoid excitation of the particles by the external light source). Also, because of their size, the PCS of micelles can be complicated by stray light, dust scattering and the presence of large particles that scatter intensely and have along decay in the auto correlation function. Therefore, the use of a calculated background (obtained from the correlator monitor channels), which the Malvern program uses, means that sample preparation (especially dust removal) had to be rigorously controlled.

6.10. Determination of clog P

The clog P of candidate compounds was estimated using the clog P for Windows® software (Version 2.0). The theoretical partition coefficient octanol/water is estimated by imputing the chemical structure of the candidate compound, from which the lowest energy structural conformation is predicted. The clog P is then estimated on the basis of chemical bond structural characteristics. For candidate compounds where an experimentally measured octanol/water partition coefficient was available, there was good agreement between the predicted and actual values (i.e. within 0.1 log units)

6.11. Data analysis

One way analysis of variance (AVOVA) and unpaired Student's t test were used to determine the statistical significance ($p < 0.05$) of calculated results among experimental groups. The relationship between variables were tested using the nonlinear curve fitting and model development program, Micromath R Scientist® for Windows® Version 1.05 (Micromath R Scientific Software). Model suitability was assessed using the Model Selection Criterion.

RESULTS AND DISCUSSION

7. Micellar Solubilization - A comparison of naturally occurring and synthetic surfactants

7.1. Introduction

For lipophilic drugs, low aqueous solubility is associated with slow dissolution, which can result in poor bioavailability. The non-polar nature of such drugs allows rapid absorption across the epithelial membrane when the drug is available in solution in the lumen of the gut, but often dissolution from crystalline particles is the rate-limiting step in absorption. One method of overcoming this problem may be the administration of the drug in lipidic solutions e.g. mixed micelles, microemulsions etc. Through normal processes of fat digestion (lipolysis), bile salt micelles are formed which solubilise the breakdown products arising from lipolysis, such as monoglycerides and fatty acids. These micelles act as a reservoir of breakdown products by maintaining them in solution in the lumen, so that when fatty acid or monoglyceride is absorbed from the aqueous contents of the gut, more material can be made available by partitioning from the mixed micelles. If a drug is administered in a lipid formulation it may undergo a similar fate, with drug also becoming solubilized at high concentration in mixed micelles, acting as a reservoir for dissolved drug. Thus the dissolution step may be avoided.

The benefits associated with delivery via lipid vehicles have been widely reported. Mixed micellar solubilization and presenting the drug in solution or dissolved in small droplets, so as to increase the interfacial surface area, are pertinent factors to enhanced bioavailability, as discussed in Chapter 3. Crucially, it appears that in order to maximise the rate of absorption of hydrophobic compounds, the drug must be solubilized throughout intestinal transit and avoid crystallisation of the drug.

Bile acids and fatty acids are known to play a role in the absorption of fats by solubilizing the water insoluble products of fat digestion into water-soluble aggregates or micelles (Carey & Small, 1970). Micellar systems have, therefore, been exploited as vehicles for oral drug delivery (O' Driscoll, 1996). In addition to enhancing the solubility of lipophilic drugs (O' Driscoll et al., 1991; Obodozie, 1997), the absorption of the drug from the gastrointestinal tract and bioavailability may also be affected by surfactant systems. The absorption enhancing action of micellar systems depends on a number of factors including the physiochemical properties of the drug, the interaction between the drug and the micellar system and the subsequent interaction of the micellised system with the

physiological environment (O' Reilly et al., 1994). However the mechanisms by which micellar systems affect absorption are complex and conflicting results have been reported indicating that these systems may enhance (Muranushi et al., 1980; O' Reilly et al., 1994), or retard transport (Amidon et al., 1982; Poelma et al., 1989 & 1990).

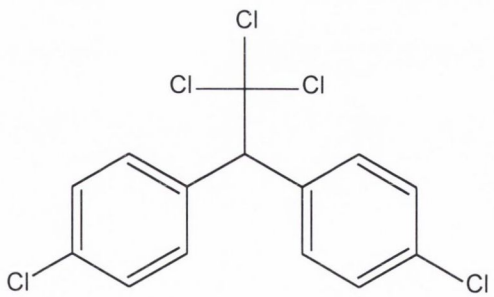
The determination of the capacity for and specificity of bile salt or surfactant micelles to solubilise drugs is an important step in developing any predictive model or approach to establish the influence of these systems on the absorption of drugs. In the present study the extent of solubilization of both DDT and saquinavir, two model lipophilic compounds, will be examined in a series of micellar vehicles, as a preliminary step to *in situ* studies. The solvent capacities of simple and mixed micellar systems, formed from both synthetic and naturally occurring surfactants, will be compared. The technique of photon correlation spectroscopy (PCS) will be used to characterize the micellar systems produced. Finally, the relationships between lipophilicity of a series of lipophilic compounds and their solubilization by bile salt micelles systems were examined, with a view to developing a predictive relationship between log P and the solubilization capacity of bile salt micelles for lipophilic compounds.

7.2. Candidate Lipophilic Compounds

7.2.1. DDT

DDT (1,1,1, - Trichloro - 2,2,- bis (4 - Chlorophenyl) ethane), popularly called Dicophane, was formerly used as an insecticide. It is a non-ionic, highly lipophilic compound and practically insoluble in water {aqueous solubility = 1.2ng/ml, Log P $_{octanol/water}$ = 6.19 (Patton et al., 1984). It has been shown to be absorbed across the intestinal mucosa unchanged (Sieber, 1974). On the basis of its low aqueous solubility and possible high membrane permeability (based on Log P), DDT belongs to the class II category of the Biopharmaceutical Drug Classification Scheme (Amidon et al., 1995). An inspection of its structure (Figure 7.1), indicates considerable hydrophobicity; it is highly fat soluble, {solubility in peanut oil = 97.5mg/ml, (Myers & Stella, 1992)}. These physiochemical properties of DDT make it an ideal model compound to investigate the factors controlling the intestinal lymphatic transport of hydrophobic drugs. Various absorption studies in the literature have reported varying extents of DDT absorption in the presence of lipid vehicles (Rothe, 1957; Pocock & Vost, 1970). DDT is transported highly through the intestinal lymphatics, with up to 19% of the administered dose, getting into the systemic circulation via this route (Myers & Stella, 1992).

(1,1,1, - Trichloro - 2,2,- bis (4 - Chlorophenyl) ethane)



Mol. Wt 354.49

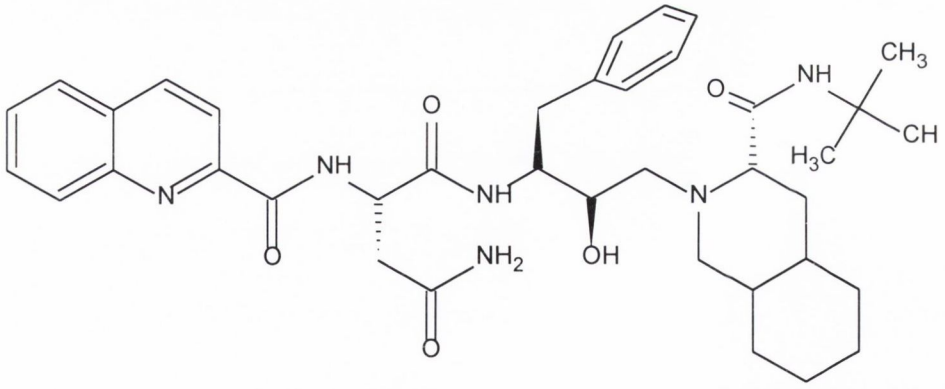
M.P. 109°C

Log P $_{o/w}$ 6.19

Figure 7.1 Structure of *p,p* DDT

7.2.2. Saquinavir

Saquinavir {N-tert-butyl-decahydro-2- (2(R)-hydroxy-4-phenyl-3- (S)-((N–(2-quinolylcarbonyl)-t-asparaginyl) amino) butyl)-4aS, 8aS-isoquinoline-3(S)-carboxamide} is a synthetic peptide like substrate analogue that inhibits the activity of HIV protease and prevents cleavage of viral polyproteins. Saquinavir is a large molecule; the free base has a molecular weight of 670 (Figure 7.2). It contains two basic centres, the pKa values of the conjugate acids of the isoquinoline and decahydroisoquinoline groups being 1.1 and 7.1 respectively. Although reasonably soluble in water under mildly acidic conditions, (2.5mg/ml in pH 3.0 phosphate/citrate buffer) when the aliphatic amino group is fully ionised, raising the pH to seven causes the drug to become only partially ionised and aqueous solubility is drastically reduced. Thus under physiological conditions saquinavir is a hydrophobic molecule, even though it contains a number of polar groups. The solubility in phosphate buffer pH 7.2 at 37^0C was determined to be 17.4 ± 3.2μg/ml. The estimated clog P is 4.5. On the basis of its low aqueous solubility and poor membrane permeability, as discussed in Chapter 5, saquinavir belongs to the class IV category of the Biopharmaceutical Drug Classification Scheme (Amidon et al., 1995).



Structure $C_{38}H_{50}N_6O_5$

Mw = 670.90 **cLog P $_{o/w}$ 4.50**

Figure 7.2 Structure of Saquinavir freebase

7.3. Results

7.3.1. Physiochemical properties of DDT and saquinavir

Some of the physiochemical properties of saquinavir and DDT are listed in Table 7.1. It is clear that DDT displays more highly lipophilic characteristics than saquinavir. Saquinavir solubility in purified water is 57 ± 12.1 μg/ml compared to 17 ± 3.2μg/ml in phosphate buffer pH 7.2. This reduced solubility reflects the increased pH, with purified water having a pH of 5.5. It is also interesting to note that the solubility in the free fatty acid, oleic acid, is markedly higher than in the triglyceride equivalent, triolein i.e. 2.48- and 11.8- fold higher for DDT and saquinavir respectively.

Table 7.1- Comparison of physiochemical properties of DDT and saquinavir

	DDT	Saquinavir
Mw	354.49	670
Log $P_{o/w}$	6.19 [B]	4.5 [C]
Solubility [A]:		
Aqueous	0.0012 [B]	57 ± 12.1
pH 7.2	n/a	17 ± 3.2
Triolein	80 (x 10^3) [D]	1.87 ± 0.48 (x 10^3)
Oleic acid	198 (x 10^3) [E]	22.4 ± 4.4 (x 10^3)

[A] (μg/ml) at 37 ^{0}C ± S.E.
[B] Patton et al., (1984)
[C] Calculated octanol/water partition coefficient
[D] Solubility (μg/g) at 23^{0}C.
[E] Obodozie, (1997)

7.3.2. Solubilization in naturally occurring and synthetic micellar systems

7.3.2.1. Natural surfactants

Bile salt simple and mixed micelles

The saturation solubility of DDT in simple and mixed bile salt micelles was measured according to the methods described previously (Chapter 6). The choice of sodium cholate (NaC), a trihydroxy conjugated bile salt, CMC 3-5mM, as candidate bile salt was based on previous experience in our laboratories, having due regard to its stability and ease of preparation (Obodozie, 1997). In addition, cholic acid is the major bile acid component of both the rat and the human hepatic bile duct, accounting for about 50% of total bile acids

present (Carey & Small, 1972). On average, the bile salt concentrations in the duodenum and upper jejunum are approximately 2 – 3 times higher post-prandially (10 – 15mM; range 3 – 35mM) (Fausa, 1974; Tangerman et al., 1986) as compared to fasting conditions (5mM, range 0-14mM) (Tangerman et al., 1986; Peeters, 1980). During digestion of lipid rich meals, concentrations in the order of 40mM can be found (Sjovall, 1959). The long chain mono-unsaturated fatty acid, oleic acid (cis-octadecanoic acid $C_{18:1}$), was chosen as it is the principal long chain fatty acid that occurs physiologically in human mixed intestinal lipids (Staggers et al., 1990). Obodozie (1997) studied a range of bile salt mixed micelles, with differing fatty acid components and found oleic acid produced the highest Papp and relative bioavailability values of DDT. The solubility of DDT in the presence of sodium cholate (40mM) micelles, at pH 7.2, with increasing concentration of oleic acid is shown in Table 7.2. Mixed micelles were formed by the incorporation of the increasing concentrations of fatty acid into 40mM NaC solution, resulting in increased saturation solubility of DDT.

Table 7.2 The saturation solubility of DDT in simple bile salt (NaC) and mixed bile salt fatty acid (NaC:OA) micelles, and relative enhancements at 37^{0}C.

System	Solubility (mM) ± SD	Relative Enhancement	
		Water	Simple Micelle
Water	0.339×10^{-5} [A]	-	-
NaC 40mM	0.151 ± 0.008	4.45×10^4	-
NaC 40mM: 10mM OA	0.146 ± 0.008	4.31×10^4	0.97
NaC 40mM: 20mM OA	0.308 ± 0.021	9.09×10^4	2.04
NaC 40mM: 40mM OA	0.693 ± 0.039	20.44×10^4	4.56

[A] Patton et al. (1984)

It can be seen that the addition to 40mM NaC, of low concentrations of fatty acid (10mM) had no significant effect. When the concentration was increased above 10mM, significant enhancements ($p < 0.05$) were observed with increased long-chain fatty acid content.

A similar pattern of increased saturation solubility with increased fatty acid content is observed for saquinavir in simple versus mixed bile salt micelles, as listed in Table 7.3.

The solubility enhancements resulting from the incorporation of 40mM oleic acid are similar for both compounds, with up to 4-fold increases in solubility between 40:40mM NaC: OA mixed micelles, compared to 40mM NaC alone.

Table 7.3 The saturation solubility of saquinavir in simple bile salt (NaC) and mixed bile salt fatty acid (NaC:OA) micelles, and relative enhancements at 37^{0}C.

System	Solubility (mM) ± SD	Relative Enhancement	
		Buffer	Simple Micelle
PBS pH 7.2	0.026 ± 0.008	-	-
NaC 40mM	0.130 ± 0.015	5.00	-
NaC 40mM: 10mM OA	0.368 ± 0.037	14.15	2.83
NaC 40mM: 20mM OA	0.463 ± 0.050	17.80	3.56
NaC 40mM: 40mM OA	0.531 ± 0.037	20.42	4.08

7.3.2.2. Synthetic surfactants

Cremophor EL simple and mixed micelles

Table 7.4 and Table 7.5 list the saturation solubility of DDT and saquinavir, respectively, in simple and mixed micellar systems prepared with the non-ionic synthetic surfactant, Cremophor EL (glycerol triricinoleal 35, Polyoxyl 35 castor oil). Cremophor EL has a CMC of 0.01% approximately. The main component of Cremophor EL is glycerol-polyoxyethylene glycol ricinoleate, which together with fatty acid esters of polyethyleneglycol represents the hydrophobic part of the product. The smaller, hydrophilic part consists of polyethylene glycols and ethoxylated glycerol. As it was not possible therefore to obtain a precise molecular weight, a 2% solution in phosphate buffer pH 7.2 was used, as has previously been reported (O' Reilly et al., 1994). Addition of oleic acid to produce mixed micelles produced significant increases in the solubilizing capacity of the micellar formulation, with increases in the saturation solubility observed for both lipophilic compounds.

Table 7.4 The saturation solubility of DDT in cremophor EL simple and cremophor EL: oleic acid mixed micelles, and relative enhancements at 37^{0}C.

System	Solubility (mM) ± SD	Relative Enhancement	
		Water	Simple Micelle
Water	0.339×10^{-5}	-	-
Cremophor EL 2%	1.53±0.042	45.13×10^4	-
Cremophor EL 2%: 10mM OA	1.83±0.028	53.98×10^4	1.20
Cremophor EL 2%: 20mM OA	2.22±0.016	65.49×10^4	1.45
Cremophor EL 2%: 40mM OA	2.62±0.079	77.29×10^4	1.71

Table 7.5 The saturation solubility of saquinavir in cremophor EL simple and cremophor EL: oleic acid mixed micelles, and relative enhancements at 37^{0}C.

System	Solubility (mM) ± SD	Relative Enhancement	
		Water	Simple Micelle
PBS pH 7.2	0.026 ± 0.008	-	-
Cremophor EL 2%	1.310 ± 0.138	50.40	-
Cremophor EL 2%: 10mM OA	4.99 ± 0.631	191.92	3.81
Cremophor EL 2%: 20mM OA	7.36 ± 1.438	286.08	5.61
Cremophor EL 2%: 40mM OA	11.478 ± 1.325	441.46	8.76

Cremophor EL simple and mixed micelles exhibit a greater solubilization capacity for both DDT and saquinavir than the corresponding bile salt systems. This observation is consistent with a previous report where the solubility of for the lipophilic compound clofazimine was examined in naturally occurring and synthetic surfactant micelles (O' Driscoll et al., 1991).

An examination of the relative enhancements for each compound, compared to solubility in cremophor EL simple micelles, reveals that the order of enhanced solubilization is

greater for saquinavir, with an 8-fold increase in the saturation solubility resulting from incorporation of 40mM oleic acid. This compares with a 1.7 fold relative enhancement for DDT in 2% cremophor EL: 40mM oleic acid mixed micelles over that observed in simple cremophor micelles.

TPGS simple and mixed micelles

D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble derivative of natural source vitamin E. It is prepared by the esterification of polyethylene glycol 1000 (hydrophilic) to the acid group of D-α-tocopheryl acid succinate (lipophilic). It is amphiphatic, miscible with water, and forms a variety of liquid crystalline phases and micelles in water, with a CMC of 0.02% by weight (Hong-Wei Wu & Hopkins, 1999). The excipient melts at 37-41^{0}C and is heat stable at temperatures <200^{0}C. The saturation solubility of DDT in simple and mixed TPGS micellar systems, formed with increasing concentrations of oleic acid, are presented in Table 7.6. As in the previous cases, increasing fatty acid content significantly increases the saturation solubility of DDT in the mixed micellar solutions.

Table 7.6 The solubility of DDT in TPGS simple and TPGS: oleic acid mixed micelles, and relative enhancements at 37^{0}C.

System	Solubility (mM) ± SD	Relative Enhancement	
		Water	Simple Micelle
Water	0.339×10^{-5}	-	-
TPGS 2%	0.686 ± 0.043	20.2×10^4	-
TPGS 2%: 10mM OA	0.858 ± 0.100	25.3×10^4	1.24
TPGS 2%: 20mM OA	1.025 ± 0.033	30.2×10^4	1.49
TPGS 2%: 40mM OA	1.495 ± 0.140	44.1×10^4	2.18

The TPGS surfactant micelles exhibit a greater solubilizing capacity for DDT than the corresponding bile salt micelles, but lower than that determined for the corresponding cremophor micelles. Cremophor EL has a hydrophilic/lipophilic balance (HLB) of 13.5, while TPGS has a HLB between 15-19. This greater hydrophilic character of TPGS micelles may explain why the saturation solubilites of these lipophilic compounds are lower than those observed for the cremophor micelles.

The trends observed in the micellar solubilization of DDT following increasing fatty acid content for the natural and synthetic surfactants examined are summarised in Figure 7.3. The increase in saturation solubility is reasonably linear for the synthetic surfactant micelle systems. For the bile salt micelle systems, the addition of a low concentration of fatty acid has no significant effect ($p<0.05$). Increasing the concentration of fatty acid thereafter, results in significant increases in DDT solubilization. The relative enhancement (i.e. versus simple micelles) afforded by addition of 40mM oleic acid is actually greatest for the bile salt micelles compared to that observed for the synthetic surfactants. These results suggest differing solubilization mechanisms with simple and mixed micelles formed from either naturally occurring or synthetic surfactants.

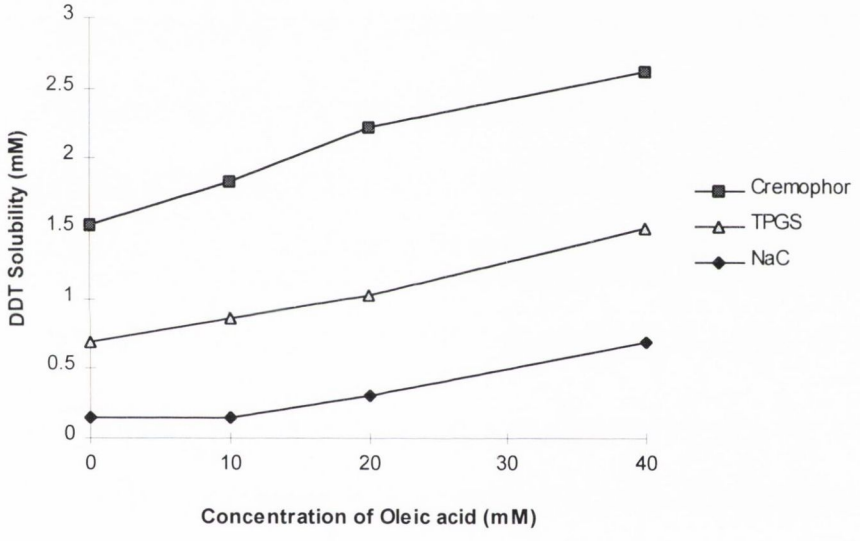


Figure 7.3 Influence of increasing fatty acid content on solubilization of DDT in 40mM NaC, 2% cremophor EL and 2% TPGS mixed micellar solutions.

This observation can be further explored by comparing the solubility increases of DDT and saquinavir as a function of oleic acid concentration for bile salts micelles (Figure 7.4) and cremophor micelles (Figure 7.5).

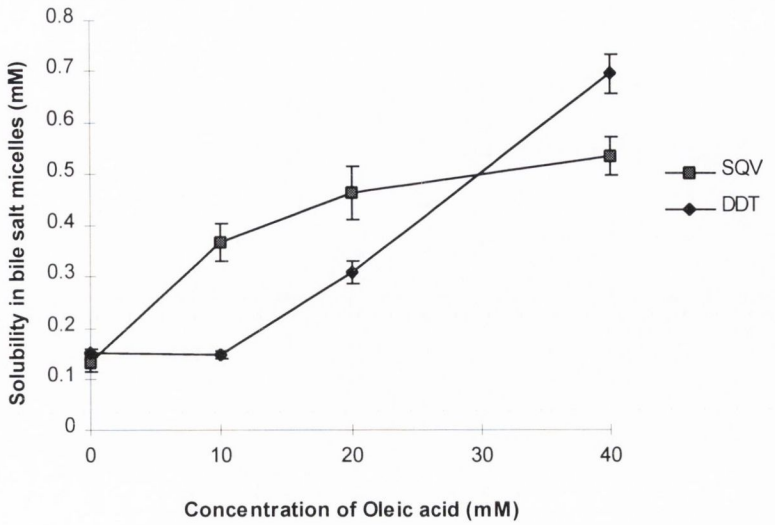


Figure 7.4 Comparison of the influence of increasing fatty acid content on solubilization of DDT and saquinavir in 40mM bile salt mixed micellar solutions.

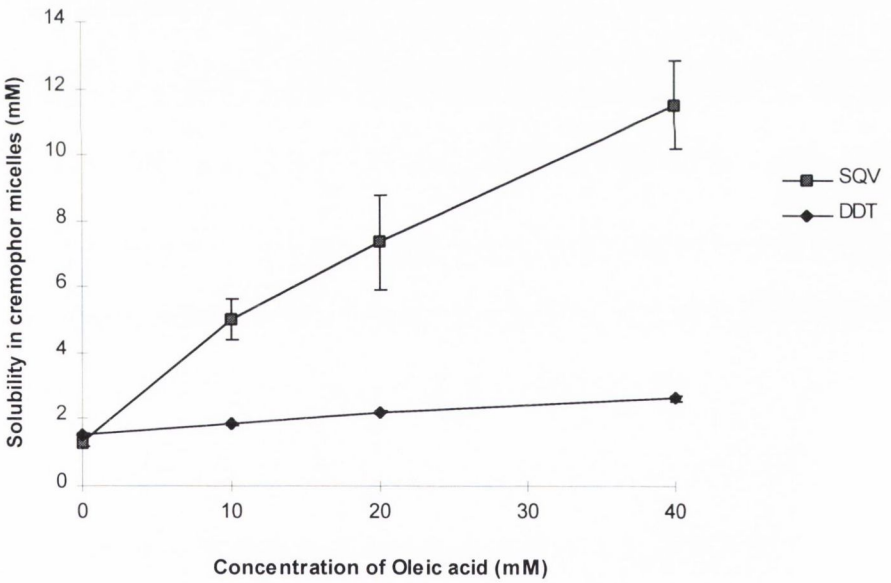


Figure 7.5 Comparison of the influence of increasing fatty acid content on solubilization of DDT and saquinavir in 2% cremophor EL mixed micellar solutions.

The solubility profile of saquinavir in bile salt mixed micelles shows a degree of negative curvature (Figure 7.4). The solubility enhancements for saquinavir in cremophor EL mixed micelles, with increasing oleic acid content, are more pronounced. The amphiphilic

index for the mixed micellar systems is calculated from the slope determined by plotting the solubility increases (over simple micelles) for the micelle system studied, against the corresponding lipid concentration. The value obtained quantitatively defines the effectiveness of an amphiphile to enhance the solubility of a solute under a fixed set of conditions (Luner et al., 1994). A large amphiphilic index indicates a substantial increase in solubility in the presence of mixed micelles relative to simple micelles and reflects the solubility increase in the presence of lipid digestion products after oral administration. The values for DDT, 0.025, 0.022 and 0.018 corresponding to cremophor EL, TPGS and NaC mixed micelles respectively, were all relatively similar. The values for saquinavir were 0.09 and 0.247 for NaC and cremophor EL mixed micelles respectively, which confirms the superior solubilization capacity of cremophor: oleic acid mixed micelles.

7.3.3. Photon Correlation Spectroscopy (PCS)

PCS has been used to characterize the bile salt and bile salt-lecithin micelles (Mazer et al., 1980; Mazer & Carey, 1983; Shankland, 1970; Schurtenberger et al., 1984). Frequently, the association pattern and kinetics in surfactant solutions have been reported to be determinants of the size of the resultant micelle, and this in turn is thought to influence the solubilization capacity and process. The average size of micelles given by micelle radius as well as the distribution of the micellar sizes around the mean, given by the polydispersity value, are important physical-chemical characteristics of the micellar solution, since they change with the association pattern, physical-chemical conditions, and surfactant concentrations in a micellar solution.

PCS studies of simple and mixed micelles produced from cremophor EL and TPGS with and without DDT, were undertaken, in order to gain insight into the equilibrium state of molecular association and DDT solubilization in these systems. The autocorrelation function produced a single exponential curve in all the micellar systems studied (both blank and DDT saturated systems), representing a monodisperse system or a sum of exponentials given by a variety of sizes. The hydrodynamic radii and polydispersities obtained for cremophor micellar system are presented in Table 7.7.

Table 7.7 PCS data for cremophor EL simple and mixed micelles, with and without DDT, prepared in phosphate buffer pH 7.2.

	DDT free Systems		DDT saturated systems	
Formulation	Zave radius (nm) ± SD	polydispersity ± SD	Zave radius (nm) ± SD	polydispersity ± SD
2%Cremophor EL	13.81 ± 1.01	0.169 ± 0.132	14.5 ± 0.5	0.084 ± 0.052
2% Cremophor EL: 10mM OA	14.416 ± 0.331	0.155 ± 0.049	14.9 ± 0.8	0.148 ± 0.106
2%: Cremophor EL 20mM OA	52.66 ± 0.60	0.115 ± 0.042	65.3 ± 0.5	0.284 ± 0.018
2% Cremophor EL: 40mM OA	67.52 ± 2.13	0.266 ± 0.021	73.8 ± 1.4	0.485 ± 0.019

There is an expected increase in micelle size as the concentration of oleic acid is increased. Between the DDT free and DDT saturated systems for each cremophor concentrations, there is a tendency towards increased micelle size on the solubilization of DDT. This marginal increase in micelle radius is more evident for the systems containing 20 and 40mM oleic acid.

The PCS studies of the TPGS micellar systems are presented in Table 7.8. The general trend in sizes observed for TPGS simple and mixed micelles is similar to that observed for cremophor micelles, with increases in micelle size with increasing oleic acid content. The micelle radii observed from simple micelles produced from these two synthetic surfactants are similar. The mixed micelles formed by incorporation of 40mM Oleic acid also produced similar sized micelles for these two surfactant systems.

Table 7.8 PCS data for TPGS simple and mixed micelles, with and without DDT, prepared in phosphate buffer pH 7.2.

	DDT free systems		DDT saturated systems	
Formulation	Zave radius (nm) ± SD	polydispersity ± SD	Zave radius (nm) ± SD	polydispersity ± SD
TPGS 2%	13.53 ± 0.826	0.206 ± 0.05	14.36 ± 0.94	0.171 ± 0.129
TPGS 2%: 10mM OA	40.92 ± 0.78	0.237 ± 0.025	48.66 ± 0.75	0.353 ± 0.011
TPGS 2%: 20mM OA	28.84 ± 0.44	0.482 ± 0.006	36.96 ± 0.115	0.52 ± 0.002
TPGS 2%: 40mM OA	65.64 ± 0.947	0.577 ± 0.005	69.2 ± 0.54	0.566 ± 0.003

The micelle size was significantly higher ($p < 0.05$) for the TPGS mixed micelles containing 10mM oleic acid than the mixed micelles containing 20mM oleic acid. This lack of a proportional increase in micelle size with increasing fatty acid content possibly reflects differing structural characteristics within the micelle. At lower fatty acid loading the TPGS micelle appears to swell dramatically. This data suggests that at a certain TPGS: fatty acid ratio (i.e. between 10 and 20mM oleic acid) a more favourable stereo-chemical 'fit' in the micelle may be attained resulting in a smaller micelle size, which increases again thereafter with increasing fatty acid content.

PCS sizing data for NaC bile salt micellar systems containing increasing concentrations of oleic acid has been previously published in our laboratory, and is included here for comparison (Table 7.9) (Obodozie, 1997).

Table 7.9 PCS data for NaC simple and mixed micelle systems, with and without DDT, prepared in phosphate buffer pH 7.2 (Obodozie, 1997).

	DDT free systems		**DDT saturated systems**	
Formulation	Zave radius (nm) ± SD	polydispersity ± SD	Zave radius (nm) ± SD	polydispersity ± SD
40mM NaC	1.1 ± 0.337	0.478 ± 0.139	0.9 ± 0.337	0.288 ± 0.082
NaC 40mM: 10mM OA	3.80 ± 0.424	0.068 ± 0.004	4.14 ± 0.44	0.344 ± 0.255
NaC 40mM: 20mM OA	4.70 ± 0.736	0.294 ± 0.220	3.84 ± 0.708	0.357 ± 0.235
NaC 40mM: 40mM OA	3.90 ± 0.604	0.395 ± 0.210	4.75 ± 0.397	0.331 ± 0.203

7.4. Discussion

Solubilization involves the 'preparation of a thermodynamically stable isotropic solution of a substance normally insoluble or very slightly soluble in a given solvent by the introduction of an additional amphiphilic component(s)' (Atwood & Florence, 1983). The ability of surfactants to promote solubilization is an important pharmaceutical property of these excipients. For drugs that are dissolution rate limited, increased solubilization and therefore an increase in dissolution rate may result in enhanced bioavailability. The solubility of, for example griseofulvin, often used as a model lipophilic drug, has been shown to increase by a factor of approximately 170 in the presence of sodium dodecyl sulfate (Marvelet al., 1964). O'Reilly et al., (1994a) reported enhancements of 4- to 60-fold in the solubility of the lipophilic drug clofazimine, (log $P_{o/w}$ 4.396, pKa = 8.51), at pH 7.2 in the presence of NaTC 10-80mM. Mixed micellar systems of NaC and fatty acids increased the solubility further relative to the simple micellar systems. The effect was dependent on fatty acid loading and the nature of the fatty acid (O' Reilly et al., 1994b). Other studies have also shown that bile salts increase the dissolution rate of lipophilic drugs in the gastrointestinal tract (Miyazhi, 1979; Gibaldi & Feldman, 1970). Meaney & Driscoll (1997) examined the solubility of dextropropoxyphene (log $P_{o/w}$ 4.18, pKa = 6.3). Simple and mixed bile salt micellar systems significantly increase the solubility of dextropropoxyphene at pH 7.4. At a pH below the pKa the solubility in aqueous solutions is increased i.e. at pH 4.5 the solubility of dextropropoxyphene was nearly 640-fold greater than at pH7.4. Simple and mixed bile salt micelles had no significant effect on the solubility of dextropropoxyphene at the lower pH.

In this study, the saturated solubility of DDT and saquinavir in a range of simple and mixed micellar systems, of both natural and synthetic origin, was therefore investigated as a prelude to carrying out *in situ* studies. The range of lipidic vehicles included simple and mixed bile salt, cremophor EL and TPGS micelles. Solubility enhancements for DDT in the simple micellar systems were over 7, 000 fold relative to the aqueous solubility while that in the mixed micellar systems were in the order of 20,000 – 40,000 fold. The aqueous solubility of saquinavir is much higher than that of DDT; however the solubility enhancement afforded by the bile salt micelles was still quite significant i.e. between 5- to 20 fold higher. All the mixed micellar systems enhanced solubility relative to solubility in simple micellar systems. Increasing the fatty acid content of mixed micellar systems

resulted in significant increases in solubilizing capacity and in general, increased micelle size.

This superior solubilization of drugs by mixed micelles is of relevance physiologically as the presence of the end products of lipid digestion (e.g. free fatty acids) in the intestine will not only stimulate bile acid secretion, but also increase the solvent capacity by formation of mixed micelles. It can be seen that addition of 40mM NaC bile salt increased the solubility of both DDT and saquinavir to 0.13-0.15mM. However the addition of 40mM oleic acid to the simple micelles increases the solubility to 0.5-0.7mM. Hence, the addition of long chain fatty acid enhanced the solubility to a greater extent. Figure 7.1 illustrates this point. Pouton and co-workers have also reported that the enhancement in solubility by the presence of lipolytic products in bile salt micelles is far greater than the solubility enhancement observed for simple micelles compared to buffer (Macgregor et al., 1997). The authors suggested that for hydrophobic drugs (i.e. log P > 4) administered as lipid formulations, digestion of the oily components of the formulations can be advantageous, as the lipolytic products enhance the solubilization capacity of intestinal bile salt micelles, thereby preventing precipitation of drug in the gut.

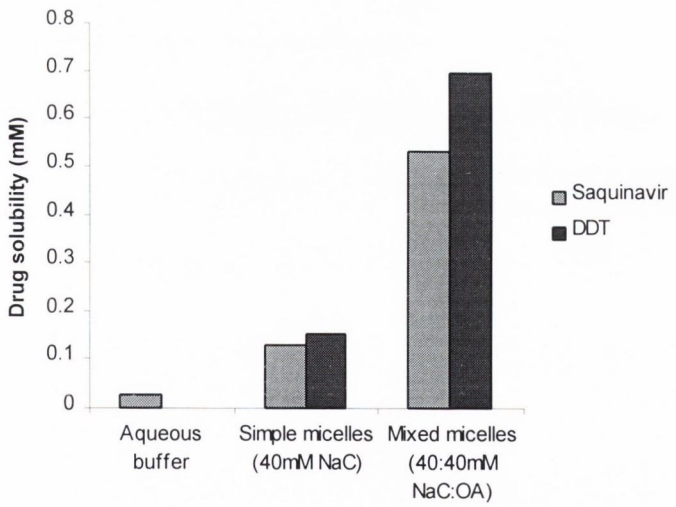


Figure 7.6 Enhanced solubility of DDT and saquinavir in simple and mixed bile salt micelles.

Cremophor EL simple micelles enhanced the solubility of DDT and saquinavir to a much greater extent than the corresponding bile salt simple micelle systems. PCS measurements confirm that the micelles produced with this surfactant range between 13- 65nm compared to 1-5nm previously reported for bile salt micelles. Interestingly, the addition of small amounts (<10mM) of long chain fatty acid had no appreciable effect on micelle size, but increasing amounts of oleic thereafter dramatically increased micelle radius. These differences reflect differing solubilization mechanisms and structural characteristics of naturally occurring and synthetic micelles. The comparisons between the solubilization trends of the two lipophilic compounds in bile salt (Figure 7.4) and cremophor (Figure 7.5) micelles as a function of increasing fatty acid content confirms the differing solubilization mechanisms of naturally occurring and synthetic mixed micelles. O' Driscoll et al., (1991) have similarly shown that the effects of naturally occurring bile salts and cremophor EL on the solubility and absorption of clofazimine differ. Interestingly the incorporation of caprylic acid into bile salt micelles resulted in increases in the solubility of clofazimine, whereas the formation of cremophor mixed micelles with caprylic acid resulted in decreases in the saturation solubility of clofazimine. These effects were likely to be due to the different structural characteristics of the micelles formed.

D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble derivative of natural source vitamin E. The product has been safely used as a nutritional supplement for more than 30 years. In recent years, it has been increasingly recognised as an effective oral absorption enhancer for improving bioavailability of poorly soluble drugs, such as cyclosporin, saquinavir and amprenavir (Yu, et al., 1999) and as a vehicle for lipid based drug delivery formulations (Hong-Wei Wu & Hopkins, 1999). For example, Sokol et al., (1991) suggested that TPGS functioned as a bile salt substitute and solubilized cyclosporin through micelle formation, improving cyclosporin absorption in children with liver transplantation and sub-optimal bile flow. The results of the present study confirm that TPGS micelles exhibit a greater solubilizing capacity than the natural bile salt micelles. While its ability to form simple micelles has been reported (Hong-Wei Wu & Hopkins, 1999) the present study demonstrates the ability of TPGS to solubilise long chain fatty acids to form mixed micelles. The mixed micelles produced are comparable, both in terms of size and solubilizing capacity, to those of cremophor EL. Significant increases in the saturation solubility of DDT were observed with increasing fatty acid loading.

The aqueous solubility of a drug is a prime determinant of its dissolution rate, and in the case of poorly soluble drugs, the aqueous solubility is less than 100μg/ml. A further parameter that is useful for identifying 'poorly soluble' drugs is the dose: solubility ratio of the drug (Horter & Dressman, 1997). The dose: solubility (D/S) ratio can be defined as the volume of gastrointestinal fluids necessary to dissolve the administered dose (Horter & Dressman, 2001). When this volume exceeds the volume of fluids available (250-1000ml), one may anticipate dissolution rate limited absorption and incomplete bioavailability of a solid oral dosage form. Griseofulvin provides a classic illustration of the utility of the D/S ratio. With an aqueous solubility of 15μg/ml at 37^0C and a dose of 500mg (Katchen & Symchowicz, 1967), griseofulvin has a D/S ratio of 33L. Thus the combination of its poor solubility and high dose constitutes a severe limitation to its oral bioavailability (Horter & Dressman, 1997). Table 7.10 shows the calculated D/S ratios values for saquinavir. Although the concentrations of bile salt and fatty acid may be higher than the reported values in the human post-prandial environment, the D/S ratios do provide an indication of the *in vivo* capacity for additional solubilization. The D/S ratios decrease with increasing concentration of amphiphile (i.e. oleic acid in this case, or lipid digestion products in the post prandial state). The reduction in D/S ratio is greater for the synthetic micelles than the naturally occurring micelles, which further justifies the use of synthetic surfactants to enhance the solubilising potential of the intestinal milieu.

Table 7.10 Calculated dose to solubility ratios in phosphate buffer pH 7.2 at 37^0C for saquinavir

Formulation	Dose-to-solubility ratio $\approx$ D/S[A]
Buffer pH 7.2	34.49L
NaC 40mM	6.86L
NaC:OA 40:40mM	1.68L
2% Cremophor	0.68L
2% Cremophor: 40mM OA	0.09L

[A] The dose is 600mg

The food effect for saquinavir is quite significant, with up to 18-fold increases in the extent of bioavailability between fed and fasted states. The effect is most pronounced after a fatty meal. One proposed reason for the improved bioavailability is increased solubilization in

post-prandial environment. The present study illustrates the extent to which increased fatty acid content can drastically increase the solubilization capacity of bile salt micelles, producing up to 20 - fold increases in the saturation solubility of saquinavir.

A relationship between the extent of solubilization and the lipophilicity of the compound has been observed by several authors (Collett & Koo, 1975; Tomida et al., 1978; Attwood & Florence, 1983). Fahelelbom et al., (1993) found a linear relationship between log P and Log Km (the distribution coefficient between the micellar and aqueous phase) of a series of rimino phenazines (n=6) in sodium dodecyl sulphate, a non-ionic surfactant, and triton-X-100 an ionic surfactant. Dressman and co-workers examined the increase in solubility of drugs as a function of bile salt concentration (Mithani et al., 1996). These authors reported that the value for the solubilization capacity (moles drug/moles bile salt) of the bile salt micelles was similar for a range of drugs whereas the solubility in water was variable and suggested that the driving force for bile salt solubilization was determined by the hydrophobicity of the drug rather than by affinity for bile salt micelles. Development of a model for predicting the extent to which bile salts can enhance the dissolution of drugs, based on physiochemical properties of the drug such as partition coefficient, melting point, aqueous solubility etc., could aid in the selection of dosing strategy and formulation design for compounds which exhibit dissolution rate limited absorption. It may also help to identify, on a priori basis, which compounds will be more bioavailable if dosed in the fed state.

The influence of the hydrophobicity of drugs on their solubilization by mixed bile salt micelles was examined using the data for DDT and saquinavir presented here, and also data for two other lipophilic compounds, clofazimine and KME-4, previously analysed under similar conditions in our laboratories (O' Reilly, 1991). This work further compliments studies previously reported by (Naylor et al., 1993; Solomon et al., 1996). In this study, however, the saturation solubility of each compound in four different micellar systems, namely 40mM NaC simple micelles and NaC:OA 40:10mM, 40:20mM and 40:40mM mixed bile salt micelles, was determined. Solubility enhancement was expressed as the ratio (S/So) of solubility in micellar solution (S) and solubility in buffer solution (So). The ratio of solubility enhancement was compared with estimated clog P for each compound (Figure 7.7) showing that in general, the extent of solubility enhancement increased with hydrophobic character.

It is clear from the plots presented below, that for the four bile salt micellar systems, the same general trend is observed; the extent of solubilization increase with increasing lipophilicity of the compound. Our results suggest that the increase in solubility in simple or mixed bile salt micelles can be approximated on the basis of the partition coefficient and the solubility of the compound. While a wider range of structurally diverse compounds would be required to determine more accurately the linearity of this relationship, it can be suggested for the plots in Figure 7.7 that the saturation solubility of saquinavir is lower than would be predicted by the other three compounds. A number of authors have reported deviations from the predicted solubilization behaviour. Hence the process of solubilization is not entirely driven by hydrophobicity, other factors must also play a role. These include molar volume, or the effect of molecular structure on the spatial orientation in the micelle. Solomon et al., (1996) reported that long chain esters of hydrocortisone, whilst displaying similar log P's to that of progesterone, could not be incorporated as efficiently as progesterone in to the micelle. Tomida et al., (1978) reported that fluorinated steroids were better solubilized than the corresponding steroids which contained no fluorine atom. The authors attributed these results to incorporation of the fluorinated drugs into the more hydrophilic outer region of the micelle.

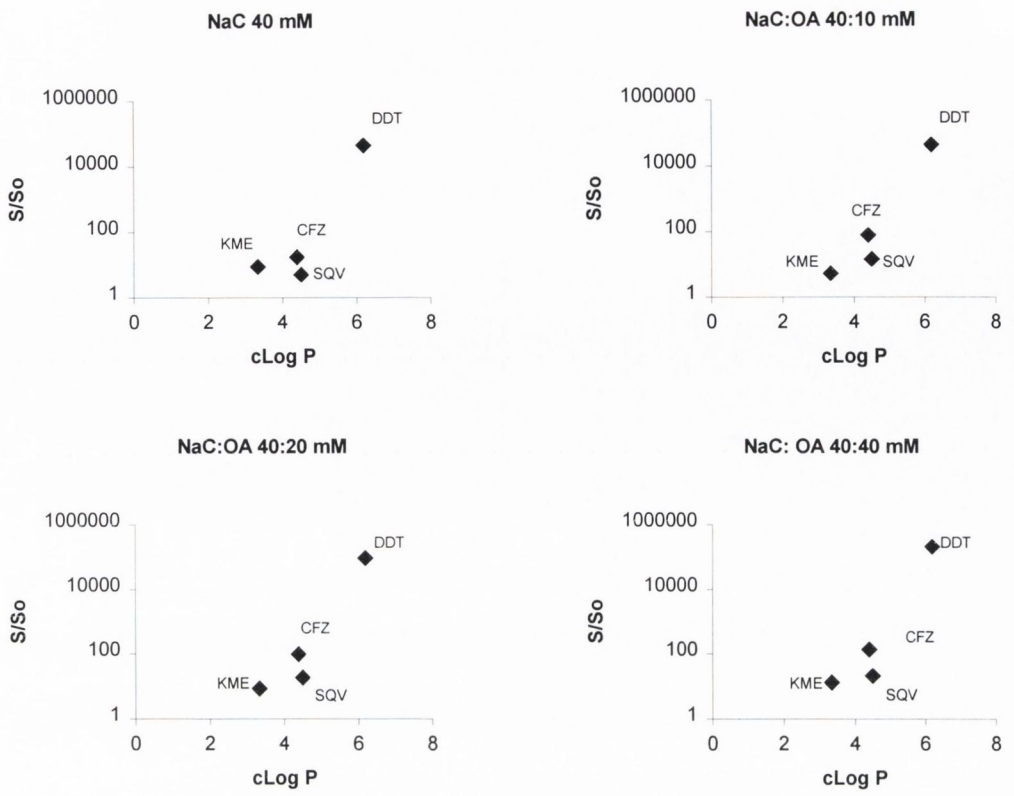


Figure 7.7 Solubility enhancement (S/So of KME-4, (KME), clofazimine (CFZ), DDT and saquinavir (SQV) in the presence of bile salt (40mM NaC) simple and mixed (NaC:OA 40:10, 40:20 and 40:40mM) micelles versus clog P.

8. Intestinal lymphatic transport of DDT from micellar systems

8.1. Introduction

The selective passage of drugs through the intestinal lymphatic route holds considerable potential in terms of drug targeting, reduced first-pass and improved bioavailability. The intestinal lymphatic system has been shown to contribute to the absorption of a number of highly lipophilic xenobiotics such as cyclosporin (Takada et al., 1986), halofantrine (Porter et al., 1996) and mepitiostane (Ichicashi et al., 1991). The mechanisms whereby lipophilic molecules are absorbed from the gastrointestinal tract and transported to the general circulation are complex and poorly understood (Porter & Charman, 1997). In this study, in order to define some of the parameters affecting the lymphatic uptake and transport of lipophilic drugs, the effects of a series of lipid micellar vehicles on the intestinal lymphatic transport of DDT were examined. DDT was chosen as a model lymphotropic agent because it is highly lipophilic, relatively metabolically stable and well transported by the intestinal lymphatic system of the rat (Noguchi et al., 1985b).

In lymphatic transport studies, an anaesthetised rat model has often been employed because it provides a controlled system for evaluating and probing the mechanistic aspects of the lymphatic transport process (Charman et al., 1986a). The mesenteric intestinal lymph duct, rather than the thoracic lymphatic duct, is the preferred site of cannulation to estimate the ability of the small intestine to lymphatically transport lipophilic molecules (Noguchi et al., 1985a). The anaesthetised rat model allows sampling of both intestinal lymph and systemic blood.

Orally administered drugs gain access to the systemic circulation via two separate and functionally distinct absorption pathways – the portal blood and the intestinal lymphatics. The physiochemical and metabolic features of the administered drug and the characteristics of the administered formulation, largely dictate the relative proportion of drug absorbed via these two pathways. Surfactants micellar systems, both of natural and synthetic type, have been shown to improve the bioavailability of poorly absorbable drugs by increasing the dissolution rate of the drug from the solid dosage form and/or by facilitating the transfer of the solute across the intestinal wall, resulting in increased absorption into portal blood. However, few studies have examined the effects of some of these highly specialized micellar vehicles on intestinal lymphatic uptake. Recent work in the Japarese literature

have examined the use of milk fat globule (MFGM) as an emulsifying agent for the improved lymphatic delivery of vitamins (D_3 and A), epidermal growth factor and insulin (Liu et al., 1995; Sato et al., 1994; Liu et al., 1991; Adachi et al., 1993; Adachi et al., 1993). Liu et al (1995) suggests that this surfactant may promote lymphatic transport; specifically by the formation of MFGM-bile salt mixed micelles *in vivo*. An increase in the lymphatic uptake of vitamin D3 from emulsions stabilised with MFGM compared to those with poly-sorbate 80 was evident; the increase, however, was not statistically significant. Further research is therefore warranted to clarify the beneficial effects of certain surfactants on lymphatic uptake.

Formulations with physiochemical characteristics representative of the final stages of lipid digestion (i.e. mixed micellar systems containing digested lipids) appear to promote the lymphatic transport of lipophilic compounds (Porter et al., 1996; Fernandez & Borgström, 1989 & 1990). The current study compares the effects on lymphatic transport of a series of lipid micellar formulations in an attempt to further elucidate the mechanisms involved in promoting lymphatic transport and thereby define the important features of a lymphotropic lipid delivery system. The lipid vehicles investigated included simple and mixed micelles, of both naturally occurring (e.g. bile salts) and synthetic (e.g. cremophor EL and TPGS) surfactants. These experiments were intended to demonstrate whether optimal delivery to the lymphatics was specifically affected by any of the formulation variables i.e. simple versus mixed micelles, natural versus synthetic surfactants and the chemical nature of the formulation excipients. In addition, the triglyceride turnover in lymph samples collected was analysed to assess the effect of the various lipid vehicles on triglyceride transport and to evaluate the relationship between lymph triglyceride transport and lymphatic transport of DDT.

8.2. Results

8.2.1. Intestinal Lymphatic transport of DDT

8.2.1.1. Naturally occurring surfactant micellar systems

Simple and mixed bile salt micelles

The lymphatic transport of DDT solubilized in 40mM Sodium Cholate (NaC) simple bile salt micelles, prepared in isotonic phosphate buffer (pH 7.2) was studied initially. Cholic acid is the major bile acid component of both the rat and the human hepatic bile duct. The choice of 40mM sodium cholate (NaC) as a concentration, although marginally higher than the expected concentration *in vivo*, was balanced on a requirement of a saturation solubility for DDT sufficient to administer a relevant dose of DDT, while minimising the toxicity observed with the use of higher concentrations (Meaney, 1997). 40mM oleic acid was incorporated into a 40mM NaC solution to give an equi-molar mixed micellar system of sodium cholate and oleic acid. Oleic acid (OA) was chosen as it is the principal long chain fatty acid that occurs in human mixed intestinal lipids (Staggers et al., 1990). In addition, long chain fatty acids have been shown to promote lymphatic transport (Palin & Wilson, 1984; Charman & Stella, 1986a).

The lymphatic transport of DDT, expressed as the cumulative percent of the administered dose, after intraduodenal administration as a 40mM sodium cholate (NaC) simple micellar system or a 40:40mM NaC:OA mixed micellar system saturated with DDT, are presented in Figure 8.1

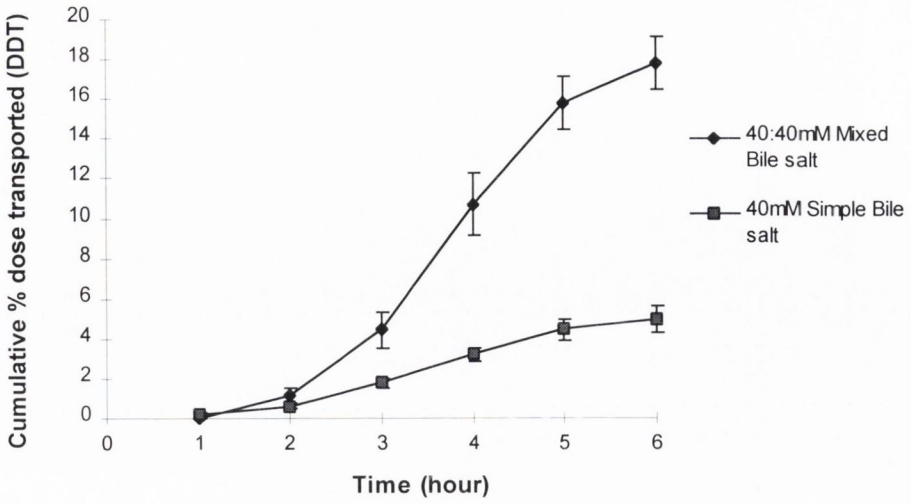


Figure 8.1 Cumulative percent dose of DDT (Mean ± SE, n ≥ 5) collected in mesenteric lymph as a function of time for 40mM NaC simple bile salt micelles and 40:40mM NaC:OA mixed bile salt micelles.

The extent of intestinal lymphatic transport of DDT from the simple bile salt micelle after 6 hours is 4.93% ± 0.63% (mean% dose ± S.E., n ≥ 5 in all groups) of the administered dose. The cumulative amount of transport of DDT in mesenteric lymph is significantly higher ($p < 0.05$) following administration in a bile salt fatty acid mixed micelle at 17.75% ± 1.3% of the dose. The intestinal lymphatic transport of DDT appears to be incomplete after 6 hours. The extent of DDT transport in the lymph from a mixed micellar system is comparable to previously published data. O' Driscoll et al., (1991) and Myers (1990) reported the extent of lymphatic transport of 19% and 15%, respectively, where DDT was administered in triglyceride emulsions in similar experiments over 12 hours.

The rate of intestinal lymphatic transport of DDT, normalised for the total amount of DDT administered, is presented in Figure 8.2. The peak rate of transport occurs 3-4 hours post initiation of drug administration for both formulations.

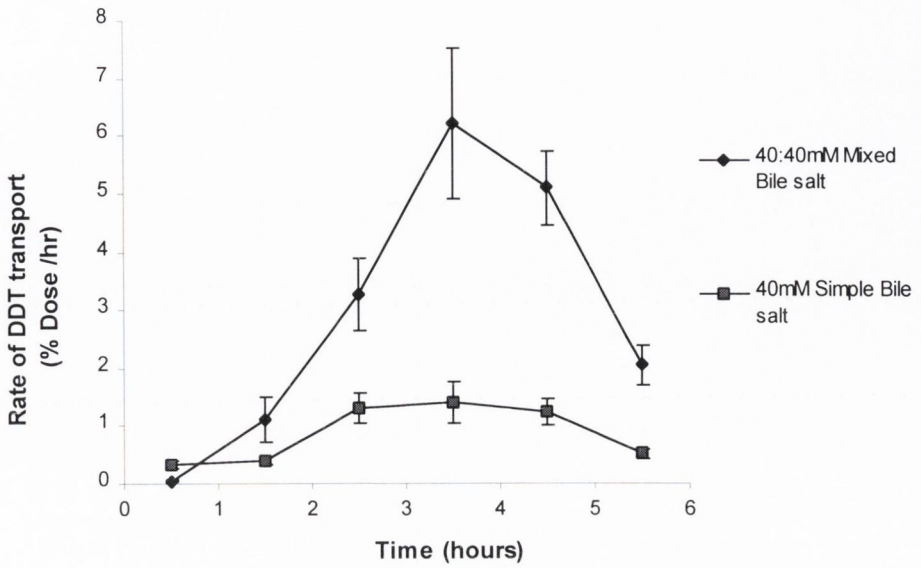


Figure 8.2 Rate of mesenteric lymphatic transport of DDT (Mean ± SE, n ≥ 5) calculated as a percentage of the total dose administered for 40mM NaC simple bile salt micelles and 40:40mM NaC:OA mixed bile salt micelles.

The maximal rate of drug transfer from mixed micelles is 5-fold greater than that observed with simple micelles. The mixed micelle is more representative of the latter stages of lipid digestion *in vivo* and appears to facilitate the rate of transfer of DDT to the intestinal lymphatics. The improved rate and extent of lymphatic transport may in part be due to the stimulation of lipoprotein biosynthesis by the long chain unsaturated $C_{18:1}$ fatty acid incorporated in the mixed micelle. As DDT is transported in association with lipoproteins, delivery in a mixed micellar state appears to be more efficient at stimulating lipoprotein biosynthesis and facilitating DDT incorporation into these lipoproteins. The increase in turbidity observed for the collected lymph samples, following administration of the mixed micelle formulation, was an indirect indicator of increased lipoprotein production. By comparison, lymph samples from rats administered the simple micelle formulation remained clear, or slightly pale. The increase in turbidity in lymph samples after administration of lipid has previously been used as relative index of chylomicron flux (Charman & Stella, 1986a). The proposed increase in lipoprotein production and triglyceride turnover will be examined further in section 8.2.3.

Phospholipid mixed bile salt micelles.

On average, three percent of average human bile is composed of phospholipid. Therefore, the phospholipid, lecithin, would be a major component of the mixed micellar aggregates formed during the digestion of lipids in the gastro-intestinal tract. In addition, the study by Obodozie, (1997), on a series of simple and mixed micellar systems, revealed that the bile salt phospholipid mixed micellar system produced the largest discrepancy between the rates of blood appearance and luminal disappearance of DDT. The extent of lymphatic transport of DDT following administration in a bile salt: phospholipid (40:40mM) mixed micellar system is presented in Figure 8.3. The bile-salt: phospholipid micelles required in excess of 72 hours to reach maximal DDT saturation and as a result the final concentration of DDT in the administered solution was 500μg/ml, which represents 90% of the saturation solubility, and was prepared in under 12 hours. The cumulative amount of DDT transported in mesenteric lymph, after 8 hours, from the bile salt: phospholipid mixed micelles was 13.37 ± 0.94% of the administered dose. Studies were now continued over 8 hours as previous studies indicated that transport was still incomplete after 6 hours. The extent of lymphatic transport after 6 hours is significantly higher ($p < 0.05$) than that observed for the simple bile salt micelles and significantly lower than the extent of transport from the bile salt: oleic acid mixed micellar formulation ($p < 0.05$), after 6 hours. The inclusion of 40mM phospholipid in the formulation appears to enhance DDT lymphatic transport, relative to the simple bile salt formulation. This effect may be specific to the phospholipid molecule itself, or as phospholipid consists of two alkyl chains of either palmitic (C_{16}), stearic(C_{18}), oleic(C18:1), linoleic($C_{18:2}$) or arachidonic acids(C_{20}) linked to a phosphatidylcholine molecule, hydrolysis in the gut lumen would liberate long chain fatty acids acyls moieties which in turn may stimulate lipoprotein production.

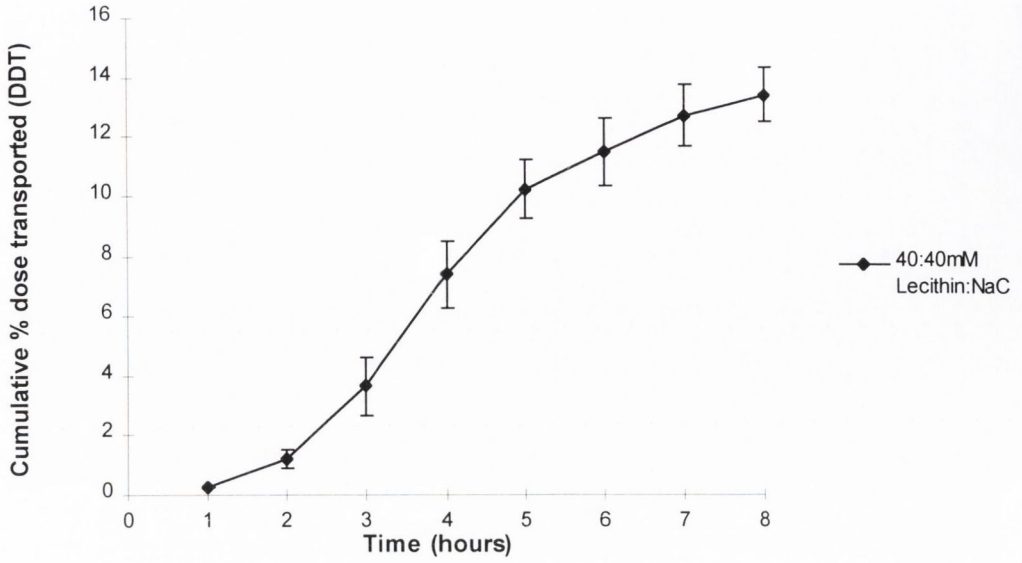


Figure 8.3 Cumulative percent dose of DDT (mean ± SE, n ≥5) collected in mesenteric lymph as a function of time for the 40:40mM NaC: phospholipid mixed micellar system

The rate profile for DDT lymphatic transport for the bile salt: phospholipid mixed micellar system is presented in Figure 8.4. The peak rate of transport occurred between 3-4 hours, with 3.73 ± 0.45% of the administered dose appearing in lymph during this time period. The trend observed in the total extent of the administered dose recovered in lymph is similar to the order observed in the maximal rate of DDT transfer for the three bile salt micellar systems, the rank order of which is as follows:

40:40mM NaC:OA > 40:40mM NaC: phospholipid > 40mM NaC

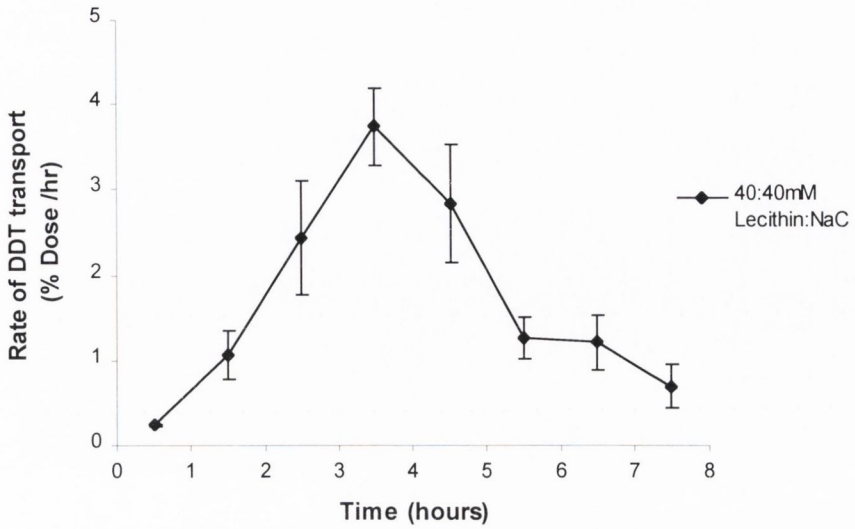


Figure 8.4 Rate of mesenteric lymphatic transport of DDT (mean ± SE, n ≥ 5) calculated as a percentage of the total dose administered for the 40:40mM NaC: phospholipid mixed bile salt micelles.

8.2.1.2. Synthetic surfactant micellar systems

Cremophor EL simple and mixed micelles

O' Driscoll et al., (1991) compared the effects of the synthetic surfactants and naturally occurring bile salts on the solubility and absorption of the poorly water soluble anti-leprotic agent, clofazimine. This study highlighted the potential merits in terms of increased solubilization capacity and enhanced intestinal permeability of simple and mixed micelles of the synthetic non-ionic surfactant, cremophor EL. DDT-saturated simple (cremophor 2%) and mixed (cremophor 2%:OA 40mM) micellar systems were administered to lymphatically cannulated rats to yield the following cumulative percent dose plot (Figure 8.5). The extent of transport after 8 hours is significantly greater for the cremophor mixed micelles compared to the simple cremophor micelle ($p < 0.05$).

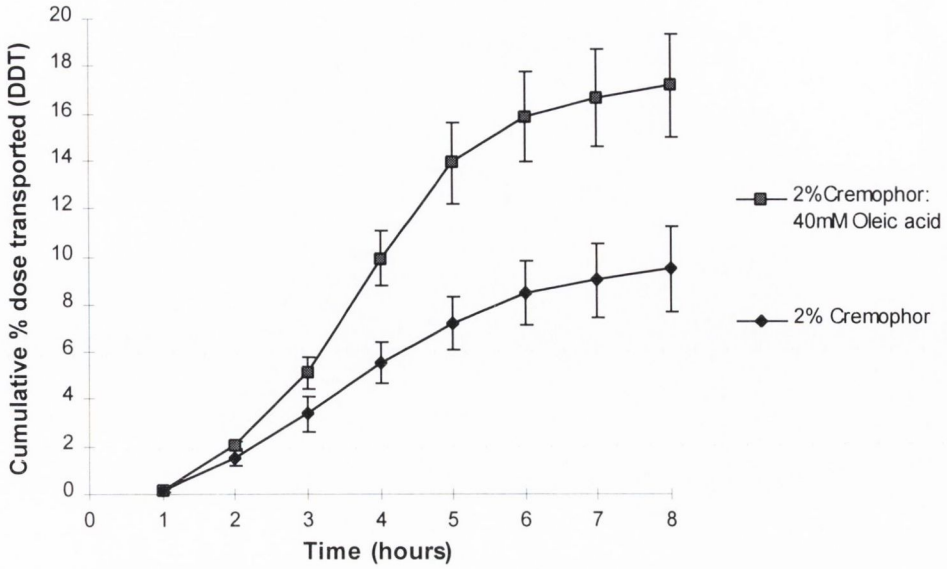


Figure 8.5 Cumulative percent dose of DDT (mean ± SE, n ≥ 5) collected in mesenteric intestinal lymph as a function of time for simple and mixed cremophor micelles.

The cumulative dose (Figure 8.5) and rate profiles (Figure 8.6) for lymphatically transported DDT following administration in simple and mixed cremophor systems exhibit similar trends to those observed for the bile salt micelles. The percent dose transported in mesenteric lymph after 8 hours for the cremophor mixed micelles was 17.13 ± 2.15%. The extent of transport after 6 hours was not statistically dissimilar to that observed with the bile salt mixed micelles. Lymphatic transport appears virtually complete after 8 hours. The extent of lymphatic transport, after 8 hours, for the simple cremophor micelles was 9.45 ± 1.79% of the administered dose. After 6 hours, the extent of transport is significantly higher for the cremophor simple micelles than that observed for the simple bile salt micelles ($p < 0.05$).

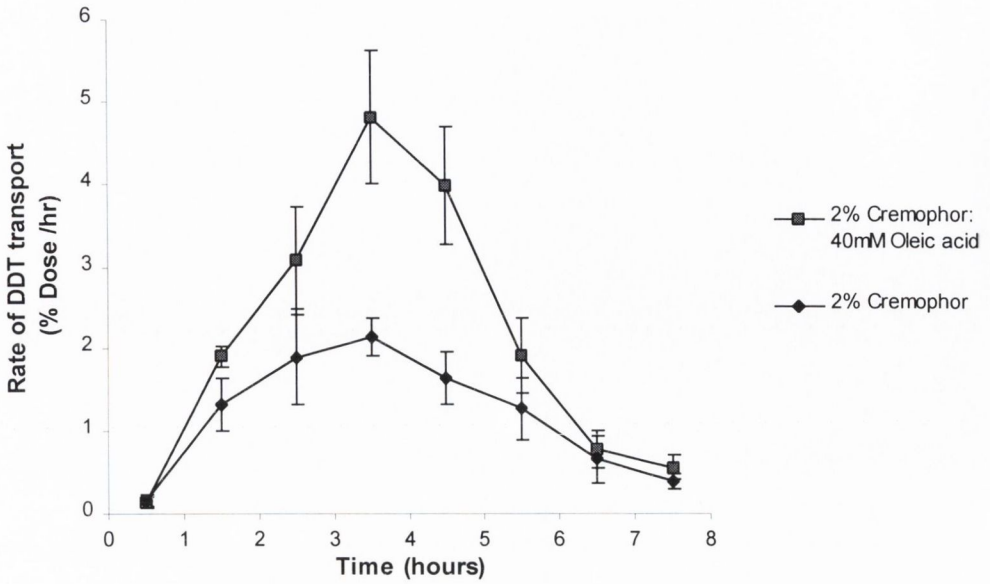


Figure 8.6 Rate of mesenteric lymphatic transport of DDT (Mean ± SE, n ≥ 5) calculated as a percentage of the total dose administered for simple and mixed cremophor micelles per collection time period.

The kinetics of DDT appearance, as indicated by lag-time and time of peak transport, from the cremophor simple and mixed micelles were similar, with peak transport occurring between 3-4 hours. However, the inclusion of the unsaturated long chain fatty acid ($C_{18:1}$) enhances the DDT peak transfer rate, as evidenced by the 2.23-fold higher dose corrected maximum rate of DDT transfer for the mixed micelles ($p < 0.05$). The dose corrected peak transfer rates for the simple and mixed cremophor micelles were 2.14 ± 0.23 and 4.81 ± 0.81 (% dose administered/hr), respectively. These compare with the dose corrected rates for the simple and mixed bile salt micelles of 1.40 ± 0.37 and 6.20 ± 1.30 (%dose administered/hr), respectively. Possible reasons for the higher rates and extents of DDT transport from cremophor simple micelles than simple bile salt micelles include an increase in chylomicron flux, whereby the cremophor stimulates chylomicron or endogenous TG turnover either directly or by acting as a triglycerogenic substrate itself. These topics will be discussed further in the section 8.3.

TPGS simple and mixed

d-Alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble form of natural source Vitamin E that has been used as a nutritional supplement for many years. Recent clinical studies suggested that TPGS could be used as an oral absorption enhancer for poorly water-soluble drugs such as cyclosporin (Chang et al., 1996). The ability of TPGS micellar systems to promote intestinal lymphatic transport of DDT was investigated. The lymphatic transport, expressed as the cumulative percent of the administered dose, after intraduodenal administration of either a 2% TPGS simple micellar formulation or a 2%TPGS: 40mM Oleic acids mixed micellar formulation is presented in Figure 8.7. The final concentration of DDT in the simple and mixed TPGS micellar systems was fixed at 200μg/ml and 500μg/ml, representing 74% and 89% of saturation solubility, respectively, as discussed in the methods section.

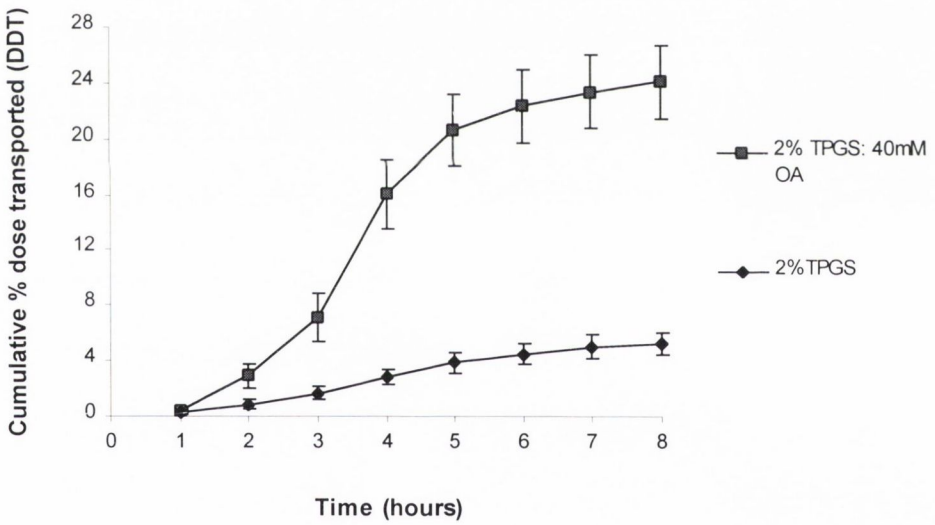


Figure 8.7 Cumulative percent dose of DDT (mean ± SE, n ≥ 5) collected in mesenteric intestinal lymph as a function of time for simple and mixed TPGS micelles.

The extent of transport from the TPGS mixed micellar system is again significantly higher than the extent of lymphatic transport for the simple micellar system. For the mixed TPGS micelles 24.05 ± 6.24% of the administered dose was transported via mesenteric lymph, which is the highest extent of transport for all the mixed micellar systems examined,

although the differences (determined after 6 hours) were not statistically significant ($p<0.05$) between NaC, cremophor and TPGS mixed micelles. For the simple TPGS micellar formulation the extent of transport after 8 hours was 5.21 ± 0.83% which is comparable to that observed for the simple bile salt micelles.

Figure 8.8 presents the dose corrected DDT lymphatic transport rate profile for the simple and mixed TPGS micellar formulations.

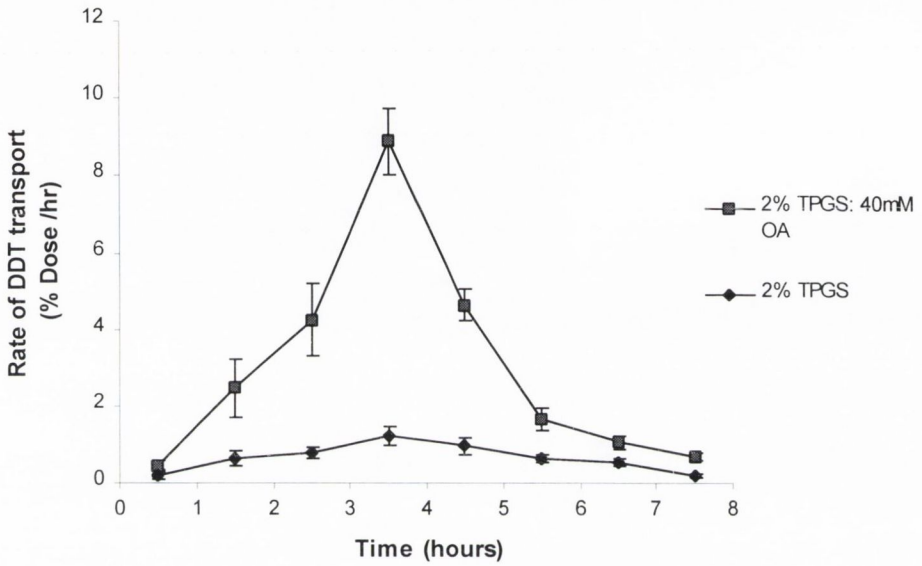


Figure 8.8 Rate of mesenteric lymphatic transport of DDT (Mean ± SE, n = 6) calculated as a percentage of the total dose administered for simple and mixed TPGS micelles per collection time period.

The rate profiles are similar to those previously observed, with peak transport occurring between 3-4 hours. The rate of transfer for the mixed micellar system is significantly higher ($p <0.05$) than for the simple micellar systems. This further confirms that incorporation of oleic acid significantly improves the lymphatic transport of DDT. The maximal transport rates for the simple and mixed TPGS micelles were 1.22 ± 0.24 (% dose administered/hr) and 8.89 ± 2.08 (% dose administered/hr), respectively.

A summary of the relevant dosage and lymphatic transport properties for the various micellar lipid formulations examined in this study, are presented in (Table 8.1).

Table 8.1 A comparison of the various micellar formulations and the extent of lymphatic transport of DDT

Parameters	Formulation excipients	Micelle size (nm)	Dose administered (mg DDT)	% Dose of DDT transported in lymph [A] (± SE)	Max DDT lymphatic transport rate (μg/hr) (± SE)
Naturally occurring surfactant micelles					
NaC 40mM	62mg NaC	1.1 [B]	0.192	4.94 [C] ± 0.63	2.68 ± 0.70
NaC: OA 40:40mM	62mg NaC 41mg OA	3.9 [B]	0.884	17.75 ± 1.30 [C]	54.83 ± 11.4
NaC: PL 40:40mM	62mg NaC 112mg PL	7.3 [B]	1.8	13.37 ± 0.938	67.22 ± 8.17
Synthetic surfactant micelles					
2% Cremophor	72mg Crem	13.81	1.954	9.45 ±1.79	41.88 ± 4.48
2% Cremophor: 40mM OA	72mg Crem 41mg OA	67.52	3.344	17.13 ± 2.16	160.43 ± 27.11
2% TPGS	72mg TPGS	13.53	0.72	5.22 ± 0.83	8.81 ± 1.74
2%TPGS: 40mM OA	72mg TPGS 41mg OA	65.64	1.8	24.05 ± 2.62	159.9 ± 15.26

[A] *After 8 hours*

[B] *Obodozie (1997)*

[C] *After 6 hours*

8.2.2. Intestinal lymphatic transport of triglycerides from micellar lipid formulations

The role of lipoproteins in the lymphatic transport of lipophilic compounds has been well established (Charman & Stella, 1986a; Fukui et al., 1989). Pocock & Vost (1974) have suggested that all DDT transported in the lymph is contained within the triglyceride core of the chylomicrons. Therefore, one of the major factors contributing to significant lymphatic transport is the co-administration of a suitable lipid source to promote lipid (e.g. fatty acids) uptake and resultant lipoprotein synthesis. In the present study, the proportion of DDT transported via the chylomicron fraction in lymph was not assessed directly, however as chylomicrons are the major transporters of triglyceride, the triglyceride levels in lymph serve as an indicator of chylomicron output. Caliph et al., (2000) have shown that the predominant triglyceride in the lymph of rats, regardless of the type of triglyceride dosed, was C_{18} long chain triglyceride. The cumulative transport of C_{18} triglyceride into mesenteric lymph and cumulative lymph flow after intraduodenal dosing in all of the lipid vehicles is shown in Table 8.2.

There were no significant differences ($p < 0.05$) in lymph flow between the different experimental groups (Table 8.2). This result is consistent with previous reports in which the effect of different lipid vehicles on the extent of lymphatic transport of DDT was manifested as changes in rate and extent of lipoprotein formation (and hence drug transport), rather than an alteration of lymph flow (Charman et al., 1986 a & b).

Initially, an estimate of endogenous triglyceride turnover was determined by perfusing the saline rehydration solution (i.e. no lipid), and analysing triglyceride levels in collected lymph samples. Endogenous triglyceride transport, was calculated to be 1.52 ± 0.11 mg/h was determined from the slope of the regression of mean cumulative lymphatic triglyceride transport over 8-hours ($r^2 = 0.98$). Porter et al., (1996) reported an endogenous triglyceride turnover of 2.67mg/hr in anaesthetized rats, while Caliph et al., (2000) reported a value of 1.06mg/hr conscious rats. The figures reported here are deemed comparable, allowing for minor deviations in experimental protocol.

Table 8.2 Cumulative transport of triglyceride into the mesenteric lymph (mean ± S.E., n≥4) and cumulative lymph flow as a function of micellar lipid vehicle after 8 hours

Formulation	Cumulative Mass of triglyceride (mg) appearing in Mesenteric lymph (Mean ± SE) [A]				Cumulative TG transport (mg) [B]	Cumulative lymph flow (ml)
	0-2 hr	0-4 hr	0-6 hr	0-8 hr		
Saline control	5.89 ± 1.03	9.16 ± 1.07	11.95 ± 1.66	14.31 ± 0.94		4.45 ± 0.35
Natural surfactant micelles						
NaC 40mM	3.97 ± 0.59	8.72 ±0.79	13.31 ± 1.31	n/a	1.36 ± 2.27	3.34 ± 0.37
NaC: OA 40:40mM	4.44 ± 0.78	20.72 ± 1.85	34.29 ± 2.63	n/a	22.33 ± 2.81	3.92± 1.05
NaC: PL 40:40mM	5.49 ± 0.65	21.06 ±2.88	33.73 ± 4.54	43.66 ± 5.35	29.35 ± 5.89	4.34 + 0.49
Synthetic surfactant micelles						
2% Cremophor	5.36 ±0.40	13.32 ± 0.68	18.95 ± 1.05	22.85 ± 1.23	8.54 ± 1.77	4.20 ± 0.41
2%Cremophor: 40mM OA	7.29 ± 0.59	24.74 ± 0.63	36.57 ± 0.66	41.03 ± 1.34	26.71 ± 1.88	3.81 ± 0.20
2% TPGS	4.36 ± 0.66	9.87 ± 1.16	14.26 ± 1.47	17.66 ± 2.07	3.35 ± 2.61	5.59 ± 0.40
2%TPGS: 40mM OA	9.17 ± 1.12	31.48 ± 4.03	42.97 ± 5.14	48.37 ± 5.47	34.06 ± 6.01	4.99 ± 0.68

[A] *Representing endogenous and exogenous lipid*
[B] *Attributable to exogenous lipid (i.e. exogenous – endogenous)*

For the natural and synthetic simple micellar systems, lymphatic transport due to exogenously administered lipid (i.e. surfactant) was determined by subtracting 'endogenous' triglyceride transport from the total triglyceride transport. Figure 8.9 presents a comparison of the triglyceride transport rate of the three simple micelle formulations. The endogenous triglyceride turnover is included for reference. The extent of lipid transport after 8 hours for animals dosed with either the bile salt or TPGS simple

micelles were not statistically different from ($p<0.05$) from the saline control. However, for the cremophor simple micelles, the cumulative extent of triglyceride transport after 8 hours was significantly higher than the saline control ($p<0.05$). This suggests that either the cremophor itself is metabolised, acting as a lipid source thereby contributing to the triglyceride turnover, or cremophor has an indirect action whereby it increases the endogenous triglyceride turnover.

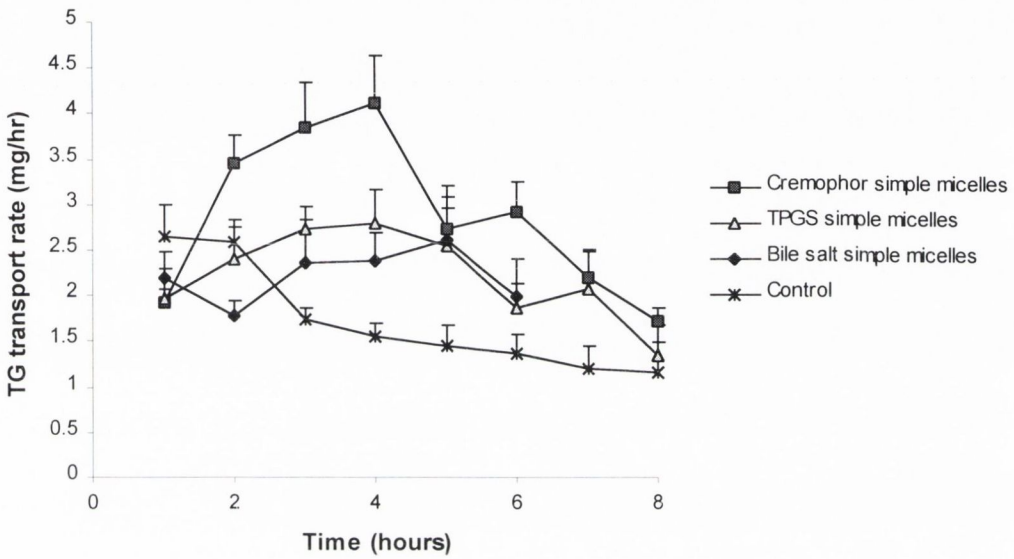


Figure 8.9 Effect of bile salt, cremophor and TPGS simple micelle formulations of the lymphatic triglyceride turnover (mean ± S.E., n ≥4).

The extent of TG lipid transport from all the mixed micellar systems was statistically significantly higher than the corresponding simple micellar systems ($p<0.05$) (Table 8.2). For the NaC, cremophor EL and TPGS mixed micellar systems, the extent of the TG transport attributable to the oleic acid incorporated in the formulations was calculated by subtracting the total TG lipid for each simple micellar system from the total TG lipid measured for each mixed micellar system. In this way any possible influence of NaC, cremophor or TPGS on TG turnover can be eliminated, rather than correcting for endogenous TG as determined with a saline control. The percentage of the administered oleic acid recovered in the mesenteric lymph after 8 hours, as re-synthesized TG ranged from 51.64 ± 9.7% for the bile salt mixed micelles, to 44.74 ± 6.32% for the cremophor mixed micelles and to 75.59 ± 18.56% for the TPGS mixed micelles. There was no significant difference in cumulative extent of triglyceride transport, after 6 hours, across the three mixed micellar groups. It should be noted that the terms endogenous and

exogenous lipid are used in a simplified manner because endogenous lipid turnover has been shown to increase in the presence of exogenous lipid (Shiau et al., 1985). Consequently, experiments utilizing radiolabeled exogenous lipid would be required to accurately distinguish between endogenous and exogenous lipid transport.

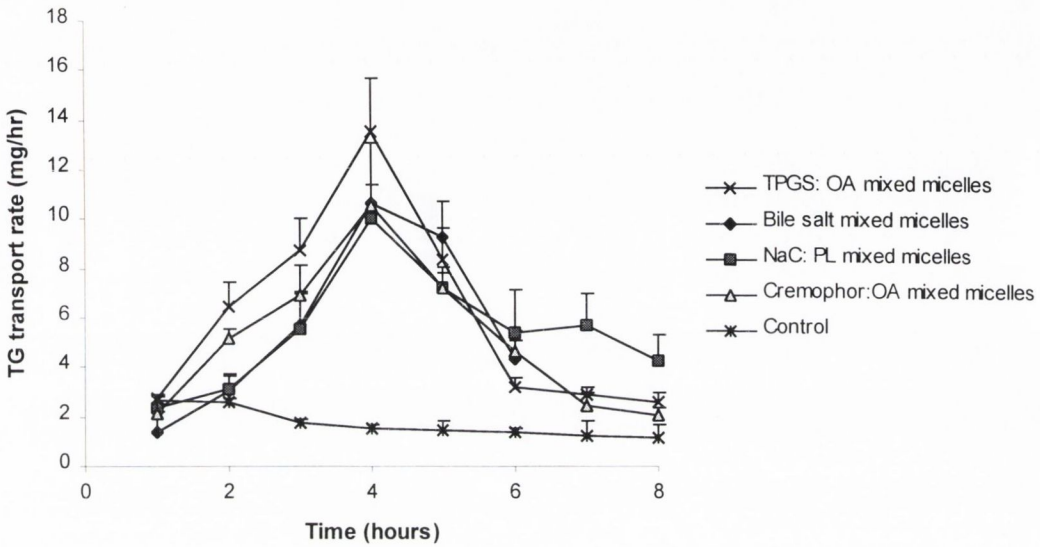


Figure 8.10 Effect of bile salt, cremophor and TPGS mixed micelle formulations on the lymphatic triglyceride turnover (mean ± S.E., n ≥4).

Examination of the triglyceride transport rate as a function of time (Figure 8.10) for the mixed micellar formulations confirms that peak triglyceride transport occurs between 3–4 hours. The triglyceride transport rate increased almost linearly over the first 4 hours of the experiment, followed by a more rapid decline between 4 and 6 hours and return to a basal level, corresponding with endogenous turnover at 7 hours. Interestingly, for the bile: salt phospholipid mixed micelles, the rate of decline in triglyceride turnover in the post-dosing phase is slower than for the other micellar systems. This may reflect a prolonged lipolysis of the phospholipid (PL) moiety, which must be partially hydrolysed prior to absorption and incorporation into lipoproteins within the enterocytes. The cumulative extent of the triglyceride transport after 8 hours for the bile salt: phospholipid was similar to that observed for the other mixed micellar systems. Phospholipids are hydrolysed in the intestinal lumen to lyso-phospholipids by pancreatic phopholipase A_1, which after absorption by the enterocytes, are reacylated prior to their central role in the stabilisation of the lipoprotein surface. These results suggest that despite the need for pre-absorptive

hydrolysis of phospholipid, the peak rate of lymphatic triglyceride turnover is not affected by comparison with the free fatty acid moiety, and in fact may prolong the period of elevated triglyceride turnover in the post-dosage phase.

8.2.3. Relationship between DDT lymphatic transport and triglyceride turnover

The relationship between DDT lymphatic transport and lipid turnover can be illustrated by examining the relationship between hourly transport of DDT and the corresponding hourly triglyceride transport for each lipid vehicle (Figure 8.11). The slopes of the lines describing these relationships represent the relative concentration, or loading of DDT per mg of lymph triglyceride.

For all micellar formulations, a similar trend was observed; namely, there is a strong positive correlation between the DDT transport rate (μg/hr) and the triglyceride turnover (mg/hr). The correlation coefficients were in excess of 0.92 for all the lipid vehicles except for the NaC and TPGS simple micelles. The greater degree of scatter for the NaC and TPGS simple micellar formulations possibly reflects the low level of TG turnover following administration; the cumulative extent of triglyceride turnover for these systems was not significantly different from that of endogenous lipid turnover, as estimated following administration of saline.

Charman et al., (1986a) compared the total lipid transport in collected intestinal lymph following administration of either 200 μl of oleic acid or peanut oil, a triglyceride equivalent. There was no significant difference in the total quantity of lipid transported over 11 hours when the two classes of lipids were administered. The apparent loading of DDT per unit of chylomicron lipid was higher when administered in an oleic acid vehicle than when administered in a triglyceride (peanut oil) vehicle. It was suggested that the two-fold higher lymphatic transport of DDT for the oleic acid vehicle was not related to differing degrees of total lipid transport, but were most likely a function of differences in kinetics of lipid and drug processing within the intestinal lumen or the enterocytes. The higher loading from the fatty acid vehicle was rationalised in terms of an increased rate of chylomicron formation; the fatty acid vehicle, which can presumably be processed towards chylomicron formation at a faster rate than a triglyceride vehicle, is able to maintain a higher concentration of the drug in the absorbed lipoidal fraction, resulting in a higher

loading of DDT per mg lymph lipid than the triglyceride vehicle. The triglyceride vehicle, taking potentially longer before chylomicron formation occurs, may lose relatively more DDT from the absorbed lipoidal fraction to the portal blood.

A comparison of the slopes obtained form the above relationships for the bile salt mixed micelles (i.e. NaC:OA and NaC: phospholipid) reveals that the NaC:OA micellar formulation produced a higher loading of DDT per mg of lymph triglyceride. This is consistent with the higher extent of DDT transport from the NaC:OA micelles than the NaC:phospholipid micelles, since both formulations produced similar total lipid turnovers after 6 hours (Table 8.2). The findings here are therefore similar to the study by Charman et al., (1986a). The fatty acid based mixed micelles allow more efficient processing of lymph triglyceride, compared to the phospholipid based mixed micellar formulation, which must undergo digestion, resulting in higher DDT loadings in intestinal lymph.

For the mixed micellar vehicles, the rank order of DDT loading per mg lymph triglyceride is similar to the rank order of the cumulative extent of DDT transport (i.e. TPGS:OA > NaC:OA > Cremophor:OA> NaC:phospholipid). For the NaC and TPGS simple micellar formulations, as the extent of triglyceride turnover remains at endogenous levels after administration of these simple micellar vehicles, slopes of the fitted lines for the above relationship should, in theory, reflect DDT loadings in endogenous triglyceride and the efficiency of transfer of DDT from these simple micelles to endogenous lipoproteins. However, the data for the NaC and TPGS simple micelles exhibits too much variability to allow accurate approximations to be made, as evidenced by the poor correlation coefficient. Interestingly, for the cremophor simple micelles the correlation describing this relationship is improved, and this system also produced the highest apparent DDT loadings (slope) of all micellar systems examined, suggesting that in addition to increasing the extent of triglyceride turnover, the cremophor simple micelles facilitate efficient transfer of DDT to lymph triglyceride.

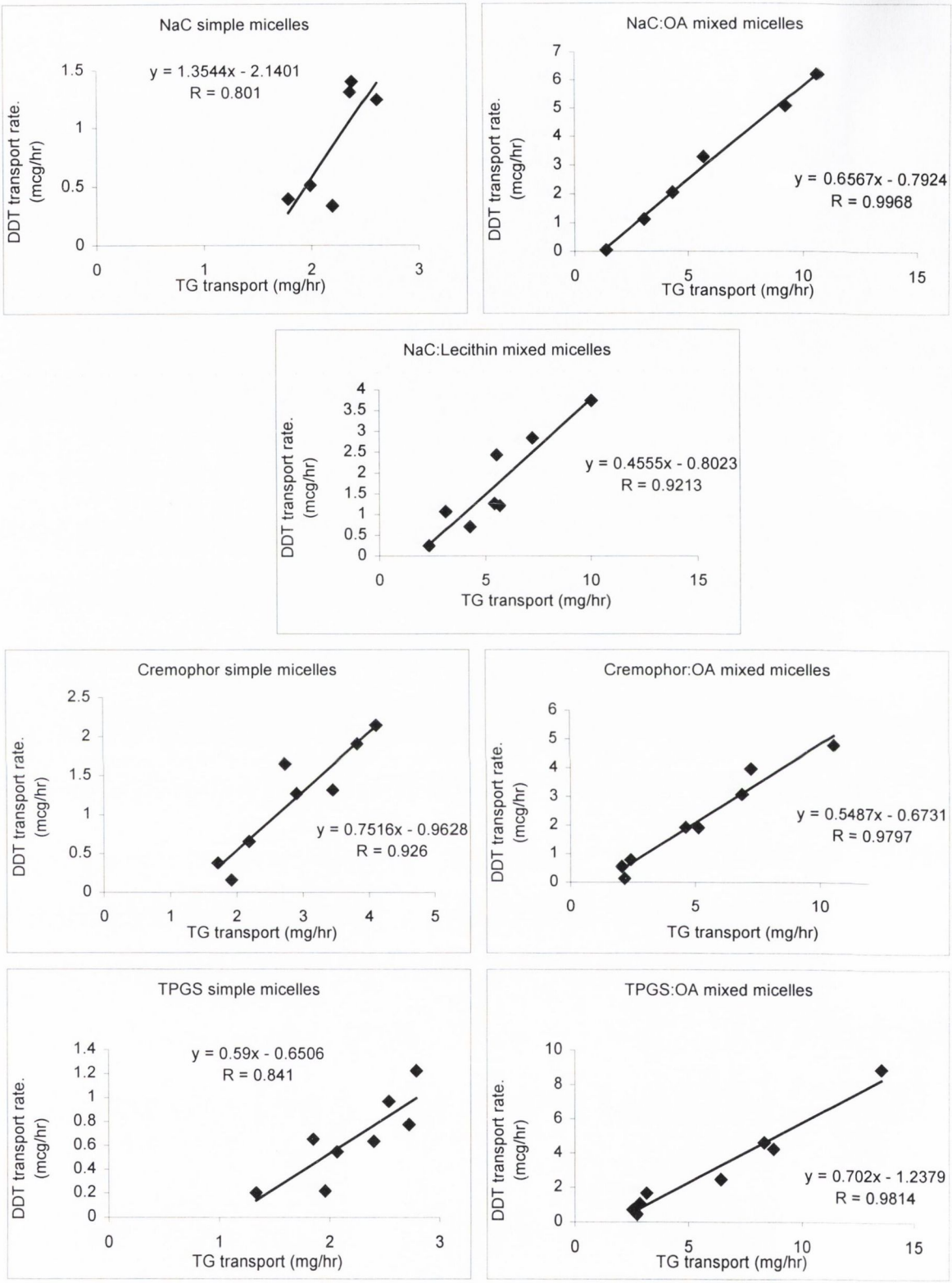


Figure 8.11 Intestinal DDT transport rate (μg/hr) versus triglyceride turnover in intestinal lymph after intraduodenal administration of DDT in a series of simple and mixed micelles.

8.3. Discussion

The present study was originally designed to assess the effects of series of lipid micellar vehicles on the rate and extent of lymphatic absorption of DDT. In so far as possible, the lipid formulations examined in the present study were formulated with oleic acid, in recognition of the potential merits of long chain fatty acids, and particularly oleic acid, in the design of a lymphotropic lipid delivery system. A number of the studies on lymphatic uptake of drugs from lipid vehicles have examined the effect of varying the type of fatty acids e.g. effect of different chain length (Caliph et al., 2000), the degree of unsaturation (Kuksis, 1987; Noguchi et al., 1985a) etc. However, few studies on the lymphatic absorption of DDT have been directed towards characterising the impact of the influence of the type of surfactant surfactants (e.g. natural vs. synthetic), or the relative effects of simple vs. mixed micelles, on the lymphatic transport of a co-administered drug.

Initially, saturated bile salt simple and mixed micelles were examined. Obodozie (1997) have previously reported that the blood concentration - time profiles following administration of DDT in bile salt micellar systems in a through and through rat gut perfusion model exhibit significant differences between simple and mixed micellar formulations. Although the rate of appearance of DDT into the blood was low for both systems, it was lower in the mixed micellar systems than in simple micellar systems. It was postulated that the reduced rate of appearance in the blood may be due to initial transfer into the lymphatics followed by redistribution into the general circulation and suggested that mixed micelles have greater potential to target the lymphatic system. Following administration in the mesenteric lymph duct cannulated anaesthetised rat model, the improved lymphatic transport from mixed versus simple bile salt micelles was confirmed with a 3.6-fold increase in the extent of DDT transported after 6 hours. The inclusion of a $C_{18:1}$ unsaturated fatty acid significantly improves the transfer of DDT to the mesenteric lymph. Lipophilic molecules, such as DDT, which are transported to a significant extent by the intestinal lymphatic system are either solubilized by, or associated with, the triglyceride core of chylomicrons, which are the major triglyceride transporting lipoproteins of the small intestine. The improved delivery from the mixed micellar formulation can be rationalised in terms of: (i) an improved drug transfer into intestinal lymph, and (ii) a stimulation of chylomicron formation within the enterocytes and resulting increased drug uptake by intestinal lipoproteins.

For all micellar systems examined, formulation in a mixed micellar state increased the intestinal lymphatic transport over that obtained for the corresponding simple micellar system. These increases in the cumulative extent of dose administered ranged from 3.6-fold for the NaC bile salt (over 6 hr), to 1.8-fold for cremophor (over 8hr) and to 4.6-fold (over 8hr) for the TPGS mixed micellar systems. The increased lymphatic transport for the mixed micellar state is related to the increased triglyceride turnover induced by the incorporation of the long chain fatty acid, oleic acid, in the formulation.

The rank order in the observed maximal lymphatic transport over 6 hours was:

TPGS:OA (22.3 ± 2.6%) > NaC:OA (17.75 ± 1.3%) > Cremophor:OA (15.8 ± 1.9%)

A similar rank order in the apparent DDT loadings per mg lymph triglyceride was observed for the three mixed micellar systems. The differences between the mixed micellar systems were not statistically significant ($p < 0.05$). Therefore, in terms of promoting DDT lymphatic transport, the synthetic surfactants appear to be as effective as the naturally occurring surfactants. In performing these comparison it must be remembered that the initial studies were performed at a bile salt concentration of 40mM, which corresponds to a 1.72% solution; whereas, due to difficulties in ascertaining an exact Mw, the subsequent studies with cremophor and TPGS were performed using a 2% solution.

The extent of lymphatic transport for the cremophor simple micellar formulation was significantly higher ($p < 0.05$) than that observed for the simple bile salt and TPGS micellar formulations. The exact mechanism whereby cremophor exerts this effect is unclear; however, the significantly higher triglyceride transport for the cremophor simple micellar systems suggests that it acts to increase triglyceride turnover. This effect may be related to a direct mechanism on the enterocytes membranes acting to displace endogenous lipids, or cremophor may act as a lipid substrate itself. Comparing the triglyceride turnover for the cremophor mixed micelles to those of the TPGS and NaC mixed micelles, the 'cremophor specific' effect is not apparent.

The concentrations of DDT in the micellar systems examined in this study were all administered at near saturation conditions; the saturation solubility being higher in the mixed micelles than the corresponding simple micelles. The actual saturation solubility (Css) of the simple cremophor micelles is 2.2-fold higher than the Css of NaC :OA mixed

micelles, and similar to the Css of the TPGS:OA mixed micelles i.e. cremophor simple micelles have a similar DDT solubilizing capacity as TPGS mixed micelles. However, the % of the dose transported lymphatically for the NaC:OA and the TPGS:OA mixed micellar systems is significantly greater, compared to cremophor simple micelles. The results presented here confirm that the increased lymphatic transport observed for the mixed micellar systems is not simply a function of increased solubilizing capacity of the mixed micellar systems promoting an increased solubilisate concentration within intestinal cells.

A strong positive correlation is evident between the DDT hourly transport rate (μg/ml) and the triglyceride turnover rate (mg/hr) for all mixed micellar systems studied. The efficiency with which the micellar formulations promote triglyceride transport appears to play a role in the extent of intestinal lymphatic transport of DDT. The extent of intestinal lymphatic transport of DDT after 6 hours is significantly higher for the NaC:OA bile salt mixed micelles than the NaC: phospholipid bile salt mixed micelles, despite similar extent of triglyceride turnover after 6 hours. This is consistent with a higher loading of DDT per mg of lymph triglyceride. The NaC:OA mixed micelles contains the free fatty acid, which can be absorbed readily, and processed into intestinal lipoproteins, whereas the phospholipid form must undergo lipolysis to release the free fatty acid in the intestine. This finding is in agreement with that of Charman et al., (1986) who compared the lymphatic transport of a fatty acid vehicle and a triglyceride equivalent vehicle as discussed in Section 8.23. Our findings suggest that the intestinal lymphatic recovery of administered lipid is higher for the fatty acid based micelles, than the phospholipid based micelles. While the molar concentration of lipid in both mixed micelles were the same, the phospholipid vehicle contains a two-fold higher long chain fatty acid content (i.e. each phospholipid molecule contributes two fatty acid moieties); however the extent of recovery of long chain lipid in the lymph was statistically similar after 6 hours. Therefore, is appears that formulation in the free fatty acid form facilitates a more efficient processing of administered lipid, in to lymph lipoproteins, which appears to improve drug transfer to the lymph, as evidenced by the higher apparent loadings and extent of transport.

9. Intestinal lymphatic transport of DDT from a SEDDS versus an oil dispersion

9.1. Introduction

In recent years, much attention has been given to the design and development of new oral dosage forms, with particular emphasis on the development of lipid-based systems. Much of the activity in this area has been focused on the development of microemulsions as drug solubilization and absorption enhancement systems (Ritschel, 1991; Constantinides, 1995). Microemulsions are thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilised by an interfacial film of surfactant molecules (Eccleston, 1994). The surfactant may be pure, a mixture of components, or combined with other additives. In the absence of water, mixtures of oil(s) and non-ionic surfactant(s) that form clear and transparent solutions are known as self-emulsifying drug delivery systems (SEDDS) and have recently been used for improving lipophilic drug dissolution and absorption (Charman et al., 1992; Shah et al., 1994). One characteristic of appropriately selected SEDDS blends is their ability to form fine oil-in-water emulsions upon mild agitation when exposed to aqueous media. Thus SEDDS represent an efficient vehicle for the *in vivo* administration of emulsions and microemulsions.

Despite a number of attempts (using for example the HLB number and critical packing parameter (CPP) of a surfactant) it is not yet possible to predict successfully which combinations of surfactant, co-surfactant and oil will produce a microemulsion, although such knowledge would be extremely valuable when formulating pharmaceutical microemulsions (Warisnoicharoen et al., 2000). To illustrate this point Lawerence and co-workers (2000) give an example of where $C_{18:1}AO$ (dimethyloleylamine-N-oxide) and its saturated counterpart, $C_{18}AO$, (dimethylstearylamine-N-oxide) possess virtually identical CPP and HLB numbers, yet only $C_{18:1}AO$ is capable of forming microemulsions at 25^0C. As a result therefore, the formulation of SEDDS remains for the most part an empirical process, though there are some useful guidelines that have emerged by characterisation of the properties of successful formulations (Pouton, 1997). Two key formulation components are the nature of the oil and the surfactant phase and their ratio. Several reports have noted a particular specificity between these formulation variables and the self-emulsifying behaviour (Pouton, 1985; Shah et al., 1994; Craig et al., 1995; Khoo et al., 1998)

In the present work SEDDS have been developed using commercially available and pharmaceutically acceptable components. They consist of an oil, a blend of a low and high HLB surfactant and an aqueous phase. There are numerous reports of SEDDS using medium and long chain triglycerides as the oil phase, however for our work the C_{18} free fatty acid form was deemed more appropriate, the justifications for which are summarised as follows:

- In the preceding chapter incorporation of oleic acid into simple micellar systems, to form mixed micelles resulted in increased lymphatic transport of co administered DDT and triglyceride turnover in intestinal lymph.
- The solubility of two model lipophilic compounds saquinavir and DDT was greater in the free fatty acid form (i.e. oleic acid) than in the triglyceride equivalent (i.e. triolein); an important parameter when considering the maximal drug loading in the SEDDS. It has been shown that the drug loading improvement of an o/w microemulsion over a micellar system appear to depend on the solubility of the drug in the dispersed oil phase, and is significant only for lipophilic drugs (Malcolmson et al., 1998; Naylor et al., 1993)
- As the potential effects of anaesthesia and bypassing the stomach on triglyceride digestion may prove difficult to establish, administration of free fatty acid as opposed to the undigested, or partially digested, mono-, di- or tri-glyceride form was deemed desirable.

Hence the preformulation work is focused on SEDDS blends containing a maximal amount of oleic acid as the lipophilic phase, while still permitting infinite dilution as a microemulsion phase. The objectives of the present work were as follows: firstly, to design SEDDS containing oleic acid based on the corresponding pseudo-ternary phase diagrams and to identify the most suitable SEDDS formulation for subsequent *in situ* testing in lymphatic transport studies. The multi-component delivery systems were optimised by evaluating their ability to self-emulsify when introduced to an aqueous medium under gentle agitation, and by determination of particle size of the resulting emulsion. Secondly, in order to examine the role of dispersibility on intestinal lymphatic transport, the lymphatic transport of DDT in the lymph cannulated rat model was assessed following administration of the optimised SEDDS formulation and compared to transport observed for an oleic acid dispersion. The lymph triglyceride levels for both experimental groups were compared. Finally, efforts were made to estimate the relative contribution of

lymphatically transported DDT to the systemic absolute bioavailability after intraduodenal administration of the self-emulsifying lipid vehicle. Plasma profiles in lymph cannulated and non-lymph cannulated rats were examined to probe aspects of mass balance of drug absorption.

9.2. Results

9.2.1. Formulation and characterisation of SEDDS

A series of self-emulsifying formulations were prepared for each of the four formulas listed in Table 9.1, with varying concentrations of oil, surfactant and co-surfactant. Pseudo-ternary phase diagrams were constructed with systems comprising four components: a long chain free fatty acid (oleic acid), a low HLB co-surfactant (Capmul MCM HLB 5.0-6.0 or Plurol Oleique HLB 10), a high HLB surfactant (Cremophor EL HLB 13.5, or Labrasol HLB 14) and as aqueous phase (water). As this system comprises four components, the ratio of surfactant to co-surfactant is kept constant so that there are only three variables, each of which can be represented by one side of the triangle. The regions of the phase diagram in which microemulsions exist were determined by titrating (dropwise) a mixture of oil, surfactant/co-surfactant blend (in a fixed ratios of 1:1, 3:1 and 6:1 surfactant: cosurfactant) against the hydrophilic phase (water), noting the points of phase separation, turbidity and transparency. Clear, transparent formulations were indicative of a stable microemulsion. Other phases produced by this system were not shown. For convenience, at these early stages a drug was not included in these pre-formulation studies. Excipients with a definite regulatory status were chosen to formulate the lipid-based formulations.

Table 9.1 Vehicle composition of various formulations of SEDDS

Formula / Component	I	II	III	IV
Lipophilic phase	Oleic acid	Oleic acid	Oleic acid	Oleic acid
Surfactant	Labrasol	Labrasol	Cremophor EL	Cremophor EL
Co-surfactant	Plurol Oleique	Capmul MCM	Plurol Oleique	Capmul MCM
Hydrophilic phase	Water	Water	Water	Water

The phase boundaries reported are accurate to 3-5%. The boundaries of the isotropically clear and heterogeneous phases were determined at 25^0C. In the phase diagrams, red and green colours designate the isotropic phases. No attempt was made to recognize microemulsion structure at different parts of the microemulsion realm but owing to the

continuity between water-poor and water-rich regions, it may be predicted that the microemulsion structure varies greatly, but progressively, as the composition varies. The red region represents an oil-in-water microemulsion and the green region a water-in-oil microemulsion. In some cases the red and green regions are connected i.e. there is a single isotropic phase on infinite dilution. In these cases the conversion between w/o to o/w was arbitrarily estimated as the point at which the addition of water drop wise, produced a temporary cloudiness in the mixture, which under gentle stirring returned to a clear isotropic system. Clear systems that did not show a change in meniscus after tilting to an angle of 900C were classified as gels.

Microemulsions were stored at ambient temperature over a one-month period. Their stability was measured by periodic visual inspection for the presence of macroscopic phase separation, as shown by cloudiness or the formation of two distinct layers. Microemulsions that exhibited no visible sign of change were considered to be stable, e.g. a single and optically clear phase.

The HLB of each surfactant system was calculated using the formula;

$$HLB_{mix} = f.\ HLB_a + (1\text{-}f).\ HLB_b$$

Where HLB_{mix} is the hydrophile/lipophile balance of the mixture

F is the fraction of component A

HLB_a is the hydrophile/lipophile balance of the component A

HLB_b is the hydrophile/lipophile balance of the component B

The influence of the ratio of surfactant to co surfactant on the area of existence of the microemulsion field was studied in the following series of partially completed pseudo ternary phase diagrams (Figure 9.1, 9.2, 9.3 and 9.4).

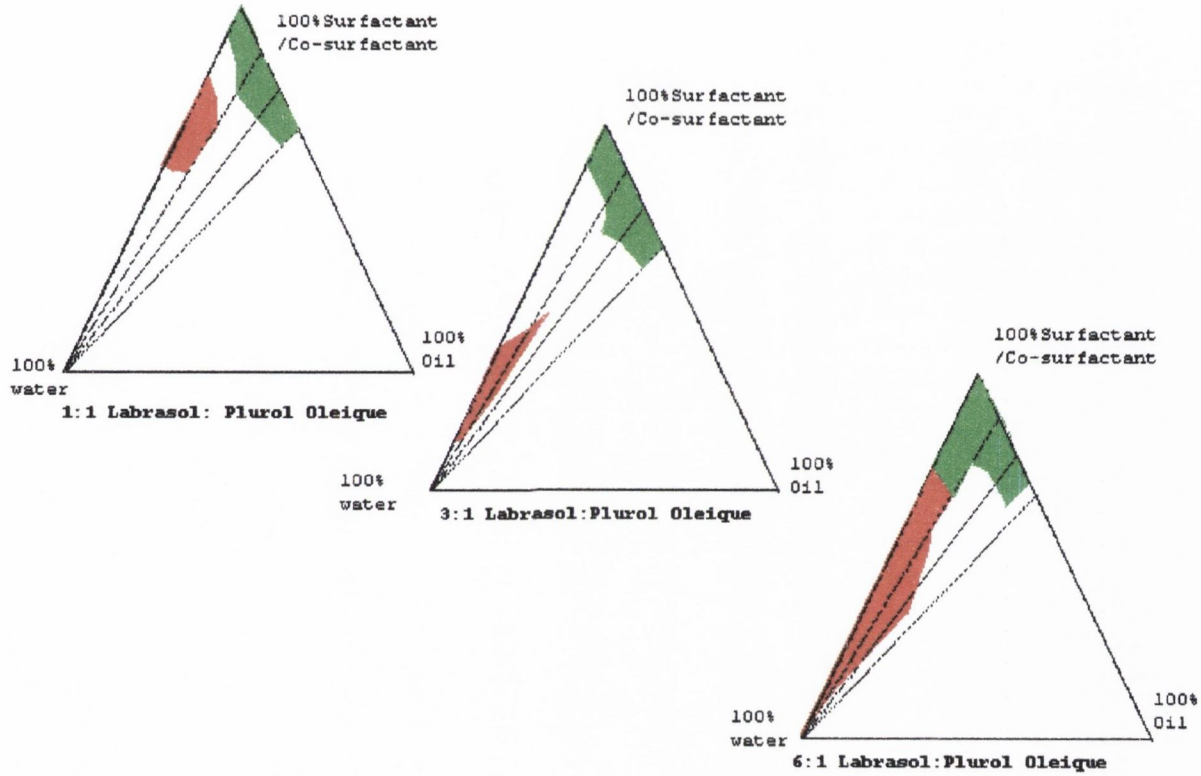


Figure 9.1 – System1. The partially completed pseudo ternary phase diagram for Labraol: Plurol Oleique: Oleic acid: water system. Red zone: This is the microemulsion existence field produced on dilution and represents efficient self-emulsification

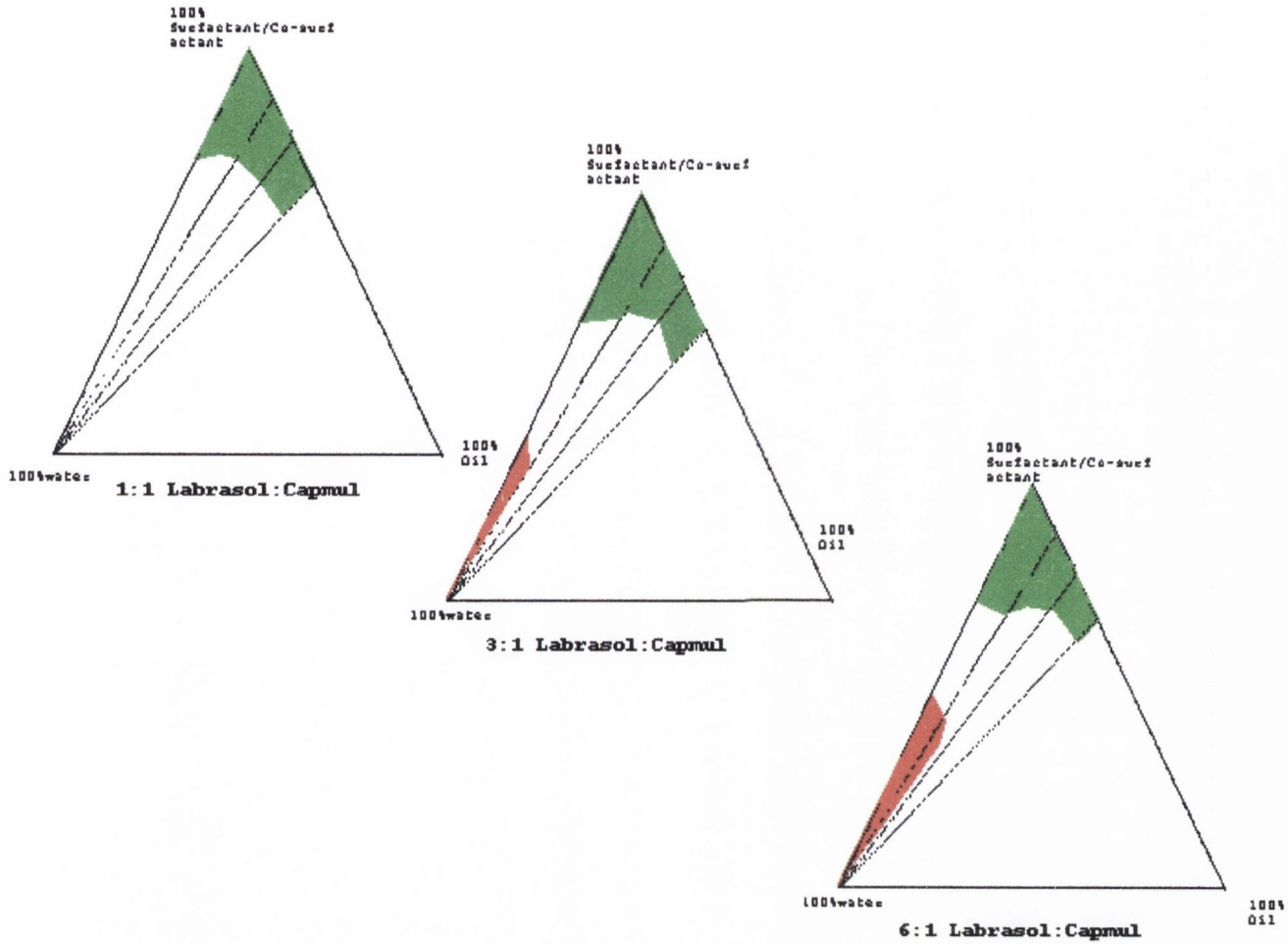


Figure 9.2 – System II. The partially completed pseudo-ternary phase diagram for Labraol:Capmul:Oleic acid: water system. The 6:1 surfactant:co-surfactant (S:CoS) ratio produces the largest area of the red zone, which appears to withstand infinite dilution with water. The 3:1 S:CoS system may also withstand infinite dilution, at low oil content, but the area of the microemulsion existence field is much smaller

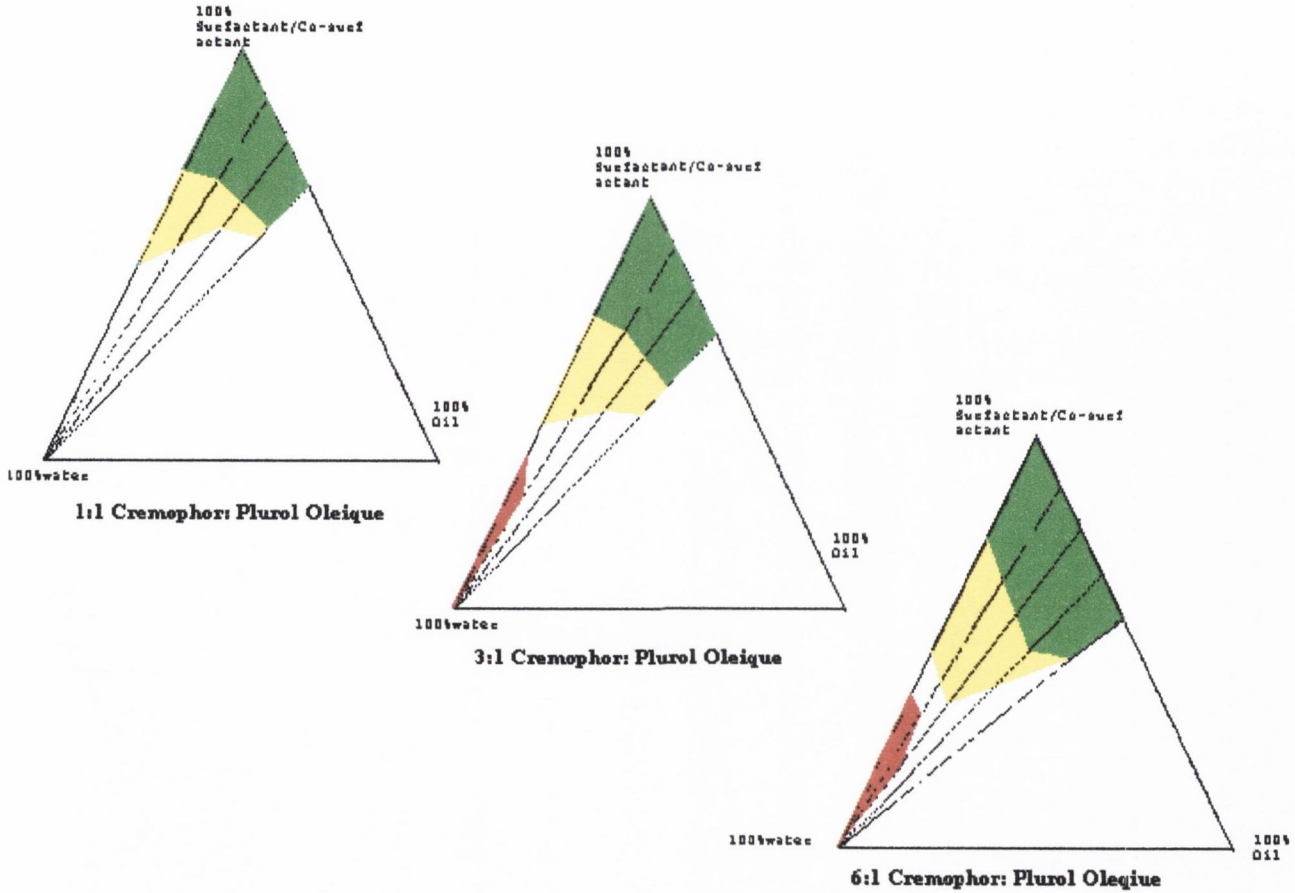


Figure 9.3 – System III. The partially completed pseudo-ternary phase diagram for Cremophor EL:Plurol Oleique: Oleic acid: water system. Yellow zone: represents the formation of transparent gel-like structures, which were more apparent at higher cremophor concentrations.

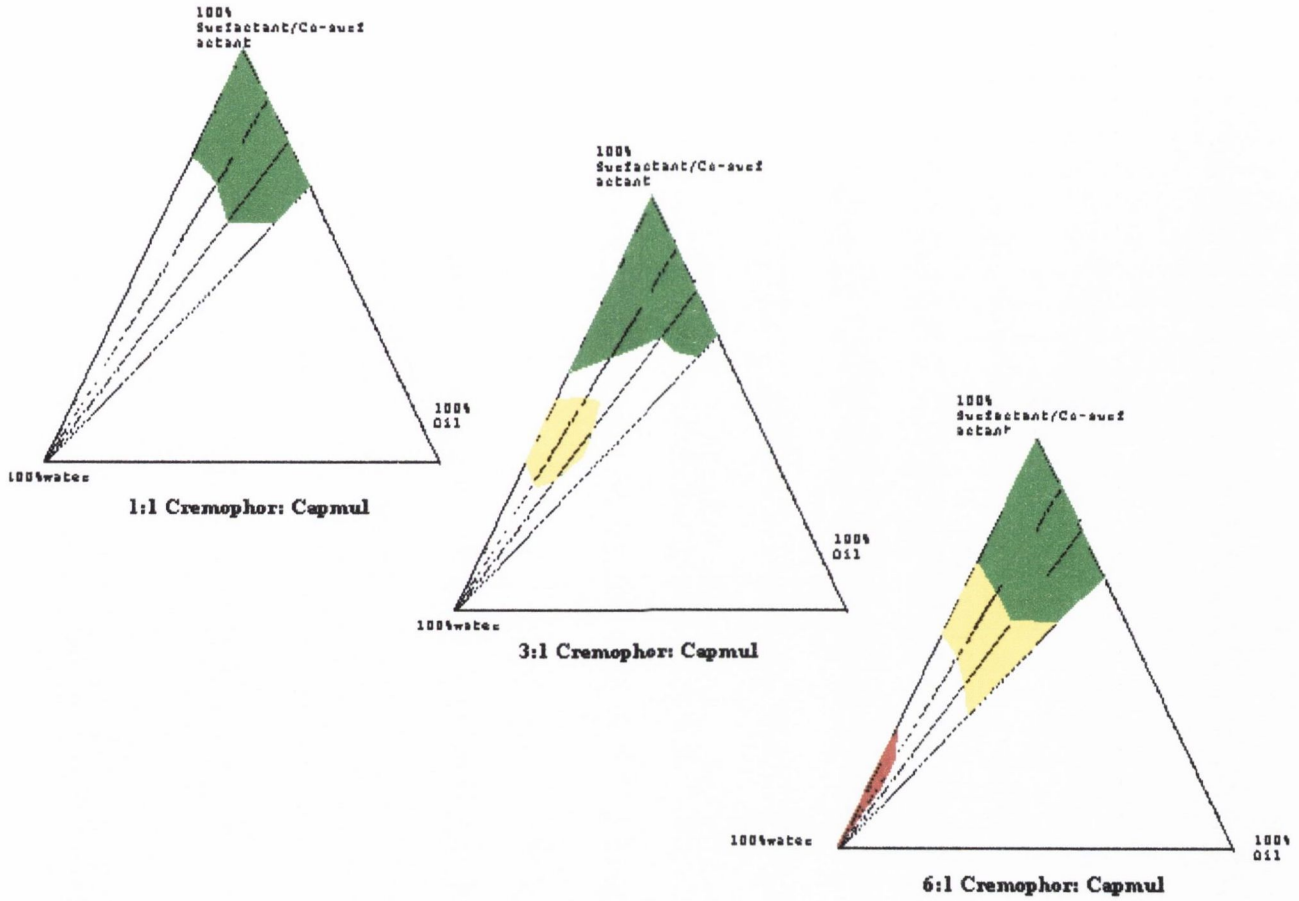


Figure 9.4 – System IV. The partially completed pseudo-ternary phase diagram for Cremophor EL:Capmul MCM: Oleic acid: water system. The area of existence of both the red zone (microemulsion) and the yellow zone (gel-like structure) increases with increasing S:CoS ratio.

The visual assessment technique used in this study is a measure of an apparent spontaneity of emulsion formation and is similar to that previously reported by Gao et al., (1998). Pseudo ternary phase diagrams were constructed to identify the self-emulsifying regions and also to establish the optimum concentrations of oil, surfactant and co surfactant. The equilibrated samples were marked as being optically clear microemulsions (single o/w or w/o microemulsions) or crude emulsions or gels. The red region is of primary interest as this is the region on the phase diagram, which corresponds to the component composition that gives a clear homogenous microemulsion system. In some cases this region appears to withstand infinite dilution, which gives an indication of the ability of this SEDDS blend to spontaneously self emulsify on dilution in the gastrointestinal tract.

Lipophilic surfactants with HLB's <10 are capable of promoting some emulsification of oil, but the resulting emulsions are normally too crude (in terms of size) to be useful. Hydrophilic surfactants with HLB's > 10 are much superior at providing fine, uniform emulsion droplets which are more likely to empty rapidly from the stomach (Macgregor et al., 1997). Furthermore, the large surface area facilitates faster and more complete absorption. However, in most cases it is the right blend of low and high HLB surfactants that leads to the formation of a stable microemulsion upon exposure to water (Constantinides, 1995). Therefore based on these considerations, two high value surfactants and two low value surfactants were selected. The two primary surfactants, Labrasol and Cremophor EL, have similar HLB values. However, SEDDS blends produced with Labrasol appear to facilitate microemulsion formation, as evidenced by the larger red regions in the phase diagrams for the systems. Capmul has a HLB in the range 5-6, compared to a HLB of 10 for Plurol Oleique. At a surfactant: cosurfactant (S:CoS) ratio of 6:1 the calculated HLB's of formula I, II, III and IV were 13.4, 13, 12.78, and 12.35 respectively. System I had the highest HLB and produces the most successful SEDDS blends in terms of the area of the o/w microemulsion region and ability to withstand infinite dilution.

When a co surfactant is added to the system, it further lowers the interfacial tension between the oil and water interface and also influences the interfacial film curvature, which thereby readily deforms around the oil droplets (Eccleston, 1994). For the four formulas examined, the areas of microemulsion and isotropic regions increased with increasing ratio of surfactant to cosurfactant. Hence, the maximum proportions of oil incorporated in microemulsions increased significantly with increasing ratio of surfactant

to cosurfactant. Similar results were reported from microemulsions using Brij 96 as surfactant and glycerin, ethylene glycol, propylene glycol as cosurfactant (Kale & Allen, 1989). These results are also similar to those of Kim et al., (1997), where a microemulsion system containing cyclosporin was developed using GLUCAM P20 as co-surfactant and Pluronic L43 as surfactant. It is thought that the addition of surfactant causes the interfacial film to condense and to be stable, while the addition of cosurfactant would cause the film to expand. From a formulation viewpoint, the increased oil (i.e. oleic acid) content in microemulsions may enhance both the solubilization capacity, and potentially the lymphotropic properties of the SEDD.

System I, which contains Labrasol/Plurol Oleique as the surfactant/cosurfactant blend, produced the largest areas of microemulsion existence field of all the blends examined. The area of microemulsion existence field increased with increasing ratio of surfactant to co-surfactant. At a 6:1 Labrasol: Plurol Oleique ratio and concentrations of up to 10% oleic acid, the SEDDS blend remained an isotropically clear microemulsion on infinite dilution with water. At higher concentrations of oil phase (i.e. oleic acid) the SEDDS blend did not remain isotropically clear on infinite dilution.

For the SEDDS blends containing cremophor EL, the formation of transparent gel-like structures upon addition of water was common. Khoo et al., (2000) have reported the formation of similar gel-like structures on dilution of SEDDS blends containing cremophor EL. Whereas the formation of microemulsion gels has shown therapeutic potential for topical drug delivery (Lawerence & Rees, 2000), the formation of a gel-like phase on dilution of a SEDDS may retard dispersibility by reducing the penetration of water and reduce the efficiency of drug release in vivo.

It is known that the particle size distribution is one of the most important characteristics determining the stability (Charman et al., 1992), the rate and extent of drug release (Shah et al., 1994) and also *in vivo* fate of microemulsions (Tarr & Yalkowsky, 1989). Table 9.2 presents the particle size and polydispersity of the SEDDS, based as intensity distribution results, following dilution of each formulation 1:200 with water. The polydispersity values varied between 0.2 and 0.5, which are comparable to reported literature values (Khoo et al., 2000; Constantinides et al., 1994). The closer to 0.0 the polydispersity value, the more homogeneous the particle population (for latex bead standard the value was 0.03). The particle size distributions were all monomodal. The *in vitro* performance of the four

SEDDS were visually assessed using a grading system similar to that previously reported by Khoo et al., (1998). In general there was good correlation between visual observations and particle size measurements. SEDDS blends that formed clear or slightly bluish microemulsions (visual grading A) had particle sizes in the range 50-100nm, microemulsions with a slightly less clear or bluish white appearance (visual grading B) had a range of 100-150nm, with the more whitish microemulsions having sizes closer to 200nm (visual grading C).

The emulsification time was not determined formally during these studies but was observed visually. The emulsification time for System I and system II, as determined by gently mixing the SEDDS blend in water by inverting the flask, was rapid i.e. under 30 seconds. For systems III and IV, the emulsification time was visibly longer, requiring up to 3 minutes for complete dispersion. This was assumed to be related to the formation of gel-like structures, as a result of the high cremophor content. It was also noticeable that the polydispersity values from freshly diluted (i.e. sized with 15 minutes of dilution) SEDDS blends of system III and IV were very high i.e. 0.55-0.65. If the samples were left to equilibrate for up to 1 hour and then sized, the polydispersity values were lower i.e. 0.2 to 0.46. Furthermore, for freshly prepared samples that were filtered through a 0.22μm filter and then sized, the polydispersity values were reduced i.e. 0.2-0.46. The polydispersity values of samples prepared from System I and II were unaffected by the time to equilibrate or filtration. Table 9.3 illustrates this point further. These results suggest the presence of larger sub-micron particles, possibly cremophor aggregates, in the microemulsions formed with cremophor, which require extended periods to adequately disperse. For the data presented in Table 9.2, the microemulsions were equilibrated for 1 hour prior to sizing.

Table 9.2 Effect of formulation composition on the dispersibility and particle size (mean ± SD, n = 3) of SEDDS blends formulated with oleic acid, following dilution 1 in 200 in water.

Composition (%w/w)		Visual grading[a]	Mean droplet size (nm)	Polydispersity
Formula	**Oleic acid (%w/w)**			
System I: *Labrasol: Plurol Oleique (6:1)*				
100	0	A	52.5 ± 1.7	0.16 ± 0.03
95	5	A	66.9 ± 0.4	0.333 ± 0.01
90	10	B	159.9 ± 1.9	0.247 ± 0.01
85	15	C	176.9 ± 5.6	0.279 ±0.05
80	20	C	184.4 ± 4.5	0.337 ± 0.12
75	25	C	181.3± 3.6	0.328 ± 0.10
System II: *Labrasol: Capmul MCM (6:1)*				
100	0	C	525.9 ± 215	0.269 ± 0.28
95	5	C	306.6 ± 6.86	0.325 ± 0.07
90	10	C	308.9 ± 53.6	0.368 ± 0.09
85	15	C	374.9 ± 195	0.145 ± 0.14
80	20	C	390.5 ± 157	0.196 ± 0.18
75	25	C	587.8 ± 32.8	0.214 ± 0.10
System III: *Cremophor EL: Plurol Oleique (6:1)*				
100	0	A	61.3 ± 2.66	0.103 ± 0.02
95	5	B	135.3 ± 1.25	0.463 ± 0.003
90	10	B	126.4 ± 1.23	0.465 ± 0.02
85	15	B	129.1 ± 0.29	0.467 ± 0.01
80	20	B	117.7 ± 0.57	0.406 ± 0.01
75	25	B	141.5 ± 1.06	0.434 ± 0.02
System IV: *Cremophor EL: Capmul MCM (6:1)*				
100	0	A	16.0 ± 1.51	0.195 ± 0.03
95	5	B	120.9 ± 0.60	0.446 ± 0.03
90	10	B	124.0 ± 1.40	0.363 ± 0.01
85	15	B	116 ± 2	0.365 ± 0.02
80	20	B	130.3 ± 3.9	0.363 ± 0.02
75	25	B	140.3 ± 4.6	0.345 ± 0.02

[a] *A, denotes a microemulsion, which was clear or slightly bluish in appearance; B, denotes a slightly less clear microemulsion, which had a bluish white appearance; C, denotes a bright white emulsion (similar to milk)*

Table 9.3 Droplet sizes and polydispersity of microemulsions formed on dilution of System IV, under various sizing conditions.

Preparation conditions	Zave	Polydispersity
Dilution and sizing in under 15 minutes unfiltered	159.76 ± 1.56	0.535 ± 0.007
Sizing in under 15 minutes filtered	130.64 ± 2.41	0.315 ± 0.002
Dilution and sizing after 60 minutes equilibration unfiltered	124.0 ± 1.40	0.363 ± 0.01
Dilution and sizing after 60 minutes equilibration filtered	124.43 ± 0.59	0.304 ± 0.006

Microemulsion droplet size was unaffected by the degree of dilution, as illustrated in Figure 9.5 (A). This is also an important feature of an efficient SEDDS (Gao et al., 1998). Furthermore, the droplet size of the microemulsion remained unaffected by saturation with the model lipophilic compound DDT (Figure 9.5 (B)).

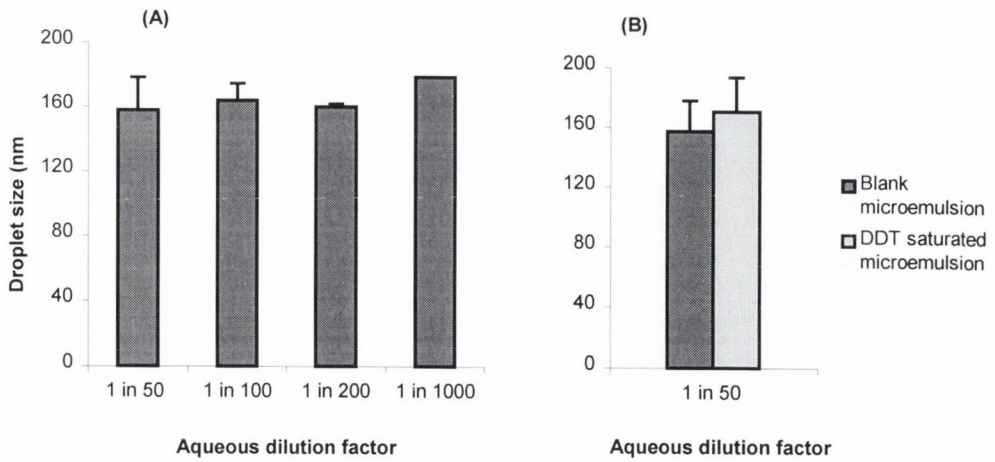


Figure 9.5 (A) Effect of dilution of the Labrasol: Plurol Oleique:oleic acid (54:9:7) SEDDS on microemulsion droplet size. (B) Effect of DDT saturation on droplet size in the 1:50 diluted microemulsion

On the basis of the partially completed pseudo-ternary phase diagrams and the particle size measurements, a SEDDS blend containing Labrasol/Plurol Oleique (System I), with a surfactant: cosurfactant ratio of 6:1, containing 10% oleic acid, was chosen for studies in the lymph cannulated rat model. As the formulation must be administered intraduodenally (i.e. bypassing the stomach), the SEDDS was diluted 1:50 prior to administration. A 1:50 dilution allows for the administration of an appropriate lipid load i.e. 2%w/v, similar to the lipid loads of mixed micellar formulations in the previous chapter. Initially the saturation solubility of DDT in the diluted SEDDS was determined. The saturation solubility of DDT was 621.19μg/ml for the 1:50 SEDDS formulation. The addition of DDT did not appear to affect the self-emulsification ability of this SEDDS, or the stability of the microemulsion formed; all samples remained as isotropically clear liquids in equilibration with excess DDT.

9.2.2. Intestinal lymphatic transport

To address the question of the role of dispersibility and oil droplet particle size on intestinal lymphatic transport, an experiment was designed where a crude oleic acid dispersion and the readily dispersible self-emulsifying lipid vehicle (referred to as SEDDS) were compared. The oleic acid and SEDDS pre-concentrate containing known quantities of DDT were diluted to a final lipid composition of 2%w/v prior to administration. The SEDDS pre-concentrate produces a stable microemulsion in water, with mild agitation, while the oleic acid dispersion required dilution in a 0.2% Tween 80 solution and homogenisation to produce a sufficiently stable crude dispersion. Since the anaesthetized rat model required slow intraduodenal infusion of the drug formulation, concerns with the reproducible dosing of small volumes of oil necessitated the dispersion of oleic acid in 0.2%Tween. The stability of this dispersion was sufficient to allow reproducibility of dosing over the 3-hour infusion period, as detailed in section 6.4.4. In a similar experiment, Charman et al., (1986a) described the combined administration of 200μl oleic acid by infusion over 2 hours, via a T-piece connector, into an intraduodenal infusion of normal saline containing 0.2%Tween, as a lipid 'solution'. Clearly neither of the terms 'solution' or 'dispersion' is strictly correct, but for the present study the term 'dispersion' seems most appropriate. Table 9.4 compares the two formulations in terms of lipid content, dose and droplet size.

Table 9.4 Formulation characteristics of the SEDDS and oleic acid dispersion

Parameters	Formulation excipients	Micelle size (nm)	Dose administered (mg DDT)
SEDDS	7.2mg Oleic Acid 9.3mg Plurol Oleique 55.5 Labrasol	166.6	2.236
Oleic acid dispersion	72mg OA 7.2mg Tween 80	<20,000	5

The cumulative percent of the administered dose of DDT versus time profile is presented in Figure 9.6.

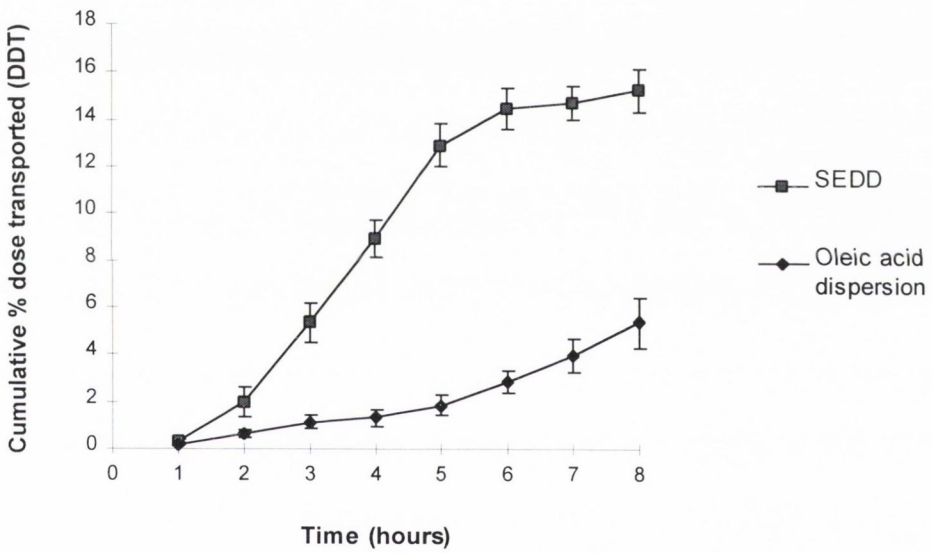


Figure 9.6 Cumulative percent dose of DDT (mean ± SE, n ≥ 5) collected in mesenteric intestinal lymph as a function of time for an oleic acid dispersion and a self-emulsifying lipid formulation.

The total extent of the administered dose of DDT recovered in intestinal lymph after 8 hours was 15.23 ± 0.90% and 5.33 ± 1.07% for the SEDDS and the oleic acid dispersion, respectively. Lymphatic transport of DDT from the oleic acid dispersion was low and very variable. Transport is still incomplete after 8 hours. The oleic acid dispersion would

require further processing within the intestine prior to drug/lipid absorption, thereby resulting in a lower and more variable extent of transport. The transfer of DDT from the oleic acid dispersion is reduced and/or delayed, possibly reflecting the processes of DDT transfer from the pure oleic acid phase, to the pre-absorptive bile salt micellar phase. In contrast, the self-emulsifying lipid microemulsion resulted in a much higher extent and rate of DDT transport, with lymphatic absorption appearing complete after 8 hours. The SEDDS formulation appears to improve these processes of DDT transfer from the microemulsion formulation, as administered, to the endogenous pre-absorptive micellar phase followed by enterocyte uptake.

The rate of intestinal lymphatic transport of DDT (Figure 9.7) demonstrated a 3.3-fold higher peak maximal rate and that peak transport occurred earlier (4-5hr) for the lipid microemulsion than the oleic acid dispersion. The rate profiles observed are similar to those observed by Porter et al., (1996), where it was postulated that poorly dispersible lipid solutions require adequate dispersion within the intestine prior to drug/lipid absorption. SEDDS would be expected to rapidly expedite the cascade of events involved in lipoprotein biosynthesis. These results presented here reflect the positive influence of increased dispersibility of a SEDDS formulation on significantly improving lymphatic transport of DDT relative to the less stable, poorly emulsified oleic acid dispersion. The results may also be rationalised by considering that portal blood flow acts as an absorption sink, competing with the intestinal lymph for the absorption of DDT. Therefore, the longer that lipid vehicle and associated drug remain in the lumen of the small intestine (i.e. as is the case for the oil dispersion), the less will be the relative concentration of the drug per unit of lipid, as DDT may be absorbed into the portal blood, and hence potentially lower the quantity of drug available for transport into the intestinal lymphatic system.

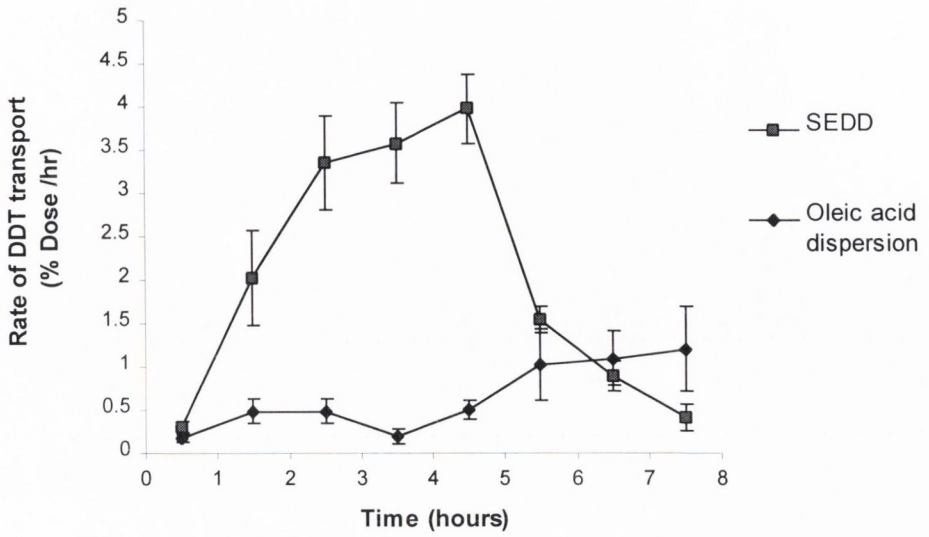


Figure 9.7 Rate of mesenteric lymphatic transport of DDT (Mean ± SE, n ≥ 5) calculated as a percentage of the total dose administered for the SEDDS and oleic acid dispersion per collection time period.

9.2.3. Intestinal lymphatic transport of triglycerides

As discussed in section 8.3, the efficiency with which a formulation promotes lymphatic transport of triglyceride also appears to play a role in the partitioning of absorbed drug into the lymph. The triglyceride transport kinetics following administration of the SEDDS and the oleic acid dispersion are shown in Table 9.5. Whereas the total lipid load for both formulations was similar (Table 9.4), the SEDDS is presented to the duodenum as a stable micro-emulsified phase with droplet sizes in the range of 100-200nm. The oleic acid emulsion forms relatively large oil droplets (<20μm) that are thermodynamically unstable.

Table 9.5 Cumulative transport of triglyceride into the mesenteric lymph (Mean ± S.E.) and cumulative lymph flow as a function of lipid vehicle, after 8 hours

Formulation	Cumulative mass of TG (μg) appearing in mesenteric lymph [A]				Cumulative TG transport[B]	Cumulative lymph flow
	0-2 hr	0-4 hr	0-6 hr	0-8 hr		
Saline control	5.89 ± 1.03	9.16 ± 1.07	11.95 ± 1.66	14.31 ± 0.94		4.45 ± 0.35
SEDDS	5.57 ± 0.53	13.68 ± 0.55	22.05 ± 0.43	27.86 ± 0.51	13.55 ± 1.05	3.95 ± 0.16
Oleic acid dispersion	5.74 ± 1.10	10.01 ± 2.31	17.99 ± 1.73	27.58 ± 4.87	13.27 ± 5.41	3.61 ± 0.22

[A] *Representing endogenous and exogenous lipid*

[B] *Attributable to exogenous lipid (i.e. exogenous – endogenous)*

Endogenous triglyceride lipid turnover, assessed in control rats administered saline over 8 hours, was 14.31 ± 0.94 mg (mean ± S.E., n= 3). Lymphatic transport due to exogenously administered lipid was determined by subtracting 'endogenous' triglyceride transport from the total triglyceride transport. The total extent of triglyceride transport at 8 hours was similar for both vehicles. The percentage of the administered oleic acid recovered in the mesenteric lymph as re-synthesized triglyceride for the oleic acid dispersion was 18.43 ± 7.5%. This figure is relatively low by comparison with the figures > 50% obtained for micellar vehicles in the preceding chapter. For the SEDDS formulation, 13.5mg of recovered triglyceride was attributable to exogenously administered lipid, which is actually greater than the amount of oleic acid administered in the SEDDS (i.e. 7.2mg). This is assumed to be derived from the lipids in the SEDDS blend, which contains 7.2mg oleic acid, 9.3mg of Plurol Oleique (Polyglycerl-6-dioleate) and 55.5mg Labrasol (C8 and C10 polyglycolysed glycerides). As the surfactants in the blend are semi-synthetic derivatives of digestible vegetable oils, the contribution that these make to overall triglyceride is difficult to quantify. It is generally accepted that the digestion products of long chain triglyceride lipids are preferentially re-synthesized in the enterocytes, assembled in lipoproteins, and secreted into mesenteric lymph, whereas shorter chain triglycerides are primarily absorbed directly into portal blood. The main potential source of long chain triglyceride is assumed to be oleic acid, however metabolism of Plurol Oleique may also liberate long chain fatty acids. Furthermore the C_8 and C_{10} glycerides in Labrasol may

influence the overall lipid turnover in the lymph. Caliph et al., (2000) reported how a medium chain triglyceride vehicle produced a 42% increase in the extent of long chain triglyceride levels collected in lymph after 12 hours. Therefore, it is unclear whether the increased long chain triglyceride observed following administration of the lipids in this SEDDS is a result of an increase in endogenous triglyceride turnover or to digestion of the surfactants themselves.

Figure 9.8 compares the rate of triglyceride turnover in lymph cannulated rats following administration of the SEDDS or the oleic acid dispersion.

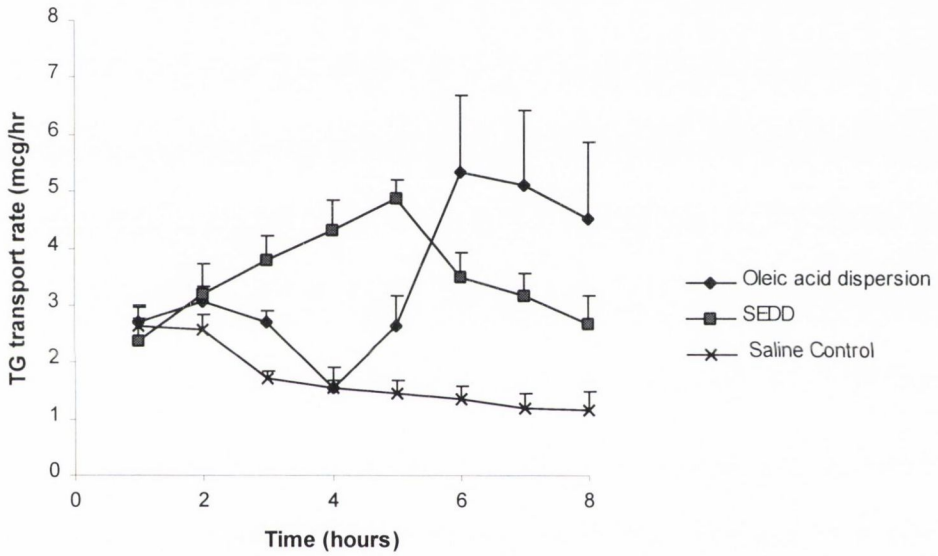


Figure 9.8 Rate of intestinal lymphatic transport of triglyceride for SEDDS and oleic acid dispersion.

The rate of triglyceride turnover for the SEDDS formulation increases linearly with time; the peak turnover occurs between 4-5 hours, displaying a similar pattern to lymphatic transport of DDT following administration of the SEDDS. The triglyceride turnover levels have returned to endogenous levels after 8 hours. For the oleic acid emulsion the rate of turnover remains at basal or endogenous levels for 4 hours followed by a rapid increase in triglyceride turnover, resulting in a peak turnover between 5-6 hours. Triglyceride levels remained elevated at the endpoint of the experiment. The maximal triglyceride transport rate was similar for both formulations. From these results it is apparent that the dispersibility of the lipid vehicle may have a significant influence on the kinetics of

lymphatic triglyceride turnover. The apparent delay in the peak triglyceride turnover for the oleic acid dispersion reflects the requirement for conversion from a poorly dispersed state, as administered, to the pre-absorptive micellar state. Formulation as a SEDDS facilitates the formation of this pre-absorptive micellar phase, as evidenced by the earlier time to peak. Hence the SEDDS formulation produces a much more efficient triglyceride turnover pattern. The time to peak in triglyceride turnover is slower than that previously observed for micellar formulations. This may reflect either the process of intestinal lipolysis of the lipids in the SEDDS formulations, or the process to conversion to the pre-absorptive micellar state.

9.2.4. Relationship between DDT lymphatic transport and triglyceride turnover

The relationship between DDT lymphatic transport and lipid turnover can be illustrated by examining the relationship between hourly transport of DDT and the corresponding hourly triglyceride transport for each lipid vehicle (Figure 9.9). The slopes of the lines describing these relationships represent the relative concentration, or apparent loading of DDT per mg of lymph triglyceride.

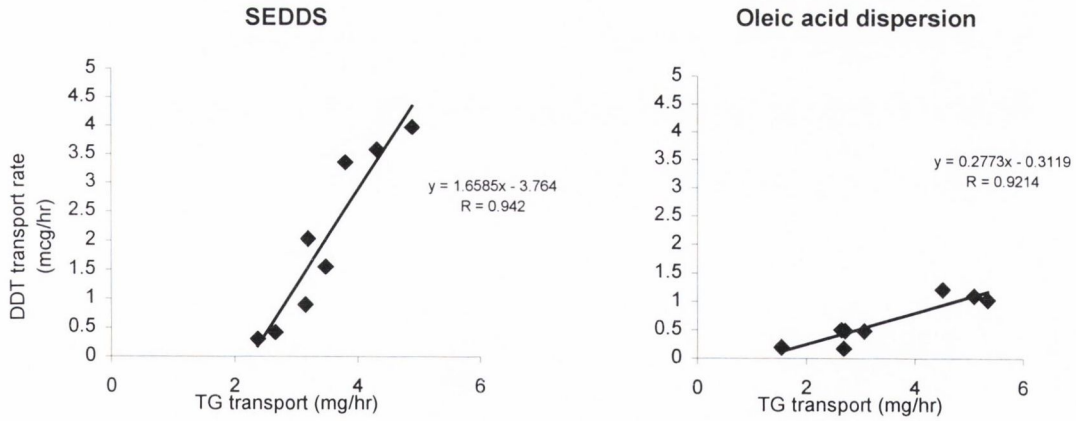


Figure 9.9 Intestinal DDT transport rate (μg/hr) versus triglyceride turnover (mg/hr) in intestinal lymph after intraduodenal administration of DDT in either a SEDDS or an oleic acid dispersion

For both lipid formulations, a similar trend was observed; namely, there is a strong positive correlation between the DDT transport rate (μg/hr) and the triglyceride turnover (mg/hr). The correlation coefficients were in excess of 0.92. An examination of the slopes of the regression lines, indicative of the apparent DDT loading per mg lipid, confirm that the oleic acid emulsion produces a lower apparent loading of DDT. The differences in the slopes of the regression lines are consistent with the difference in cumulative lymph transport of DDT between the two vehicles i.e. since there is similar triglyceride transport, the higher apparent loading of DDT per mg triglyceride for the SEDDS is reflected in the higher total lymph transport after 8 hours for the SEDDS.

9.2.5. Comparison of DDT plasma concentrations in lymph cannulated and non lymph cannulated rats.

DDT plasma concentrations-time profiles (0-8hr) in lymph cannulated and sham operated non-lymph cannulated rats are presented in Figure 9.10 ($n \geq 5$). DDT (2.236mg) was administered by intraduodenal infusion in a saturated self-emulsifying lipid system. These data must be viewed with the understanding that in lymph-drained animals, DDT input is composed of transport from only the portal blood since the mesenteric lymph was collected (drained) and analysed separately. In the non-cannulated animals (no lymph drainage), plasma levels reflect absorption directly into the portal blood and indirectly via the lymphatics. Therefore it may be possible to assess, independently of lymph levels, the possible contribution to lymph transport by the various vehicles. That is, if lymph transport constitutes a significant component of the total amount of drug reaching systemic circulation, differences in the areas under the blood concentration time curves should result. The purpose of these experiments was to investigate the effect of redistribution from lymph to blood, and the influence of lymphatically transported DDT to overall bioavailability levels.

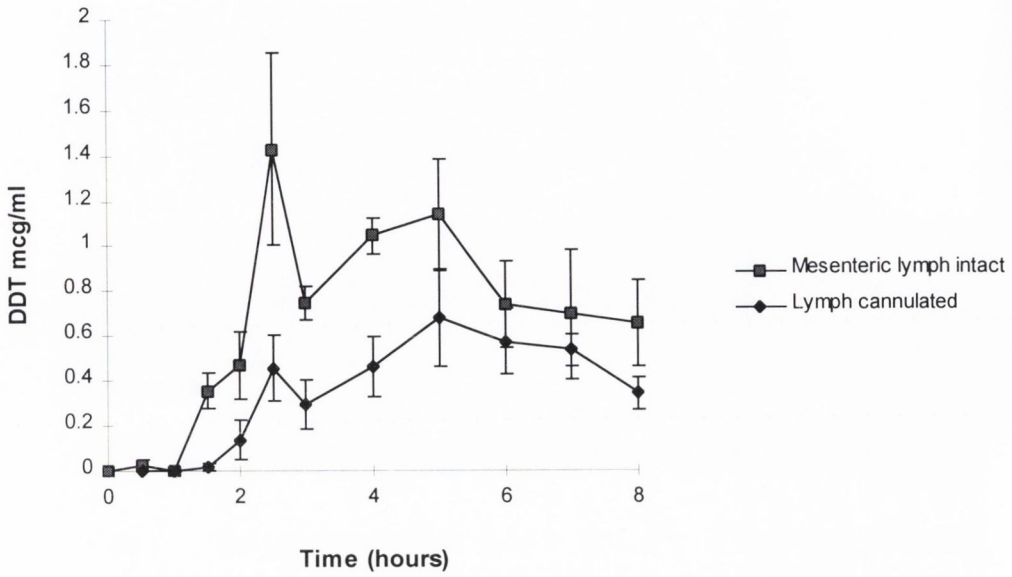


Figure 9.10 DDT plasma concentration-time profile following intraduodenal administration of the SEDDS to rats with mesenteric lymph duct intact (n=5) and cannulated (n=6).

After administration of DDT in the SEDDS, the plasma $AUC_{0\text{-}8hr}$ in non-lymph cannulated rats was significantly greater ($p < 0.05$) than that in lymph cannulated rats, demonstrating the importance of lymphatic transport in contributing to systemic plasma levels after administration in this vehicle (Table 9.6). The higher mean values obtained are due to redistribution of transported drug via the lymph into the blood.

Table 9.6 Selected pharmacokinetic parameters (± SE) after intraduodenal administration of DDT (2.236mg) in a SEDDS formulation to rats with mesenteric lymph duct intact (n=5) and cannulated (n=6).

Experiment	C max (μg/ml)	T max (hours)	$AUC_{0\rightarrow 8h}$ ($\mu g\ h\ ml^{-1}$)
Lymph cannulation	0.681 ± 0.217	5	3.08 ± 0.47
Non-lymph cannulation	1.425 ± 0.423 [A]	2.5	5.10 ± 0.73[A]

[A] Significantly greater ($p < 0.05$) than lymph cannulated rats

9.2.6. Comparison of DDT availability in lymph-cannulated and bioavailability in nonlymph cannulated animals

There is little information in existing literature, as regards the absorption kinetics and pharmacokinetic profile of DDT. Most of the available reports are on the characterization of the lymphatic transport (Pocock & Vost, 1970; Sieber, 1974; Charman & Stella, 1986a; O' Driscoll et al., 1991). In order, therefore, to quantitatively relate the extent of systemic absorption, pharmacokinetic parameters such as elimination rate constant and volume of distribution, were required. To obtain reference pharmacokinetic parameters of DDT from an intravenous administration, therefore, the data of O' Driscoll et al., (1991) was fitted and analysed for such parameters. In their study, DDT was administered as a bolus i.v. dose (5mg) in soybean oil emulsion and blood samples were taken to obtain the data sets in the Appendix 1.

The plasma concentration-time data for DDT following intravenous administration to sham operated animals was fitted using the SCIENTIST® non-linear curve-fitting program. The best fit was expressed by a bi-exponential equation, characterizing bolus intravenous input and first order output, with macro constants, for a two compartmental model, as described by Equation 9.1.

$$C_{plasma} = Ae^{-\alpha.time} + Be^{-\beta.time} \qquad \textbf{Equation 9.1}$$

while the blood level profile is shown in Appendix 1.

From the fit obtained with this data set, the following values were given for the pre-exponential and exponential constants;

$$A=51.812; \quad B = 5.9059; \quad \alpha = 1.2793; \quad \beta = 0.13544$$

Therefore the bi-exponential equation describing the model was given as:

$$C_{plasma} = 51.812.e^{-1.2793.time} + 5.9059.e^{-0.13544.time} \qquad \textbf{Equation 9.2}$$

The $AUC_{0\rightarrow\infty}$ was estimated to be 84.105μghrml^{-1} and the clearance (CL_R) calculated as 59.5ml/hr. The apparent biological $t_{1/2}$ was 5.1hr. It was also interesting to note in the study by O' Driscoll et al (1991), that the amount of DDT detected in lymph, following i.v. administration was low with approx 1% of the dose cumulatively appearing in the mesenteric lymph over 11 hours.

In lymph cannulated animals total DDT availability was defined as the mass transported directly into the lymph plus the proportion of the dose absorbed via the blood into the systemic circulation (relative to an i.v. control). DDT bioavailability was also estimated in non-lymph cannulated rats (i.e. lymph intact animals) using a standard i.v. /oral bioavailability study design and by sampling only systemic blood. The oral bioavailability calculated in this way is compared with the total availability estimated from lymph plus blood data in lymph cannulated animals in Table 9.7. The data from O' Driscoll et al., (1991) is also included for comparison.

Table 9.7 Comparison of total DDT availability (mean % dose ± SE, n ≥ 5) in lymph cannulated and bioavailability in lymph cannulated rats after intra-duodenal administration in a self-emulsifying lipid formulation. Data from O' Driscoll et al., (1991) is included for comparison.

Experiment	Lymph cannulated rats	Non-lymph cannulated rats	Lymph cannulated rats (O' Driscoll et al., 1991)	Non-lymph cannulated rats
Lymphatic absorption [A]	15.23 ± 0.9		19.37 ± 4.1	
Plasma Availability [B]	17.77 ± 2.52	26.79 ± 5.13	12.27 ± 5.9	21.16 ± 4.37
Total (lymph + blood) Availability/ Bioavailability	33.00 ± 2.67	26.79 ± 5.13	31.64 ± 7.18	21.16 ± 4.37

[A] *Cumulative mass of DDT recovered over 8 hours in mesenteric lymph calculated as a percentage of dose*

[B] *The percentage dose of DDT absorbed directly into the blood was calculated as* $(AUC_{oral}/D_{oral})/AUC_{i.v.}/D_{i.v.})$

The two data sets exhibit similar trends (i.e. lack of mass balance); a comparison of the extent of DDT total availability determined in lymph cannulated and bioavailability in non lymph-cannulated animals reveals a trend towards underestimation of total exposure, by using simple bioavailability data. However, in both studies, the difference between the estimated bioavailability in non-lymph cannulated rats and total availability in lymph cannulated was not statistically significant ($p<0.05$). Caliph et al., (2000), performed similar comparative experiments for halofantrine, and reported a statistically significant lack of mass balance, which will be discussed in Section 9.3.

9.3. Discussion

The ideal characteristics of a self-emulsifying formulation would be an oil/surfactant blend, which would rapidly disperse to form a fine microemulsion with minimal agitation and droplets within the nanometre range (10-200nm). The purpose of this section was to determine the conditions required for the formation of a SEDDS that maintains a microemulsion structure on infinite dilution. In published literature, both long and medium chain triglycerides oils with different degrees of saturation have been used as the lipophilic phase for the design of SEDDS. The majority of the recommended oils tend to be modified or hydrolysed vegetable oils, which tend to have inherent self-emulsifying properties themselves. In fact, in many cases, a lipophilic surfactant may play the role of the hydrophobic oil in the formulation (Shah et al., 1994, Constantinides, 1995). For this study it was deemed desirable to form a lipophilic phase with the long chain free fatty acid form. Oleic acid is a C_{18} unsaturated fatty acid, and its inclusion in micellar systems in the preceding chapter was found to enhance lymphatic transport. A number of studies have highlighted the potential of oleic acid-containing formulations to promote lymphatic transport. For example, the lymphotropic potential of oleic acid is the main constituent of the HALO ™ (Hepatic Avoidance using Lymphatic Output) drug delivery system (Barnwell & Attwood, 1996). This system presents an enteric-coated biphasic rapid and sustained release liquid filled hard gelatin capsule containing a drug dissolved in oleic acid. The proposed mechanism of action of the HALO™ delivery system is quoted as "preventing inhibition of lymphatic absorption by lipophilic drugs. The initial rapid release of oleic acid is believed to activate the lymphatic system for lipid absorption, while the sustained release component of the formulation maintains lymphatic delivery and prevents saturation of its transport capacity". In an unrelated study, Constantinides et al., (1994) reported how the incorporation of caprylic acid into w/o microemulsions enhanced the permeability of Calcein, compared to microemulsions without this medium chain fatty acid. The mechanisms involved may be different to our study, but this study highlights the potential for utilising formulation components to affect the absorption route.

Equilibrium phase behaviour cannot reveal the true nature of the interfacial disruption which gives rise to spontaneous emulsification, but at least such studies enable prediction of the phases which are likely to form on dilution of SEDDS with water (Pouton, 1997). This study has investigated the ability of a series of surfactants and cosurfactant blends to form microemulsions with oleic acid, both by visual assessment and particle size analysis.

The two techniques gave largely complementary results and confirmed previous observations (e.g. Pouton et al., 1985; Craig et al., 1995) that the emulsification process may be highly dependent on the composition of the oil-surfactant mixture. The surfactant to cosurfactant ratio of the SEDDS blend affected the extent of the microemulsion existence field; increasing S:CoS ratio increased the area of the microemulsion existence field. A stable microemulsion with small droplet size, which was unaffected by addition of DDT, was selected with the following ratio of Oleic acid: Labrasol: Plurol Oleique of 7:54:9. At and below 10% oleic acid this system approached the behaviour of a stable system capable of infinite dilution with the aqueous phase.

Pouton has recently proposed a classification system for self-emulsifying lipid formulations (Pouton, 2001). The oleic acid: Labrasol: Plurol Oleique (7:54:9) SEDDS blend chosen in our study is classified as Type IIIA. The particle sizes of microemulsions in the category are 100-250nm. There is a suggestion of some loss of solvent capacity on dilution for SEDDS in this classification. However the risk of precipitation is difficult to predict. Theoretically, the hydrophilic surfactants (HLB>12) will tend to part from the oil during dispersion, and become dissolved in the aqueous phase, forming a micellar solution in the continuous phase. It is unclear whether this results in an overall loss in solvent capacity for the drug, and at present there is no established techniques available to help formulators assess the risk of precipitation (Pouton, 2001). To allow for this, in our studies DDT was administered intraduodenally in a pre-diluted microemulsion form (2%w/v in water), at concentrations representing the saturation solubility of DDT. Digestion is not deemed to be crucial for Type III systems, as the diluted microemulsion form should facilitate the subsequent assimilation with the relevant bile salt micellar structures or perhaps act independently of bile as has been shown for Neoral (Mueller et al., 1994; Trull et al., 1995). There is evidence to suggest that the presence of surfactants can inhibit digestion of the oil within the formulation (Solomon et al., 1996; Macgregor et al., 1997). Patel et al., (1999) reported that polyglycolysed glyceride oils, such as Labrasol, display poor digestibility characteristics in the *in vitro* lipolysis model, possibly due to stearic occlusion of the lipase/co lipase complex by the PEG side chain. Labrasol produced some initial inhibition of lipolysis and was only very slowly digested, however hydrophilic surfactants such as Tween 80 were found to irreversibly inhibit lipolysis. The extent to which such *in vitro* effects will be manifested in vivo is unclear; presentation of the oil phase in a pre-digested form (i.e. oleic acid) is therefore deemed beneficial.

The potential for the optimised SEDDS to promote the lymphatic transport of DDT was examined in the mesenteric lymph-duct cannulated rat model. The extent of DDT transported by the intestinal lymph is comparable to that obtained with the mixed micelles in the preceding chapter. By comparison lymphatic transport from the oleic acid dispersion is poor, more prolonged with a delayed peak transport and incomplete absorption after 8 hours. Dispersion of lipid droplets into an emulsion of high surface area is an essential step in the efficient intestinal absorption of lipids. Previous authors have reported a similarly delayed peak transport rate for lipid solutions, which as mentioned previously are comparable in formulation terms to this oleic acid dispersion (Porter et al., 1996; Hauss et al., 1998). Hauss et al., (1998) attributed this apparent delay to the requirement for pre absorptive lipolysis of the triglyceride vehicle but in this experiment the lipid is presented in unconjugated fatty acid form. The results presented here therefore suggest that this apparent lag phase is related to the need for conversion to a more disperse lipidic state.

The relative rate of intestinal lymphatic transport of DDT from the SEDDS, the crude emulsion and a NaC: OA bile salt mixed micellar formulation (Figure 9.11), demonstrates that although similar peak relative rates achieved for the different lipid formulations, peak transport occurred earlier (3-4hours) for the micellar formulations compared with the oleic acid emulsion. The SEDDS has a peak rate at 4-5 hours; the delay possibly due to conversion from the microemulsion form as administered to a pre absorptive micellar state. The above relative rate profiles are not dissimilar from those reported by Porter et al., (1996). Peak transport of halofantrine occurred earlier (3-4hours) for the micellar and emulsified formulations compared with the lipid solution (6-8hours). The authors proposed that this data indicated that formulations that better resembled the latter stages of lipid dispersion/digestion in vivo enhanced lymphatic transport.

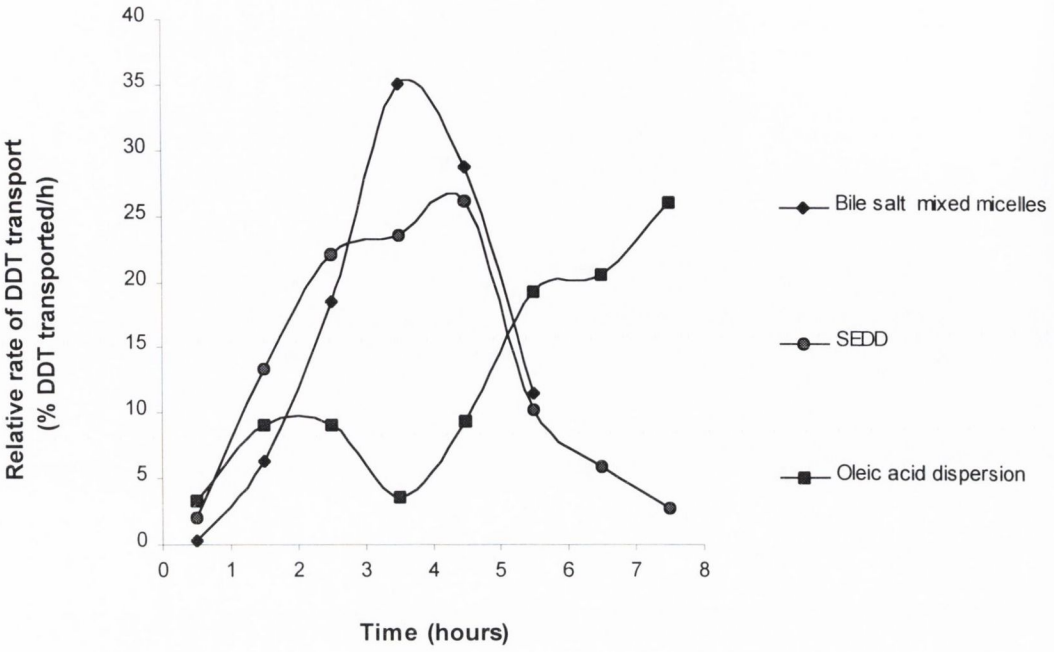


Figure 9.11 The relative rate of intestinal lymphatic transport of DDT (calculated as the percentage of the total DDT transported for each formulation per collection time point) as a function of the different lipid formulations.

Lipid formulations that promote triglyceride turnover appear to increase the extent of lymphatic DDT transport as evidenced by the linear relationship between DDT transport and triglyceride transport in lymph. Clearly, the efficiency with which a formulation promotes lymphatic triglyceride transport, and not just the amount of lipid administered, also appears to play a role in the partitioning of absorbed drug into lymph. Theoretically, the SEDDS formulation in this study contained a similar lipid load to the oleic acid emulsion and the total amount of triglyceride collected after 8 hours in the lymph was similar for both formulations (27.58 ± 2.17mg for the oleic acid emulsion vs. 27.85 ± 0.51mg). However the SEDDS formulations produced a greater extent of DDT lymphatic transport and a greater loading of DDT per mg lymph triglyceride. The kinetics of triglyceride turnover are improved following administration of the SEDDS as evidenced by the steady increase in triglyceride turnover to a peak lipid turnover of 4.88 ± 0.30mg/hr occurring between 4-5 hours, and a return to endogenous levels at 8 hours. By comparison, the triglyceride turnover remains at basal levels for 4 hours after administration of the oleic acid emulsion, followed by a peak lipid turnover of 5.33 ± 1.35mg/hr between 5–6hours and levels remain elevated at the end of the experiment (Figure 9.8). The more efficient triglyceride turnover for the SEDDS facilitates the DDT

transfer from the intestinal lumen to the intestinal lipoproteins, resulting in higher apparent DDT loadings per mg of lymph triglyceride. These findings are therefore similar to those in Chapter 8, where significant differences in the extent of DDT transport were observed, despite similar extents of triglyceride turnover after 6 hours, for the NaC: OA and NaC: PL bile salt mixed micelles.

Hauss et al., (1998) have also reported a higher loading of drug per mg of chylomicron triglyceride following administration of SEDDS formulations. In this study, ontazolast, a poorly water-soluble LTB4 inhibitor, was administered by gavage to conscious, minimally restrained mesenteric lymph duct cannulated rats. Maximum lymphatic transport of ontazolast occurred with a Peceol SEDDS and an emulsion formulation. The most efficient lymphatic triglyceride transport occurred following dosing of a Peceol SEDDS solution, which also resulted in the highest concentration of ontazolast in the chylomicron triglyceride. It was suggested that SEDDS formulations, which promote rapid absorption of drug, result in higher concentrations within the enterocytes during absorption and hence, may simultaneously improve lymphatic drug transport by a concentration-partitioning phenomenon.

The final goal of this chapter was to determine the contribution of lymphatic transport and absorption directly into the portal blood to the overall bioavailability of a model lipophilic compound, DDT. Comparison of the plasma AUC's for lymph cannulated and non-lymph cannulated facilitated a mass balance across the two animal models and therefore an appreciation of the altered clearance and/or disposition of appreciably lymphatic transported drugs. The data presented in this study confirm that extensive lymphatic transport can have a drastic effect on plasma AUC, contributing to as much as 46% of the total DDT availability. The data exhibits a similar trend to that from O' Driscoll et al., (1991), suggesting that the extent of DDT availability (blood plus lymph) estimated in lymph cannulated animals is higher than that predicted from bioavailability estimates in non-lymph cannulated animals. The differences were not statistically significant, possibly reflecting the extensive variability in DDT plasma profiles in both animal groups.

A significant lack of mass balance between availability and bioavailability values, has been reported for Halofantrine (Hf), another highly lipophilic compound, with significant lymphatic transport (Caliph et al., 2000). The contribution of lymphatic transport and absorption directly into the portal blood to the overall oral bioavailability of Hf, was

determined in lymph-cannulated, conscious, unrestrained rats after administration in lipidic vehicles with different fatty acid chain lengths. After administration in the long chain TG lipid vehicle (i.e. where lymphatic transport accounted for up to 70% on total availability), Hf bioavailability measured in non lymph-cannulated animals was significantly lower than the extent of total availability measured in lymph-cannulated animals (estimated as percent appearing in the intestinal lymph plus percent transported directly into the blood). While the plasma availability in lymph cannulated rats was higher than the plasma bioavailability following administration of the medium chain and short chain TG vehicles the differences were not statistically significant ($p < 0.05$). The authors proposed that drug entering the systemic circulation via the lymph, in association with lymph chylomicrons may be more rapidly cleared than drug entering via the portal blood, leading to a lower plasma AUC's in the non-lymph cannulated animals. Importantly, systemic availability was the same in both models when the extent of lymphatic transport was low, indicating that the discrepancies between the two data sets reflect a lymph specific mechanism and not a simple methodological difference.

Similar discrepancies have previously been reported between the extent of absorption of a lipophilic lipid regulator (CI-976)(log P 5.83) when assessed in lymph-cannulated and non-cannulated animals (Hauss et al., 1994). An increased lymphatic transport of CI-976 did not result in proportionate increases in CI-976 plasma AUC. In the study with CI-976, the difference between percentage bioavailability for animals with and without lymph cannulation after administration of an emulsified preparation was 43%, however the total amount of CI-976 collected in mesenteric lymph was 0.4% of the administered dose. An increased deposition of drug in adipose tissue after administration in lipidic vehicles (which promoted lymphatic transport) was observed, leading the authors to speculate that drug delivery to the systemic circulation in association with lymph lipoproteins (and in particular chylomicrons) led to altered systemic clearance of the drug, and rapid sequestration in the adipose tissue. The authors concluded that plasma concentration-time data alone might not fully reflect relative bioavailability and pharmaco-dynamic potential of lipophilic drugs dosed in lipid vehicles.

A number of authors have reported that the association pattern of lipophilic drugs can markedly affect drug pharmacokinetic and distribution patterns (Humberstone et al., 1998; Wasan & Cassidy, 1998). In a practical sense, these observations may also draw into question the use of the volume of distribution (Vd) obtained from intravenous

administration of a drug, which exhibits appreciable lymphatic transport. This may lead to an underestimation of the Amax (total amount in the body Amax = Kel. Vc. AUCoral). For example, reports in the literature indicate that there is a poor distribution of intravenously administered DDT from the blood to lymph, (0.6-1% of administered dose, Noguchi et al., 1985; O'Driscoll et al., 1991). However, there is a high distribution of intraduodenally administered DDT in the lymph prior to the reaching the systemic circulation (up to 24% in this study). Therefore the Vd from the oral route is likely to be higher than that from the intravenous route. The implications of these collective findings are that changes in formulation of lipophilic compounds may not only provoke changes in the route of absorption, for example formulating with long versus medium chain lipids, but may also subsequently provoke changes in the clearance and deposition profiles. The therapeutic and toxicological ramifications of these proposals are considerable, particularly as these changes may not be apparent from conventional (plasma) bioavailability data.

10. Intestinal lymphatic transport of saquinavir

10.1. Introduction

Gastrointestinal absorption may occur via the portal system, through lymphatic uptake via the lacteals, or by a combination of both pathways. Highly lipophilic drugs possessing good solubility in triglycerides are potential candidates for significant lymphatic transport (Charman & Stella, 1991). There are a number of biopharmaceutical advantages associated with significant drug transport via the intestinal lymphatics. Transport via the intestinal lymphatics circumvents first-pass metabolism and may therefore enhance the bioavailability of drugs that exhibit high first-pass metabolism (O'Driscoll, 1992). The process of intestinal lymphatic drug transport also continues over longer time periods than that typically observed for drug absorption via the portal vein and may be utilized to extend the time course of drug absorption (Porter & Charman, 1997). In terms of directed or targeted delivery, the relatively high concentrations of B- and T-lymphocytes in the lymph provide appealing targets for cytokines such as interferon and immunomodulators in general.

Saquinavir (SQV) is a highly lipophilic compound with a theoretical cLog P = 4.5 and limited aqueous solubility (solubility in water at 37^0C = 17μg/mL for the free base). However, the bioavailability of saquinavir is relatively low, 4% for a hard gelatin capsule. This has been increased to 12% with the introduction of the new soft gelatin capsule containing a SEDDS formulation of saquinavir (Fortovase®), which contains medium chain mono- and diglycerides, povidone, and alpha tocopherol (Perry & Noble, 1998). The bioavailability is increased in the postprandial state. The poor bioavailability may be due to a combination of factors including poor solubility, extensive intestinal and hepatic first pass metabolism. Saquinavir is a substrate for efflux by P-gp and metabolism by CYP 3A4, however the contribution of these physiological processes to the oral bioavailability of saquinavir has yet to be established.

The lymphatic system is a primary target for early anti-immunodeficiency virus drug therapy and modes of enhancing the delivery of anti-HIV agents to this tissue are being explored (Manouilov et al., 1995 & 1997; Bibby et al., 1996). Recent investigations utilizing a highly sensitive and quantitative polymerase chain reaction technique have shown that a large viral burden exists in the lymphoid tissue of asymptomatic infected patients and, in fact, the lymphatic system serves as a major reservoir for HIV during the

early course of the infection and that the lymph system is the primary site of HIV replication *in vivo* (Pantaleo et al., 1993). In the circulation system, most of the lymphocytes pass through the thoracic lymph duct several times a day (O'Driscoll, 1992). Therefore, if a new dosage form from which saquinavir directly penetrates the thoracic lymph duct is developed, the therapeutic efficiency of saquinavir should be improved further.

Saquinavir is an ideal candidate for lymphatic targeting for the following reasons: (i) highly lipophilic, (ii) extensive first pass metabolism, (iii) increased bioavailability following co-administration of fatty meal and (iv) the therapeutic benefit of targeting this potent HIV protease inhibitor to the lymphatic system. In Chapter 8 and 9, the potential merits of a series of lipid formulations at promoting intestinal lymphatic transport of DDT, and increasing drug loading in lymph triglyceride, were outlined; the conclusions from which suggested that mixed micellar and SEDDS formulations offer advantages over the simple micellar formulations and oily dispersions. Consequently, studies were undertaken to examine the rate and extent of lymphatic transport of saquinavir in anaesthetised rats from two mixed micellar formulations and a SEDDS formulation. The concentrations of saquinavir achieved in lymph and plasma were compared. Analysis of triglyceride levels in collected lymph was performed to determine the apparent loadings of drug per mg triglyceride. The lymphatic transport of the mesylate salt of saquinavir, which is more water-soluble than the free base form, was also examined. Finally, plasma profiles in lymph cannulated and non-lymph cannulated were examined to probe aspects of mass balance of drug absorption and to examine the relative contribution of lymphatic transport and absorption directly into the portal blood to the overall bioavailability of saquinavir.

10.2. Results.

10.2.1. Intestinal lymphatic transport of saquinavir

10.2.1.1. Saquinavir (freebase) in cremophor EL: oleic acid mixed micelles

The dose of saquinavir used in these studies was 5mg. The recommended daily dose of saquinavir SGC is 1200mg t.i.d. and assuming a dose conversion of 1:200 for rats to humans, the dose used in this study is equivalent to 1000mg. Kempf et al., (1997) have performed pharmacokinetic studies on a range of protease inhibitors, and used a dose of 10mg/kg body weight for saquinavir administered orally to rats (equivalent to 3mg for an average sized rat).

The extent of lymphatic transport of saquinavir was determined after intraduodenal administration, over two hours, in a 2% cremophor: 40mM oleic acid mixed micellar formulation. The cumulative extent of saquinavir transported via the mesenteric lymph after 8 hours was 1.34μg ± 0.3μg. This represents 0.027% of the administered dose (Figure 10.1).

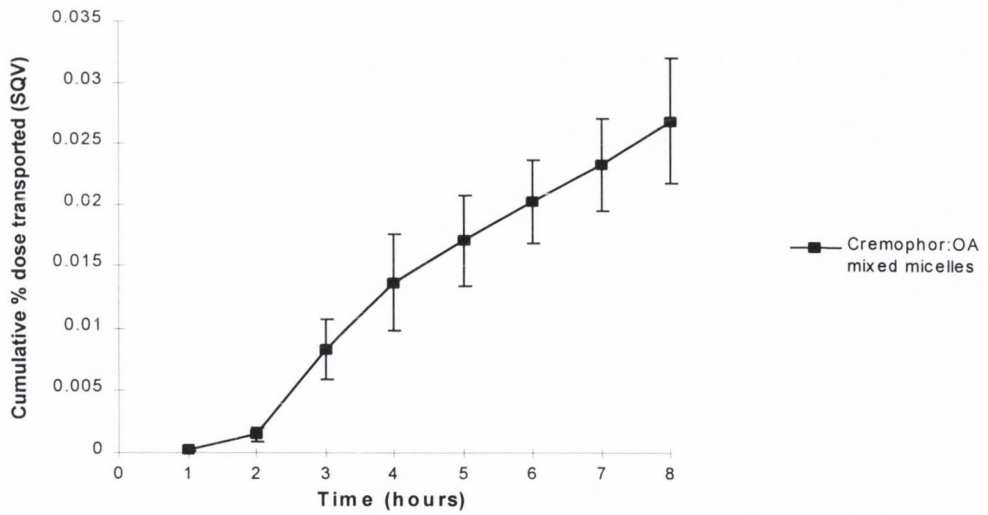


Figure 10.1 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as a mixed micellar vehicle (2% Cremophor EL: 40mM Oleic acid).

The rate of intestinal lymphatic transport as a function of time following administration of the cremophor: oleic acid mixed micellar vehicle is presented in Figure 10.2. The maximum rate of transport occurred 2-3 hours post-initiation of drug administration. The extent of lymphatic absorption is still incomplete after 8 hours. Intestinal lymphatic transport of DDT from a similar mixed micellar formulation was essentially complete after 8 hours. This may suggest that lymphatic transport, triggered by absorption from the distal regions of the intestine may be contributing to this extended phase of lymphatic transport.

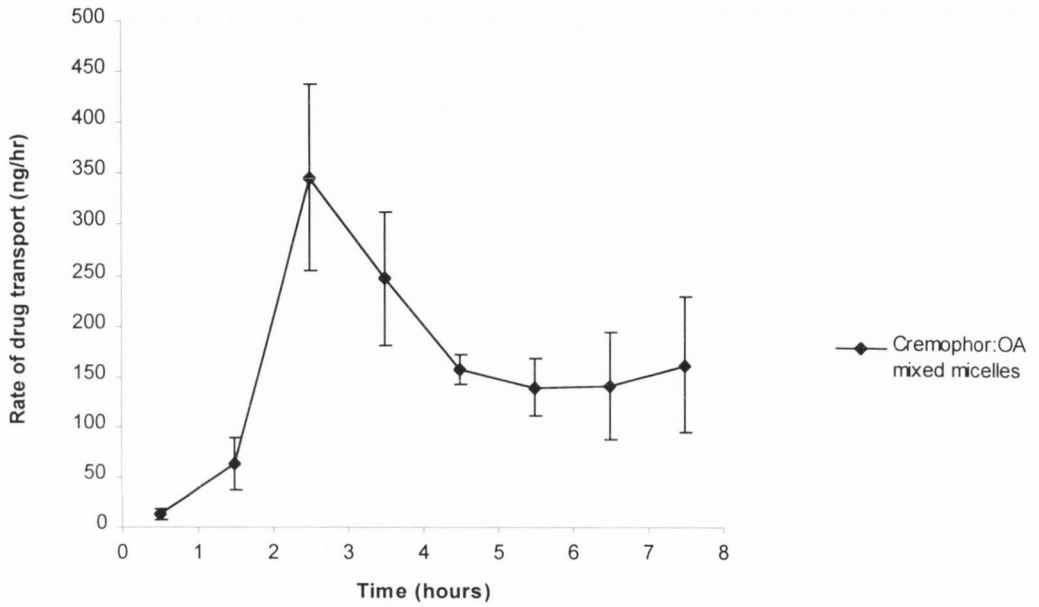


Figure 10.2 Rate of transport of saquinavir into the intestinal lymph (mean ± SE) (n≥5). Saquinavir (5mg) was administered intraduodenally as a mixed micellar vehicle (2% Cremophor EL: 40mM Oleic acid).

10.2.1.2. Saquinavir in a TPGS:OA mixed micelle formulation

The extent of intestinal lymphatic transport from a TPGS: oleic acid mixed micellar system was examined. As discussed methods section 6.4.2, the 2%TPGS: 40mM oleic acid mixed micellar formulation requires in excess of 12 hours equilibration to ensure complete mixed micellar formation. This was believed to be due to the slow dissolution of TPGS aggregates in the buffer solution. In an effort to facilitate the dissolution of TPGS, a 6:1 blend of TPGS: PEG was prepared, which was subsequently formulated with 40mM oleic

acid to prepare a mixed micellar solution. The cumulative extent of transport following intraduodenal administration of 5mg in this TPGS: PEG:OA mixed micellar formulation is presented in Figure 10.3. The cumulative extent of SQV transported in the intestinal lymph over 8 hours was 1.26 ± 0.38μg, representing 0.025% of the administered dose, which was similar to the result obtained for the cremophor: oleic acid mixed micellar formulation.

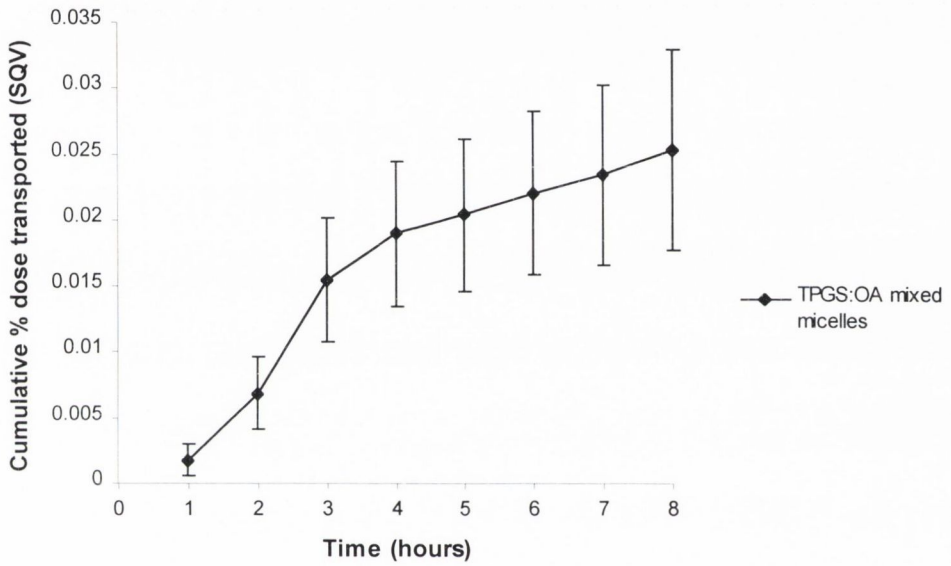


Figure 10.3 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as a mixed micellar vehicle (2%TPGS: 40mM Oleic acid: 0. 28% PEG).

The rate of mesenteric lymph transport for the TPGS:OA mixed micellar vehicle exhibited similar trends to the cremophor: oleic acid mixed micellar formulation, with maximal rate of transport occurring between 2-3 hours (Figure 10.4).

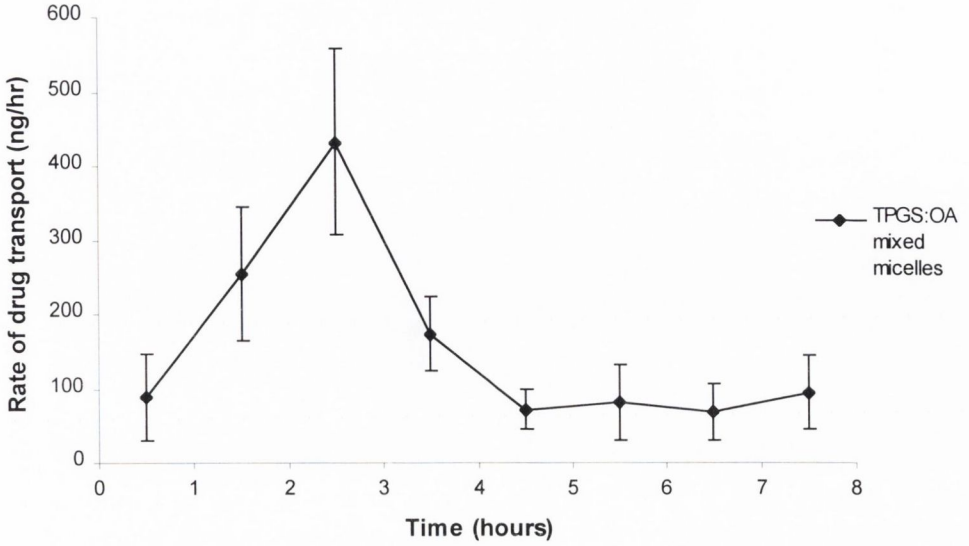


Figure 10.4 Rate of transport of saquinavir into the intestinal lymph (mean ± SE) (n≥5). Saquinavir (5mg) was administered intraduodenally as a mixed micellar vehicle (2%TPGS:0. 28% PEG: 40mM Oleic acid).

10.2.1.3. Saquinavir in a SEDDS formulation

The cumulative extent of lymphatic transport following administration of 5mg saquinavir in a self-emulsifying lipid formulation is presented in Figure 10.5. The total amount of the administered dose collected in the mesenteric lymph after 8 hours was 2590 ± 660μg, representing 0.052% of the administered dose. Although this represents a two-fold increase on the extent of transport observed for the cremophor: OA and TPGS:OA mixed micellar systems, the difference was not statistically significant at the 95% confidence interval. This lack of statistical significance reflects the extensive variability observed within each set of data.

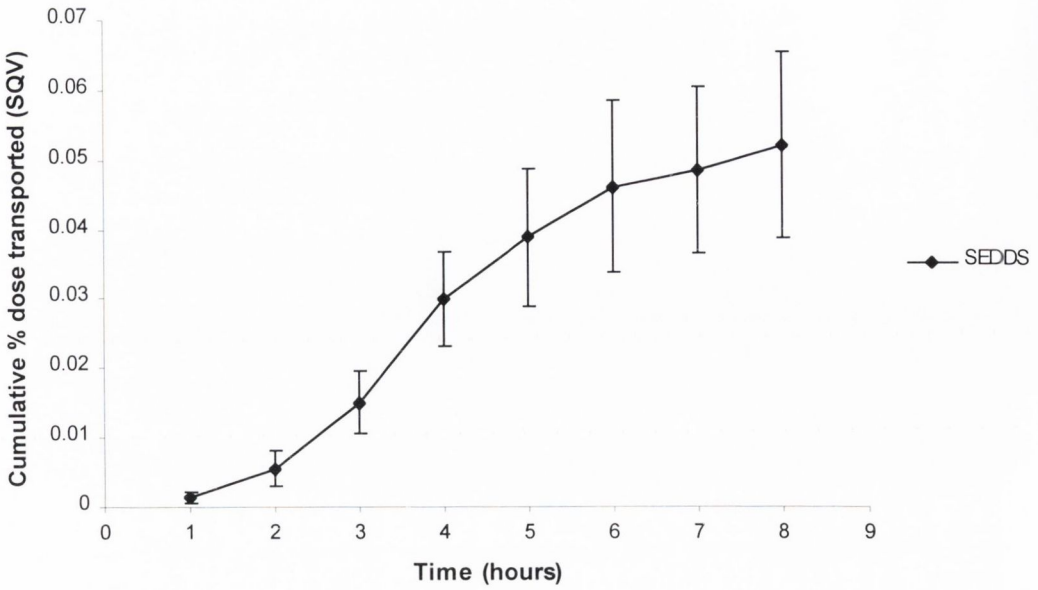
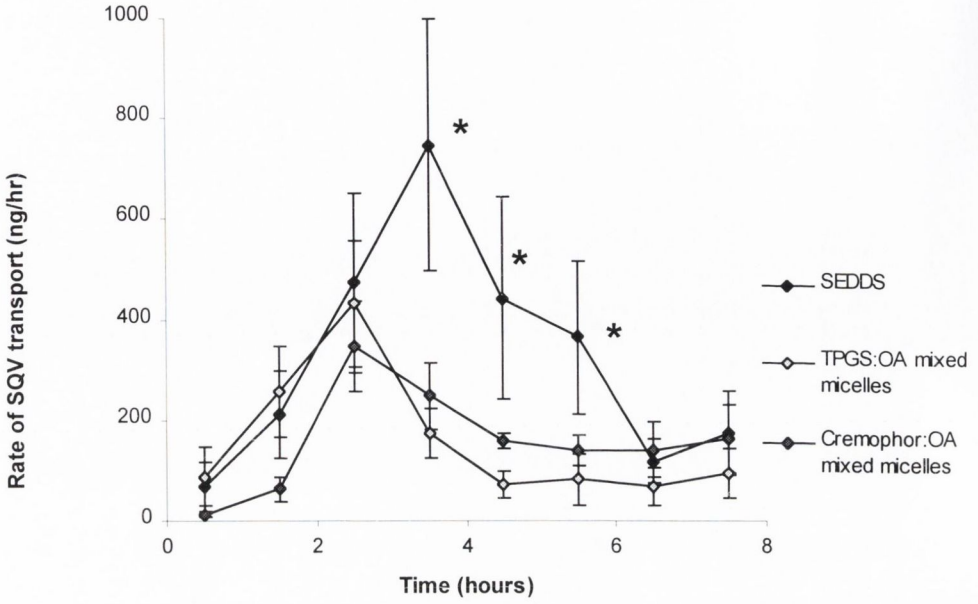


Figure 10.5 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as a SEDDS diluted 1:50 with water.

The rate profile of the mesenteric lymph transport of saquinavir following administration of the SEDDS formulation is compared with the rate profiles for the cremophor and TPGS mixed micellar formulations in Figure 10.6. The maximal rate of transport occurs between 3-4 hours post initiation of drug administration. This relative delay in peak intestinal lymphatic transport over that observed for the mixed micellar systems is consistent with the findings in Chapter 8. This confirms that administration as a SEDDS produces a delay in the maximal rate of lymphatic transport, possibly reflecting the conversion of the SEDDS from a microemulsion phase, as administered, to a pre-absorptive mixed micellar phase.

In terms of targeted lymphatic transport, the SEDDS in this experiment appears to prolong the period of intestinal lymphatic transport. The maximal rate of lymphatic transport at the 3-4, 4-5 and 5-6 hours is significantly higher, at the 95% confidence interval, for the SEDDS than for the mixed micellar formulations.



* Statistically significant ($p<0.05$)

Figure 10.6 Rate of transport of saquinavir into the intestinal lymph (mean ± SE) (n≥5). Saquinavir (5mg) was administered intraduodenally either as a SEDD, as a cremophor EL:OA or a TPGS:PEG:OA mixed micellar formulation.

10.2.2. Intestinal lymphatic transport of saquinavir mesylate

Saquinavir mesylate was the saquinavir salt in Invirase®, the hard gel formulation that has now been replaced with Fortovase®, a lipid-based formulation containing saquinavir freebase. The mesylate salt is a more water-soluble form of saquinavir (aqueous solubility 2.2mg/ml) and has a lower clog P (2.12). A comparison of the effect of these differing physiochemical properties on the rate and extent of mesenteric lymph transport was performed.

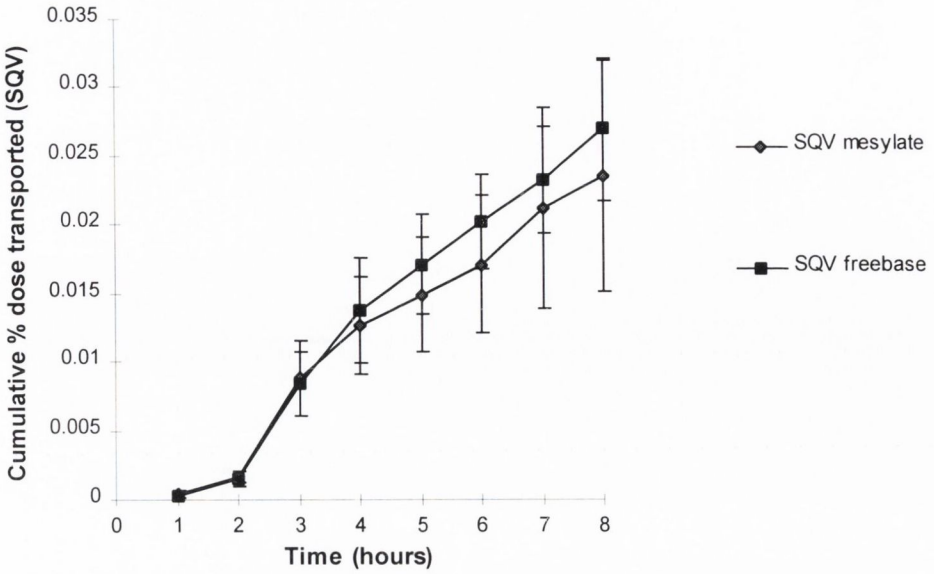


Figure 10.7 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally either as a mesylate salt or as a free base in a cremophor EL: OA mixed micellar formulation.

The cumulative extent of lymphatic transport following administration of the mesylate salt of saquinavir in a cremophor mixed micellar formulation was 1.175 ± 0.419μg, equivalent to 0.024% of the administered dose. The rate profiles for saquinavir mesylate and freebase are also similar (Figure 10.8). The extent and rate of lymphatic transport is similar whether saquinavir is administered as the highly lipophilic free base or as the less lipophilic mesylate salt. This suggests the partial conversion of the saquinavir mesylate to the saquinavir free base following administration in the gastrointestinal tract.

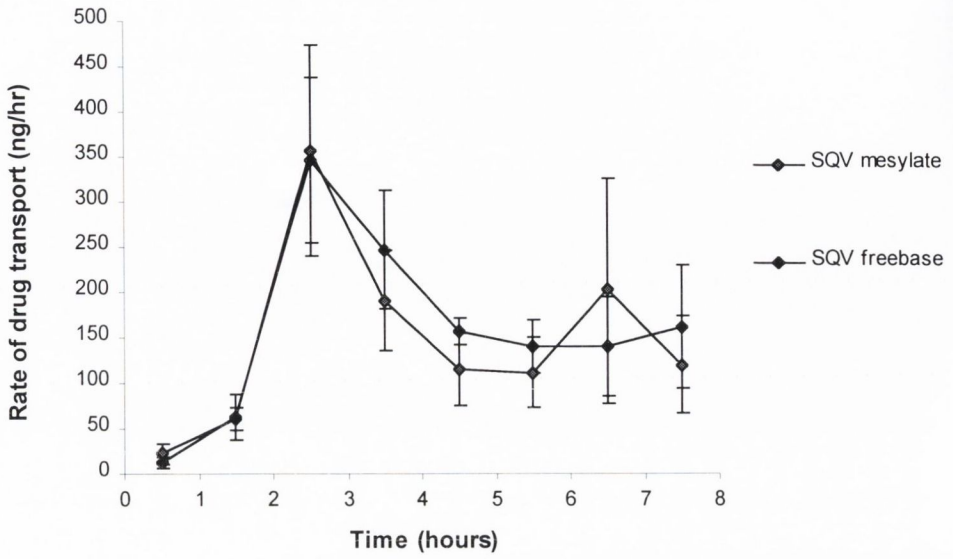


Figure 10.8 Rate of transport of saquinavir into the intestinal lymph (mean ± SE) (n≥5). Saquinavir (5mg) was administered intraduodenally either as a mesylate salt or as a free base in a cremophor EL: OA mixed micellar formulation.

10.2.3. Intestinal lymphatic transport of triglycerides

The cumulative C_{18} triglyceride transport data after intraduodenal dosing in the lipid formulations examined in this study is summarised in Table 10.1. The cremophor EL and TPGS mixed micellar vehicles produced statistically similar extents and rates of triglyceride transport. After endogenous (blank) triglyceride correction, the percentage of the administered (exogenous) dose of oleic acid (33.85mg) recovered as re-synthesized long chain triglyceride in intestinal lymph after 8 hours was 82.75 ± 13.23% and 62.92 ± 7.00%, for the cremophor EL and TPGS mixed micellar systems respectively. These figures are comparable to those previously reported for micellar vehicles in Chapter 8, but as noted in that section, these approximations do not take into account any potential effect the co-administered surfactants may have on triglyceride turnover. These results suggest that the administration of saquinavir does not influence the triglyceride turnover in intestinal lymph. The SEDDS blend consisted of 10% Oleic acid and 90% surfactant/co-surfactant blend, which after administration, results in total lipid dosing of 6mg OA, 7.7mg Plurol Oleique and 46.3mg Labrasol. The total amount of exogenous lipid detected in

intestinal lymph was 6.23 ± 2.71mg. However, to assume an almost quantitative recovery of administered oleic acid is most likely incorrect, as many of the surfactants may potentially either increase endogenous triglyceride turnover, or act as lipid substrates themselves, as discussed in Chapter 9.

Table 10.1 Cumulative transport of triglyceride into the mesenteric lymph (mean ± S.E., n≥4) and cumulative lymph flow as a function of lipid vehicle after 8 hours.

Formulation	Cumulative Mass of triglyceride (mg) appearing in Mesenteric lymph (Mean ± SE) [A]				TG transport (mg) [B]	Cumulative lymph flow (ml)
	0-2 hr	0-4 hr	0-6 hr	0-8 hr		
Saline control	2.93 ±0.06	6.58 ± 0.24	10.26 ± 0.34	15.06 ± 0.85		4.45 ± 0.35
Cremophor EL: OA mixed micelles	7.27 ± 2.05	26.73 ± 3.24	37.21 ± 3.99	43.07 ± 3.84	28.01 ± 4.50	4.86 ± 0.64
TPGS:OA mixed micelles	7.56 ± 1.70	25.57 ± 2.46	31.74 ± 1.76	36.36 ± 1.71	21.30 ± 2.37	4.67 ± 0.66
SEDDS	3.75 ± 0.38	11.92 ± 1.33	18.07 ± 1.58	21.29 ± 2.05	6.23 ± 2.71	5.29 ± 0.34
SQV mesylate in Cremophor EL:OA mixed micelles	6.71 ± 0.36	23.04 ± 1.87	31.70 ± 2.69	38.61 ± 4.08	23.55 ± 4.74	4.53 ± 0.47

[A] Representing endogenous and exogenous lipid
[B] Attributable to exogenous lipid (i.e. exogenous – endogenous)

Figure 10.9 presents the transport rate of C_{18} triglyceride in intestinal lymph following administration in either cremophor EL or TPGS mixed micelles, a SEDDS or as a saline rehydration solution. The maximal rate of triglyceride transport following administration of the SEDDS formulation occurs between 3-4 hours compared with 2-3 hours for the micellar vehicles. A similar delay in peak triglyceride turnover for the SEDDS formulation compared to mixed micelles was observed in Chapter 8 and 9. This may

reflect either the process of intestinal lipolysis of the lipids in the SEDDS formulations, or the process to conversion to the pre-absorptive micellar state.

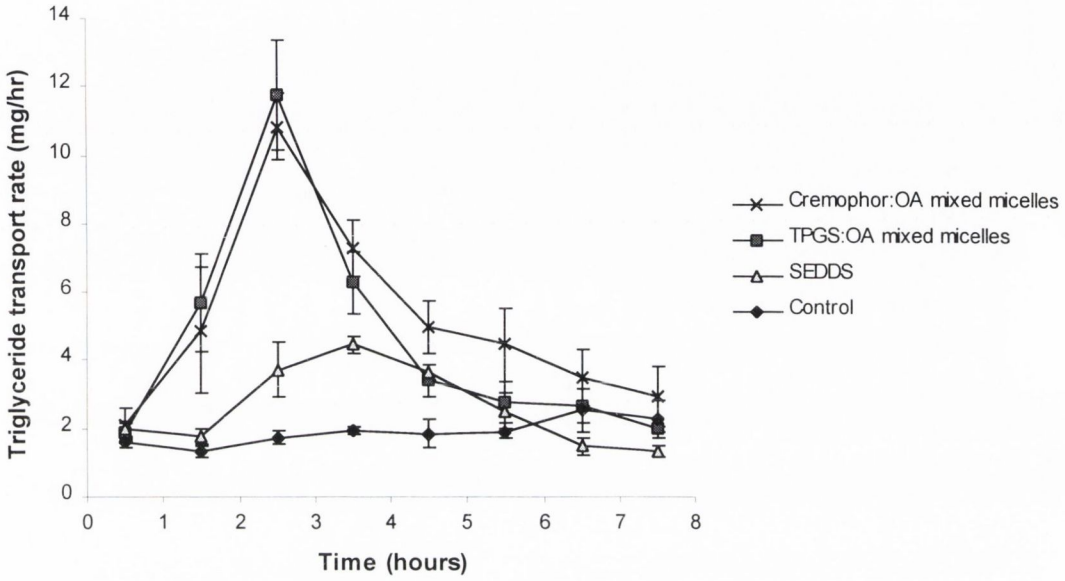


Figure 10.9 Effect of cremophor EL and TPGS mixed micelles and SEDDS formulations on the lymphatic triglyceride turnover (mean ± S.E., n ≥4).

10.2.4. Relationship between DDT lymphatic transport and triglyceride turnover

In the preceding chapters there was strong positive correlation between the DDT transport rate (μg/hr) and the triglyceride turnover (mg/hr) for all the lipid systems examined. The relationship between hourly transport of saquinavir and the corresponding hourly triglyceride transport for each lipid vehicle turnover was therefore explored (Figure 10.10). The slopes of the lines describing these relationships represent the relative concentration, or apparent loading of saquinavir per mg of lymph triglyceride.

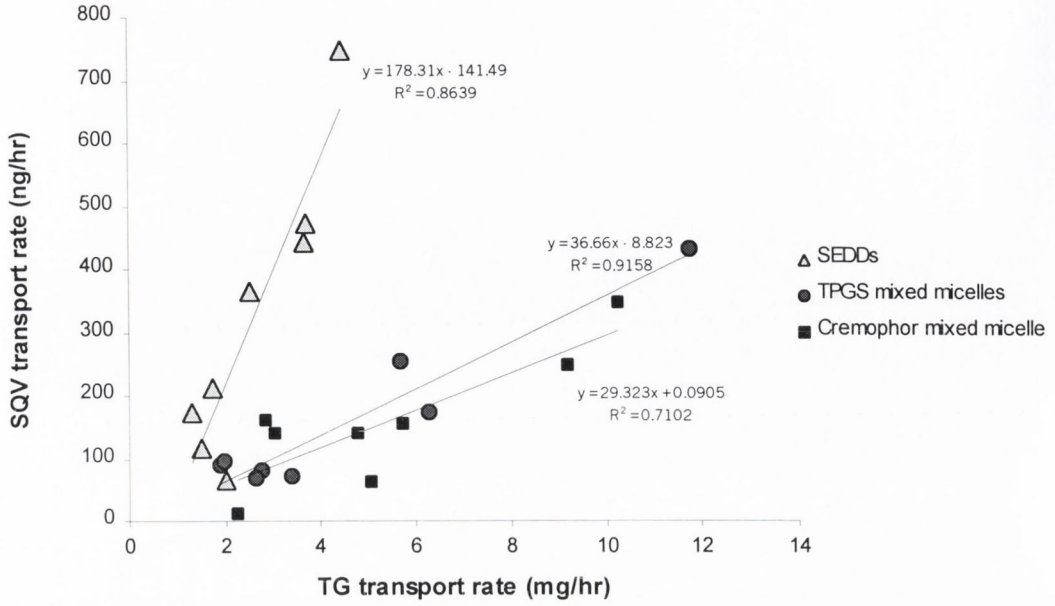


Figure 10.10 Intestinal saquinavir transport rate (ng/hr) versus triglyceride turnover (mg/hr) in intestinal lymph after intraduodenal administration of saquinavir in either the cremophor EL:OA, the TPGS:OA mixed micelles or the SEDDS formulations.

The SEDDS formulation produces a higher apparent drug loading per mg of lymph triglyceride, while the two mixed micellar systems produced similar apparent loadings. This is consistent with the findings of preceding chapters, where the SEDDS formulation produced the highest apparent loading of DDT per mg lymph triglyceride. The exact mechanism as to how this higher loading is achieved is unclear, although it appears that formulation as a SEDDS facilitates transfer of drug from the intestine, through the enterocytes and into the newly synthesized intestinal lipoproteins. Whether this is linked to the attainment of higher concentrations of drug within the enterocytes or a more efficient absorption and processing of lipid, when presented as a SEDDS, is unknown. The data for the three lipid formulations examined in this study exhibits a greater degree of scatter, as evidenced by the poorer degree of correlation, than that reported for DDT in the preceding chapters.

10.2.5. Comparison of saquinavir concentrations in lymph and plasma of lymph cannulated rats.

Although this total extent of lymphatic transfer as a percentage of the dose administered may appear relatively low, it may prove more relevant to examine the concentration of saquinavir in mesenteric lymph as a function of time for each lipid vehicle, as presented in Figure 10.11. Porter & Charman, (2001) noted that form a lymph targeting perspective, a relatively small extent of absolute lymphatic transport (when assessed as a fraction of absolute bioavailability) may be adequate to provide the desired pharmacological effect. During the initial clinical latency period of human immunodeficiency virus (HIV) infection a significant amount of virus is not detected in blood, but histopathological changes in lymph nodes are observed. The rapid lymph transfer of drug is of clinical relevance, because a primary clinical hallmark of AIDS is the depletion of CD4 lymphocytes and pathology of associated lymphoid tissues. Enhancement of saquinavir lymph levels is particularly relevant in light of the rapid dynamics and high mutability of HIV, (Ho et al., 1995; Finzi et al., 1997), attributes that dictate the inevitable emergence of resistance in the presence of inadequate drug concentrations. Redirection of the absorption pathway of orally administered anti-HIV compounds from the portal blood to the HIV rich intestinal lymphatics may therefore significantly enhance therapy against HIV.

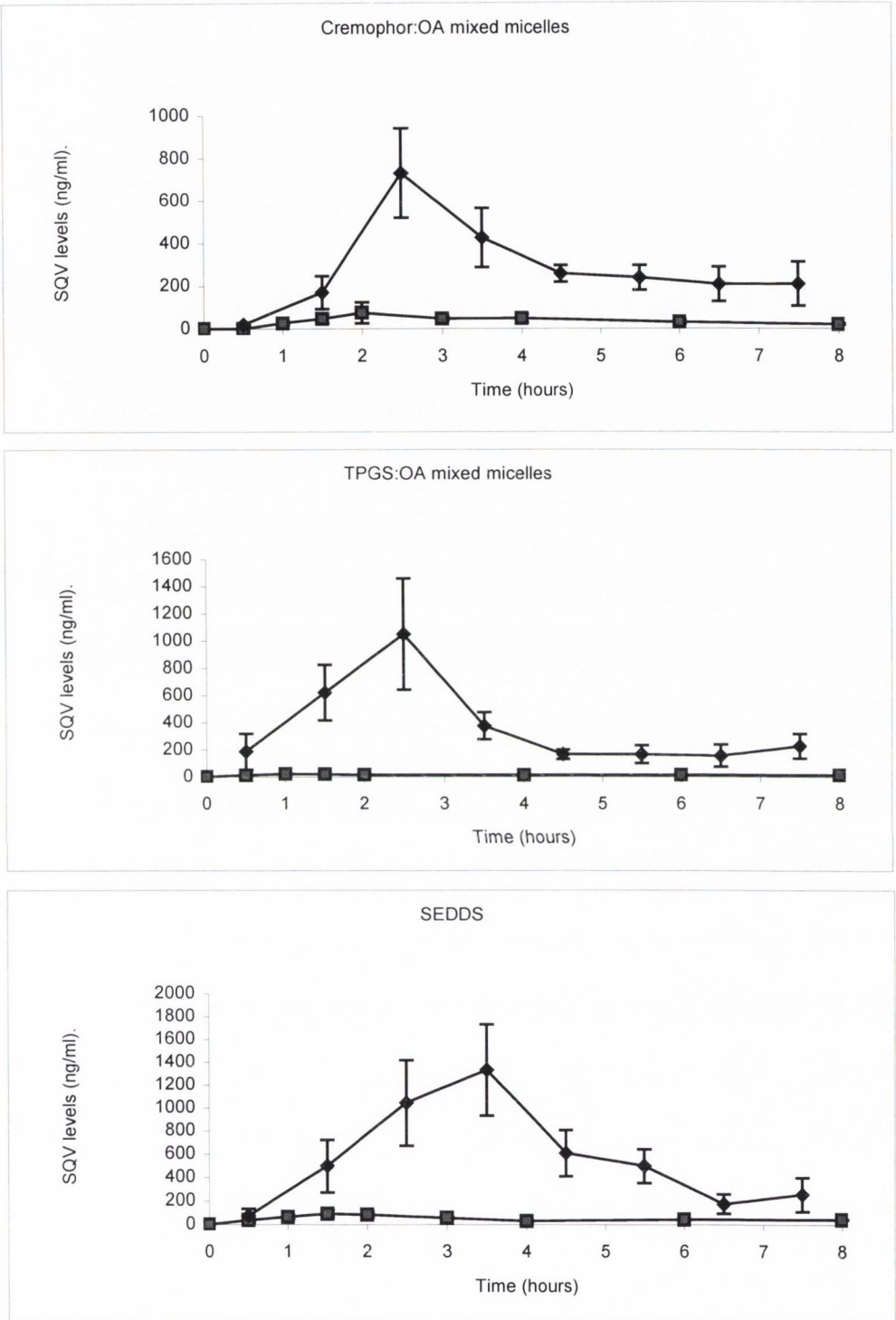


Figure 10.11 Concentrations of saquinavir in the plasma (■) and mesenteric lymph (◆) after intraduodenal administration to mesenteric lymph cannulated rats of 5mg saquinavir in either cremophor EL:OA, TPGS:OA mixed micelles or in a SEDDS formulations.

For the three lipid systems examined the concentrations of saquinavir achieved in the mesenteric lymph is significantly higher than systemic plasma levels. The peak concentrations in lymph were approximately 10-fold, 26-fold and 15-fold higher than the corresponding peak plasma concentrations for the cremophor EL mixed micelle, the TPGS mixed micelle and the SEDDS formulations, respectively. The peak concentration in the mesenteric lymph, which occurs between 2-3 hours, is more delayed than that observed in systemic plasma, which occurs between 1.5-2 hours. This possibly reflects a more prolonged absorption process from the mesenteric lymphatics.

10.2.6. Saquinavir plasma concentrations in lymph cannulated and non-lymph cannulated rats

Saquinavir plasma concentration-time profiles (0-8h) after intra-duodenal administration of 5mg saquinavir in three lipid based formulations to lymph cannulated rats and sham operated non-lymph cannulated rats ($n \geq 5$ for each formulation) are presented in Figure 10.12. This data must be viewed with the understanding that for lymph cannulated animals, systemic plasma concentrations do not reflect contributions from saquinavir lymphatic transport, (as mesenteric lymph was collected), whereas plasma levels in the non-lymph cannulated (lymph intact) rats result from saquinavir absorption directly into the blood and indirectly into the blood via the lymph.

After administration of saquinavir in the cremophor EL mixed micelle formulation, the plasma $AUC_{0\text{-}8hr}$ of saquinavir in nonlymph-cannulated rats was significantly greater than that in lymph-cannulated rats ($p < 0.05$). This potentially demonstrates the influence of intestinal lymphatic transport to systemic plasma levels after administration of saquinavir in a cremophor EL mixed micelle vehicle. However, the plasma profiles were not significantly different after administration of saquinavir in the SEDDS, which appears to contradict the findings for the cremophor EL mixed micelle system. The plasma concentrations for the TPGS were too close to, or lower than, the limit of quantitation and as a result it was not possible to accurately estimate the AUC for each experiment. The data is included to illustrate a similar plasma profile for lymph cannulated and nonlymph-cannulated rats. Possible reasons for the differing effects of these two lipid formulations will be discussed in section 10.3. Plasma profiles for the cremophor EL mixed micelles containing saquinavir mesylate salt were below the limit of quantitation of the assay.

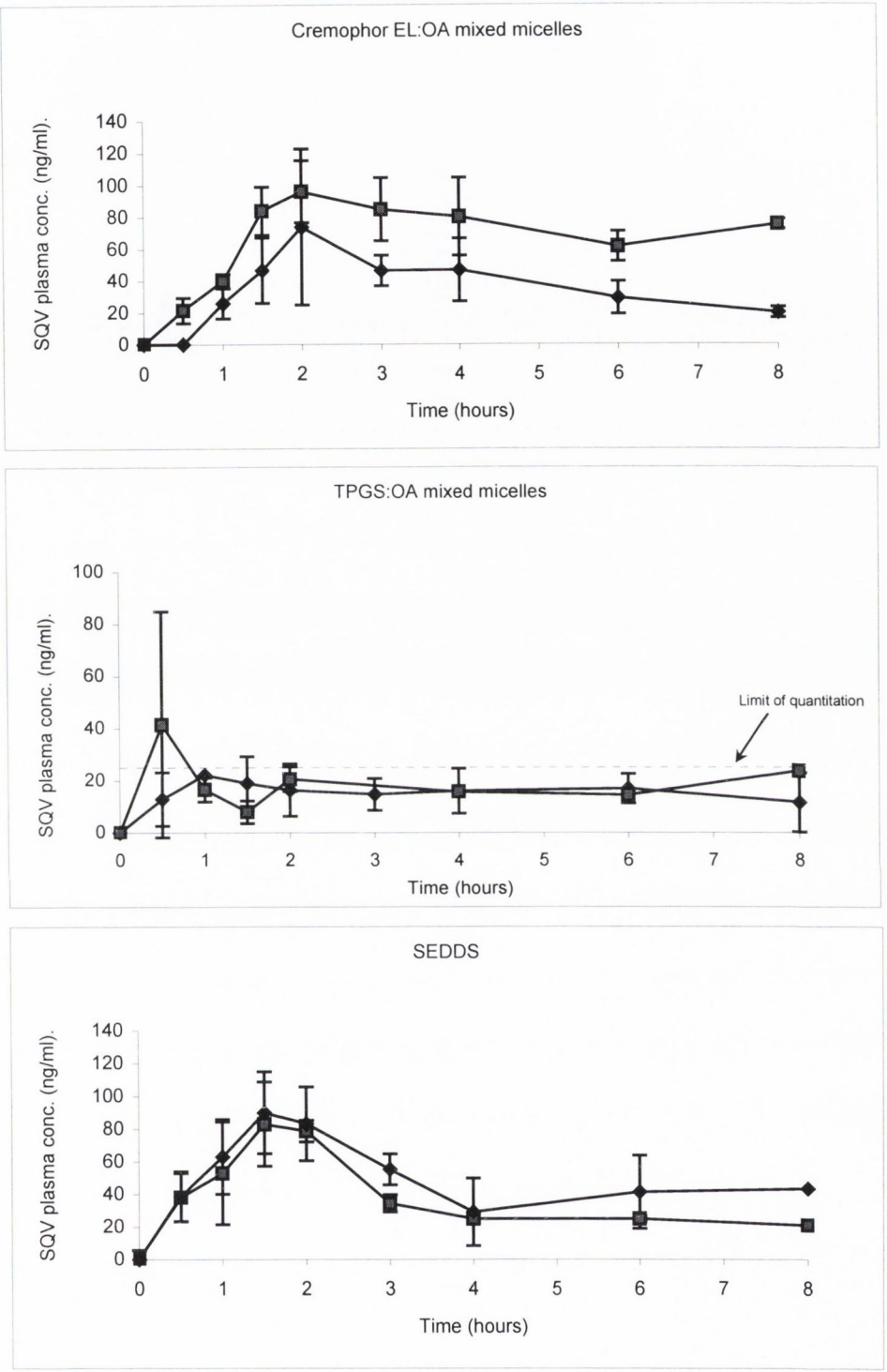


Figure 10.12 Plasma concentration – time profiles of saquinavir (mean ± SE, n≥5) in non-lymph cannulated (■) and lymph cannulated rats (◆) after intraduodenal administration of 5 mg of saquinavir in three lipid-based formulations (cremophor EL mixed micelles, TPGS mixed micelles and a SEDDS).

10.2.7. Comparison of saquinavir availability in lymph-cannulated and bioavailability in nonlymph cannulated animals

The goal of these comparisons presented in Figure 10.12 was to estimate the proportion of drug absorbed via non-lymphatic routes (as a function of the different formulations) and to compare these profiles with the observed effect of the different micellar vehicles on lymphatic transport. In lymph cannulated animals total saquinavir availability was defined as the mass transported directly into the lymph plus the proportion of the dose absorbed via the blood into the systemic circulation (relative to an i.v. control). Saquinavir bioavailability was also estimated in non lymph-cannulated rats using a standard i.v. / oral bioavailability study design and by sampling blood only. The oral bioavailability calculated in this way is compared with the total availability estimated from lymph plus blood data in lymph-cannulated animals.

Figure 10.13 below presents rat plasma saquinavir concentration as a function of time following an i.v. bolus of 1mg, which was analysed using a non-linear curve-fitting program as described in the methods. Pharmacokinetic parameters were determined for each animal and then averaged. The experimental data points are represented by symbols while the solid line is the least squares fit to a triexponential equation. Plasma concentrations were again fitted to a triexponential equation using values of A,B,C, alpha, beta and gamma, estimated in the original fit, and the dose constants and estimating the area under the curve (AUC). For the mean i.v. blood concentration, an $AUC_{0\text{-}\infty hr}$ of 1361.55ng.hr.ml^{-1} was obtained, the clearance, calculated as dose/AUC was 0.734L/hr and the apparent biological half-life was 1.71hr.

Williams et al., (1992), have previously utilized a triexponential disposition process to describe the saquinavir plasma concentration-time profile following i.v. administration of 12 mg of saquinavir in human volunteers. The clearance and terminal half-life were estimated as 98.8L/hr and 13.2h, respectively.

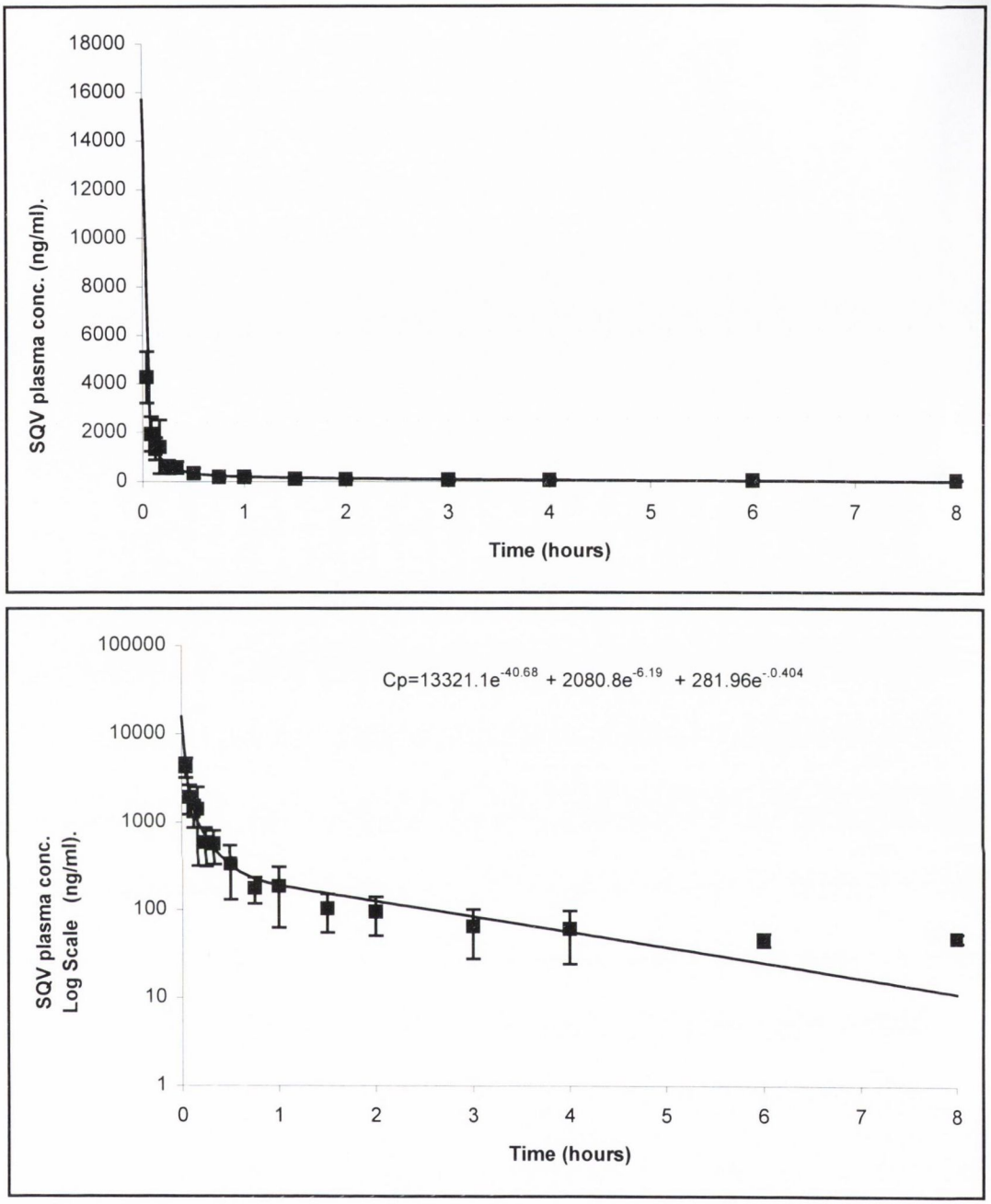


Figure 10.13 Saquinavir plasma concentrations following i.v. administration of 1mg fitted to a triexponential equation plotted on normal and log scales (n=7).

Table 10.2 Comparison of total saquinavir availability (mean % dose ± SE, n ≥ 5) in lymph cannulated and bioavailability in non-lymph cannulated rats after intra-duodenal administration in a cremophor EL:OA mixed micelle or a SEDDS formulation.

Experiment	Cremophor EL mixed micelles		SEDDS	
	Lymph cannulated rats	Non-lymph cannulated rats	Lymph cannulated rats	Non-lymph cannulated rats
Lymphatic transport [A]	0.027 ± 0.005		0.052 ± 0.013	
Plasma Availability [B]	4.98 ± 1.72	8.53 ± 1.71	6.41 ± 2.03	3.88 ± 0.73
Total (lymph and blood) Availability/ Bioavailability	5.01 ± 1.72	8.53 ± 1.71	6.46 ± 1.23	3.88 ± 0.73

[A] *Cumulative mass of saquinavir recovered over 8 hours in mesenteric lymph calculated as a percentage of dose*

[B] *The percentage dose of saquinavir absorbed directly into the blood (i.e. nonlymphatic absorption) was estimated based on the plasma* $AUC_{0\rightarrow\infty h}$, *relative to an i.v. control* $(AUC_{oral}/D_{oral})/AUC_{i.v.}/D_{i.v.})$

There were no statistically significant differences between saquinavir availability and bioavailability values, which potentially confirms the lack of substantial lymphatic transport. The saquinavir bioavailability, determined from the $AUC_{0\rightarrow\infty h}$ calculated from the plasma profiles in non lymph cannulated rats, did not differ significantly between the SEDDS and cremophor EL mixed micelles. The plasma concentrations from the cremophor EL mixed micelles and SEDDS were consistently higher than those of the TPGS mixed micelle formulation confirming a higher extent of bioavailability. Potential mechanisms by which lipid based formulations may have enhanced drug absorption include the effects on the gastro intestinal membrane permeability, increased solubilizing capacity, or an effect on the metabolism of saquinavir.

10.3. Discussion

Saquinavir is a highly lipophilic, poorly water-soluble HIV protease inhibitor that exhibits poor bioavailability in humans ranging from 4-12% depending on the formulation and conditions. The potential merits of formulation in specialised lipid vehicles (e.g. mixed micelles, SEDDS) at promoting intestinal lymphatic transport of the model lipophilic compounds DDT were highlighted in the preceding chapters. The utility of formulation of saquinavir in similar lipid formulations has been assessed in the present study.

Figure 10.14 compares the cumulative extent of lymphatic transport vs. time for the three lipid formulations administered in this study. The extent of transport after 8 hours was greatest for the SEDDS formulations, however, given the extensive variability within each group of data, the differences were not statistically significant. The total extent of transport was less than expected given the high lipophilicity of saquinavir (clog P = 4.5, solubility in triolein 1.87mg/ml) and limited aqueous solubility (17μg/ml). The estimated systemic bioavailability of these formulations was also low (i.e. 4-8%). By expressing the extent of lymphatic transport as a percentage of the bioavailability values calculated in Table 10.2, intestinal lymphatic transport constitutes 0.32% and 1.34% of the bioavailable dose of saquinavir for the cremophor EL mixed micelles and SEDDS formulations respectively. The results of this study suggest that lipophilicity alone was not the only factor governing intestinal lymphatic transport.

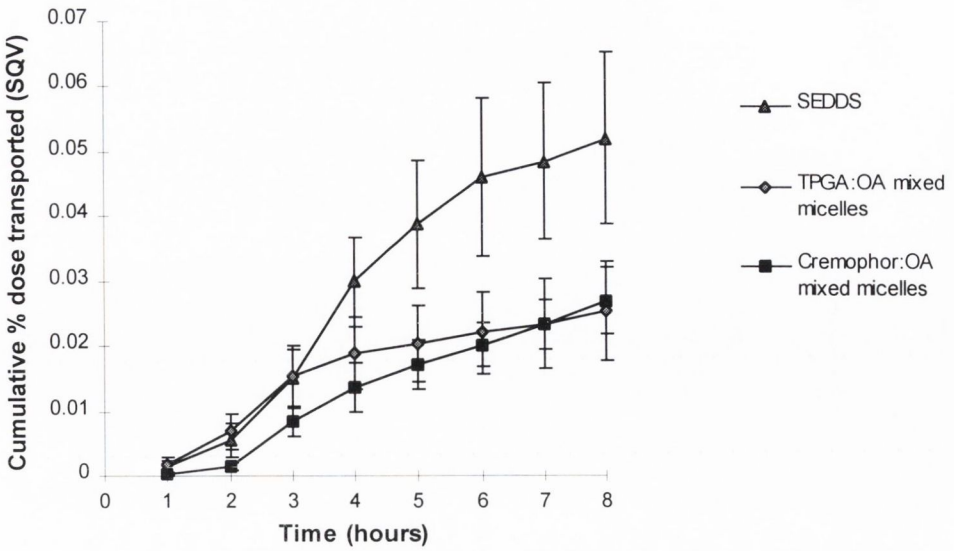
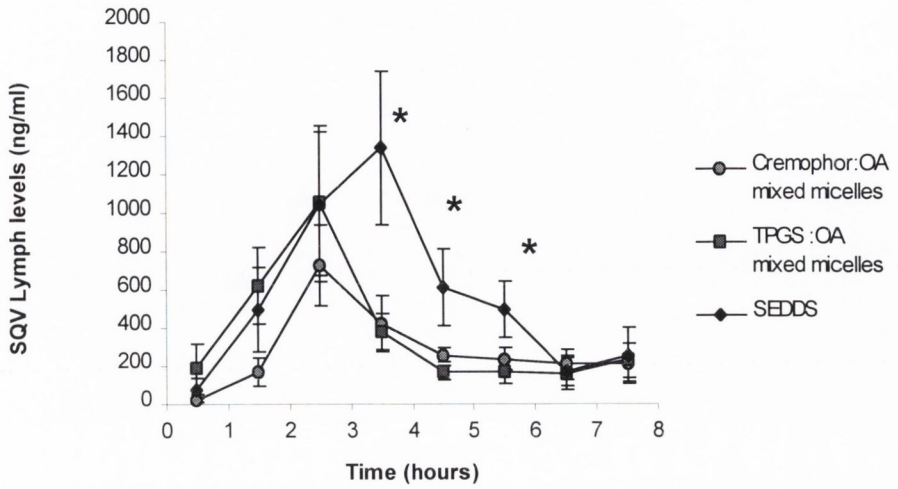


Figure 10.14 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally in a cremophor EL mixed micellar formulation, a TPGS mixed micellar formulation or as a SEDDS.

The pathological progression of HIV infection and AIDS suggests that therapeutic benefit may be gained by redirecting a proportion of the dose of compounds with significant anti-HIV activity, such as saquinavir, to the lymphatic tissue and the lymphoid tissue in general. A comparison of the concentrations in lymph and plasma (Figure 10.11) suggests that while the total extent of drug transported lymphatically is low, the concept of promoting the higher concentration in intestinal lymph compared to those detected in plasma through a formulation effect is possible for saquinavir, which may be clinically significant. Hauss et al., (1994) have also reported on the concept of targeted lymphatic transport by formulation of lipophilic compounds using lipid vehicles.

The SEDDS formulation produced higher lymph concentrations than the micellar systems (Figure 10.15), an effect that was not clearly evident from the plasma concentration-time profile, and assuming a t.i.d dosage regime, the therapeutic benefits may be considerable. The SEDDS formulations also appeared to prolong the period of lymphatic transport. Consequently, when choosing the optimal formulations for drug delivery, screening for changes in both the lymph and plasma absorption patterns is crucial particularly for compounds with significant lipophilicity and when lymphatic transport is advantageous.



* Statistically significant ($p<0.05$)

Figure 10.15 Lymph saquinavir concentration-time profile after intraduodenal administration of 5mg saquinavir in either cremophor EL:OA mixed micelles, TPGS:OA mixed micelles or a SEDDS formulation.

Another example of where quantification of drug concentrations in the lymph may provide a more accurate indication of drug concentrations at the site of action than the routine assay of drug concentrations in the plasma is the lipophilic immunosuppressant, Cyclosporin A (Takada et al., 1988). A polyoxy 60 castor oil (HCO-60) micellar formulation increased the lymphatic concentration of CsA by approximately 20 times compared to the conventional olive oil preparation (Takada et al., 1985 & 1986). Moreover, a basic pharmacological study using the rat heart transplantation study showed that the mean survival time (in day) of the transplanted rat heart was significantly longer for the new formulation with the proposed selective lymphatic transporting ability, than with the olive oil formulation (Yasumura et al., 1986; Takada et al., 1987). However, as the micellar formulation also increased systemic bioavailability, the possibility of the intensified immunosuppressive effect being related to increased systemic concentrations could not be overlooked.

The higher apparent loadings of saquinavir per mg lymph triglyceride provide further evidence for the ability of this SEDDS to produce higher lymph concentrations than the mixed micellar formulations (Figure 10.10). This higher apparent loading of saquinavir

from the SEDDS formulation is similar to the findings for DDT as detailed in Chapter 9. This effect is believed to be related to either a facilitation of higher drug concentrations within the enterocyte, or a more efficient processing of intestinal lipid into intestinal lipoproteins, and facilitating the transfer of solubilized drug from the intestine into intestinal lipoproteins. The SEDDS formulation in this study also appears to prolong the process of triglyceride turnover; an effect may be viewed as providing for sustained higher lymph concentrations.

The concentrations in the lymph for either the cremophor EL or TPGS mixed micelles were not dissimilar. In contrast, the plasma profiles for the TPGS mixed micellar system exhibited lower saquinavir plasma concentrations than the cremophor EL mixed micelle formulation. The plasma concentration following administration of the TPGS mixed micellar were too low to allow accurate comparison. The implications here are that changes in formulations can produce differing effects on the lymph and portal absorption routes of saquinavir, which may not be evident from conventional plasma versus time profiles.

A comparison of the $AUC_{0\rightarrow 8h}$ for plasma profiles in lymph cannulated and non-cannulated rats, following administration of the cremophor EL mixed micellar formulation, suggest a significant contribution of lymphatically transported saquinavir to systemic plasma profiles. The AUC for rats with intact lymph was 375.49 ± 73.50ng.h.ml^{-1} compared with an AUC of 155.41 ± 21.22ng.h.ml^{-1} for rats with mesenteric lymph removed. These results suggest that removal of the portion of drug transported by the mesenteric lymph produced a 58% drop in the extent of systemic absorption after 8 hours. However, the amount of saquinavir detected in the lymph (0.03% dose administered) did not reflect this. A similar disproportionate discrepancy has previously been reported between the extent of absorption of a lipophilic lipid regulator (CI-976) (log P 5.83) when assessed in lymph-cannulated and non-lymph cannulated animals (Hauss et al., 1994). By comparing the plasma $AUC^{0\rightarrow 14h}$ values for both experimental groups administered either a suspension or an emulsion formulation, the proportion of the plasma $AUC^{0\rightarrow 14h}$ value contributed by lymphatic transport was found to be significant for both, with plasma AUC in lymph-cannulated rats being 43-57% lower. Absolute bioavailability values were not obtained, as an i.v. study was not performed. The total amount of drug transported through the lymph was low (<1% of dose). The authors suggested that, particularly for lipophilic compounds

with low bioavailability, small amounts of drug absorption via the lymph may have a significant impact on the total plasma AUC value.

When the plasma bioavailability and availability values are compared for the cremophor EL mixed micelles (which take into account the elimination phase or tail-area of the Cp vs. t. plot i.e. $AUC_{8hr \rightarrow \infty h}$), the extent of systemic absorption in lymph-cannulated and non-lymph cannulated rats is no longer significant. Similarly, there were no statistically significant differences between the total availability values estimated in lymph cannulated and bioavailability values in non-lymph cannulated groups following administration of the SEDDS. A comparison of the plasma profiles for the three lipid formulations suggest the interplay of multiple factors on the gastrointestinal absorption of this compound; however, lymphatic transport does not appear to be a significant contributor to oral bioavailability.

The bioavailability calculated for saquinavir did not differ significantly between the cremophor EL mixed micelles and the SEDDS. The saquinavir plasma levels for these systems compare favourably with those previously reported by Roberts et al., (1992), where, the oral bioavailability in rats was approximately 3%. Anti-viral tests in vitro with SQV have typically given IC_{50} and IC_{90} values in the range 1-10nM and 5-50nM respectively. In pharmacokinetic studies in the rat a single dose of 10mg/kg p.o. gave a mean peak plasma level of about 100ng/ml (150nM) and plasma levels remained above 10ng/ml beyond six hours (Roberts et al., 1992). In our study, the plasma levels at 8 hours remained above the antiviral IC_{90} ($11.6ng/ml^{-1}$), following administration of 5mg saquinavir in either cremophor EL mixed micelle (75.3 ± 5.8ng/ml) or SEDDS (21.7 ± 1.7ng/ml) formulations.

Plasma levels following administration of the mesylate salt were negligible or below the limit of quantitation compared to an estimated bioavailability of 8% for the free base formulation. The mesylate salt was the salt form used in the original saquinavir formulation that reached FDA approval within 9 months of initial submission in 1995, Invirase®. This formulation has now been replaced with a soft gelatin capsule form, Fortovase®, which contains the more lipophilic free base form of the drug, in a lipid-based formulation. The newer formulation significantly improves bioavailability, although the mean absolute bioavailability has not been reported. It is estimated that a single 600mg dose of saquinavir SGC increase the bioavailability 3.3-fold compared to a single dose of saquinavir HGC (Perry & Noble, 1998). It is unclear why the manufacturers chose to use

the mesylate form of saquinavir initially, but may be related to its higher aqueous solubility, which would presumably improve drug dissolution characteristics. The aqueous solubility of the mesylate salt is 2.2mg/ml, compared to an aqueous solubility of 0.017mg/ml for the free base form. Our findings confirm that formulation of the free base form in a lipid vehicle increases the system bioavailability over that observed for the mesylate form. In addition, the similar lymphatic transport pattern for the saquinavir mesylate salt and the free base form possibly reflects conversion of the mesylate salt to the free base form within the intestine, however further studies would be required to address this.

In a similar study, Porter et al., (1996a &b) compared the intestinal lymphatic transport of Halofantrine (Hf) free base (triglyceride solubility >50mg/ml) to that of the more hydrophilic hydrochloride salt (Hf.HCL) (triglyceride solubility ~1mg/ml). Potential explanations for the higher than expected lymphatic transport in rats (4.6% of the dose for Hf.HCL vs. 18% for free base), of the Hf.HCl salt include the partial conversion of the free base within the intestinal lumen and/or a specific interaction between Hf.HCl and lymph lipoproteins. The lymphatic transport of the two salts was further examined in lymphatic cannulated conscious dog model (Khoo et al., 1999). When Hf.HCl 100mg was administered in a postprandial dog model, lymphatic transport accounted for a surprisingly high 47% of the administered dose, compared to 54% for the free base form. Interestingly the kinetics of lymphatic transport displayed an ~1-2h lag phase compared with post prandial administration of the free base, and lymphatic transport was largely complete within 6 h. Subsequent studies have suggested that lymphatic transport after administration of Hf.HCl was driven by its conversion to the highly lipid soluble free base form (Porter & Charman, 2001). Although the equilibrium fraction of Hf base at intestinal pH values after administration of Hf.HCl would be very low (Hf is a tertiary amine with an estimated pKa of at least 10), two factors driving conversion to the free base within the post prandial environment are its high lipid solubility and the extensive post-prandial solubilization of Hf.HCl.

This study confirms that the absorption kinetics of saquinavir in rats exhibits large variability. For example, in calculating the extent of lymphatic transport as a % dose administered, the coefficients of variability for the cremophor EL: OA, TPGS:OA and SEDD formulations examined were 43%, 74% and 62% respectively. Substantial inter-individual variability has also been reported for saquinavir in human studies (Lunn &

Aarons, 1998). Point estimates for inter-individual variance terms associated with saquinavir disposition range between ~0.3 and ~0.6. These values give rise to approximate coefficients of variation between 58 and 85%, and correspond to roughly 9-fold and 21-fold differences between upper and lower limits of approximate 95% predictive ranges in humans. High inter subject variability is a common characteristic for lipophilic drugs which exhibit low bioavailability combined with high first pass effect (Thummel et al., 1997).

11. Effect of P-glycoprotein and cytochrome P450 modulators on intestinal lymphatic transport of saquinavir

11.1. Introduction

Saquinavir was the first protease inhibitor to be licensed for the treatment of HIV infections and has established itself as a leading component of the multidrug therapy now used for the disease (Perry & Noble, 1998). However, the bioavailability of saquinavir is still quite poor, ranging from 4-12% depending on the formulation. This arises as a consequence of multiple factors, including the poor solubility characteristics, but also extensive intestinal and hepatic first pass metabolism, primarily mediated by CYP 3A4, and decreased absorption due to the presence of efflux proteins in the intestinal epithelium (Fitzsimmons & Collins, 1997). Previous studies indicate that saquinavir interacts with the multidrug transport system, P-glycoprotein (P-gp) (Alensz et al., 1998, Kim et al., 1998). P-gp is a plasma membrane efflux pump that is expressed in normal tissues, including the apical membrane of the intestinal and renal epithelia and the endothelial cells of the blood-brain barrier (Thiebaut et al., 1987; Cordon Carlo et al 1989). Expression of MDR1 in CD4 T-lymphocytes, the major target for HIV infection, has also been reported (Bommhardt et al., 1994). P-gp substrates are generally hydrophobic and have partition coefficients greater than 2 (Germann et al., 1993).

Focusing on the fact that the substrates of CYP 3A4 and P-gp are mutually overlapping, as is the case with saquinavir, Benet et al., (1996) have proposed that these proteins act synergistically to present a barrier to absorption from the small intestine (Wacher et al., 1995; Benet et al., 1996; Wacher et al., 1998). It is hypothesized that P-gp has the effect of recycling the substrate, increasing enterocyte residence time and therein increasing susceptibility to metabolism by CYP 3A4. The extent of intestinal metabolism can be very significant and in the case of cyclosporin more than two-thirds of the oral dose is metabolised in the human intestine (Wu et al., 1995). Thus, intestinal CYP 3A4-dependent metabolism and the P-gp counter-transport processes may play a significant role in limiting portal absorption of the saquinavir into the systemic circulation. The extent of contribution from the intestinal metabolism to poor oral bioavailability of saquinavir has not yet been fully established.

In the case of HIV protease inhibitors, high plasma concentrations are preferred because the treatment objective is to completely inhibit viral proliferation, an effect which is

proportional to drug concentrations. Virus surviving drug exposure is likely to eventually mutate and become resistant to that drug, and possibly related drugs. Therefore, it is essential to successful therapy of HIV disease for orally administered drugs to be adequately and consistently absorbed. Co administered food with saquinavir SGC, and in particular, a high fat meal, may increase saquinavir plasma concentrations by up to three to four fold, and also decrease the rate of absorption (Noble & Faulds, 1996). Some authors have observed a second peak following a single dosage administration, and related this to further food intake (Kenyon et al., 1998). A number of authors have proposed that lipid digestion products may specifically interfere with saquinavir CYP3A4 metabolism and/or block intestinal drug transporters. Kenyon et al., (1998) suggested that the second peak at later time points may reflect reduction in P-gp action from the distal small intestine, or as a result of food interacting with the pump activity. Interestingly, the increased potential for lymphatic transport, as a result of ingested lipids promoting lipoprotein turnover in the intestinal lymph, was not proposed. The capsule formulation of saquinavir contains glyceride fatty acid esters. Aungst et al., (1999) suggested that these formulation excipients may affect P-gp activity *in vivo*, and thus influence the oral bioavailability of saquinavir.

Some tissues such as the brain and testes are possibly protected from exposure to HIV protease inhibitors due to drug entry being limited by P-gp, located in the capillary endothelium. Choo et al., (2000) designed a study to determine whether pharmacological modulation of P-gp activity would alter the distribution of HIV protease inhibitors into the brain and the testes. Intravenous administration of the novel and potent P-gp inhibitor LY-335979 to mice (1-50mg/kg) increased brain and testes concentrations of ^{14C}C-Nelfinavir, up to 37- and 4- fold, respectively, in a dose-dependent fashion. Similar effects in brain levels were observed with ^{14}C-labeled amprenavir, indinavir and saquinavir. The authors concluded that their findings provide 'proof of concept' for increasing HIV inhibitor distribution into pharmacological 'sanctuary sites' by targeted inhibition of P-gp using selective and potent agents and proposed a new therapeutic strategy to reduce HIV-1 viral replication.

There are numerous reports indicating that the lymphoid tissue is the major storage and/or replication site of HIV in vivo (Gunthard et al., 2001; Pantaleo et al., 1993; Zhang et al., 1999). In the preceding chapters the concept of targeted lymphatic transport of lipophilic compounds by administration in lipid vehicles containing long chain fatty acids to

stimulate lipoprotein production, has been discussed. The present chapter is a follow on from this hypothesis. There have been several studies demonstrating saquinavir mediated transport by P-glycoprotein *in vitro* and the effect of specific modulators of P-gp and/or CYP, such as verapamil, ketoconazole and cyclosporin on transepithelial transport (Alsenz et al., 1998; Profit et al., 1999). Furthermore, a number of studies have examined the influence of intestinal metabolism on portal blood levels of P-gp/CYP susceptible compounds, such as saquinavir (Cuvelier et al., 1998) in the rat, and in humans, cyclosporin (Kolars et al., 1991) and midazolam (Paine et al., 1996). However, to date no study has examined the potential effects of the P-gp/CYP elimination mechanism on the intestinal lymphatic absorption pathway. In the present study the functional contribution of P-gp/CYP mediated elimination *in vivo* was evaluated in terms of its effect on intestinal lymphatic transport and systemic plasma profiles in the rat. We hypothesized that concomitant oral administration of P-gp/CYP modulators might also increase the intestinal lymphatic transport of orally administered saquinavir from the intestinal lumen and have tested this in the mesenteric lymph duct cannulated rat model. The design of this study is similar to that described in Chapter 10, with saquinavir being administered in a cremophor mixed micellar formulation. Of the three lipid formulations examined in Chapter 10, cremophor EL:OA mixed micelles was the preferred choice based on its superior solubilizing potential, and also as this lipid formulation promoted a maximal increase in intestinal triglyceride levels. Cremophor EL may also act as a P-gp/CYP modulator itself (Nerurkar et al., 1997). The administered formulation will also contain a P-gp/CYP modulator co-solubilized in the vehicle. The objectives of this study were three fold: (a) to demonstrate a proposed P-gp/CYP mediated effect on the intestinal lymphatic transport of saquinavir; (b) to assess the effect of co-administration of the P-gp/CYP modulators, verapamil, ketoconazole and cyclosporin, on increasing intestinal lymphatic transport and (c) to compare this with the observed effect of co-administration on systemic concentrations of saquinavir.

11.2. Results

11.2.1. Intestinal lymphatic transport of saquinavir

11.2.1.1. Co-administration of verapamil

The lymphatic transport of saquinavir following administration in a cremophor EL: oleic acid mixed micellar systems was examined in the chapter 10. The extent of transport was 0.03% of the administered dose, with peak lymphatic transfer occurring between 2-3 hours. The influence of co-administration of verapamil was studied initially, as it is a potent inhibitor of P-gp activity but also acts as a substrate/inhibitor of CYP 3A4 (Saitoh & Aungst, 1995; Thummel et al., 1997). Verapamil is well-absorbed following oral administration to human, dogs, and rats (Mc Tavish & Sorkin, 1989). If verapamil is a substrate of intestinal P-gp and is efficiently secreted by the efflux system, the *in vivo* absorption might be expected to be low. However it is possible that verapamil is not a direct substrate for P-gp (competitive inhibitor), but is a non-transportable (non-competitive) inhibitor of the normal intestinal P-gp. Alternatively, the intestinal efflux system mediated by P-gp could be easily saturated after typical oral doses, and contribute little relative to passive diffusion (Saitoh & Aungst, 1995). Saitoh & Augnst (1995) confirmed that the rat upper intestine has a potent efflux system, transporting verapamil effectively from the serosal side to the mucosal side. Aungst & Saitoh, (1996) reported that verapamil exhibits a maximal inhibitory effect on P-gp at around 0.25mM in cell culture models. The majority of studies utilizing verapamil as a metabolic inhibitor were performed in cell culture models, with few *in situ* models to allow direct comparison. Sandstrom et al., (1998) performed a series of experiments in the human gut on the *in vivo* absorption properties of verapamil in the concentration ranges of 0.08 to 0.8mM and observed a non-linear increase in fraction absorbed with increasing concentration of verapamil consistent with a saturation of P-gp mediated efflux. The decision on a final concentration of 1mM, which was equivalent to a 1.743mg dose in the 3ml dose volume, was based on the concentrations used in these studies, with a view to producing maximal inhibitory effect.

The cumulative extent of lymphatic transport of saquinavir following co-administration of verapamil is shown in Figure 11.1. The extent of intestinal lymphatic transport after 8 hours of saquinavir is 5.74μg ± 1.48μg, representing 0.115% of the administered dose.

Co-administered verapamil (1mM) significantly increases ($p<0.05$) the lymphatic transport of saquinavir 4.2-fold over control (i.e. in absence of modulator).

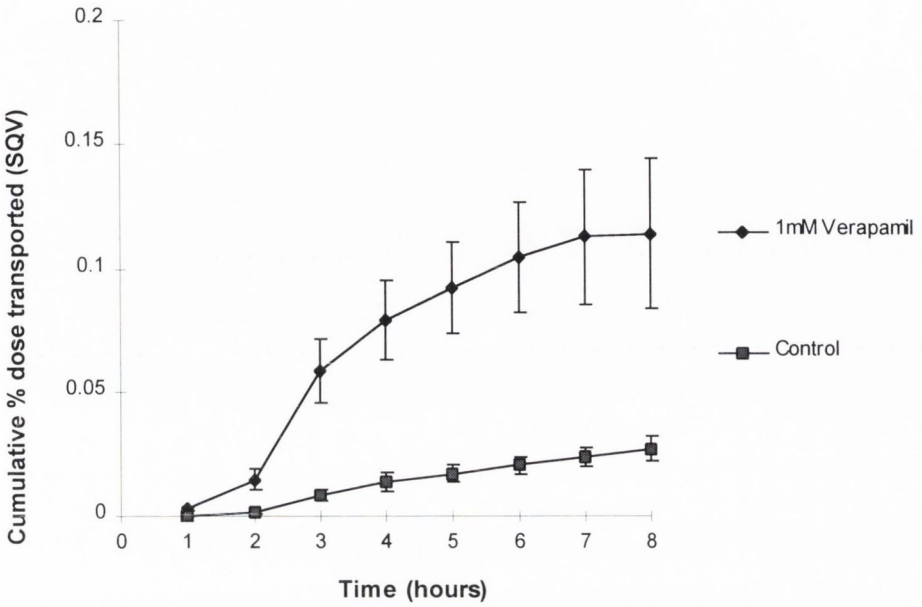


Figure 11.1 Cumulative percent of dose of saquinavir (mean ± SE., n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as a mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) either alone (control) or containing 1mM Verapamil.

11.2.1.2. Co-administration of ketoconazole

Ketoconazole, an azole anti-fungal agent commonly implicated in drug-drug interactions, is a well-characterized non-competitive inhibitor of CYP 3A4 (Maurice et al., 1992; Gibbs et al., 2000). It is not believed to be a good substrate for P-gp, although it is likely to affect other efflux transporters (Kim et al., 1999). However, ketoconazole has been shown to inhibit P-gp in a vinblastine-resistant cell line (KB-V1) (Siegsmund et al., 1994). With the objective being maximal suppression of P-gp/CYP activity in the intestine, a final concentration of 1mM, which is equivalent to a 1.59mg in the final dosage volume, was chosen for this formulation based on the following observations. Typical oral dosing of ketoconazole in humans is 200mg/day, which is the dose routinely used to assess drug interactions in human studies, would equate to a 1mg dose in rats, assuming a conversion factor of 1:200 for human to rats. Zhang et al., (1998) studied the effect of a 20mg/kg oral dose of ketoconazole (equivalent to 6mg in a 300g rat) on the bioavailability of K02, a

novel protease inhibitor. Salphati & Benet, (1998), used a higher dose of ketoconazole of 80mg/kg in rats when assessing the effect on digoxin in the conscious rat. Sandstrom et al., (1999) used a concentration of 0.075mM ketoconazole, equivalent to a dose of 8mg, in examining the effect on human jejenum, opting for a low dose so as to minimize a potential effect on liver metabolism.

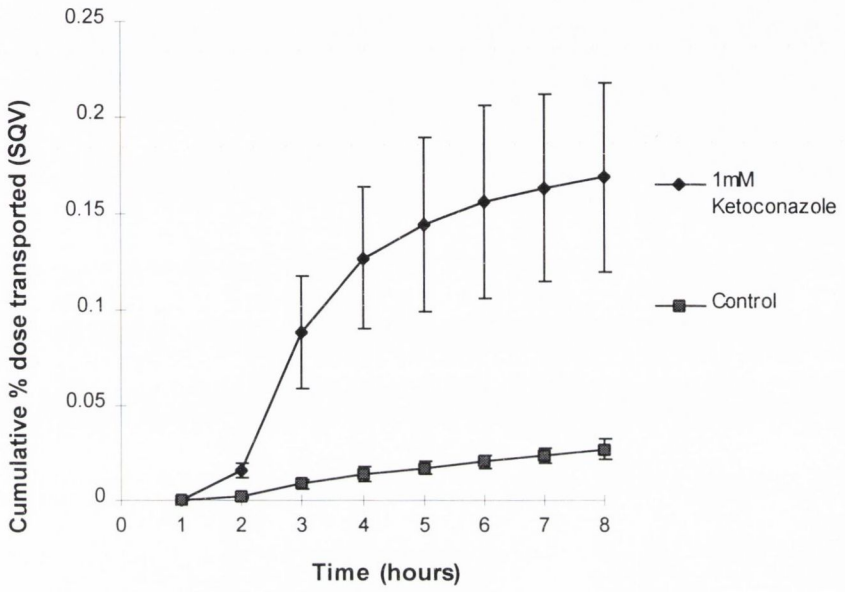


Figure 11.2 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) either alone (control) or containing 1mM Ketoconazole.

The extent of transport after co-administration of ketoconazole (1mM) was 8.43μg ± 2.43μg, representing 0.17% ± 0.05% of the administered dose collected in the mesenteric lymph after 8 hours (Figure 11.2). This represents a statistically significant 6.3 -fold increase on the extent of transport in the absence of the metabolic modulator (i.e. control)

11.2.1.3. Co-administration of cyclosporin

Cyclosporin, a lipophilic fungal undecapeptide, is a potent immunosuppressant, which is currently the drug of choice in preventing graft rejection and graft versus host disease in organ transplantation (Borel, 1988; Venkataramanan et al., 1989). Cyclosporin is an effective inhibitor of P-gp with a relatively low inhibitory concentration of approximately

0.08μM in cell culture lines (Naito & Tsuruo, 1989). Alensz et al., (1998) reported that the saturation concentration in the active transport system in Caco-2 cell culture studies occurred at concentrations in excess of 1μg/ml. Cyclosporin also acts as a substrate for CYP3A. Hughes et al., (1993) examined the effect of erythromycin on the disposition of cyclosporin A in an *in situ* perfused rat model. An oral dose of 2.5mg cyclosporin was used. The majority of drug-drug interactions studies for cyclosporin in humans utilize a 10mg/kg dose, which corresponds to a 3.3 mg dose for the 300g rat. A final concentration of 1mM, which will result in an equivalent dosing of 3.6mg to each rat was chosen.

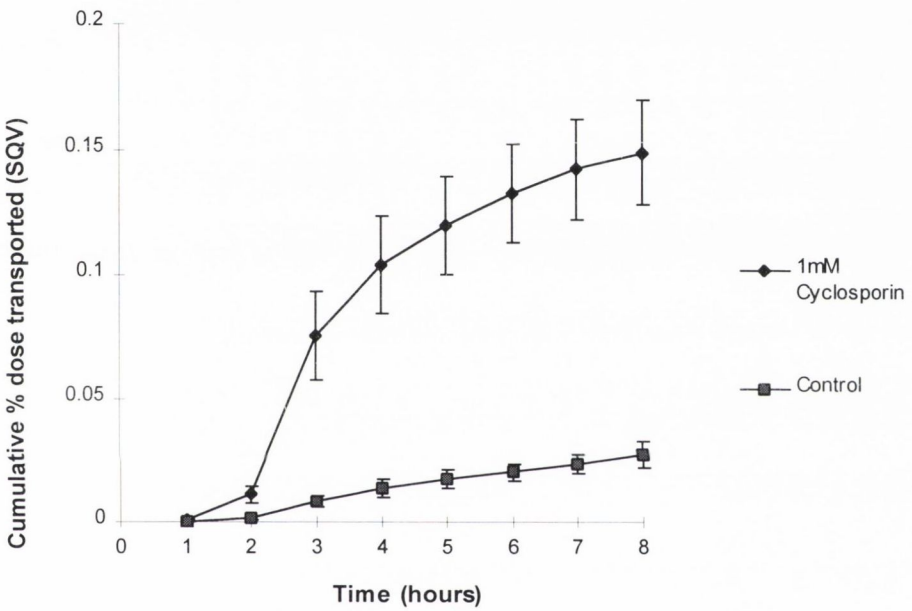


Figure 11.3 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) either alone (control) or containing 1mM cyclosporin A.

The extent of transport after co-administration of cyclosporin (1mM) was 7.42μg ± 1.04μg, representing 0.15% ± 0.02% of the administered dose collected in the mesenteric lymph after 8 hours (Figure 11.3). This represents a statistically significant 5.5-fold increase on the extent of transport in the absence of the metabolic inhibitor (i.e. control).

11.2.1.4. Co administration of ketoconazole (1mM) in combination with verapamil (1mM)

One of the difficulties in ascertaining the relative effect of inhibition of P-gp transporters, or the CYP3A enzyme on intestinal lymphatic transport is that neither of the modulators used in this study has selectivity for either P-gp or CYP3A4. However, it is generally accepted that verapamil acts primarily as a P-gp inhibitor, whereas ketoconazole acts as a potent non-competitive CYP3A4 inhibitor. Achira et al., (1999) performed a comparative study to determine the selectivity of a series of modulators for P-glycoprotein and CYP3A4, in cell culture lines and intestinal microsomes, respectively. The ratio of IC_{50} values for P-gp to that of CYP3A4 was between 60-150 for ketoconazole, and 1.5 for verapamil, confirming that for mechanistic studies of this kind neither modulator allows exclusive selectivity, however verapamil and ketoconazole exert their maximal effects at P-gp and CYP3A4 respectively. As the effect of both inhibitors on the intestinal lymphatic transport were similar, this may suggest that inhibition of either P-gp activity or CYP3A4 metabolism in the intestine exerts a similar effect on lymphatic transport. To test this hypothesis, experiments were performed where both modulators were co-administered in the cremophor mixed micelle formulation containing 5mg saquinavir.

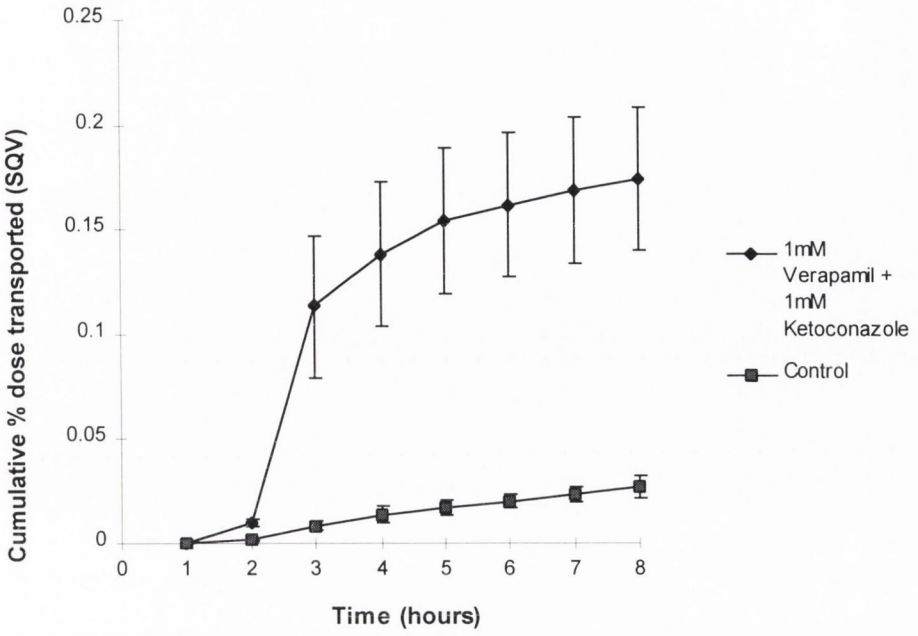


Figure 11.4 Cumulative percent of dose of saquinavir (mean ± SE) (n≥5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) either alone (control) or containing 1mM verapamil plus 1mM ketoconazole.

The extent of transport after co-administration of verapamil (1mM) and ketoconazole (1mM) in combination was 8.67μg ± 1.72μg, representing 0.17% ± 0.03% of the administered dose collected in the mesenteric lymph after 8 hours (Figure 11.4). As these values for the cumulative extent of lymphatic transport were statistically similar to those obtained when either modulator was administered individually, these results suggest that there is no additive or synergistic effects produced following co administration of verapamil and ketoconazole at the concentrations studied.

The relative rate of intestinal lymphatic transport of saquinavir, as a function of the different formulations (Figure 11.5) demonstrates that peak transport occurred between 2-3 hours for all the systems examined. The relative maximal rates of transport were statistically significantly higher ($p<0.05$) for the systems containing P-gp/CYP modulators versus control (i.e. SQV alone), with the highest relative maximal rate of transfer of saquinavir to intestinal lymph obtained for the formulation containing verapamil and

ketoconazole in combination. The differences between the systems containing P-gp/CYP modulator(s) however were not statistically significant ($p<0.05$).

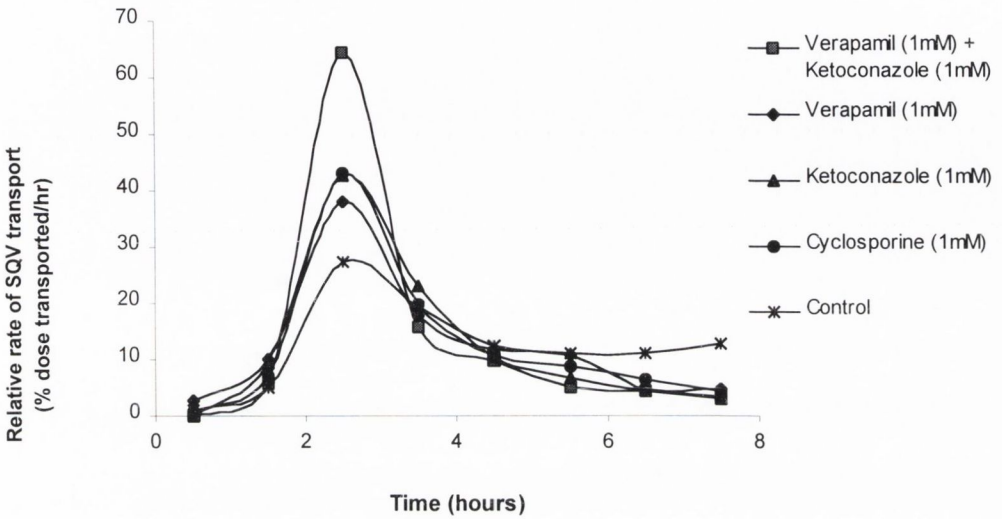


Figure 11.5 The relative rate of transport of SQV into the intestinal lymph (calculated as the percentage of the total drug transported for each formulation per collection time period).

11.2.2. Intestinal lymphatic transport of triglycerides

Table 11.1 summarises the cumulative C_{18} triglyceride transport and lymph flow data after intraduodenal dosing in all of the different formulations examined in this study. Each formulation contained the exact same lipid content, with an equivalent dose of 33.85mg of oleic acid and 60 mg of cremophor administered to each animal. Hence any differences in lipid turnover are attributable to the effects of the co-administered modulator. The lipid turnover for the formulations containing either 1mM verapamil alone, and 1mM ketoconazole alone was similar to the lipid turnover in the absence of a modulator i.e. saquinavir alone. However following concomitant administration of 1mM verapamil and 1mM ketoconazole, the observed triglyceride transport, as determined by the cumulative extent of recovery after 8 hours, was significantly reduced ($p<0.05$). Hence, while neither verapamil nor ketoconazole alone, exerted an observable effect on the extent of triglyceride turnover, at the 1mM concentrations used, when the two agents are combined they appear to reduce the extent of triglyceride turnover in intestinal lymph. Furthermore,

administration of 1mM cyclosporin appears to produce a similar effect, with a statistically significant reduction in the total extent of triglyceride recovered in intestinal lymph after 8 hours. The rank order of the percentage of the administered (exogenous) dose of oleic acid (33.85mg) recovered as re-synthesized triglyceride in intestinal lymph after 8 hours, after endogenous (blank) triglyceride correction, was as follows:

Ketoconazole (92.13 ±13.46%) ≥ Verapamil (82.88 ± 10.71%) >
Verapamil/ketoconazole (42.62 ± 7.93%) ≥ Cyclosporin (36.8 ± 11.1%)

Table 11.1 Cumulative transport of triglyceride into the mesenteric lymph (Mean ± S.E.) and cumulative lymph flow for each formulation. Each vehicle was formulated in 2% cremophor: 40mM oleic acid.

Formulation	Cumulative Mass of triglyceride (mg) appearing in Mesenteric lymph (Mean ± SE) [A]				Cumulative TG transport (mg) [B]	Cumulative lymph flow (ml)
	0-2 hr	0-4 hr	0-6 hr	0-8 hr		
Saline (endogenous control)	2.93 ± 0.06	6.58 ± 0.24	10.26 ± 0.34	15.06 ± 0.85		4.45 ± 0.35
SQV alone (control)	7.27 ± 2.05	26.73 ± 3.24	37.21 ± 3.99	43.07 ± 3.84	28.01 ± 4.50	4.86 ± 0.64
SQV + Verapamil	9.20 ± 1.59	27.15 ± 2.54	36.67 ± 2.03	43.12 ± 2.96	28.05 ± 3.63	4.35 ± 0.83
SQV + Ketoconazole	8.81 ± 0.64	27.57 ± 2.73	38.25 ± 3.36	46.25 ± 3.90	31.18 ± 4.55	6.67 ± 0.51
SQV +Cyclosporin	4.50 ± 1.28	16.87 ± 2.12	22.93 ± 2.48	27.52 ± 3.08	12.45 ± 3.74 [C]	5.08 ± 0.69
SQV + verapamil + ketoconazole	4.54 ± 0.24	18.34 ± 1.84	25.08 ± 1.67	29.49 ± 2.02	14.43 ± 2.68 [C]	5.16 ± 0.54

[A] *Representing endogenous and exogenous lipid*

[B] *Attributable to exogenous lipid (i.e. exogenous – endogenous)*

[C] *Statistically significant difference ($p < 0.05$) than the cremophor mixed micelle formulation containing SQV alone (i.e. no modulator)*

11.2.3. Relationship between DDT lymphatic transport and triglyceride turnover

While it appears that, at the concentrations studied, cyclosporin and the combined verapamil/ketoconazole formulation reduced triglyceride turnover, the extent of saquinavir lymphatic transport was not significantly affected, which may imply that that these systems produced a higher loading per mg of lymph triglyceride. This was investigated by examining the relationships between saquinavir transport rate (ng/hr) and triglyceride transport rate (mg/hr) (Figure 11.6). The slopes of these lines represent the relative concentration, or apparent loading of saquinavir, per mg of lymph triglyceride.

A strong positive correlation exists between hourly saquinavir transfer rates and the triglyceride turnover, with r^2 values in excess of 0.91 for all systems examined. The four formulations containing modulators of P-gp/CYP produced higher apparent loadings than control (i.e. SQV alone). The higher apparent loadings produced by the formulations containing either cyclosporin or verapamil/ketoconazole combined is confirmed.

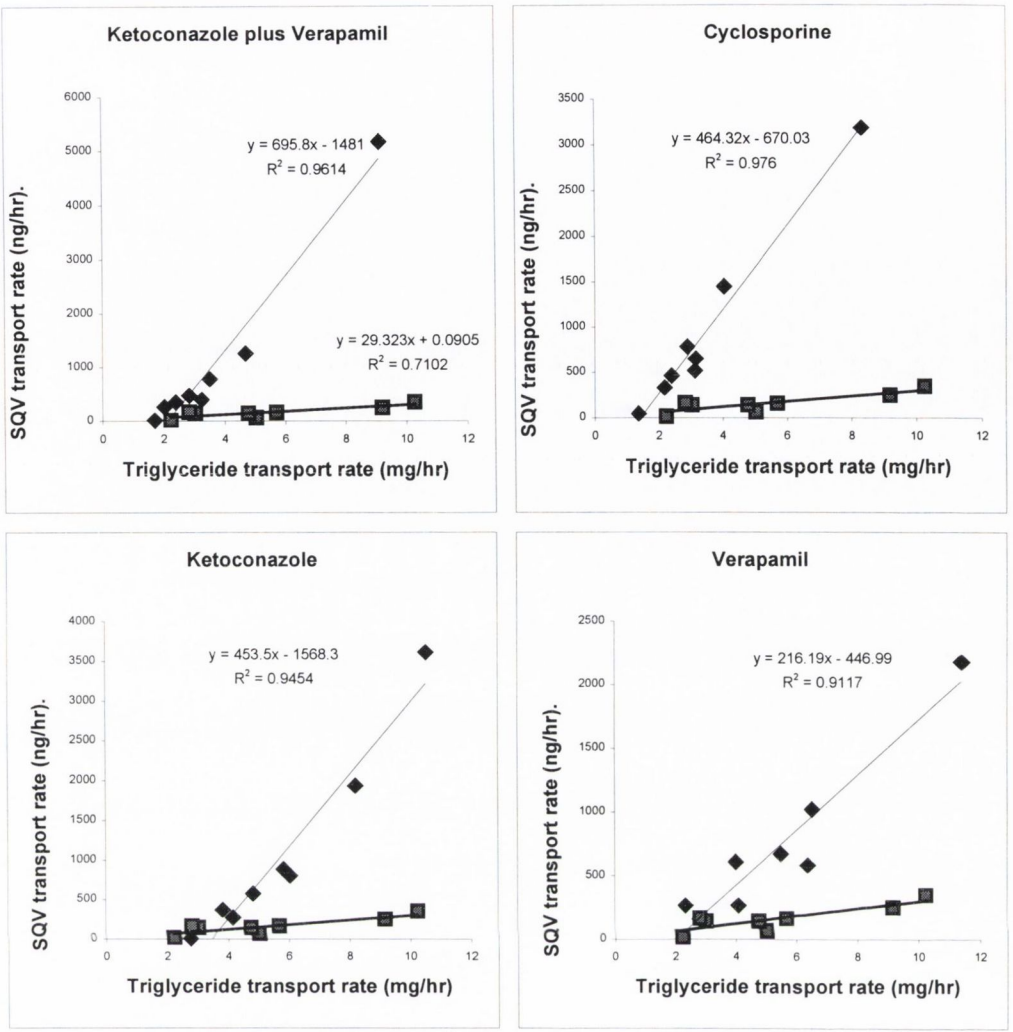


Figure 11.6 Intestinal saquinavir transport rate (ng/hr) versus triglyceride turnover (mg/hr) in intestinal lymph. Saquinavir (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) either alone (control (■) or containing 1mM verapamil, 1mM ketoconazole, 1mM cyclosporin or 1mM verapamil plus 1mMketoconazole in combination (◆).

The higher apparent loadings produced by formulations containing P-gp/CYP modulators can be further explored by plotting the amount of saquinavir per mg of recovered lymph triglyceride as a function of time. Figure 11.7 confirms that the peak transfer of saquinavir to lymph triglyceride occurs between 2-3 hours. Co-administration of P-gp/CYP3A modulators produced significantly higher concentrations of saquinavir in lymph triglyceride than the control (i.e. SQV alone)($p<0.05$), with peak concentrations of 567ng/mg of saquinavir in lymph triglyceride obtained for the formulation containing the combination of verapamil and ketoconazole.

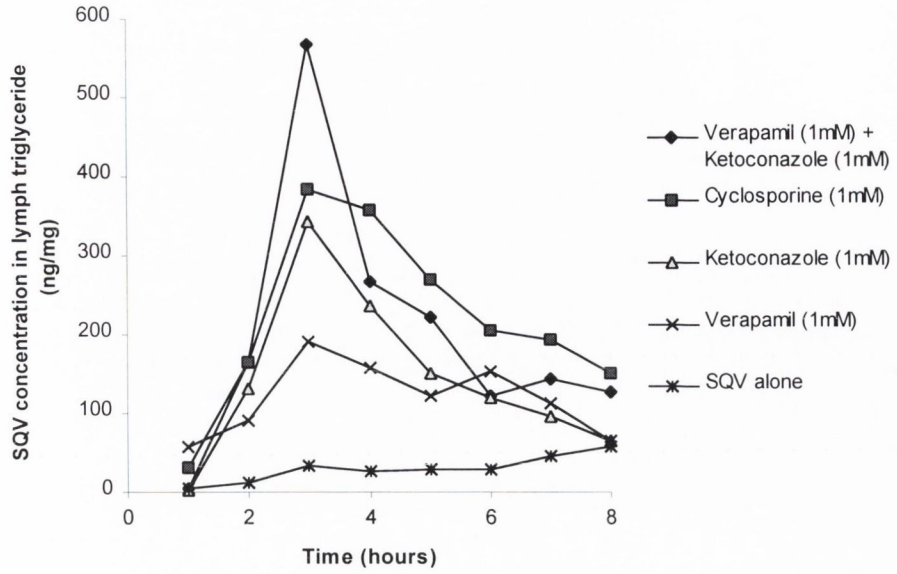


Figure 11.7 Effect of formulation on the concentration of saquinavir in lymph triglyceride

11.2.4. Comparison of saquinavir concentrations in lymph and plasma in lymph cannulated rats.

As mentioned above, maintenance of high concentrations of saquinavir is crucial in preventing viral proliferation, resistance and enhancing therapeutic efficacy. Traditionally, the degree of lymphatic transport is expressed as a percentage of the dose administered. However, if targeting to the lymphatic system for enhanced therapeutic efficacy is the objective, the concentration of drug in the lymph at any one time may be a more relevant parameter. The concentrations of saquinavir in mesenteric lymph and systemic plasma lymph cannulated rats, over 8 hours, are shown in Figure 11.8.

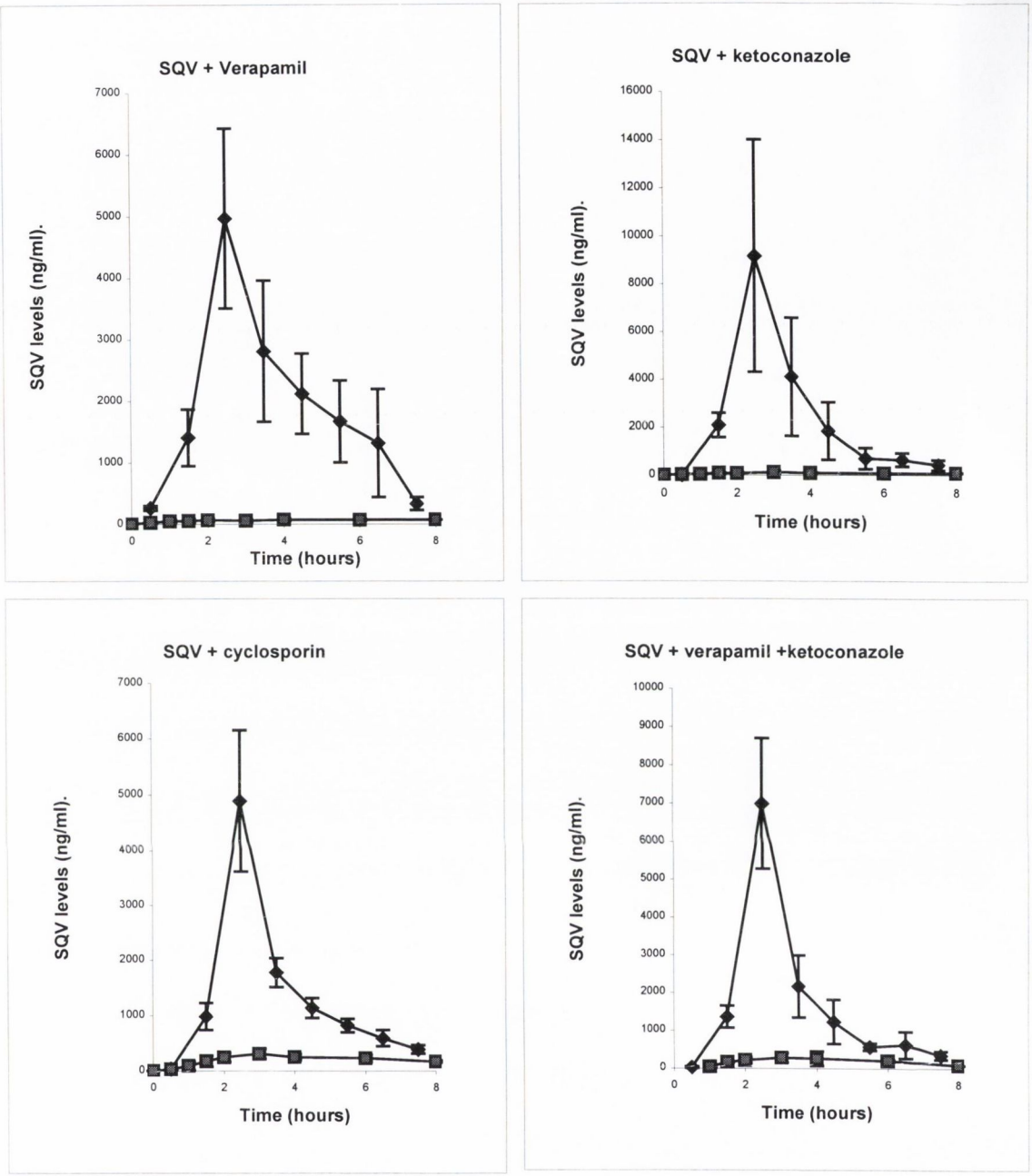


Figure 11.8 Concentrations of SQV in the plasma (■) and mesenteric lymph (◆). SQV (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) containing either 1mM verapamil, 1mM ketoconazole, 1mM cyclosporin or 1mM verapamil plus 1mM ketoconazole in combination

In section 10.2.5, the peak lymph concentration of saquinavir in lymph was 10-fold higher than the corresponding peak plasma concentration following administration in a cremophor mixed micellar formulation. For the formulations containing P-gp and CYP modulators, the differences between systemic plasma levels and mesenteric lymph concentrations are more pronounced (Table 11.2).

Table 11.2 Comparison of peak saquinavir concentrations in lymph and plasma for each formulation in lymph cannulated rats.

Formulation	$Cmax_{, plasma}$ (ng/ml)	$Cmax_{, lymph}$ (ng/ml)	Relative enhancement [A]
SQV alone	73.82 ± 48.88	729.86 ± 209.39	9.89
SQV + Verapamil	73.76 ± 7.71	4967.87 ± 1455.71	67.4
SQV + Ketoconazole	130.64 ± 58.79	9152.59 ± 4841.30	70.1
SQV + Cyclosporin	306.74 ± 42.26	4885.26 ± 1269.85	15.92
SQV + Ketoconazole + Verapamil	299.56 ± 35.67	6989.55 ± 1702.80	23.3

[A] ($Cmax_{, lymph}$/ $Cmax_{, plasma}$)

11.2.5. Saquinavir plasma concentrations in lymph cannulated and non-lymph cannulated rats

The plasma concentrations of saquinavir in lymph-cannulated and nonlymph-cannulated rats following administration of the cremophor mixed micelle formulation containing 5mg saquinavir, with and without a series of modulators, is presented in Figure 11.9. This data must be viewed with the understanding that for lymph cannulated animals, systemic plasma concentrations do not reflect contributions from saquinavir lymphatic transport, (as mesenteric lymph was collected), whereas plasma levels in the non-lymph cannulated (lymph intact) rats result from saquinavir absorption directly into the blood and indirectly into the blood via the lymph.

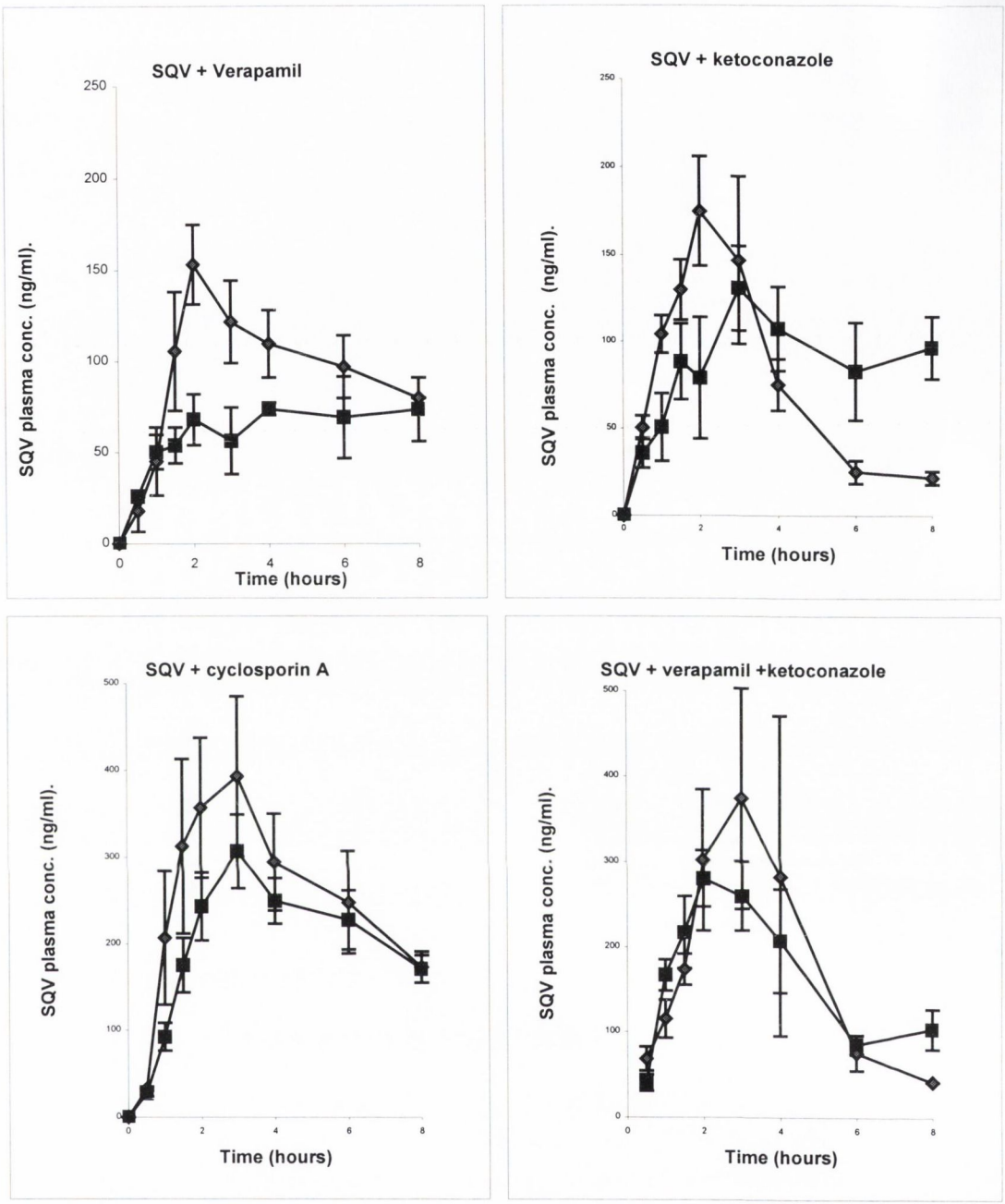


Figure 11.9 Plasma concentration – time profiles of saquinavir (mean ± SE, n≥5) in non-lymph cannulated (◆) and lymph cannulated rats (■). SQV (5mg) was administered intraduodenally as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) containing either 1mM verapamil, 1mM ketoconazole, 1mM cyclosporin or 1mM verapamil plus 1mM ketoconazole in combination

In the present study, administration of a series of formulation containing P-gp/CYP modulators increases the plasma levels of saquinavir both in lymph cannulated and non-

lymph cannulated rats. The relevant pharmacokinetic parameters for each formulation in each experimental group are presented in Table 11.3. The rank order in the relative enhancement in the $AUC_{0\rightarrow 8h}$ produced by inclusion of P-gp/CYP modulators in non-lymph cannulated rats, compared to control (i.e. no modulator) is as follows:

Cyclosporin (5.2-fold)>ketoconazole/verapamil (2.3-fold)
> verapamil (1.6-fold) > ketoconazole (1.3-fold)

For the lymph cannulated rats the rank order of relative enhancement in the $AUC_{0\rightarrow 8h}$ versus control (i.e. SQV alone) is as follows:

Cyclosporin (6.7-fold) > ketoconazole/verapamil (5.8-fold)
> ketoconazole (4.0-fold) > verapamil (2.7-fold)

Table 11.3 A comparison of the pharmacokinetic parameters of SQV in cannulated and non-cannulated rats after intraduodenal administration of each formulation

Formulation	**Lymph cannulated**	**Non-lymph cannulated**
	$AUC_{0\rightarrow 8h}$ (ng.h.ml^{-1}) ± S.E	
SQV alone	155.41±21.22	375.79±79.40
SQV + Verapamil	426.21 ± 48.15	616.58 ± 123.89
SQV + Ketoconazole	627.55 ± 143.46	480.63 ± 38.59
SQV + Ketoconazole + Verapamil	911.60 ± 214.28	859.64 ± 214.28 [A]
SQV + Cyclosporin	1050.43 ± 108.58	1955.61 ± 389.08 [A]
	Cmax (ng/ml) ± S.E	
SQV alone	73.82 ± 48.88	96.03 ± 19.40
SQV + Verapamil	73.76 ± 7.71	153.04 ± 21.85
SQV + Ketoconazole	130.64 ± 58.79	174.63 ± 31.11
SQV + Ketoconazole + Verapamil	299.56 ± 35.67	374.32 ± 128.68 [A]
SQV + Cyclosporin	306.74 ± 42.26	392.59 ± 92.09 [A]

[A] *Statistically significant higher ($p < 0.05$) than SQV alone (i.e. no modulator)*

The data presented in Table 11.3 confirm an increased systemic absorption of saquinavir following administration of the P-gp/CYP modulators used in this study. The formulation containing cyclosporin and verapamil/ketoconazole combined produced statistically significant ($p<0.05$) increases in the $AUC_{0\rightarrow 8h}$ and peak plasma concentration in non-lymph cannulated animals. The increased plasma concentrations induced by concomitant administration of P-gp/CYP modulators most likely reflect inhibition of intestinal P-gp and CYP3A4 resulting in increased portal absorption. It is also possible, that the cyclosporin formulation and the verapamil/ketoconazole formulation exert a more potent influence on hepatic metabolising enzymes. In section 11.2.1 an increased extent of lymphatic transport induced by these modulators was observed; however, the extent of increase is unlikely to be a major determinant of systemic bioavailability. A comparison between lymph-cannulated and non-lymph cannulated pharmacokinetic data in Table 11.3 potentially confirms this; while there is a general trend towards higher peak plasma concentrations (Cmax) in non-lymph cannulated rats, the differences in $AUC_{0\rightarrow 8h\ between}$ lymph-cannulated and non-lymph cannulated groups were not statistically significant.

11.2.6. Total saquinavir availability in lymph-cannulated and bioavailability in nonlymph cannulated animals

In lymph-cannulated animals, total saquinavir availability was defined as the mass transported directly into the lymph plus the proportion of the dose absorbed via the blood into the systemic circulation (relative to an i.v. control). Saquinavir bioavailability was also estimated in non-lymph cannulated rats using a standard i.v/oral bioavailability study design and by sampling systemic blood only. The oral bioavailability calculated in this way is compared with the total availability estimated from lymph plus blood in lymph cannulated animals in Table 11.4.

Comparing the estimated bioavailability values in non lymph cannulated rats, co-administration of cyclosporin and verapamil/ketoconazole combined produced statistically significant 4.1- and 2.7-fold increases in systemic bioavailability versus control (i.e. SQV alone), respectively. The overall contribution of lymphatic transport to systemic bioavailability was 0.4% and 0.7% of the dose for these two formulations, respectively.

Table 11.4 Comparison of saquinavir availability (mean % dose ± SE, n≥5) in lymph-cannulated and saquinavir bioavailability in non-lymph cannulated rats for each formulation.

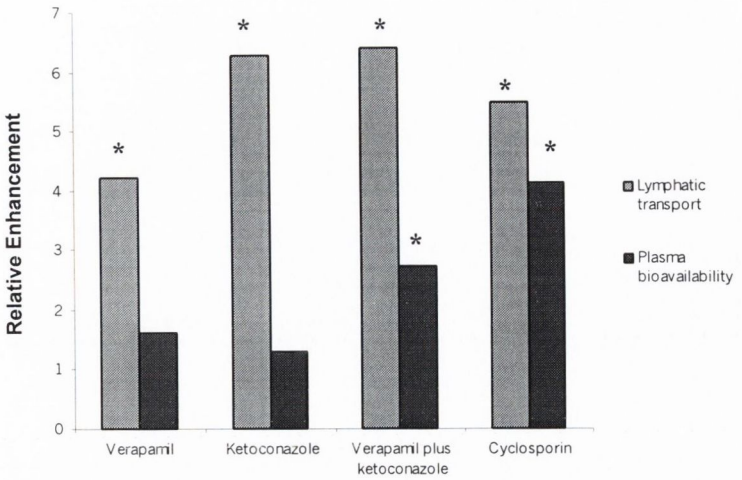
Formulation	Lymph-cannulated Rats			Nonlymph-cannulated Rats
	Lymphatic Transport[A]	Plasma Availability[B]	Total (Lymph + Blood) Availability	Plasma Bioavailability[C]
SQV alone	0.027 ± 0.005	4.98 ± 1.30	5.00 ± 1.30	8.50 ± 1.71
SQV + Verapamil	0.114 ± 0.030	8.96 ± 1.17	9.07 ± 1.18	13.82 ± 2.29
SQV + ketoconazole	0.169 ± 0.049	11.89 ± 2.64	12.06 ± 2.67	10.92 ± 2.94
SQV + verapamil + ketoconazole	0.173 ± 0.034	22.81 ± 3.70	22.98 ± 3.72	23.21[D] ± 7.38
SQV + cyclosporin	0.148 ± 0.021	23.00 ± 2.16	23.15 ± 2.17	34.97[D] ± 5.86

[A] *Cumulative mass of SQV recovered over 8 hours in mesenteric lymph calculated as a percentage of dose*

[B] *The percentage dose of saquinavir absorbed directly into the blood (i.e. non-lymphatic absorption) was estimated based on the plasma* $AUC_{0\rightarrow\infty h}$ *, relative to an i.v. control* $(AUC_{oral}/D_{oral})/AUC_{i.v.}/D_{i.v.})$

[C] *Bioavailability calculated as* $(AUC^{oral}{}_{0\rightarrow\infty h}/D_{oral}) / (AUC^{i.v.}{}_{0\rightarrow\infty h}/D_{i.v.})$

[D] *Statistically significant difference* ($p < 0.05$) *in SQV bioavailability compared to SQV alone*



* significantly increased ($p<0.05$) versus control (i.e. SQV alone)

Figure 11.10 The relative enhancements in lymphatic transport (%dose administered) and plasma bioavailability (AUC^{oral}/D_{oral}) / ($AUC^{i.v}/D_{i.v}$) of saquinavir compared to control (i.e. no modulator). Saquinavir (5mg) was administered in a mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) alone (i.e. control) or containing either 1mM verapamil, 1mM ketoconazole, 1mM cyclosporin or 1mM verapamil plus 1mM ketoconazole in combination

Figure 11.10 shows the relative enhancement in lymphatic transport and plasma bioavailability for the various formulations compared to control (i.e. SQV alone). There is a greater relative enhancement in the extent of lymphatic transport compared to the corresponding relative enhancement in plasma bioavailability, for the formulations containing verapamil, ketoconazole and verapamil with ketoconazole. This can be further demonstrated by examining the extent of lymphatic transport as a percentage of total bioavailability obtained for each formulation in non-lymph cannulated rats. For the micellar formulation containing saquinavir alone, the overall contribution of the lymphatic route to the total bioavailability was 0.32%. Following co-administration of either verapamil alone or ketoconazole alone, the lymphatic contribution increases to 0.824% and 1.55% of the bioavailable dose, respectively. Hence, at the concentrations studied, the effects of these modulators on P-gp and/or CYP produce greater relative increases on lymphatic transport than portal absorption and in this way indicate a degree of preferential targeting to the lymphatic system.

11.3. Discussion

The extent of intestinal lymphatic transport of saquinavir (as % dose), as determined in Chapter 10, was deemed to be low, considering the highly lipophilic nature of the compound. It was postulated that susceptibility to the P-gp/CYP counter transport system may limit the extent of intestinal lymphatic transport of saquinavir. This study was designed to test this hypothesis and to determine whether pharmacological modulation of P-gp and or CYP activity would alter the distribution of saquinavir into the lymphatics. The pathological progression of HIV infection and AIDS suggests that therapeutic benefit may be gained by redirecting a proportion of the dose of compounds with significant anti-HIV activity, such as saquinavir, to the lymphatic system and the lymphoid tissue in general. Recent data has identified lymphoid tissue as a critical repository and conduit for the human immunodeficiency virus during infection and the progression of AIDS (Pantaleo et al., 1991, 1993). The majority of lymphocytes (the target for HIV) populate the lymphoid tissue, only about 2% being resident in peripheral blood (Westermann & Pabst, 1990). Of these peripheral lymphocytes, only about 1% are infected throughout the course of an HIV infection. Redirection of the absorption pathway of orally administered anti-HIV compounds from the portal blood to the HIV rich intestinal lymphatics may therefore significantly enhance therapy against HIV.

The intestinal lymphatic transport of saquinavir co-administered with a series of P-gp/CYP modulators in cremophor mixed micellar formulations was studied in the mesenteric lymph duct cannulated anaesthetized model. This is the first reported mechanistic study of its kind and the utility of this animal model, providing for sampling of both lymph and plasma and facilitating the co-administration of two or more compounds intraduodenally, has thus been demonstrated.

Co-administration of P-gp/CYP modulators significantly increased the extent of intestinal lymphatic transport. Hence, it is apparent that the P-gp/CYP intestinal counter transport mechanism acts to markedly restrict the transport of saquinavir through the intestinal lymphatic route. The increase in the total fraction of the administered dose recovered in mesenteric lymph compared to control (i.e. saquinavir administered in cremophor micelles alone) were between 4.2- and 6.4-fold, and were all statistically significant ($p<0.05$). For the three modulators co-administered with saquinavir in this study, the rank order in the

cumulative extent of intestinal lymphatic transport over 8 hours (i.e. potency) was as follows:

Ketoconazole > Cyclosporin > verapamil

The combined administration of ketoconazole and verapamil produced a marginal increase in the extent of lymphatic transport, compared to either agent alone. Whereas it may be postulated that verapamil acts almost exclusively on P-gp, and ketoconazole acts preferentially on CYP3A4, the combined formulation might have been expected to produce a greater increase in the intestinal lymphatic transport. This result suggests that the respective effects of verapamil and ketoconazole are not additive at the concentration of modulator used in this study. Higher concentrations of modulator would be required to prove whether this effect is related to a maximal lymphatic transporting capacity, or due to overlapping and competing structural specificities to P-glycoprotein and CYP3A4. The differences between the four formulations containing modulators were not statistically significant. These results confirm that P-gp and or CYP elimination processes significantly reduce the intestinal lymphatic concentrations of saquinavir, although it is unclear therefore whether the P-gp effect or the CYP effect is a more crucial determinant of intestinal lymphatic concentrations.

Peak concentrations in lymph are 67- and 70-fold higher than plasma peak concentrations for the formulations containing either verapamil or ketoconazole, respectively. Peak lymph concentrations are 15- and 23-fold higher than plasma peak concentrations for the cyclosporin and combined verapamil plus ketoconazole formulations. This may suggest that at lower doses of modulator, the increase in lymphatic transport may be more prevalent (i.e. a significant increase in lymphatic transport is observed with no comparable increase in systemic plasma levels); whereas at higher doses, and/or the use of more potent inhibitors, which have a more marked effect on portal absorption and/or hepatic metabolism, the increase in transport via the lymphatic route may not be as evident. A comparison of the relative enhancement of lymphatic transport and plasma bioavailability compared to control (i.e. SQV alone) confirms a greater effect for verapamil or ketoconazole on the amount of drug transported by the lymphatic route (Figure 11.10). These findings suggest that selective increase in intestinal lymphatic levels of saquinavir can be achieved by targeted pharmacological inhibition of P-gp and/or CYP.

Although the mechanisms by which lipophilic drugs gain access to the intestinal lymphatics via the enterocytes are not fully elucidated, the majority of lymphatically transported drugs are associated with the triglyceride core of the chylomicron. In the present study, the cremophor mixed micellar formulation acts as a lipid source to 'drive' intestinal lipoprotein synthesis and hence increase triglyceride uptake by enterocytes. There is a relatively good correlation between the lymphatic triglyceride turnover and the rate saquinavir transport in the intestinal lymph, and so it is reasonable to assume saquinavir is transported in association with lymph triglyceride. An unexpected finding of this study was that administration of cyclosporin and the combined verapamil/ketoconazole formulations produced a statistically significant reduction in the total extent of triglyceride recovered in intestinal lymph after 8 hours. The triglyceride turnover appears to be unaffected by the administration of either verapamil or ketoconazole alone, at the concentrations used in this study. While it is unclear how these effects were manifested, the therapeutic ramifications of a reduced triglyceride turnover are considerable.

All of the formulations containing modulators produced significant increases in the apparent loading of saquinavir per mg of lymph triglyceride, compared to control (i.e. SQV alone). Furthermore, the formulations containing cyclosporin or verapamil plus ketoconazole combined, produced higher apparent loadings per mg of lymph triglyceride, than the formulations containing verapamil or ketoconazole alone. This effect appears to compensate for a lower triglyceride turnover observed for the former two formulations, i.e. despite a reduction in intestinal triglyceride transport, the rate of drug transfer to triglyceride is higher. The higher apparent loadings obtained suggest that further increases in the extent of lymphatic transport may be possible e.g. by using higher concentrations of modulator, or by more appropriate selection for modulators which do not reduce triglyceride turnover.

Cuvelier et al., (1998) examined the absorption mechanisms of saquinavir in rat intestinal loops. The $AUC_{0\rightarrow 1h}$ determined from sampling portal plasma, over 60 minutes, following co-administration of cyclosporin (i.v.), ketoconazole and ritonavir, were 4.3, 3.9 and 7.1-fold higher, respectively, than with saquinavir alone. By comparison in the present study saquinavir systemic bioavailability is significantly increased 4.1-fold in following co-administration of 1mM cyclosporin. The lack of a significant effect for ketoconazole on saquinavir levels in the present study suggest that a higher dose of ketoconazole is required

to produce a significant increase in systemic plasma levels in the rat. A threefold increase in bioavailability has been reported for saquinavir and ketoconazole in humans (Noble & Faulds, 1996).

12. General Discussion

The advent of combinatorial chemistry and high throughput screening has resulted in the rapid identification of many highly potent new chemical entities. Coincident with the increasing use of these technologies, however, has been a developing trend towards the identification of lead compounds with higher molecular weights and log P values and lower aqueous solubilities. Whilst these attributes conspire to provide optimised drug-receptor binding characteristics, they also tend to result in poor drug dissolution (high log P) and poor membrane permeability characteristics (high Mw). Formulation of these highly lipophilic drugs thus presents considerable challenges to the pharmaceutical scientist.

The co-administration of lipid-based delivery systems and/or the presence of food often result in increased oral absorption of lipophilic drugs. These effects are traditionally ascribed to improved drug dissolution in the lipid rich postprandial environment, or the potential effect of co-administered lipids on membrane permeability. In some cases an increase in lymphatic transport may play a significant role in the absorption enhancement process. The main advantages of intestinal lymphatic absorption include, avoidance of first pass metabolism, and the potential for site-specific delivery of therapeutic compounds to the lymphatics.

Lymphatic transport of a range of drugs including cyclosporin, mepitiostane, probucol, halofantrine and a range of lipophilic prodrugs, has been investigated with varying degrees of success (Porter & Charman, 1997). While all the factors influencing lymphatic transport have still to be elucidated it is known that the physiochemical properties of the drug, the interaction of the drug with the physiochemical milieu in the intestinal lumen during lipid absorption, and the chemical nature of any co-administered vehicle are crucial. To date most of the work on lymphatic transport has been done using rat models and the maximum percentage transported in the lymph, on co-administration of various lipid vehicles, tends to be limited to approximately 15-20% of the administered dose (Porter & Charman, 1997). However recent data in a fed versus fasted dog model using the lipophilic drug halofantrine, showed much higher levels (54%) of lymphatic transport in the fed state (Khoo et al., 1999). These studies, in the more physiologically relevant dog model have indicated a greater potential for this route of transport than was previously

thought possible and, coupled with the increasing trend towards highly potent, lipophilic drug candidates, has resulted in a renewed interest in this research field.

This project aimed at investigating the effects of a range of lipid vehicles on the intestinal lymphatic transport, and mechanisms thereof, for two model lipophilic compounds, DDT and saquinavir, with a view to evaluating the ideal characteristics of a lymphotropic lipid formulation.

12.1. Solubilization of lipophilic compounds

In the small intestine, co-administration of food and/or lipids, leads to the secretion of bile and digestive enzymes that produce a complex system of intestinal colloidal phases, designed to facilitate the digestion and absorption of dietary lipids. The terminal phases of this digestive mixture consist of mixed micelles of bile salts and various lipids. The elevated levels of bile salt and dietary lipids mixed micelles can improve the wetting of poorly soluble drugs and increase their effective solubility via solubilization. Since a drug must be soluble in the aqueous environment of the gastrointestinal tract in order to be absorbed, the effect of the micellar systems on the solubility of DDT and saquinavir was first determined. The range of lipidic vehicles included simple and mixed bile salt, cremophor EL and TPGS micelles. The solubilising capacity of mixed micelles is significantly higher than that of simple micelles for both lipophilic compounds. Increasing the fatty acid content of mixed micellar systems resulted in significant increases in both solubilizing capacity and also increased micelle size.

The enhancement in solubility by the presence of 40mM oleic acid in bile salt micelles is far greater than the solubility enhancement observed for simple micelles compared to buffer. This suggests that the presence of lipolytic end products may be a more crucial determinant of the solubilising capacity of the intestinal milieu, than the concentrations of bile salts obtained in the intestinal post prandial environment. However, this affect is highly dependent on the type of fatty acid. In the present study, the long chain fatty acid, oleic acid serves to increase the solubilising capacity in all the mixed micellar systems. A decrease in solubility of DDT with increasing caprylic acid content was observed in 40mM NaC bile salt micelles (Obodozie, 1997). This effect was not merely a function of the solubility of DDT in the free fatty acid as the solubility of DDT is greater in caprylic (297.97 ± 3.25 mg/ml) than in oleic acid (197.74 ± 2.32mg/ml) (Obodozie, 1997).

O'Reilly (1991) noted that the saturation solubility of the lipophilic compound, clofazimine, in a micellar solution of cremophor decreased with increasing concentration of caprylic acid. That the medium chain fatty acid mixed micelles did not enhance the solubility of DDT or clofazimine may imply that medium chain fatty acid mixed micelles have a poorer capacity to solubilise, non-polar, poorly soluble compounds, than their corresponding long chain fatty acid systems. Similarly, Takahi & Underwood (1974) found that the aqueous solubilization of α-tocopherol was 3 to 7 fold less when lipid of medium chain length was incorporated into a bile salt micellar system, than a corresponding long-chain mixture. Further studies are therefore warranted to compare the solubilising power of medium chain versus long chain fatty acid mixed micelles for highly lipophilic compounds (log P > 4).

The simple and mixed micelles formed from the synthetic surfactants, cremophor EL and TPGS, exhibit greater solubilizing capacity for both lipophilic compounds than the corresponding NaC bile salt micellar systems. This effect may be related to the larger micelle size of synthetic surfactants micelles compared to the bile salt micelles. Interestingly, the solubilization mechanisms for both lipophilic compounds in naturally occurring and synthetic surfactant micelles appear to differ. Lipid based formulations, formed with synthetic surfactants such as these, may therefore serve to supplement/enhance the solubilizing action of the naturally secreted bile salts following administration. For example the theoretical dose to solubility ratio of saquinavir is reduced from 34L in buffer pH 7.2 to 1.68L in 40:40mM NaC: OA bile salt mixed micelles. Dressman & Reppas, (2000) suggested that a dose/solubility ratio > 0.25L indicates that conditions in the gastrointestinal tract are less than optimal for drug dissolution, since it is probable that sink conditions do not prevail. Cremophor EL: OA mixed micelles produce a far greater reduction in this theoretical volume to 0.09L.

Furthermore, appropriately designed lipid based formulations may act to reduce a potential fed versus fasted effect. The present study illustrates the extent to which increased fatty acid content (i.e. equivalent to ingesting a lipid rich meal) can drastically increase the solubilization capacity of bile salt micelles, producing between 5- to 20 - fold increases in the saturation solubility of saquinavir, analogous to the 4-fold reduction in the volume of gastrointestinal fluids necessary to dissolve the administered dose (i.e. D/S ratio). Hence in the absence of co-administered food, the lipids and surfactants in a lipid-based delivery systems may serve to increase the solubilizing capacity of the intestinal milieu, thereby

reducing a potential food effect. Hence, these two beneficial features, namely, the ability of the surfactants present to supplement/enhance the action of naturally secreted bile salts, and the capacity of lipids present in the formulation to maintain the co-administered drug in solution in the gastrointestinal milieu, are important characteristics of appropriately designed lipid based formulations. In keeping with these hypotheses is the observation that plasma cyclosporin levels following administration of 'Neoral®' have been shown to be less dependent on both bile levels in the intestine and the presence of food, than the original 'Sandimmune®' formulation (Mueller et al., 1994; Trull et al., 1995; Kahan et al., 1995).

The relationship between the extent of solubilization and the lipophilicity of the compound was examined for four lipophilic compounds studied under the same experimental conditions in our laboratories (Section 7.3). Generally high S/So correlated with high apparent log P, for all micellar systems. Apparent relationships such as these may be used to approximate the increase in solubility as a function of bile salt: fatty acid concentration and may be used to predict which drugs will exhibit fed versus fasted differences in drug absorption (Mithani et al., 1996). In addition to partitioning behaviour, other factors may be important in determining the extent of solubilization, as evidenced by the lower than predicted solubility of saquinavir. The molecular size and shape of the drug can come into play, because the volume available to the drug in the micelle is limited, especially in the case of simple bile salt micelles. Saquinavir, with a Mw of 670, is the highest of the four compounds studied, compared to Mw's of 300, 354 and 472 for KME-4, DDT and clofazimine, respectively, which may explain, in part, its lower than predicted solubility. Specific interactions may also be important. For example, Fahelebom et al., (1993) have investigated the relationship between log P and Log Km (the distribution coefficient between the micellar and aqueous phase) for a series of rimino phenazines (n=6) in sodium dodecyl sulphate (non-ionic) and triton-X-100 (ionic). The relationship is reasonably linear for five of the compounds; however the point for clofazimine was above the trends line with both surfactants. The authors suggested that clofazimine was not solubilized in the same region of these synthetic micelles as the other compounds. While the solubility enhancement for clofazimine in bile salt simple and mixed micelles appears to fit the trends observed in Figure 7.7, the limitation of such predictions, due to the limited amount of data collected to date in the present study, should be recognised.

12.2. Intestinal lymphatic transport from lipid formulations

The effects of a range of lipid systems on DDT and saquinavir intestinal lymphatic absorption was investigated, with a view to elaborating potential dosage form strategies for increasing intestinal lymphatic drug transport. Obodozie (1997) reported that the calculated blood appearance rates following administration of DDT in bile salt micellar systems in a through and through rat gut perfusion model exhibit significant differences between simple and mixed micellar formulations. An estimated 4-fold higher extent of diversion of DDT into the blood for simple micelles than for mixed micellar systems was observed. It was postulated that the lower blood appearance rate was due to a formulation-induced diversion of DDT into the lymphatics by the mixed micellar system. The predicted difference in blood and lymph transfer is proven reasonably accurate, as our results confirm a 3.6-fold higher degree of DDT transported by the lymphatic route after 6 hours between mixed and simple bile salt micelles. Taken together, the results from these two studies reveal how changes in formulation can significantly affect the partitioning of DDT between lymph and blood (i.e. simple micelles producing higher portal absorption and mixed micelles promoting a higher extent of transport via the lymphatic route). Ichihashi et al., (1992) have similarly reported how formulation changes can affect the intrinsic lymphatic partition rate (ILPR, calculated as $[((Dose_{lymph}/(Dose_{lymph} + Dose_{blood}) * 100\%)]$ of mepitiostane (MP), by performing an experiment where both thoracic lymph and portal blood are sampled. The ILPR of MP was significantly higher following administration in sesame oil than in a suspension formulation.

For the three simple and mixed micellar systems, formulation in a mixed micellar state increased the intestinal lymphatic transport over that obtained for the corresponding simple micellar system. These increases in the cumulative extent of dose administered ranged from 3.6-fold for the NaC bile salt (over 6 hr), to 1.8-fold for cremophor (over 8hr) and to 4.6-fold (over 8hr) for the TPGS mixed micellar systems, relative to simple micelles. The rank order in the observed maximal lymphatic transport over 6 hours in mixed micelles was:

TPGS > NaC > Cremophor

Our results suggest that the inclusion of long chain lipids to form a mixed micellar state is crucial for optimal intestinal lymphatic uptake. However, it has been reported that appreciable lymphatic transport may occur in the absence of co-administered lipid. For

example, a 2-fold enhancement in the lymphatic transport of retinyl palmitate was reported after administration of an aqueous polysorbate 80 micellar solution compared with an emulsion of lipid solution (Nishigaki et al., 1976). The intestinal lymphatic transport of cyclosporin has been enhanced up to 5-10 fold by simple micellar solutions in comparison to mixed micellar systems and lipid solutions (Takada et al., 1986), leading the authors to propose that the surfactant, HCO 60, in these simple micelles exhibits a 'selective lymphatic transporting ability' (Takada et al., 1988). These studies suggest that drug transport into the mesenteric lymphatics may be facilitated by formulation in the absence of exogenous lipid. However, it is important to note that while lymph transport has been reported in the absence of co-administered lipid, the administered formulations have often contained relatively high concentrations of surfactants (e.g. 8% solutions) and the effect of these compounds on membrane integrity and enterocyte functionality has generally not been addressed. In support of this is the finding that a 2% cremophor simple micellar increased the endogenous lymphatic lipid turnover in our study. Furthermore, in house data suggests that Tween 80 increases the lipoprotein production in a modified intestinal Caco-2 cell line (Seeballuck et al., 2001 unpublished).

The extent of lymphatic transport following administration of DDT in simple micellar systems in this study was 5-9% of the administered dose. For the TPGS and bile salt simple micelles, the lipid turnover remained at basal/endogenous levels, and so it is assumed DDT lymphatic transport occurs by drug partitioning into endogenous lipid turnover pathways. A further example of transport in the absence of co-administered lipid exists with the examination of halofantrine hydrochloride (Hf.HCL) lymphatic transport (Porter et al., 1996 a & b). The extent of intestinal lymphatic transport of Hf.HCL was 4-5% of dose, and was unaffected by co-administration of exogenous lipid. The proportion of Hf recovered from the chylomicron fraction of lymph was significantly higher when administered with exogenous lipid compared with the suspension formulation (86% vs. 68%), and there was a corresponding decrease in the proportion of Hf transported by VLDL fraction (12% vs. 26%). Chylomicrons are primarily responsible for transport of exogenous lipid via the mesenteric lymph and VLDL play a larger role in the lymphatic transport of endogenous lipids (Tso et al., 1987). Thus, in the absence of co-administered lipid, lymphatic drug transport may occur by partitioning of drug into endogenous lipid turnover pathways resulting in transport primarily via association with VLDL. However, the limited turnover of lipid via the VLDL pathway in the fasted state may limit the potential capacity of this pathway. There are three potential mechanisms whereby these

simple micellar systems may be exerting this effect: (i) an overall enhanced drug absorption occurring as a result of efficient solubilization in the surfactant micelles (ii) enhanced enterocyte drug concentrations facilitated by the surfactants acting to increase membrane permeability or (iii) partitioning of drugs into lymph lipoproteins synthesised from endogenous lipids (Porter & Charman, 2001). The extent of transport for the simple NaC and TPGS micellar systems was low but similar, at 4.94 and 5.22% of the administered dose, respectively. This potentially confirms the limited capacity of intestinal lymphatic transport, in the absence of exogenously administered fatty acid material, analogous to administration in the fasted state.

The results presented in Chapter 9 & 10 confirm the potential for SEDDS to promote the lymphatic absorption of lipophilic molecules. The extent of DDT and saquinavir transported by the intestinal lymph is not statistically dissimilar to that obtained with the mixed micelles. The maximal rate of transfer is more delayed than the mixed micellar formulation (Figure 9.11 & 10.6). This possibly reflects the delay involved in converting from a microemulsion phase to a pre-absorptive phase represented by the bile salt mixed micelles. By comparison lymphatic transport from the oleic acid dispersion is poor, more prolonged with a delayed peak transport and incomplete absorption after 8 hours. Dispersion of lipid droplets into an emulsion of high surface area is an essential step in the efficient intestinal absorption of lipids.

Porter et al., (1996a) observed significant formulation-related differences in the extent of lymphatic transport of halofantrine which reflected the extent of lipid dispersion in the formulation i.e. the rank order of the vehicles for the promotion of lymphatic transport was micellar (17.7%) > emulsion (11.8%) > lipid solution (4 to 10%). A similar rank order is observed in the present experiment by comparing the extent of lymphatic transport from micellar formulation (Chapter 8) with the transport from the SEDDS and oleic acid dispersion (Chapter 9). Porter et al., (1996a) observed a statistically significantly higher extent of transport between the micellar vehicle and the emulsion formulation. In the present study there was no significant difference in the extent of lymphatic transport of DDT, between the mixed micelle formulations and the SEDDS formulation. The conclusions from both studies suggest that the formation of a micellar state is crucial for optimal lymphatic transport. However, in our study formulation as a readily dispersible SEDDS produced comparable transport, while also offering advantages in terms of relative

ease of formation, compared with the prolonged stirring and/or sonication required for formulation of micellar vehicles.

The findings of Chapter 10, confirm the potential advantages of a SEDDS formulation, over mixed micellar formulations. The extent of saquinavir lymphatic transport after 8 hours was two fold higher than from mixed micellar formulations, however these differences were not statistically significant due to extensive variability observed for the saquinavir data. This study also revealed potential advantages in terms of targeted intestinal lymphatic delivery of saquinavir, with the SEDDS formulation resulting in significantly higher and more sustained concentrations in the mesenteric lymph, as depicted in Figure 10.15.

12.3. Intestinal lymphatic transport of triglycerides

Intestinal lymphatic transport of lipophilic compounds has been shown to occur in association with re-synthesized long chain lipids in the core of the lymph lipoproteins. For all the lipid formulations there was a strong positive correlation between the DDT transport rate (μg/hr) and the triglyceride turnover (mg/hr) (Figure 8.11 and Figure 9.9). It was noticeable, in our study that the trends observed in the extent of DDT lymphatic transport after 6 hours, for all the lipid formulations described in chapter 8 and 9, closely resembled the overall extent of triglyceride turnover observed for each formulation after 6 hours. This led us to examine the relationship between total DDT lymphatic transport and lymph C_{18} triglyceride transport for the 9 lipid vehicles examined in this study with DDT (Figure 12.1). A strong positive correlation exists (correlation coefficient = 0.90). This result confirms that absorption and processing of formulation lipid through the enterocytes leads to an increase in lymphatic transport of DDT. Hence an important characteristic of an ideal lymphotropic lipid formulation is the ability to stimulate a maximal extent of lymphatic lipid turnover.

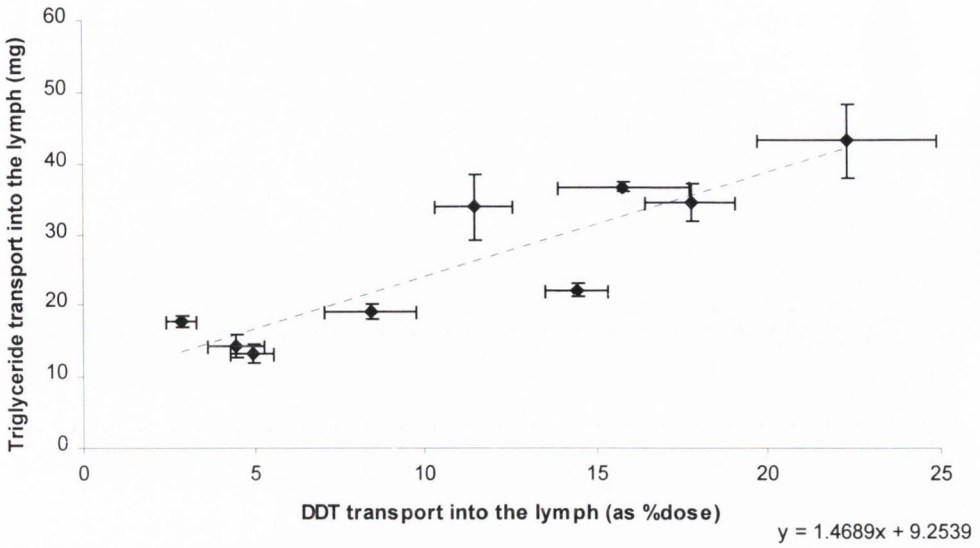


Figure 12.1 Correlation between cumulative mass of lymph C_{18} triglyceride lipid (mean ± SE; n≥5) and DDT lymphatic transport (mean % dose ± SE; n≥5) after 6 hours, after intraduodenal administration of a series of lipid formulations (correlation coefficient =0.90)

It is important to point out however that the above relationship does not automatically suggest that increasing the amount of exogenously administered lipid will result in increases in the % dose transported by the lymph. Clearly, the efficiency of lymph triglyceride turnover and the apparent loading of drug per mg of lymph triglyceride are important features. For example, comparing the SEDDS and oleic acid dispersion, both formulations contained similar amounts of total lipid per dose, but the amount of C_{18} lipid is greater in the oleic acid dispersion. The total amount of triglyceride collected after 8 hours in the lymph was similar for both formulations (27.58 ± 2.17mg for the oleic acid emulsion vs. 27.85 ± 0.51mg for SEDDS). However the SEDDS formulations produced a greater extent of DDT lymphatic transport. The rate of triglyceride turnover is improved following administration of the SEDDS as evidenced by the steady increase in triglyceride turnover to a peak lipid turnover of 4.88 ± 0.30mg/hr occurring between 4-5 hours, and a return to endogenous levels at 8 hours. By comparison, the triglyceride turnover remains at basal levels for 4 hours after administration of the oleic acid emulsion, followed by a peak lipid turnover of 5.33 ± 1.35mg/hr between 5 – 6hours and levels are elevated at the end of the experiment (Figure 9.8). The SEDDS formulations appears to induce a more efficient triglyceride turnover pattern which in turn facilitates DDT transfer from the

intestinal lumen to the intestinal lipoproteins. Alternatively, as has been suggested by Hauss et al., (1998), the SEDDS formulation may promote rapid absorption of the drug, resulting in higher concentrations within the enterocyte during absorption and hence, may simultaneously improve lymphatic drug transport by a concentration-partitioning process.

Likewise, the NaC: OA bile salt mixed micelles produce a significantly higher extent of lymphatic transport, after 6 hours, than the bile salt: phospholipid mixed micelles, despite similar extents of triglyceride turnover and a higher long chain fatty acid loading in the NaC: PL mixed micelles. Therefore, is appears that formulation in the free fatty acid form facilitates a more efficient processing of administered lipid, into lymph lipoproteins, which appears to improve drug transfer to the lymph, as evidenced by the higher apparent loadings and extent of transport. These findings are similar to the study by Charman et al., (1986a), where it was postulated that a fatty acid vehicle, which can be presumably processed towards chylomicron formation at a faster rate than a triglyceride vehicle, is able to maintain a higher drug loading in lymph lipid than the triglyceride vehicle.

In light of the data presented here, the relationship between lymphatic drug transport and triglyceride turnover can be summarised as follows. Lipid formulations that promote triglyceride turnover appear to increase the extent of lymphatic drug transport as evidenced by the linear relationship between DDT transport and triglyceride transport in lymph (Figure 12.1). Clearly, efficiency with which a formulation promotes lymphatic triglyceride transport also appears to play a role in the partitioning of absorbed drug into lymph. Portal blood flow acts as an absorption sink, competing with the intestinal lymph for the absorption of DDT. The portal blood flow is approximately 500 times greater than the flow of intestinal lymph (Bollman et al., 1948; Reininger & Saperstein, 1957). Therefore, the longer that lipid and associated drug remain in the lumen of the small intestine, the less will be the relative concentration of the drug per unit of lipid and potentially lower quantity of drug transport in the intestinal lymphatic system.

The reason for the higher apparent loading of both DDT and saquinavir per mg of triglyceride in the SEDDS formulation over that observed for the mixed micellar systems is less clear. The differing lipid components in the SEDDS and the mixed micelles hinder a direct comparison of the efficiencies of exogenous triglyceride transport between mixed micelles and SEDDS. The surfactant blend in the SEDDS increases the lymphatic turnover of triglyceride. However, whether this is due to an increase in endogenous

triglyceride turnover (e.g. through displacing endogenous lipids in biological membranes) or to digestion of the surfactants themselves is unclear. The total extent of triglyceride turnover after 8 hours is lower for the SEDDS formulation, than the mixed micellar systems. Therefore the most likely mechanisms whereby SEDDS promote a higher apparent loading, and similar extents of lymphatic transport for both DDT and saquinavir, is through promoting higher drug concentrations within the enterocyte, as suggested by Hauss et al., (1998).

The loading, as a % by weight of DDT, in the lymph triglyceride can also be quantified by dividing the recovered quantity of DDT by the amount of triglyceride transported over the duration of the experiment. The values obtained are listed in Table 12.1, and are in agreement with a report by Charman et al., (1986b) where loadings of DDT in chylomicrons was approximately 0.5-2% by weight. The solubility of DDT in triolein (a triglyceride which would be similar to that found in the core of a chylomicron) is approximately 8% by weight (Patton et al., 1984). Therefore, excluding the low loadings for the NaC and TPGS simple micelles, DDT is present at between 5.7% and 17.5% of its saturated solubility in long chain triglyceride. This is a good indication of how efficient DDT transport by chylomicron can be under relatively optimal conditions in the rat model.

Table 12.1 The cumulative %w/w of DDT in lymph triglyceride as a function of formulation.

Formulation	% w/w of DDT in lymph triglyceride after 8 hours	% of the saturation solubility in lymph triglyceride [A]
NaC Simple micelles	0.0748 ± 0.0116 [B]	0.94
NaC:OA mixed micelles	0.4579 ± 0.0487 [B]	5.72
NaC:Lecithin mixed micelles	0.5553 ± 0.0783	6.94
Cremophor simple micelles	0.8101 ± 0.1581	10.12
Cremophor:OA mixed micelles	1.3960 ± 0.1813	17.45
TPGS simple micelles	0.2126 ± 0.0418	2.65
TPGS:OA mixed micelles	0.8950 ± 0.1405	11.19
SEDDS	1.2912 ± 0.0794	16.14
Oleic acid emulsion	0.9341 ± 0.2082	11.67

[A] assume saturation solubility of DDT in triolein is 8%w/w (Patton et al., 1984)

[B] after 6 hours

Another interesting feature of the plots of the DDT transport rate (μg/hr) and the triglyceride turnover (mg/hr) (Figure 8.11 and Figure 9.9) are the negative intercepts in the regression equations. Charman et al., (1986b) reported a similar observation and suggested that this reflected the appearance of particles in the lymph (potentially chylomicrons and/or other intestinal lipoproteins), which did not contain DDT. Interestingly, the average of all the intercepts in these two plots is 1.26 mg/hr, which corresponds well with the estimated endogenous turnover estimated with a saline control experiment (i.e. 1.53mg/hr). This observed minimal or low capacity of endogenous lipid to transport DDT can be further illustrated by examining the % loadings for the NaC and TPGS simple micellar formulations (Table 12.1). For these systems the rate of triglyceride turnover is not significantly different from endogenous lipid turnover. As such, intestinal lymphatic transport of DDT reflects the portion of DDT transported to endogenous lipid, which is believed to be primarily synthesized into VLDL as opposed to chylomicrons, which have a greater role in exogenous lipid transport (Tso et al., 1987). VLDL therefore have a greater role to play in the fasted state. The lower loadings for the NaC and TPGS simple micellar systems, reflecting 0.94% and 2.66% of the saturated solubility in long chain triglyceride respectively, confirm a less efficient uptake of administered DDT by endogenous lipoproteins. This is analogous to a lower extent of lymphatic transport in the fasted state and confirms the hypothesis that absorption of co-administered of lipid by the enterocytes facilitates the transfer of drug from the intestinal lumen, into the enterocytes followed by subsequent uptake by intestinal lipoproteins.

12.4. Estimating the maximal potential for saquinavir lymphatic transport

Although the physiochemical properties of saquinavir suggested it was a potential candidate for appreciably lymphatic transport, the results of this study using a rat model showed that this was not the case. The extent of transport from a series of lipid vehicles was between 0.025 to 0.05% of the dose administered. Lymphatically transported saquinavir amounted to less than 0.3-1.3% of the bioavailable dose. Possible reasons for this will be discussed in the context of the near-optimal lymphatic transport of DDT from similar lipid vehicles. The loading, as a % by weight of saquinavir, in the lymph triglyceride following administration can be estimated by dividing the recovered quantity of saquinavir by the amount of triglyceride transported after 8 hours (Table 12.2)

Table 12.2 The %w/w of saquinavir in lymph triglyceride as a function of formulation.

Formulation	% w/w of SQV in lymph triglyceride after 8 hours	% of the saturation solubility in lymph triglyceride
Cremophor:OA mixed micelles	0.0029	1.45
TPGS:OA mixed micelles	0.0035	1.75
SEDD	0.012	6

[A] assume saturation solubility of SQV in triolein is 0.2%w/w

The loadings of DDT per mg of lymph triglyceride (Table 12.1) were 480-, 255- and 107-fold higher than the corresponding saquinavir loadings for the cremophor mixed micelles, the TPGS mixed micelles and the SEDDS formulation, respectively. DDT is approximately 40-fold more soluble in triolein than saquinavir (Table 7.1). Hence, saquinavir loadings are approximately 12-, 6.4- and 2.7-fold lower in lymph triglyceride, allowing for the difference in triolein solubility. It is also possible to predict the maximal amount of saquinavir to be transported, following administration of each formulation, from the saturation solubility of saquinavir in lymph triglyceride, the total amount of triglyceride transported and the maximum efficiency of drug transfer to intestinal lipid using DDT as a model compound (as estimated in Table 12.1). For the cremophor EL: OA mixed micellar formulation, by assuming the maximum efficiency of drug transfer to lymph triglyceride is 17% of the saturation solubility in lymph triglyceride, the solubility of saquinavir in triolein is 0.2% w/w and the extent of triglyceride turnover was 43mg (Table 10.1), this equates to a value of 14.62μg, or 0.29% of the administered dose compared to the actual value of between 0.027% (Figure 10.1). Naturally, these values have been over simplified, as there are other factors that will influence saquinavir lymphatic transport, most noteablely Log P. However, the calculations do give a rough estimate of the maximal extent of lymphatic transport that can be predicted for saquinavir, under relatively optimal conditions. Possible reasons for the lower than expected lymphatic transport of saquinavir include will be discussed as follows.

The importance of partition coefficient from a purely mass transport perspective can be readily seen if chylomicron flow to blood flow are compared, as reported by Charman & Stella (1986). Portal blood flow to intestinal lymph flow in the rat is approximately 500:1

and it can be assumed that the concentration of chylomicron lipid is ~1% of the intestinal lymph after ingestion of a long chain fatty acid (Shiau et al., 1985; Tso et al., 1982). For a drug, completely absorbed from the gastrointestinal tract, to be transported in to equal extents by the portal blood and intestinal lymph, on flow considerations alone, the molecule would require a partition coefficient of at least 50,000 in favour of chylomicron lipid (and not 1-octanol). The logarithm of this value is 4.7, which is consistent with the minimal partition coefficient values which Noguchi et al., (1985a) have suggested are required for significant lymphatic transport. An apparent relationship between the log P of a series of drug and its lymphatic transport has been reported, among others, for a series of DDT analogues (Sieber, 1976), and testosterone esters (Noguchi et al., 1985a). The calculated Log P for saquinavir is marginally below this threshold figure (i.e. clog P =4.5).

The apparent relationship between the extent of DDT transport and triglyceride transport (Figure 12.1) suggests that triolein solubility of the lipophilic candidate may be a more appropriate predictor of maximal lymphatic transport. Charman & Stella, (1986b) compared the cumulative extent of lymphatic transport of two model compounds, with similar log p values, (DDT, log P= 6.19) and HCB (Log P= 6.53). The extent of transport after 10 hours, expressed as a percentage of the administered dose varied considerably, with 33% DDT collected versus 2.3% of HCB. Interestingly the ratio between the cumulative 10hr lymphatic transport of these two compounds (% dose DDT transported/% dose HCB transported =14.6) and their solubilities in peanut oil (DDT solubility/HCB solubility - 13.0) are similar, which indicates the importance of lipid solubility in determining the ultimate lymphatic transport of drugs of high partition coefficients. The limited solubility of saquinavir in triolein, compared to DDT, (Table 7.1) was one of the contributing factors for the low extent of intestinal lymphatic transport observed.

Myers & Stella (1992) highlighted how extensive red blood cell binding may limit intestinal lymphatic transport of penclomidine, a lipophilic cytotoxic agent (log P 5.36, solubility in triolein 175mg/ml). The extent of transport was lower than expected, given these physical characteristics, with 1.5% of the administered dose being recovered in intestinal lymph after 12h. Although penclomidine displays highly lipophilic characteristics, and should readily associate with lymph triglyceride, the red blood cell affinity may cause a skewing or shift in the pseudo-equilibrium between chylomicron bound and unbound drug, by providing a sink towards which the drug is drained (i.e. red blood cells). The situation may be similar for saquinavir. In other words, for a highly

plasma protein bound drug, such as saquinavir (>98%), to undergo appreciable lymphatic transport, not only must the drug have a high affinity for chylomicrons, but it must also overcome the added affinity for plasma protein binding.

Given the susceptibility of saquinavir to the P-gp/CYP counter transport system, which has been mostly demonstrated in cell culture lines, we proposed that these efflux and/or elimination pathways may also act to reduce intestinal lymphatic transport, and tested this hypothesis by co-administering P-gp and CYP modulators. The results in Chapter 11 confirm the extent to which modulation of PGP/CYP can drastically affect the intestinal lymphatic transport of saquinavir. Table 12.3 compares the loading the saquinavir in the lymph triglyceride following administration of each formulation/modulator(s).

Table 12.3 The %w/w of saquinavir in lymph triglyceride as a function of formulation. SQV (5mg) was administered as mixed micellar vehicle (2% Cremophor: 40mM Oleic acid) alone or containing either 1mM verapamil, 1mM ketoconazole, 1mM cyclosporin or 1mM verapamil plus 1mM ketoconazole in combination

Formulation	% w/w of SQV in lymph triglyceride after 8 hours	% of the saturation solubility in lymph triglyceride
SQV alone	0.0029	1.45
SQV + verapamil	0.013	6.5
SQV + ketoconazole	0.018	9
SQV + cyclosporin	0.027	13.5
SQV + verapamil + ketoconazole	0.029	14.5

The loadings of saquinavir in intestinal triglyceride, expressed as a % of the saturation solubility in lymph triolein, range between 6.5-14.5%. These values correspond well to the range reported for the DDT lymphatic transport experiments. These results confirm that P-gp and CYP in the intestine act to severely limit the amount of saquinavir transported by the lymphatic route, and that modulation of either P-gp and/or CYP in the intestine leads to significant increases in intestinal lymphatic transport. If it is assumed that the drug is unlikely to be present at greater than 17% of its saturated solubility in lymph triglyceride in this animal model, as determined under relatively optimal conditions for DDT, the

loadings obtained in the presence cyclosporin and the combined ketoconazole plus verapamil formulation represent near maximal lymph-targeting values.

Co-administration of ketoconazole produces a 6.2-fold increase in the extent of lymphatic transport of saquinavir compared to a 4.2-fold increase following co-administration of verapamil. Although the differences between the two formulations were not statistically significant, given the higher relative potency of ketoconazole to inhibit CYP than P-gp, this may suggest that inhibition of intestinal CYP is more effective at promoting intestinal lymphatic transport. Future work may be directed at confirming the relative effects of P-gp and CYP on intestinal lymphatic transport, through the use of more selective modulators.

The combined administration of ketoconazole and verapamil produced a marginal, but not statistically significant, increase in the extent of lymphatic transport, compared to either agent alone. This suggests that the respective effects of verapamil and ketoconazole, on P-gp and CYP, are not additive at the concentration of modulators used in this study. However on closer examination, while the total extent of transport after 8 hours does not reveal an additive effect, a comparison of the loadings for each formulation in Table 12.3 or the peak transfer rates of saquinavir in Figure 11.5, or the apparent loadings in Figure 11.6, the rate of saquinavir transfer to intestinal lymph is elevated for the combined verapamil/ketoconazole formulation compared to either agent alone. The differences, while not statistically significant, potentially confirm a more potent effect when both agents are co-administered. While the loadings of saquinavir are improved, the reduced triglyceride turnover observed for the combined verapamil/ketoconazole formulation, may explain why the higher loadings do not result in a higher extent of lymphatic transport after 8 hours.

An unexpected finding of this study was that administration of cyclosporin and the combined verapamil/ketoconazole formulations produced a statistically significant reduction in the total extent of triglyceride recovered in intestinal lymph after 8 hours. The triglyceride turnover appears to be unaffected by the administration of either verapamil or ketoconazole alone, at the concentrations used in this study. While it is unclear how these effects were manifested, the effect is undesirable not only from a lymph-targeting perspective, but also the therapeutic ramifications of a reduced triglyceride turnover are considerable.

Fields and co-workers (Field et al., 1995; Field & Mathur, 1995) have identified a possible role of p-glycoprotein or a related member of the p-glycoprotein ATP-binding cassette family in normal cholesterol trafficking and triglyceride rich lipoprotein secretion in intestinal cells. In their study, progesterone, verapamil and trifluroperazine, all recognised inhibitors of P-gp, decreased cholesterol transport and apo-lipoprotein B and lipid secretion in Caco 2 cell lines at concentrations of between 5-50μM of inhibitor. In contrast, methotrexate, an antimetabolite that does not interact with P-gp had no effect. It was postulated that P-gp might function to maintain the acidic environment of transport vesicles and therefore could play a role in the transport of lipids in the intestine. It is possible therefore that the observed decrease in triglyceride turnover induced by cyclosporin, or the combined verapamil/ketoconazole formulations in our results, may be related to a P-gp mediated reduced lipoprotein synthesis.

12.5. Intestinal lymphatic targeting by co-administration of P-gp/CYP modulators

The concept of assessing the therapeutic efficacy of an antiviral regime based on the immunological and virological status in lymphoid tissue, as opposed to the traditional immunological and virological markers of the disease in plasma (e.g. plasma HIIV RNA levels) has been discussed in Chapter 4. Gunthard et al., (2001) suggested in patients receiving potent antiretroviral therapy for 2 years resulting in sub-quantifiable viral load in plasma, the greatest viral burden may continue to be in lymphoid tissue rather than the CNS or genitourinary compartments. Choo et al., (2000) have reported on the potential of a new therapeutic strategy to reduce HIV-1 viral replication, by increasing HIV protease inhibitor distribution into pharmacological 'sanctuary sites' in the brain and testes, by targeted inhibition of P-gp using selective and potent agents.

The results of our study have shown that the intestinal P-gp/CYP counter transport process severely restricts saquinavir penetration into the intestinal lymphatics in the rat. Therefore, we propose that this approach could be extended to the treatment of HIV infection by pharmacological modulation of intestinal P-gp and/or CYP activity and hence alter the distribution of saquinavir into the lymphatics and lymphoid tissue.

A comparison of the relative enhancement in lymphatic transport (% dose administered) and bioavailability values between formulations with P-gp modulators and the control

formulation (i.e. SQV alone) demonstrates the proposed targeting concept (Figure 11.10). For the formulations containing either verapamil, ketoconazole or verapamil/ketoconazole combined the relative enhancement for lymphatic transport is greater than the corresponding relative enhancement in plasma; whereas, for the formulation containing co-administered cyclosporin, the relative enhancement in both absorption routes is similar. To further illustrate this, a single point comparison between peak lymph and plasma concentrations in lymph-cannulated rats was performed (Table 11.2). In the control (i.e. SQV alone) formulation, the difference between lymph and plasma peak concentrations is 9-fold. This difference is more pronounced for the formulations containing either verapamil or ketoconazole, with 67- and 70-fold higher lymph than plasma peak concentrations, respectively. The peak concentration ratio is not as great for the cyclosporin (15-fold) and verapamil/ketoconazole (23-fold) formulation. This may suggest that at lower doses of modulator, the relative enhancement on lymphatic transport may be more prevalent (i.e. a significant increase in lymphatic transport is observed with no comparable increase in systemic plasma levels); whereas at higher doses, and/or the use of more potent inhibitors, which have a more marked effect on portal absorption, the relative enhancement on transport via the lymphatic route may not be as evident. It is also possible that the use of the more potent inhibitors, such as cyclosporin, may be exerting an effect on liver metabolism, thereby reducing the extent of hepatic first pass effect. This could also explain the greater increase in plasma concentrations observed for this formulation.

The implications of this study are that targeted pharmacological inhibition of P-gp and/or CYP can produce differing effects on the lymph and portal absorption routes of saquinavir, which may not be evident from conventional plasma versus time profiles. These findings provide a rationale for formulation approaches to improve the intestinal lymphatic targeting of saquinavir, the proposed mechanisms for which include the following. As previously mentioned, it is commonly believed that the drug transporter P-gp and cytochrome P450 3A are functionally linked components of a xenobiotic detoxification cascade that limits bioavailability of a number of drugs, P-gp acting to 're-cycle' its substrates thereby increasing the susceptibility to CYP mediated metabolism. By disrupting this process, the concentration of drug compound within the enterocytes will be elevated. Coupled with the co-administration of an appropriate lipid vehicle to drive triglyceride uptake by the intestinal lymphatics, and assuming a simple partition/partition process, these two effects combined will serve to maintain a higher concentration of the

drug in the absorbed lipoidal fraction, resulting in a higher loading of saquinavir per mg triglyceride. By contrast if the P-gp/CYP counter transport process remains fully functional, or in the absence of co-administered lipid, relatively more saquinavir will be either lost to intestinal metabolism or absorption by portal blood.

A significant increase in lymph concentrations is evident when verapamil or ketoconazole are used alone, whereas to induce significant increases in systemic plasma concentrations, the use of more potent inhibitor(s) e.g. cyclosporin, is necessary. There are a number of possible explanations for this. Firstly, a modest disruption of P-gp/CYP in intestinal cells is sufficient to increase the concentration of drug within the cell that results in increases in the rate of transfer to intestinal lymph in association with intestinal lipoproteins by a simple mass transfer process. While the concentration of drug within the enterocyte may be elevated, portal blood concentrations may not be similarly increased due to on-going efflux/elimination activity. It is possible that saquinavir 'trafficked' through the lipoidal/lipoprotein route, may be less susceptible to ongoing P-gp/CYP activity (Khoo et al., 1998a). Secondly, increased enterocyte concentrations of drug may produce similar relative increases in both lymph and portal blood. However, drug carried through the portal blood may undergo first pass metabolism in the liver, and as such the relative increase in systemic circulation may not be as pronounced. Thirdly, the cyclosporin and ketoconazole/verapamil combination formulations appear to reduce lymphatic lipid turnover. It is possible therefore, that assuming near-maximal saturation of lymph triglyceride, the reduced triglyceride turnover serves to limit the amount of drug that can be transported lymphatically. Under these conditions, portal blood absorption will be favoured.

Certain excipients (e.g. surfactants), which are commonly added to pharmaceutical formulations, may function to enhance intestinal permeability of drugs by inhibiting efflux mechanisms (Nerurkar et al., 1997; Dintaman & Silverman, 1999). It is possible therefore that the surfactants in the lipid formulations used to promote lymphatic transport of saquinavir in Chapter 10 inhibit the P-gp/CYP mechanism. It seems likely, however, that the modulators used in Chapter 11 (e.g. 1mM verapamil, ketoconazole, cyclosporin) are more potent than the 40mM concentrations of surfactants.

An added pharmacokinetic benefit of combination therapy is a reduction in inter-subject variance. Ritonavir reduced the percent coefficient of variability for saquinavir

pharmacokinetic parameters from about 70% to about 30%. Differential expressions of intestinal CYP 3A contributes to high inter subject variability in the pharmacokinetics of drugs like saquinavir that undergo extensive first-pass metabolism (Kolars et al., 1994; Thummel et al., 1997). Eliminating this pathway as a significant contributor to saquinavir clearance would be expected to reduce pharmacokinetic variance, as is the case. In addition to the approximate 60-fold increase in saquinavir AUC (Merry et al., 1997), the present study potentially unveils another benefit to ritonavir-saquinavir combination therapy; namely increased lymphatic concentrations of the protease inhibitor. The relative importance of these factors needs to be addressed in future experiments; particularly in the light of the profound pharmacokinetic interaction between ritonavir and saquinavir, one of the largest reported in literature, wherein ritonavir converts saquinavir into a drug exhibiting relatively low first pass metabolism, as detailed in Chapter 4. In conclusion the finding that the intestinal lymphatic transport of saquinavir is substantially increased by the concomitant administration of CYP/P-gp modulators warrants further research with a view to increasing clinical efficacy of the oral formulations.

12.6. Comparison of availability in lymph-cannulated and bioavailability in non lymph-cannulated animals for both DDT and saquinavir

In Chapter 9, a comparison of DDT bioavailability and availability values suggests that the extent of DDT availability (blood plus lymph) estimated in lymph cannulated animals is higher than that predicted from bioavailability estimates in non-lymph cannulated animals, although the difference was not statistically significant. These findings are similar to those reported by Caliph et al., (2000) for halofantrine bioavailability and availability in non-lymph cannulated and lymph cannulated conscious rats. The authors have suggested that these results reflect either pre-systemic drug clearance within the lymphatics or an altered systemic clearance pattern for lymphatically transported drug. The potential for changes to systemic clearance for chylomicron associated, and lymphatically transported drugs is consistent with previous suggestions by Hauss et al., (1994) and in line with a large body of work which suggests that association of lipophilic drugs with lipoproteins can markedly affect drug pharmacokinetics and distribution patterns (Humberstone et al., 1998; Wasan & Cassidy, 1998).

Comparing the saquinavir availability in lymph cannulated rats (lymph + blood) to the bioavailability calculated in non-lymph cannulated for the six formulations used in this

study (Table 10.2 and Table 11.4), the absence of any major difference between availability and bioavailability values confirms the lack of substantial lymphatic transport seen with the lymphatic transport data.

The general conclusions that can be drawn from a comparison of the bioavailability and availability values for these two compounds is as follows. For extremely lipophilic compounds, such as DDT, which display a high triolein solubility and metabolic stability, intestinal lymphatic transport may be a significant contributor to overall bioavailability. The implications from these studies with DDT are that subtle changes in formulation, for example SEDDS versus oily dispersion or changes in dosing strategy, for example fasted vs. post-prandial administration, may provoke a change to the proportion of the drug transported via the lymph, and the extent of total exposure (assessed as blood plus lymph) that may not be evident in simple plasma bioavailability studies. For compounds, which do not display such extreme lipophilicity, such as saquinavir, the extent of lymphatic transport, as a percentage of dose, may be low but is still formulation-dependent. However, the physiochemical properties that indicate the potential for intestinal lymphatic transport (i.e. lipophilicity) also dictate that the extent of absorption by the portal blood may also be low, for reasons such as poor dissolution, permeability through the aqueous boundary layer, and susceptibility to P-gp, which has a propensity for hydrophobic compounds (Ford et al., 1990; Zamora et al., 1988; Germann et al., 1993)(P-gp has been previously described as a 'hydrophobic vacuum cleaner' (Raviv et al., 1990)). Consequently, for compounds with significant lipophilicity, great care must be taken to screen for changes in the route of transport (and potential changes in drug deposition profile associated with these altered delivery routes) when changing lipid formulation components.

12.7. Conclusions

The schematic diagram in Figure 12.2 presents the potential mechanisms for gastrointestinal drug absorption from lipid-based formulations via the portal and lymphatic route.

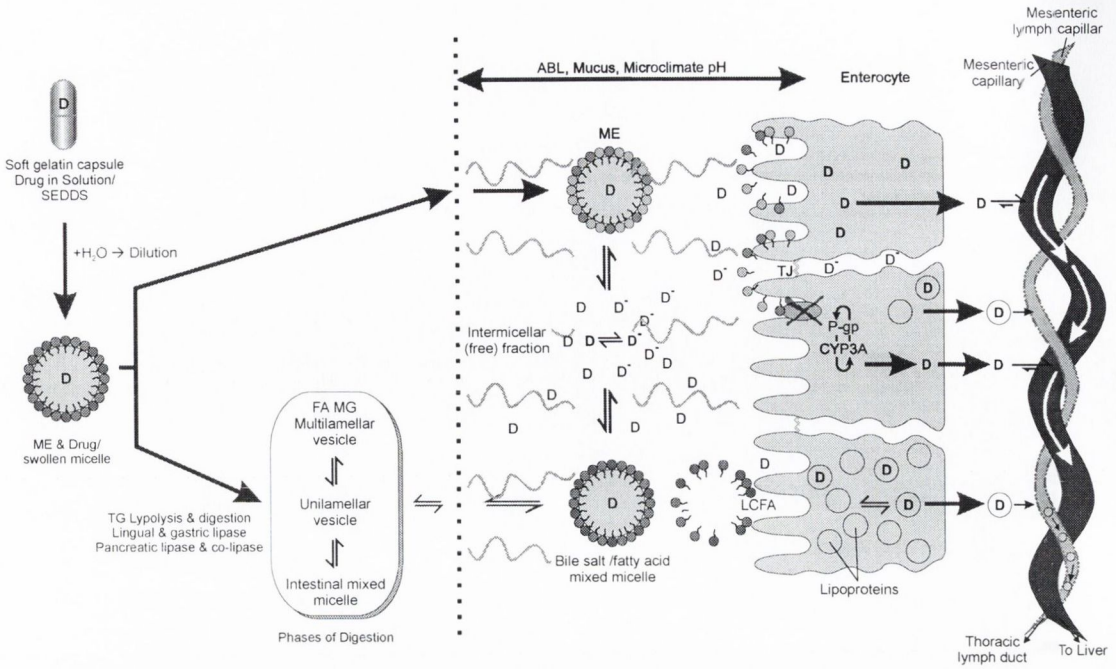


Figure 12.2 Schematic representation of the mechanisms of drug absorption from lipid-based formulations via the portal and lymphatic route.

SEDDS formulations provide for precise and convenient unit dosage forms of lipid formulations, and when appropriately designed allow for rapid assimilation with the relevant bile salt structures on dilution/digestion in the intestine, thereby overcoming dissolution rate limitations for lipophilic drugs. The route taken to the pre-absorptive state will depend on the digestibility of the formulation, which poses important considerations in terms of the solubility of the drug candidate in the respective phases on dilution and/or digestion in the intestine, the potential for drug precipitation, and the potential for lipid surfactants to inhibit lipolysis. There are a number of potential mechanisms whereby co-administered lipids may influence the absorption processes: (i) increase membrane permeability, (e.g. transcellular (membrane effect) or paracellular (tight junction function)) (ii) inhibit efflux/elimination mechanisms (e.g. P-gp) (iii) increase production of lipoproteins. Following absorption, blood and lymph capillaries compete for the transport of drug molecules to the systemic circulation. The portal blood represents the major absorption pathway for the vast majority of orally administered drugs as it has a high capacity to transport both water soluble and poorly water soluble compounds. Specifically, access to the intestinal lymph (as opposed to the portal blood) is provided for by the more 'open' structure of lymph vessels. The majority of drug transported via intestinal lymph is associated with lipoproteins secreted by the enterocyte.

In this thesis, three specific mechanisms were examined in greater detail, namely, the potential for lipid/surfactant based formulations to stimulate drug uptake via the lymphatic route; the effect of co-administration of P-gp/CYP modulators on drug uptake via both absorption routes; and the relationship between the lymphatic drug uptake and lipid turnover in intestinal lymph. The following is a summary of the major findings of this thesis:

- The solubility of the drug candidate in lymph triglyceride, and the ability of the formulation to efficiently stimulate triglyceride turnover are crucial determinants of lymphatic transport.
- Mixed micelle and SEDDS formulations, containing oleic acid, made confer lymph-targeting advantages in terms of promoting lymphatic drug transport and high apparent drug loadings in intestinal lymph triglyceride.
- P-gp/CYP eliminations mechanisms in the rat intestine severely limit the intestinal lymphatic transport of saquinavir.
- Selective increases in intestinal lymphatic levels of saquinavir can be achieved, *in situ,* by targeted pharmacological inhibition of P-gp and/or CYP and could be viewed as a novel therapeutic strategy to reduce HIV replication within the lymphatic system.
- The reduction in triglyceride lymph turnover following administration of certain P-gp/CYP modulator(s) provides *in situ* data indicative of a link between P-gp-like mechanisms and lipoprotein production.

APPENDIX AND REFERENCES

13. APPENDIX 1

Blood concentration levels, following intravenous administration of DDT (5mg) in soy oil emulsion (Data obtained from Dr. Caitriona O' Driscoll, ref. O' Driscoll et al., 1991)

	DDT Blood Concentrations (μg/ml) ± SE
Time (hr)	Data set A
0.00	56.00
0.25	45.692 ± 3.081
0.5	32.777 ± 2.090
0.75	24.586 ± 1.089
1	20.698 ± 0.519
1.5	11.806 ± 0.618
2	6.751 ± 0.249
4	1.874 ± 0.437
6	0.879 ± 0.388
8	0.613 ± 0.472
10	0.965 ± 0.421
11	0.549 ± 0.441

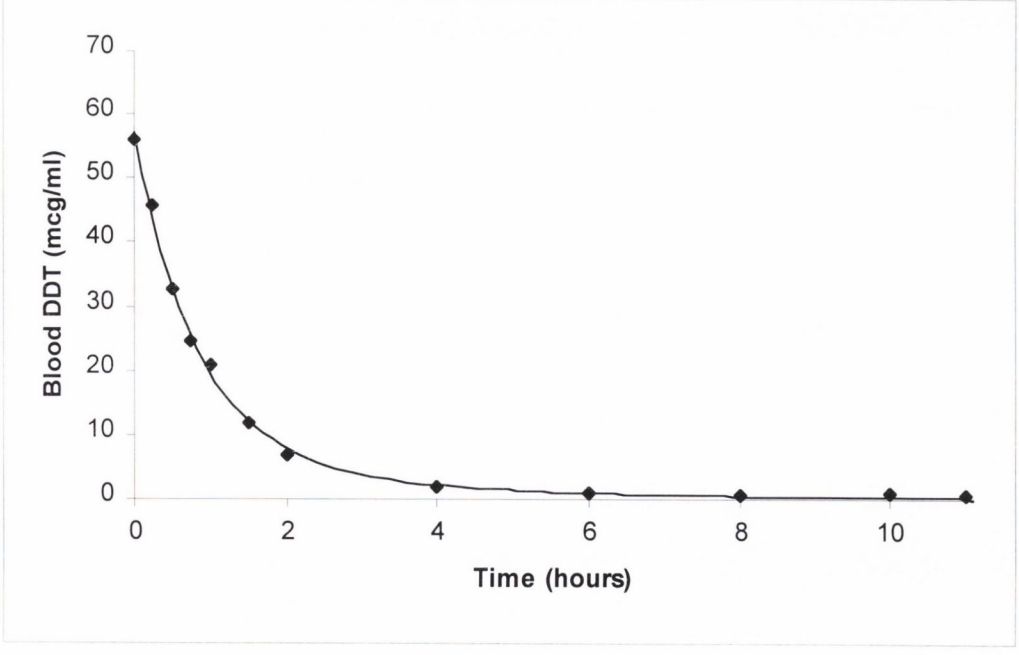


Figure 13.1 DDT blood level profile following i.v. administration of DDT(5mg)

REFERENCES

Abrams, L. S., H. S. Werntraub, J. E. Patrick, and J. L. Mc Guire. 1978. "Comparative bioavailability of a lipophilic steroid." *Journal of Pharmaceutical Sciences* 67 1287-1290.

Achira, M., H. Suzuki, K. Ito and Y. Sugiyama. "Comparative studies to determine the selective inhibitors for P-glycoprotein and cytochrome P4503A4. 1999. *AAPS Pharm. Sci.* 1(4): article 18

Adachi, I., H. X. Liu, I. Horikoshi, M. Ueno, and H. Sato. 1993. "Possibility of lymphatic absorption of epidermal growth-factor from intestine." *Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan* 113(3): 256-63.

Allen, T. M., C. B. Hansen, and L. S. S. Guo. 1993. "Subcutaneous administration of liposomes - a comparison with the intravenous and intraperitoneal routes of injection." *Biochimica et Biophysica Acta* 1150(1): 9-16.

Alpers, D. H., N. M. Bass, M. J. Engle, and K. Deschryver-Kecskemeti. 2000. "Intestinal fatty acid binding protein may favour differential apical fatty acid binding in the intestine." *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids* 1483(3): 352-62.

Alsenz, J., H. Steffen, and R. Alex. 1998. "Active apical secretory efflux of the HIV protease inhibitors, Saquinavir and Ritonavir, in Caco-2 Cell monolayers." *Pharmaceutical Research* 15(3): 423-8.

Amidon, G. E., W. I. Higuchi, and N. F. Ho. 1982. "Theoretical and experimental studies of transport of micelle-solubilized solutes." *Journal of Pharmaceutical Sciences* 71(1): 77-84.

Amidon, G. L., H. Lennernas, V. P. Shah, and J. R. Crison. 1995. "A theoretical basis for a biopharmaceutic drug classification - the correlation of *in-vitro* drug product dissolution and *in-vivo* bioavailability." *Pharmaceutical Research* 12(3): 413-20.

Anderberg, E. K., T. Lindmark, and P. Artursson. 1993. "Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route." *Pharmaceutical Research* 10(6): 857-64.

Aramaki, Y., H. Tomizawa, T. Hara, K. Yachi, H. Kikuchi, and S. Tsuchiya. 1993. "Stability of liposomes *in-vitro* and their uptake by rat Peyer patches following oral-administration." *Pharmaceutical Research* 10(8): 1228-31.

Attwood, D. 1994. "Microemulsions." In: Kreuter (Ed.), Colloidal Drug Delivery Systems, Marcel Dekker, Inc., NY. 3-71.

Attwood, D. and A. T. Florence. 1983. "Pharmaceutical aspects of solubilization." In: Surfactant Systems. Chapman and Hall, London. 298-99.

Aungst, B. J. 1993. "Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or pre-systemic metabolism." *Journal of Pharmaceutical Sciences* 82(10):979-87.

Aungst, B. J. and H. Saitoh. 1996. "Intestinal absorption barriers and transport mechanisms, including secretory transport, for a cyclic peptide, fibrinogen antagonist." *Pharmaceutical Research* 13(1): 114-19.

Aungst, B. J., N. H. Nguyen, N. J. Rogers, S. M. Rowe, M. A. Hussain, S. J. White, and L. Shum. 1997. "Amphiphilic vehicles improve the oral bioavailability of a poorly soluble HIV protease inhibitor at high doses." *International Journal of Pharmaceutics* 156:79-88.

Barancik, M., L. Polekova, T. Mrazova, a. Breier, T. Stankovicova, and J. Slezak. 1994. "Reversal effects of several Ca^{2+}-entry blockers, neuroleptics and local-anaesthetics on P-glycoprotein-mediated Vincristine resistance of L1210/Vcr mouse leukemic-cell Line." *Drugs Under Experimental and Clinical Research* 20(1): 13-18.

Barbui, T., a. Rambaldi, L. Parenzan, M. Zucchelli, N. Perico, and G. Remuzzi. 1992. "Neurological symptoms and coma associated with doxorubic in administration during chronic cyclosporin therapy." *Lancet* 339(8806): 1421.

Barnwell, S. G. and D. Attwood. 1996. "HALO®: A semi-solid biphasic release drug delivery system designed to enhance oral bioavailability." *Bull. Tech Gattefosse* 89. 71-80.

Barnwell, S. G., T. Laudanski, M. J. Story, C. B. Mallinson, R. J. Harris, S. K. Cole, M. Keating, and D. Attwood. 1992. "Improved oral bioavailability of Propranolol in healthy-human volunteers using a liver bypass drug delivery system containing oleic-acid." *International Journal of Pharmaceutics* 88(1-3): 423-432.

Barrowman, J. A. 1978. "Transport of absorbed lipids" In: *Physiology of the Gastrointestinal Lymphatic System*, Barrowman, J.A., Ed., Cambridge University Press, Cambridge, 165.

Barry, M. G., C. Merry, J. Lloyd, K. Halifax, P. Carey, F. Mulcahy, and D. J. Back. 1998. "Variability in trough plasma saquinavir concentrations in HIV patients - a case for therapeutic drug monitoring." *British Journal of Clinical Pharmacology* 45(5): 501-2.

Baugaut, M. and H. Carlier. 1987. "Role of intestinal hydrolases, endogenous substrates and chyloportal partition in fat absorption. " In: A. Kuksis (Ed.), Fat Absorption, Vol.1 CRC Press, Boca Raton. 197-232.

Benoit, J.N., and D.C. Zawieja 1994 "Gastrointestinal lymphatics". In: *Physiology of the gastrointestinal tract.* 3rd Eds. Raven Press NY. 1669-92.

Bender, A. R., H. von Briesen, J. Kreuter, I. B. Duncan, and H. Rübsamen Waigmann. 1996. "Efficiency of nanoparticles as a carrier system for antiviral agents in Human Immunodeficiency Virus-infected human monocytes/ macrophages in vitro." *Antimicrob Agents Chemother* 40(6): 1467-71.

Bender, A., V. Schafer, a. M. Steffan, C. Royer, J. Kreuter, H. Rubsamenwaigmann, and H. Vonbriesen. 1994. "Inhibition of HIV in-vitro by antiviral drug-targeting using nanoparticles." *Research in Virology* 145(3-4): 215-20.

Benet, L. Z., C. Y. Wu, M. F. Hebert, and V. J. Wacher. 1996. "Intestinal drug metabolism and antitransport processes: a potential paradigm shift in oral drug delivery." *Journal of Controlled Release* 39(2-3): 139-43.

Berg, S. L., a. Tolcher, J. A. Oshaughnessy, a. M. Denicoff, M. Noone, F. P. Ognibene, K. H. Cowan, and F. M. Balis. 1995. "Effect of R-verapamil on the pharmacokinetics of Paclitaxel in women with breast-cancer." *Journal of Clinical Oncology* 13(8): 2039-42.

Bergstrom, K and B Werner. 1966. "Proteins in human thoracic duct lymph. Studies on the distribution of some proteins between lymph and blood." *Acta Chir. Scand* 131: 413.

Bernard, A., B. Echinard, and H. Carlier. 1987. "Differential intestinal absorption of two fatty acid isomers: Eladic and Oleic acids." *American Journal of Physiology* 253: G751-59.

Bernback, S., L. Blackberg, and O. Hernell. 1989. "Fatty acids generated by gastric lipase promote human milk triacylglycerol digestion by pancreatic colipase-dependent lipase." *Biochim. Biophys. Acta.* 1001 286-93.

Betageri, G. V., C. D. V. Black, J. Szebeni, L. M. Wahl, and J. N. Weinstein. 1993. "Fc-receptor-mediated targeting of antibody-bearing liposomes containing Dideoxycytidine Triphosphate to human monocyte macrophages." *Journal of Pharmacy and Pharmacology* 45(1): 48-53.

Bhargava, H.N, A. Narurkar, and L.M. Lieb 1987. "Using microemulsions for drug delivery". *Pharmaceutical Technology* 11 (2): 46-52.

Bibby, D. C., W. N. Charman, S. A. Charman, M. N. Iskander, and C. J. H. Porter. 1996. "Synthesis and evaluation of 5' alkyl ester prodrugs of zidovudine for directed lymphatic delivery." *International Journal of Pharmaceutics* 144(1): 61-70.

Billich, S., M. T. Knoop, J. Hansen, P. Strop, J. Sedlacek, R. Mertz, and K. Moelling. 1988. "Synthetic peptides as substrates and inhibitors of Human Immune Deficiency Virus-1 protease." *Journal of Biological Chemistry* 263(34): 17905-8.

Boden, D. and M. Markowitz. 1998. "Resistance to human immunodeficiency virus type 1 protease inhibitors." *Antimicrobial Agents and Chemotherapy* 42(11): 2775-83.

Bollman, J. L., J. C. Cava, and J. H. Grindley. 1948. "Techniques for the collection of lymph from the liver, small intestine and thoracic duct of the rat." *Journal of Laboratory Clinical Medicine* 331: 349-52.

Bommhardt, U., J. C. Cerottini, and H. R. MacDonald. 1994. "Heterogeneity in P-glycoprotein (Multidrug Resistance) activity among murine peripheral T Cells: correlation with surface phenotype and effector function." *Eur J Immunol* 24. (12): 2974-81.

Boote, D. J., I. F. Dennis, P. R. Twentyman, R. J. Osborne, C. Laburte, S. Hensel, J. F. Smyth, M. H. Brampton, and N. M. Bleehen. 1996. "Phase I study of etoposide with SDZ PSC 833 as a modulator of multidrug resistance in patients with cancer." *Journal of Clinical Oncology* 14(2): 610-618.

Borel, J.F. 1988. "Immunosuppression: building on Sandimmune (cyclosporin)." *Transplant Proc.* 20: 149-153.

Borgstrom, B. 1980. "Importance of phospholipids, pancreatic phospholipase A_2 and fatty acid for the digestion of dietary fat." *Gastroenterology* 789: 54-62.

Borgstrom, B., A. Dahlquist, G. Lundh, and J. Sjovall. 1957. "Studies of intestinal digestion and absorption in the human." *Journal of Clinical Investigation* 361: 121-36.

Buckingham, LE, M. Balasuramanian, R.M. Emanuele, K.E. Clodfether and J.S.Coon 1995. Comparison of solutol HS15, cremophor EL and novel ethoxylated fatty acid surfactants as multidrug resistance modification agents. *Int. J. Cancer* 62: 436-42

Busbee, D. L., J. S. H. Yoo, J. O. Norman, and C. O. Joe. 1985. "Polychlorinated biphenyl uptake and transport by lymph and plasma components." *Proceedings of the Society for Experimental Biology and Medicine* 179: 116-22.

Caliph, S. M., W. N. Charman, and C. J. Porter. 2000. "Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats." *Journal of Pharmaceutical Sciences* 89(8): 1073-84.

Carey, M. C. and D. M. Small. 1970. "The characteristics of mixed micellar solutions with particular reference to bile. " *American Journal of Medicine.* 495: 90-608.

Carey, M. C. and D. M. Small. 1972. "Micelle formation by bile salts." *Arch. Intern. Med* 130: 506-27.

Carey, M. C., D. M. Small, and C. M. Bliss. 1983. "Lipid digestion and absorption." *Annu. Rev. Physiol.* 45(651).

Carpenter, C. C. J., M. A. Fischl, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, D. A. Katzenstein, J. S. G. Montaner, D. D. Richman, M. S. Saag, R. T. Schooley, M. A. Thompson, S. Vella, P. G. Yeni, and P. A. Volberding. 1997. "Antiretroviral therapy for HIV infection in 1997 - updated recommendations of the international aids society USA panel." *Jama-Journal of the American Medical Association* 277(24):1962-69.

Carrigan, P. J. and T. R. Bates. 1973. "Biopharmaceutics of drugs administered in lipid containing dosage forms. I: GI Absorption of griseofulvin from an oil in water emulsion in the rat." *Journal of Pharmaceutical Sciences* 62: 328-34.

Casley-Smith, J. R. 1980. "The fine structure and functioning of tissue channels and lymphatics." *Lymphology* 12: 183.

Cavert W, Notermans DW, Staskus K, Wietgrefe SW, Zupancic M, Gebhard K, Henry K, Zhang ZQ, Mills R, McDade H, Goudsmit J, Danner, SA, and Haase AT. 1997. "Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection." *Science* 276(5314): 960-4.

Chakrabarti, S. and F. M. Belpaire. 1978. "Bioavailability of phenytoin in lipid containing dosages forms in the rat." *Journal of Pharmacy and Pharmacology* 30: 330-331.

Chan, W. K., L. T. Nguyen, V. P. Miller, and R. Z. Harris. 1998. "Mechanism-based inactivation of human Cytochrome P450 3A4 by grapefruit juice and red wine." *Life Sciences* 62(10): PL135-PL142.

Chang T, Benet LZ, and Hebert MF . 1996. " The effect of water-soluble Vitamin E on cyclosporine pharmacokinetics in healthy volunteers." *Clin Pharmacol Ther* 59(3): 297-303.

Charman, S. A., A. M. Segrave, G. A. Edwards, and C. J. H. Porter. 2000. "Systemic availability and lymphatic transport of Human Growth hormone administered by subcutaneous injection." *Journal of Pharmaceutical Sciences* 89(2): 168-77.

Charman, S. A., W. N. Charman, M. C. Rogge, T. D. Wilson, F. J. Dutko, and C. W. Pouton. 1992. "Self-emulsifying drug delivery systems: Formulation and biopharmaceutic evaluation of an investigational lipophilic compound" *Pharmaceutical Research* 9(1): 87-93.

Charman, W. N. 1998. "Lipids and oral bioavailability: are there yet-to-be realised opportunities?" *Bull. Tech. Gattefosse* 91: 55-63.

Charman, W. N. and V. J. Stella. 1986a. "Effect of lipid class and lipid vehicle volume on the intestinal lymphatic transport of DDT." *International Journal of Pharmaceutics* 33: 165-72.

Charman, W. N. and V. J. Stella. 1986b. "Estimating the maximal potential for intestinal transport of lipophilic drug molecules." *International Journal of Pharmaceutics* 34: 175-78.

Charman, W. N. and V. J. Stella. 1991. "Transport of lipophilic molecules by the intestinal lymphatic-system." *Advanced Drug Delivery Reviews* 7(1): 1-14.

Charman, W. N., M. C. Rogge, A. W. Boddy, and B. M. Berger. 1993. "Effect of food and a monoglyceride emulsion formulation on danazol bioavailability." *Journal of Clinical Pharmacology* 33(4): 381-6.

Charman, W. N., T. Noguchi, and V. J. Stella. 1986a. "An experimental system designed to study *in situ* lymphatic transport of lipophilic drugs in the anaesthetized Rat." *International Journal of Pharmaceutics* 33: 155-64.

Charman, W. N., T. Noguchi, and V. J. Stella. 1986b. "Testing potential dosage form strategies for intestinal lymphatic drug transport: studies in the rat." *International Journal of Pharmaceutics* 33: 173-79.

Chen, C. J. J.E. Chin, K. Ueda, D. Clark, I. Pastan, M.M. Gottesman, I.B. Robinson 1986. "Interal duplication and homology with bacterial transport processes in the mdr1 (P-glycoprotein) gene from multi-drug resistant cells." *Cell* 47: 381-389.

Choo, E. F., B. Leake, C. Wandel, H. Imamura, a. J. J. Wood, G. R. Wilkinson, and R. B. Kim. 2000. "Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes." *Drug Metabolism and Disposition* 28(6): 655-60.

Clarke, R. M., L. Yao, L. She, and H. C. Furr. 2000. "A comparison of lycophene and astaxanthin absorption from corn oil and olive oil emulsions." *Lipids* 1073-78.

Collett, J. H. and L Koo. 1975. "Interaction of substituted benzoic acids with polysorbate 20 micelles." *Journal of Pharmaceutical Sciences* 641: 253-55.

Collier, a. C., R. W. Coombs, D. A. Schoenfeld, R. L. Bassett, J. Timpone, a. Baruch, M. Jones, K. Facey, C. Whitacre, V. J. Mcauliffe, H. M. Friedman, T. C. Merigan, R. C. Reichman, C. Hooper, and L. Corey. 1996. "Treatment of Human Immunodeficiency Virus infection with saquinavir, zidovudine, and zalcitabine." *New England Journal of Medicine* 334(16): 1011-17.

Constantinides, P. P. 1995. "Lipid microemulsions for improving drug dissolution and oral absorption - physical and biopharmaceutical aspects." *Pharmaceutical Research* 12(11): 1561-72.

Constantinides, P. P., C. M. Lancaster, J. Marcello, D. C. Chiossone, D. Orner, I. Hidalgo, P. L. Smith, a. B. Sarkahian, S. H. Yiv, and a. J. Owen. 1995. "Enhanced intestinal-absorption of an RGD peptide from water-in-oil microemulsions of different composition and particle-size." *Journal of Controlled Release* 34(2):109-16.

Constantinides, P. P., G. Welzel, H. Ellens, P. L. Smith, S. Sturgis, S. H. Yiv, and A. B. Owen. 1996. "Water-in-oil microemulsions containing medium-chain fatty acids/salts: Formulation and intestinal absorption enhancement evaluation." *Pharmaceutical Research* 13 (2): 210-5.

Constantinides, P. P., J. P. Scalart, C. Lancaster, J. Marcello, G. Marks, H. Ellens, and P. L. Smith. 1994. "Formulation and intestinal-absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides." *Pharmaceutical Research* 11(10): 1385-90.

Cordon Cardo, C., J. P. O' Brien, D. Casals, L. Rittman Grauer, J. L. Biedler, M. R. Melamed, and J. R. Bertino. 1989. "Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites." *Proc Natl Acad Sci. U S A* 86 (2): 695-8.

Cordoncardo, C., J. P. O' Brien, J. Boccia, D. Casals, J. R. Bertino, and M. R. Melamed. 1990. "Expression of the multidrug resistance gene-product (P-glycoprotein) in human normal and tumor-tissues." *Journal of Histochemistry & Cytochemistry* 38(9): 1277-87.

Craig, D. Q. M., M.J. Patel and M. Ashford. 2000. "Administration of Emulsions to the Gastrointestinal Tract." *In: Pharmaceutical Emulsions and suspensions.* Eds. by F. Nielloud and G. Marti-Mestres, Marcel Decker, 323-348.

Craig, D. Q. M. 1993. "The use of self-emulsifying systems as a means of improving drug delivery." *Bull. Tech. Gattefosse* 86(21-31).

Craig, D. Q. M., H. S. R. Lievens, K. G. Pitt, and D. E. Storey. 1993. "An investigation into the physicochemical properties of self-emulsifying systems using low-frequency dielectric-spectroscopy, surface-tension measurements and particle-size analysis." *International Journal of Pharmaceutics* 96(1-3): 147-55.

Craig, D.Q.M., A.K. Chan, N.A. Khan 1996. "A comparison of self-emulsifying properties of polyglycolysed glyceride oils." *Pharm Sci.* 1: 559-562.

Craig, D. Q. M., S. A. Barker, D. Banning, and S. W. Booth. 1995. "An investigation into the mechanisms of self-emulsification using particle-size analysis and low-frequency dielectric-spectroscopy." *International Journal of Pharmaceutics* 114(1): 103-10.

Crison, J.R. and G.L. Amidon. 1999 "Method of formulation for increasing the bioavailability of poorly water soluble drugs" US patent, No. 5 993-858.

Curatolo, W. and R. Ochoa. 1994. "Safety assessment of intestinal permeability enhancers". In: A.G. De Boer (Ed.), Drug Absorption Enhancement. Harwood Academic, Switzerland. 367-89.

Cuvelier, a. C., F. Berlioz, S. C. Dautrey, C. Roze, C. Carbon, and R. Farinotti. 1998. "Intestinal absorption of saquinavir in the rat: insights into possible mechanisms." *Gastroenterology* 114(4): A957.

Daneshmend, T. K. 1990. "Diseases and drugs but not food decrease ketoconazole bioavailability." *British Journal of Clinical Pharmacology* 29(6): 783-84.

Dantzig, a. H., R. L. Shepard, J. Cao, K. L. Law, W. J. Ehlhardt, T. M. Baughman, T. F. Bumol, and J. J. Starling. 1996. "Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, Ly335979." *Cancer Research* 56(18): 4171-79.

Dantzig, a. H., R. L. Shepard, K. L. Law, L. Tabas, S. Pratt, J. S. Gillespie, S. N. Binkley, M. T. Kuhfeld, J. J. Starling, and S. A. Wrighton. 1999. "Selectivity of the multidrug resistance modulator, LY 335979,for P-glycoprotein and effect on cytochrome P-450 activities." *Journal of Pharmacology and Experimental Therapeutics* 290(2): 854-62.

Darimont, C., N. Gradoux, and A. De Pover. 1999. "Epidermal growth factor regulates fatty acid uptake and metabolism in Caco-2 cells." *American Journal of Physiology-Gastrointestinal and Liver Physiology* 39(3): G606-G612.

Darke, P. L. 1994. "Stability of dimeric retroviral proteases." In: Retroviral Proteases, vol. 241 104-127

Darke, P. L., S. P. Jordan, D. L. Hall, J. A. Zugay, J. A. Shafer, and L. C. Kuo. 1994. "Dissociation and association of the HIV-1 protease dimmer subunits - equilibria and rates." *Biochemistry* 33(1): 98-105.

Davidson, N. O. 1994. "Cellular and molecular mechanisms of small intestinal lipid transport" In: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, N.Y. 1909-34.

Davidson, N. O., M. J. Drewek, J. I. Gordon, and J. Elovson. 1988. "Rat intestinal apoliprotein B gene expression. Evidence for integrated regulation of bile salt, fatty acid and phospholipid flux." *Journal of Clinical Investigation* 82: 300-308.

Deeks, S. G., M. Smith, M. Holodniy, and J. O. Kahn. 1997. "Hiv-1 protease inhibitors - a review for clinicians." *Journal of the American Medical Association* 277(2): 145-53.

Desormeaux, A., P. Harvie, S. Perron, B. Makabipanzu, D. Beauchamp, M. Tremblay, L. Poulin, and M. G. Bergeron. 1994. "Antiviral efficacy, intracellular uptake and pharmacokinetics of free and liposome-encapsulated 2',3'-Dideoxyinosine." *Aids* 8(11): 1545-53.

Dintaman, J. M. and J. A. Silverman. 1999. "Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethyleneglycol 1000 succinate (TPGS)." *Pharmaceutical Research* 16(10): 1550-1556.

Dipali, S. R., Y. J. Lin, W. R. Ravis, and G. V. Betageri. 1997. "Pharmacokinetics and tissue distribution of long circulating liposomal formulation of 2', 3'-Dideoxyinosine." *International Journal of Pharmaceutics* 152(1): 89-97.

Dodic, N., B. Dumaitre, a. Daugan, and P. Pianetti. 1995. "Synthesis and activity against multidrug-resistance in chinese-hamster ovary cells of new acridone-4-carboxamides." *Journal of Medicinal Chemistry* 38(13): 2418-26.

Dressman, J. B. and C. Reppas. 2000. "In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs." *European Journal of Pharmaceutical Sciences.* 11: S73-S80.

Drevon, C. A. 1991. "Absorption, transport and metabolism of vitamin E." *Free Radical Research Communications* 14(4): 229-46.

Drewe, J., H. Gutmann, G. Fricker, M. Torok, C. Beglinger, and J. Huwyler. 1999. "HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporin analog SDZ PSC 833." *Biochemical Pharmacology* 57(10): 1147-52.

Eccleston, J. 1994. "Microemulsions." In: J. Swarbrick and J.C. Boylan. (Eds.), Encyclopaedia of Pharmaceutical Technology, Vol. 9, Marcel Dekker, N.Y. 375-421

Edwards, G. A., C. J. H. Porter, S. M. Caliph, S Khoo, and W. N. Charman. 2001. "Animal models for the study of intestinal lymphatic drug transport." *Advanced Drug Delivery Reviews* 50(1-2): 45-60.

Embretson, J., M. Zupancic, J. L. Ribas, a. Burke, P. Racz, K. Tennerracz, and a. T. Haase. 1993. "Massive covert infection of helper T-lymphocytes and macrophages by HIV during the incubation period of Aids." *Nature* 362(6418): 359-62.

Fahelelbom, K. M., R. F. Timoney, and O. I. Corrigan. 1993. "Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants." *Pharmaceutical Research* 10(4): 631-4.

Fausa, O. 1974. "Duodenal bile acids after a test meal." Scand. J. of Gastroenterology. (9): 567.

Fernandez, E. and B. Borgström. 1989. "Effects of tetrahydrolipstatin, a lipase inhibitor, on absorption of fat from the intestine of the rat." *Biochim Biophys Acta* 1001 (3): 249-55.

Fernandez, E. and B. Borgstrom. 1990. "Intestinal Absorption of retinal and retinyl palmitate : effects of tetrahydrolipstatin." *Lipid.* 25: 549-52.

Ferry, D. R., H. Glossman, and A.J. Kaumann 1985. "Relationship between the stereo selective negative isotropic effects of verapamil enantiomers and their binding to putative calcium channels in human heart. " *British Journal of Pharmacology* 84(4): 811.

Field, F. J. and S. N. Mathur. 1995. "Intestinal lipoprotein synthesis and secretion." *Progress in Lipid Research* 34(2): 185-98.

Field, F. J., E. Albright, and S. N. Mathur. 1988. "Regulation of triglyceride-rich lipoprotein secretion by fatty acids in Caco-2 cells." *Journal of Lipid Research* 29(11): 1427-37.

Field, F. J., E. Born, and S. N. Mathur. 1995. "Triacylglycerol-rich lipoprotein cholesterol is derived from the plasma membrane in Caco-2 cells." *Journal of Lipid Research* 36(12): 2651-60.

Field, F. J., E. Born, H. S. Chen, S. Murthy, and S. N. Mathur. 1995. "Esterification of plasma-membrane cholesterol and triacylglycerol-rich lipoprotein secretion in Caco-2 cells - possible role of P-Glycoprotein." *Journal of Lipid Research* 36(7): 1533-43.

Finzi, D., M. Hermankova, T. Pierson, L. M. Carruth, C. Buck, R. E. Chaisson, T. C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M. Markowitz, D. D. Ho, D. D. Richman, and R. F. Siliciano. 1997. "Identification of a reservoir for Hiv-1 in patients on highly active antiretroviral therapy." *Science* 278(5341): 1295-300.

Finzi, D., J. Blankson, J.D. Siliciano, J. Margolick, K. Chadwick, T. Pierson, et al. 1999. Latent infection of CD4+ T cells provides a mechanism for life long persistence of HIV-1, even in patients on effective combination therapy. *Nature Medicine.* 5(5): 512-17.

Fischl, M. A. 1989. "State of antiretroviral therapy with zidovudine." *Aids* 3: S137-S143.

Fischl, M. A., D. D. Richman, C. Flexner, M. F. Para, R. Haubrich, a. Karim, P. Yeramian, J. Holdenwiltse, and P. M. Meehan. 1997. "Phase I/II Study of the toxicity, pharmacokinetics, and activity of the HIV protease inhibitor Sc-52151." *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 15(1): 28-34.

Fitzsimmons, M. E. and J. M. Collins. 1997. "Selective biotransformation of the Human Immunodeficiency Virus protease inhibitor, saquinavir, by human small-intestinal cytochrome P4503A4: potential contribution to high first- pass metabolism." *Drug Metab Dispos* 25(2): 256-66.

Flexner, C. 1998. "HIV-Protease inhibitors." *New England Journal of Medicine* 338(18): 1281-92.

Flexner, C. 2000. "Dual protease inhibitor therapy in HIV-Infected patients: pharmacologic rationale and clinical benefits." *Annual Review of Pharmacology and Toxicology* 40:649-74.

Flexner, C., S. S. Broyles, P. Earl, S. Chakrabarti, and B. Moss. 1988. "Characterization of human immunodeficiency virus Gag/Pol gene products expressed by recombinant." *Virology* 166(2): 339-49.

Ford, J. M. and W. N. Hait. 1990. "Pharmacology of drugs that alter multidrug resistance in cancer." *Pharmacological Reviews* 42(3): 155-99.

Ford, J. M., E. P. Bruggemann, I. Pastan, M. M. Gottesman, and W. N. Hait. 1990. "Cellular and biochemical-characterization of thioxanthenes for reversal of multidrug resistance in human and murine cell-lines." *Cancer Research* 50(6): 1748-56.

Friche, E. P.B. Jensen, M. Sehested, J.F. Demant, N.N.Nissen 1990. "The solvents cremophor EL and polysorbate 80 modulate daunorubicin resistance in the multidrug resistant ehlich ascites Tumor". *Cancer Commun.* 2: 297-303

Fromm, M. F., D. Busse, H. K. Kroemer, and M. Eichelbaum. 1996. "Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin." *Hepatology* 24(4): 796-801.

Fukui, E., H. Kurohara, A. Kageyu, Y. Kurosaki, T. Nakayama, and T. Kimura. 1989. "Enhancing effect of medium-chain triglycerides on intestinal absorption of D-α-Tocopherol acetate from lecithin-dispersed preparations in the rat." *J. Pharmacobio-Dyn.* 12: 80-86.

Gallo Torres, H. E. 1970. "Intestinal absorption and lymphatic transport of D,1-3, 4-3H2-a-Tocopheryl nicotinate in the rat." *Int J Vitamin Nutr. Res.* 40(4): 505-14.

Gallo Torres, H. E. 1970. "Obligatory role of bile for the intestinal absorption of vitamin E." *Lipids* 5: 379-84.

Gao, Z. G., H. G. Choi, H. J. Shin, K. M. Park, S. J. Lim, K. J. Hwang, and C. K. Kim. 1998. "Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A." *International Journal of Pharmaceutics* 161(1): 75-86.

Garzon-Aburbeh, A., J. H. Poupaert, M. Claesen, and P. Dumont. 1986. "A lymphotropic prodrug of L-Dopa." *Journal of Medicinal Chemistry* 29: 687-91.

Garzon-Aburbeh, A., J. H. Poupaert, M. Claesen, P. Dumont, and G. Atassi. 1983. "1,3-Dipalmitoylglycerol ester of chlorambucil as a lymphotropic, orally administrable anti-neoplastic agent." *Journal of Medicinal Chemistry* 26(8): 1200-3.

Geelen, J., A. Coert, R. Meijer, and J. van der Vies. 1977. "Comparison of metabolism of testosterone undecanoate and testosterone in the gastrointestinal wall in rat in vitro and in vivo." *Acta Endocrinologica* 86: 216-24.

Germann, U. A., I. Pastan, and M. M. Gottesman. 1993. "P-glycoproteins: mediators of multidrug resistance." *Sem. Cell Biology* 4: 63-76.

Gibaldi, M. and S. Feldman. 1970. "Mechanisms of surfactant effects on drug absorption." *Journal of Pharmaceutical Sciences* 59(5): 579-89.

Gibbs, M. A., M. T. Baillie, D. D. Shen, K. L. Kunze, and K. E. Thummel. 2000. "Persistent inhibition of Cyp3A4 by ketoconazole in modified caco-2 cells." *Pharmaceutical Research* 17(3): 299-305.

Glynn, S. L. and M. Yazdanian. 1998. "In vitro blood-brain barrier permeability of nevirapine compared to other HIV antiretroviral agents." *Journal of Pharmaceutical Sciences* 87(3): 306-10.

Gollapudi, S. and S. Gupta. (1990). Human immunodeficiency virus I – induced expression of P-glycoprotein. *Biochem. Biophys. Res Commun.* 171: 1002-1007.

Gomez, D. Y., V. J. Wacher, S. J. Tomlanovich, M. F. Hebert, and L. Z. Benet. 1995. "The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporin." *Clinical Pharmacology & Therapeutics* 58(1): 15-19.

Gottesman, M. M. and I. Pastan. 1993. "Biochemistry of multidrug-resistance mediated by the multidrug-transporter." *Annual Review of Biochemistry* 62:385-427.

Granger, D. N., N. A. Mortillaro, R. Kvietys, G Rutili, J. C. Parker, and A. E. Taylor. 1980. "Role on interstitial matrix during intestinal volume absorption." *American Journal of Physiology* 238: G183.

Green, P. G. and J. Hadgraft. 1987. "Facilitated transfer of cationic drugs across a lipoidal membrane." *International Journal of Pharmaceutics* 37: 251-55.

Grimus, R. C. and I. Schuster. 1984. "The role of the lymphatic transport in the enteral absorption of naftifine by the rat." *Xenobiotica* 14(4): 287-94.

Groothius, D.R. and R.M. Levy. 1997. "The entry of antiviral and antiretroviral drugs into the central nervous system". *J. Neurovirol.* 3: 387-400

Groves, M. J., R. M. A. Mustafa, and J. E. Carless. 1974. "Phase studies of mixed phosphated surfactants N-hexane and water." *Journal of Pharmacy and Pharmacology* 26: 616-23.

Gupta, S., C.H. Kim, T. Tsuruo and S. Gollapudi. 1992. "Preferential expression and activity of multidrug resistance gene 1 product (P-glycoprotein), a functional active efflux pump, in human CD8+ T cells: A role in cytotoxic effector function." *J. Clin. Immunol.* 12: 203-210.

Gunthard, H.F., D.V. Havlir, S. Ficus, Z.Q.Zhang, J. Eron, J. Mellors, et al. 2001. "Residual HIV-1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years." *Journal of Infectious Diseases* 183(9): 1318-27

Haase, a. T., K. Henry, M. Zupancic, G. Sedgewick, R. A. Faust, H. Melroe, W. Cavert, K. Gebhard, K. Staskus, Z. Q. Zhang, P. J. Dailey, H. H. Balfour, a. Erice, and a. S. Perelson. 1996. "Quantitative image analysis of Hiv-1 infection in lymphoid tissue." *Science* 274(5289): 985-89.

Hargrove, J. T., W. S. Mason, and A. C. Wentz. 1989. "Absorption of oral progesterone is influenced by vehicle and particle size." *Am. J. Obstet. Gynecol* 161: 948-51.

Harvie, P., A. Desormeaux, N. Gagne, M. Tremblay, L. Poulin, and D. Beauchamp. 1995. "Lymphoid tissues targeting of liposome-encapsulated 2',3'-Dideoxyinosine." *AIDS* 9(7): 701-7.

Hauss, D. J., S. C. Mehta, and G. W. Radebaugh. 1994. "Targeted lymphatic transport and modified systemic distribution of Ci-976, a lipophilic lipid-regulator drug, via a formulation approach." *International Journal of Pharmaceutics* 108(2): 85-93.

Hauss, D. J., S. E. Fogal, J. V. Ficorilli, C. A. Price, T. Roy, A. A. Jayaraj, and J. J. Keirns. 1998. "Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor." *Journal of Pharmaceutical Sciences* 87(2): 164-9.

Hebert, M. F. 1997. "Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporin and tacrolimus oral drug delivery." *Advanced Drug Delivery Reviews* 27(2-3): 201-14.

Hebert, M. F., J. P. Roberts, T. Prueksaritanont, and L. Z. Benet. 1992. "Bioavailability of cyclosporin with concomitant rifampin administration is markedly less than predicted by hepatic enzyme-induction." *Clinical Pharmacology & Therapeutics* 52(5): 453-57.

Higgins, C. F. 1992. "ABC transporters, from microorganisms to man." *Annu, Rev. Cell Biol.* pp110-113

Higgins, C. F. and M. M. Gottesman. 1992. "Is the multidrug transporter a Flippase?" *Trends in Biochemical Sciences* 17(1):18-21.

Hirnle, P. 1997. "Liposomes for drug targeting in the lymphatic system." *Hybridoma* 16(1):127-32.

Hirsch, M. S. and R. T. Daquila. 1993. "Therapy for human-immunodeficiency-virus infection." *New England Journal of Medicine* 328(23): 1686-95.

Ho, D.D., A.U. Neumann, A.S. Perelson, W. Chen, J.M. Leonard and M. Markowitz. 1995. "Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. " *Nature* 373: 126-6.

Hoffman, N. E. 1970. "The relationship between uptake in vitro of oleic acid and micellar solubilization." *Biochimica Et Biophysica Acta* 196: 193-203.

Hofmann, A. F. and B. Borgstrom. 1964. "The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained During Fat Digestion and Absorption." *Journal of Clinical Investigation* 43:247-57.

Hofmann, A. F. and B. Borgstrom.. 1962. "Physio-chemical state of lipids in intestinal content during their digestion and absorption." *Fed. Proc.* 21: 43-50.

Hollt, V., M. Kouba, M. Dietel, and G. Vogt. 1992. "Stereoisomers of calcium-antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein." *Biochemical Pharmacology* 43(12): 2601-8.

Holmberg, I., L. Aksnes, T. Berlin, B. Lindback, J. Zemgals, and B. Lindeke. 1990. "Absorption of a pharmacological dose of vitamin-D3 from 2 different lipid vehicles in man - comparison of peanut oil and a medium chain triglyceride." *Biopharmaceutics & Drug Disposition* 11(9): 807-15.

Homan, R., J. E. Grossman, and H. J. Pownall. 1991. "Differential effects of eicosapentanoic acid and oleic acid on lipid synthesis and secretion by HepG2 cells." *Journal of Lipid Research* 32: /231-41.

Hong-Wei Wu, S. and W.K. Hopkins. 1999. " Characteristics of D-α-Tocopheryl PEG 1000 succinate for applications as an absorption enhancer in drug delivery systems". Pharmaceutical Technology, 23(10): 52-67

Horio, M., E. Lovelace, I. Pastan, and M. M. Gottesman. 1991. "Agents which reverse multidrug-resistance are inhibitors of [H-3]Vinblastine transport by isolated vesicles." *Biochimica et Biophysica Acta* 1061(1): 106-10.

Horst, H. J., W. J. Holte, M. Dennis, A. Coert, J. Geelen, and K. D. Voigt. 1976. "Lymphatic absorption and metabolism of orally administered testosterone undecanoate in man." *Klin. Wschr* 54: 875-79.

Horter, D. and J. B. Dressman. 1997. "Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract." *Advanced Drug Delivery Reviews* 25(1): 3-14.

Horter, D. and J. B. Dressman. 2001. "Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract." *Advanced Drug Delivery Reviews* 46(1-3): 75-87.

Hsing, S., Z. Gatmaitan, and I. M. Arias. 1992. "The function of Gp170, the multidrug-resistance gene product, in the brush border of rat intestinal mucosa." *Gastroenterology* 102(3): 879-85.

Hsu, A., G. R. Granneman, G. L. Cao, L. Carothers, T. El-Shourbagy, P. Baroldi, K. Erdman, F. Brown, E. Sun, and J. M. Leonard. 1998. "Pharmacokinetic interactions between two human immunodeficiency virus protease inhibitors, Ritonavir and Saquinavir." *Clinical Pharmacology & Therapeutics* 63(4): 453-64.

Hughes, C. M., J. G. Swanton, and P. S. Collier. 1993. "Cyclosporin A and Erythromycin: a study of a drug interaction in the *in situ* perfused rat liver model." *Biopharm Drug Dispos* 14 (7): 615-25.

Humberstone, a. J. and W. N. Charman. 1997. "Lipid-based vehicles for the oral delivery of poorly water-soluble Drugs." *Advanced Drug Delivery Reviews* 25(1): 103-28.

Humberstone, A. J., A. F. Cowman, J. Horton, and W. N. Charman. 1998. "Effect of altered serum lipid concentrations on the IC50 of halofantrine against plasmodium." *Journal of Pharmaceutical Sciences* 87(2): 256-8.

Humberstone, a. J., C. J. H. Porter, and W. N. Charman. 1996. "A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine." *Journal of Pharmaceutical Sciences* 85(5): 525-29.

Hunter, J. and B. H. Hirst. 1997. "Intestinal Secretion of Drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption." *Advanced Drug Delivery Reviews* 25(2-3): 129-57.

Hunter, J., B. H. Hirst, and N. L. Simmons. 1991. "Epithelial secretion of vinblastine by human intestinal adenocarcinoma cell (Hct-8 and T84) layers expressing P-glycoprotein." *British Journal of Cancer* 64(3): 437-44.

Hurst, M. and D. Faulds. 2000. "Lopinavir." *Drugs* 60(6): 1371-79.

Hussain, M. M. 2000. "A proposed model for the assembly of chylomicrons." *Atherosclerosis* 1481-15.

Ichihashi, T., H. Kinoshita, Y. Takagishi, and H. Yamada. 1991. "Intrinsic lymphatic partition rate of mepitisotane, epitiostanol and oleic acid absorbed from rat intestine." *Pharmaceutical Research.* 8: 1302-6.

Ichihashi, T., H. Kinoshita, Y. Takagishi, and H. Yamada. 1992. "Effect of bile on absorption of mepitiostane by the lymphatic-system in rats." *Journal of Pharmacy and Pharmacology* 44(7): 565-69.

Ichihashi, T., H. Kinoshita, Y. Takagishi, and H. Yamada. 1992. "Effect of oily vehicles on absorption of mepitiostane by the lymphatic-system in rats." *Journal of Pharmacy and Pharmacology* 44(7): 560-564.

Ichihashi, T., Y. Takagishi, and H. Yamada. 1992. "Factors determining the intrinsic lymphatic partition rate of epitiostanol and mepitiostane." *Pharmaceutical Research* 9(12): 1617-21.

Jensen, L. T., H. P. Olesen, J. Risteli, and I. Lorenzen. 1990. "External thoracic-duct venous shunt in conscious pigs for long-term studies of connective-tissue metabolites in lymph." *Laboratory Animal Science* 40(6): 620-624.

Johnson, J. M. 1976. "Triglyceride biosynthesis in the intestinal mucosa." In: K. Rommel, H. Goebell and R. Bohmer (Eds.), Lipid Absorption, Biochemical and Clinical Aspects, MTP Press, Lancester. 85-94.

Jurimaromet, M., K. Crawford, T. Cyr, and T. Inaba. 1994. "Terfenadine metabolism in human Liver - *in-vitro* inhibition by macrolide antibiotics and azole antifungals." *Drug Metabolism and Disposition* 22(6): 849-57.

Kahan, B. D., J. Dunn, C. Fitts, D. Vanburen, D. Wombolt, R. Pollak, R. Carson, J. W. Alexander, M. Choc, R. Wong, and D. S. Hwang. 1995. "Reduced intersubject and intrasubject variability in cyclosporin pharmacokinetics in renal-transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals." *Transplantation* 59(4): 505-11.

Kale, N.J. and L.V. Allen. 1989. "Studies on microemulsions using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as cosurfactants." *International Journal of Pharmaceutics*, 57: 87-93.

Kartner, N., J.R. Riordan and V Ling. 1983. Cell surface p-glycoprotein is associated with multidrug resistance in mammalian cell lines. *Science* 221: 1285-88.

Katchen, B. and S. Symchowicz. 1967. "Correlation of dissolution rate and griseofulvin absorption in man." *Journal of Pharmaceutical Sciences* 511: 108-10.

Kenyon, C. J., F. Brown, G. R. Mcclelland, and I. R. Wilding. 1998. "The use of pharmacoscintigraphy to elucidate food effects observed with a novel protease inhibitor (saquinavir)." *Pharmaceutical Research* 15(3): 417-22.

Khoo, S. M., C. J. Porter, G. A. Edwards, and W. N. Charman. 1998a. "Metabolism of halofantrine to its equipotent metabolite, Desbutylyhalofantriene, is decreased when orally administered with ketoconazole." *Journal of Pharmaceutical Sciences* 87(12): 1538-41.

Khoo, S. M., A. J. Humberstone, C. J. H. Porter, G. A. Edwards, and W. N. Charman. 1998b. "Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine." *International Journal of Pharmaceutics* 167(1-2): 155-64.

Khoo, S. M., C. J. Porter, and W. N. Charman. 2000. "The formulation of halofantrine as either non-solubilizing PEG 6000 or solubilizing lipid." *International Journal of Pharmaceutics* 205(1-2): 65-78.

Khoo, S. M., G. A. Edwards, C. J. H. Porter, and W. N. Charman. 1999a. "A novel triple cannulated conscious dog model for assessment of the absorption, enterocyte-based metabolism and lymphatic transport of candidate lipophilic drugs." *Pharm Sci. 1 S4193 (Abstract).*

Khoo, S.M., C. J. H. Porter, G. A. Edwards, and W. N. Charman. 1999b. "Intestinal lymphatic transport is the primary absorption route for halofantrine after oral post-prandial administration." *Pharm Sci. 1 S-642 (Abstract).*

Kim, A. E., J. M. Dintaman, D. S. Waddell, and J. A. Silverman. 1998. "Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein." *Journal of Pharmacology and Experimental Therapeutics* 286(3): 1439-45.

Kim, C. K., Y. J. Choi, S. J. Lim, M. G. Lee, S. H. Lee, and S. J. Hwang. 1993. "Lymph-node targeting and pharmacokinetics of $^{[H-3]}$methotrexate-encapsulated neutral large unilamellar vesicles and immuno-liposomes." *International Journal of Pharmaceutics* 98(1-3) : 9-18.

Kim, R. B., M. F. Fromm, C. Wandel, B. Leake, a. J. J. Wood, D. M. Roden, and G. R. Wilkinson. 1998. "The drug transporter P-glycoprotein limits oral absorption and brain entry of Hiv-1 protease inhibitors." *Journal of Clinical Investigation* 101(2):289-94.

Kim, R. B, C. Wandel, B. Leake, M.Cvetkovic, M.F. Fromm, P.J. Dempsey et al 1999. Interrelationship between substrate and inhibitors of human CYP3A and P-glycoprotein. *Pharmaceutical Research* 16 (3): 408-12

Kim, C-K, Sang-A Ryuu, K-M Park, S.J. Lim and S-J Hwang. 1997. "Preparation and physio-chemical characterization of phase inverted water/oil microemulsions containing cyclosporin." *.International Journal of Pharmaceutics* 147: 131-134

Kitchen, V. S., C. Skinner, K. Ariyoshi, E. A. Lane, I. B. Duncan, J. Burckhardt, H. U. Burger, K. Bragman, A. J. Pinching, and J. N. Weber. 1995. "Safety and activity of saquinavir in HIV infection." *Lancet* 345(8955): 952-5.

Kiyasu, J. K., B. Bloom, and I. L. Chaikoff. 1952. "The portal blood absorption of absorbed fatty acids." *Journal of Biological Chemistry* 199: 415-19.

Kohl, N. E., E. A. Emini, W. A. Schleif, L. J. Davis, J. C. Heimbach, and R. A. Dixon. 1988. "Active human immunodeficiency virus protease is required for viral infectivity." *Proceedings of the National Academy of Sciences of the United States of America.*

Kolars, J. C., K. S. Lown, P. Schmiedlinren, M. Ghosh, C. Fang, S. A. Wrighton, R. M. Merion, and P. B. Watkins. 1994. "Cyp3a gene-expression in human gut epithelium." *Pharmacogenetics* 4(5): 247-59.

Kolars, J. C., P. L. Stetson, B. D. Rush, M. J. Ruwart, P. Schmiedlin-Ren, and E. A. Duell. 1992. "Cyclosporin metabolism by P450IIIA in rat enterocytes." *Transplantation* 53(3): 596-602.

Kolars, J. C., P. Schmiedlin-Ren, J. D. Schuetz, C. Fang, and P. B. Watkins. 1992. "Identification of rifampin-inducible P450iiia4 (Cyp3A4) in human small bowel." *Journal of Clinical Investigation* 90(5): 1871-8.

Kolars, J. C., W. M. Awni, R. M. Merion, and P. B. Watkins. 1991. "First-pass metabolism of cyclosporin by the gut." *Lancet* 338(8781): 1488-90.

Komiya, I., J.U. Park, N.F.H. Ho, W.I. Higuchi. 1980. "Quantitative mechanistic studies in simultaneous fluid flow and intestinal absorption using steroids as model solutes." *International Journal of Pharmaceutics* 4: 249-262

Kommuru, T. R., B. Gurley, M. A. Khan, and I. K. Reddy. 2001. "Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q(10): formulation development and bioavailability assessment." *International Journal of Pharmaceutics* 212(2): 233-46.

Kommuru, T. R., M. Ashraf, M. A. Khan, and I. K. Reddy. 1999. "Stability and bioequivalence studies of two marketed formulations of coenzyme Q(10) in beagle dogs." *Chemical & Pharmaceutical Bulletin* 47(7): 1024-28.

Kortz, W. J., B. D. Schirmer, C. M. Mansbach 2nd, F. Shelburne, M. R. Toglia, and Quarfordt. 1984. "Hepatic uptake of chylomicrons and triglyceride emulsions in rats fed diets of differing fat." *Journal of Lipid Research* 25(8): 799-804.

Kovarik, J. M., E. A. Mueller, J. B. Vanbree, W. Tetzloff, and K. Kutz. 1994. "Reduced interindividual and intraindividual variability in cyclosporin pharmacokinetics from a microemulsion formulation." *Journal of Pharmaceutical Sciences* 83(3): 444-46.

Kroemer, H. K., J. C. Gautier, P. Beaune, C. Henderson, C. R. Wolf, and M. Eichelbaum. 1993. "Identification of P450 enzymes involved in metabolism of verapamil in humans." *Naunyn-Schmiedebergs Archives of Pharmacology* 348(3): 332-37.

Kuksis, A. 1987. "Absorption of fat soluble vitamins." In: A Kuksis (Ed.), Fat Absorption Vol 2. CRC Press Boca Raton. 65-86.

Kupferschmidt, H. H. T., H. R. Ha, W. H. Ziegler, P. J. Meier, and S. Krahenbuhl. 1995. "Interaction between grapefruit juice and midazolam in humans." *Clinical Pharmacology & Therapeutics* 58(1): 20-28.

Kupferschmidt, H. H., K. E. Fattinger, H. R. Ha, F. Follath, and S. Krähenbühl. 1998. "Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man." *Br J Clin Pharmacol* 45(4): 355-9.

Kvietys, P. R., R. D. Speran, M. B. Grisham, and P. Tso. 1991. "Jejunal mucosal injury and restition: role of hydrolytic products of food digestion." *American Journal of Physiology* 264: G384-91.

Labroo, R. B., K. E. Thummel, K. L. Kunze, T. Podoll, W. F. Trager, and E. D. Kharasch. 1995. "Catalytic role of cytochrome P4503a4 in multiple pathways of alfentanil metabolism." *Drug Metabolism and Disposition* 23(4): 490-496.

Laher, J. M., M. W. V. R. D. Rigler, J. A. Barrowman, and J. S. Patton. 1984. "Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat." *Journal of Lipid Research* 251: 337-42.

Lamka, J. and J. Kvetina. 1990. "On the limiting factors affecting the distribution of drugs from blood into the lymphatic system." *Biopharmaceutics and Pharmacokinetics.*

Lampen, A., U. Christians, F. P. Guengerich, P. B. Watkins, J. C. Kolars, and A. Bader. 1995. "Metabolism of the immunosuppressant tacrolimus in the small intestine." *Drug Metabolism & Disposition* 23(12): 1315-24.

Lang, V. B., P. Langguth, C. Ottiger, H. WunderliAllenspach, D. Rognan, B. RothenRutishauser, J. C. Perriard, S. Lang, J. Biber, and H. P. Merkle. 1997. "Structure-permeation relations of Met-Enkephalin peptide analogues on absorption and secretion mechanisms in caco-2 monolayers." *Journal of Pharmaceutical Sciences* 86(7): 846-853

Lawrence, M. J. 1996. "Microemulsions as drug delivery vehicles." *Current Opinion in Colloid & Interface Science* 1(6): 826-32.

Lawrence, M. J. and G. D. Rees. 2000. "Microemulsion-based media as novel drug delivery systems." *Advanced Drug Delivery Reviews* 45(1): 89-121.

Leak, L. V. 1980. "Lymphatic removal of fluids and particles in the mammalian lung." *Environmental Health Perspectives* 35(55).

Lee, J. S. "Lymphatic Contractility". In: Physiology of the Lymphatic Circulation, Shepard, A.P., and Granger, D.N., Eds., Raven Press, N.Y., 201, 1984. .

Lennernas, H., S. Nylander, and a. L. Ungell. 1997. "Jejunal permeability: a comparison between the ussing chamber technique and the single-pass perfusion in humans." *Pharmaceutical Research* 14(5): 667-71.

Lennernas, H. S. 1997. "Human jejunal effective permeability and its correlation with preclinical drug absorption models". *J. Pharm. Pharmacol.* 49: 627-38.

Leroux, J., R. Cozens, J. L. Roesel, B. Galli, F. Kubel, E. Doelker, and R. Gurny. 1995. "Pharmacokinetics of a novel HIV-1 protease inhibitor incorporated into biodegradable or enteric nanocapsules following intravenous and oral administration to mice." *Journal of Pharmaceutical Sciences* 84 (12).

Levin, M. S., V. D. Talkad, J. I. Gordon, and W. F. Stenson. 1992. "Trafficking of exogenous fatty-acids within Caco-2 cells." *Journal of Lipid Research* 33(1): 9-19.

Levy, E., M. Mehran, and E. Seidman. 1995. "Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion." *Faseb Journal* 9(8): 626-35.

Li, X. L. and W. K. Chan. 1999. "Transport, metabolism and elimination mechanisms of anti-HIV agents." *Advanced Drug Delivery Reviews* 39(1-3): 81-103.

Lipton, S.A. 1998. "Neuronal injury associated with HIV-1: approaches to treatment." *Pharmaceutical Research* 15: 1182-1188.

Lin, J.H., M. Chiba, S.K. Balani, I.W. Chen, G. Kwei, K.J. Vastag and J.A. Nishime. 1996. Species differences in the pharmacokinetics and metabolism of Indinavir, a potent HIV protease inhibitor. *Drug Metabolism and Disposition* 24(10): 1111-15.

Lin, J. H. 1997. "Human immunodeficiency virus protease inhibitors - from drug design to clinical studies." *Advanced Drug Delivery Reviews* 27(2-3): 215-33.

Lin, J. H., D. E. Storey, I. W. Chen, and X. Xu. 1996. "Improved oral absorption of L-365 260, a poorly soluble drug." *Biopharmaceutics & Drug Disposition* 17(1): 1-15.

Lin, J. H., I. Chen, and H. Lievens. 1991. "The effect of dosage forms on the oral absorption of L-365260, a potent CCKb receptor antagonist in dogs." *Pharmaceutical Research* 8 S272.

Lipton, S. A. and H. E. Gendelman. 1995. "Dementia associated with the acquired-immunodeficiency-syndrome." *New England Journal of Medicine* 332(14): 934-40.

Liu, H. X., I. Adachi, I. Horikoshi, and M. Ueno. 1991. "Promotion of intestinal drug absorption by milk fat globule membrane." *Yakugaku Zasshi* 111:510-514.

Liu, H. X., I. Adachi, I. Horikoshi, and M. Ueno. 1995. "Mechanism of promotion of lymphatic drug absorption by milk-fat globule-membrane." *International Journal of Pharmaceutics* 118(1): 55-64.

Lown, K. S., D. G. Bailey, R. J. Fontana, S. K. Janardan, C. H. Adair, and L. A. Fortlage. 1997. "Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal extraction." *Journal of Clinical Investigation* 99(10): 2545-53.

Lown, K. S., J. C. Kolars, K. E. Thummel, J. L. Barnett, K. L. Kunze, S. A. Wrighton, and Watkins. 1994. "Interpatient heterogeneity in expression of Cyp3a4 and Cyp3a5 in small bowel." *Drug Metabolism & Disposition* 22(6): 947-55.

Lown, K. S., R. R. Mayo, A. B. Leichtman, H. L. Hsiao, D. K. Turgeon, and P. Schmiedlin-Ren. 1997. "Role of intestinal P-glycoprotein (Mdr1) in interpatient variation in the oral bioavailability." *Clinical Pharmacology & Therapeutics* 62(3): 248-60.

Lucas, M. L., W. Schneider, F. J. Harberich, and J. A. Blair. 1975. "Direct measurement by pH microelectode of the pH microclimate in rat proximal jejenum." *Proceedings of the Royal Society of London, Series B: Biological Sciences* 19239-48.

Lum, B. L., G. A. Fisher, J. Hausdorf, M. Gottwald, H. Collins, N. A. Brophy, M. N. Ehsan, J. Halsey, and B. I. Sikic. 1995. "The effect of SDZ-Psc-833, a cyclosporin-D analog, on the pharmacokinetic behaviour of etoposide during a phase-I trial to modulate multidrug-resistance." *Clinical Pharmacology & Therapeutics* 57(2): 175.

Lum, B. L., S. Kaubisch, a. M. Yahanda, K. M. Adler, L. Jew, M. N. Ehsan, N. A. Brophy, J. Halsey, M. P. Gosland, and B. I. Sikic. 1992. "Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporin in a phase-I trial to modulate multidrug resistance." *Journal of Clinical Oncology* 10(10): 1635-42.

Luner, P. E., S. R. Babu, and G. W. Radebaugh. 1994. "The effects of bile-salts and lipids on the physicochemical behaviour of gemfibrozil." *Pharmaceutical Research* 11(12): 1755-60.

Lunn, D. J. and L. Aarons. 1998. "The pharmacokinetics of saquinavir: a Markov Chain Monte Carlo population analysis." *J Pharmacokinet Biopharm* 26(1): 47-74.

Macgregor, K. J., J. K. Embleton, J. E. Lacy, E. A. Perry, L. J. Solomon, H. Seager, and C. W. Pouton. 1997. "Influence of lipolysis on drug absorption from the gastro-intestinal tract." *Advanced Drug Delivery Reviews* 25(1): 33-46.

Malcolmson, C., C. Satra, S. Kantaria, a. Sidhu, and M. J. Lawrence. 1998. "Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions." *Journal of Pharmaceutical Sciences* 87(1): 109-16.

Manouilov, K. K., I. I. Fedorov, F. D. Boudinot, C. A. White, L. P. Kotra, R. F. Schinazi, C. I. I. Hong, and C. K. Chu. 1995. "Lymphatic targeting of anti-Hiv nucleosides - distribution of 3'-Azido-3'-Deoxythymidine (Azt) and 3'-Azido-2',3'-Dideoxyuridine (Azdu) after administration of Dipalmitoylphosphatidyl Prodrugs to Mice." *Antiviral Chemistry & Chemotherapy* 6(4): 230-238.

Manouilov, K. K., Z. S. Xu, F. D. Boudinot, R. F. Schinazi, and C. K. Chu. 1997. "Lymphatic targeting of anti-Hiv nucleosides: distribution of 2',3'-Dideoxyinosine." *Antiviral Research* 34(3):91-9.

Marvel, J.R., D.A.Schlichting, C. Denton, E.J. Levy and M.M. Chan. 1964. *J. Invest. Dermatol.* 42: 197

Mathur, S. N., E. Born, S. Murthy, and F. J. Field. 1997. "Microsomal triglyceride transfer protein in caco-2 cells: characterization and regulation." *Journal of Lipid Research* 38(1): 61-67.

Maurice, M., L. Pichard, M. Daujat, I. Fabre, H. Joyeux, J. Domergue, and P. Maurel. 1992. "Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture." *FASEB J* 6(2): 752-8.

Mazer, N. A. and M. C. Carey. 1983. "Quasi-elastic light-scattering studies of aqueous biliary lipid systems. cholesterol solubilization and precipitation in model bile solutions." *Biochemistry* 22(2): 426-42.

Mazer, N. A., G. B. Benedek, and M. C. Carey. 1980. "Quasielastic light-scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions." *Biochemistry* 19(4): 601-15.

Mc Tavish, D. and E. M. Sorkin. 1989. "Verapamil: an updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension." *Drug* 38: 19-76.

McDonald, G. B. and M Weidman. 1987. "Partitioning of polar fatty acids into lymph and portal vein after intestinal absorption in the rat." *Q.J.Exp.Phsiology* 72: 153-59.

McDonald, G. B., D. R. Saunders, M. Weidman, and L. Fisher. 1980. "Portal venous transport of long-chain fatty acids absorbed from rat intestine." *American Journal of Physiology* 239: G141-50.

Meaney, C. 1997. "Use of intestinal cell culture models to assess the absorption enhancing potential of micellar systems". *PhD Thesis* University of Dublin, Trinity College Dublin.

Meijer, D. K. F., G. Molema, F. Moolenaar, D. Dezeeuw, and P. J. Swart. 1996. "(Glyco)-Protein drug carriers with an intrinsic therapeutic activity: the concept of dual targeting." *Journal of Controlled Release* 39(2-3): 163-72.

Meinzer, A., J. Mueller, and J. Vonderscher. 1995. "Microemulsion-a suitable galenical approach for the absorption enhancement of low soluble compounds?" *Bull. Tech. Gattefosse* 88: 21-26.

Merry, C., M. G. Barry, F. Mulcahy, J. F. Tjia, K. L. Halifax, J. Heavey, C. Kelly, and D. J. Back. 1998. "Ritonavir pharmacokinetics alone and in combination with saquinavir in HIV-infected patients." *Aids* 12(3): 325-27.

Mishima, M., S. Kobayashi, S. Abe, and C. Yamato. 1990. "Metabolic fate of indomethacin farnesil, a prodrug of indomethacin: characteristic biotransformation of indomethacin farnesil in rats." *Xenobiotica* 20:135-46.

Mithani, S. D., V. Bakatselou, C. N. Tenhoor, and J. B. Dressman. 1996. "Estimation of the increase in solubility of drugs as a function of bile salt concentration." *Pharmaceutical Research* 13(1): 163-67.

Miyamoto, E. A. Tsuji and T. Yamana. 1983. "Effects of surfactants on the G.I. absorption of β lactam antibiotics in rats". *J. Pharm. Sci.* 72: 651-654.

Miyazaki, S., H. Inoue, T. Yamahira and T. Nadai. 1979. "Interaction of drugs with bile components. I. Effects of bile salts on the dissolution behaviour of indomethacin and phenylbutazone." *Chem. Pharm. Bull.* 27: 2468-2472

Moberly, J. B., T. G. Cole, D. H. Alpers, and G. Schonfield. 1990. "Oleic acid stimulation of Apo-lipoprotein B secretion from Hep G2 Cells and caco2 cells occurs post-transcriptionally." *Biochimica Et Biophysica Acta* 104: 270-80.

Molema, G., R. W. Jansen, R. Pauwels, E. Declercq, and D. K. F. Meijer. 1990. "Targeting of antiviral drugs to lymphocytes-T4 - anti-HIV activity of neoglycoprotein Azt-mp conjugates *in-vitro*." *Biochemical Pharmacology* 40(12): 2603-10.

Molla, a., M. Korneyeva, Q. Gao, S. Vasavanonda, P. J. Schipper, H. M. Mo, M. Markowitz, T. Chernyavskiy, P. Niu, N. Lyons, a. Hsu, G. R. Granneman, D. D. Ho, C. A. B. Boucher, J. M. Leonard, D. W. Norbeck, and D. J. Kempf. 1996. "Ordered accumulation of mutations in HIV protease confers resistance to ritonavir." *Nature Medicine* 2(7): 760-766.

Moyle G and Gazzard B. 1996. "Current knowledge and future prospects for the use of HIV protease inhibitors." *Drugs* 51(5): 701-12.

Moyle, G. J., M. Sadler, and N. Buss. 1999. "Plasma and cerebrospinal fluid saquinavir concentrations in patients receiving combination antiretroviral therapy." *Clinical Infectious Diseases* 28(2): 403-4.

Mueller, E. A., J. M. Kovarik, and K. Kutz. 1994. "Minor influence of a fat-rich meal on the pharmacokinetics of new oral formulation of cyclosporin." *Transplantation Proceedings* 26(5):2957-58.

Mueller, E. A., J. M. Kovarik, J. B. Vanbree, J. Grevel, P. W. Lucker, and K. Kutz. 1994. "Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporin in a crossover comparison with the market formulations." *Pharmaceutical Research* 11(1): 151-55.

Mueller, E. A., J. M. Kovarik, J. B. Vanbree, W. Tetzloff, J. Grevel, and K. Kutz. 1994. "Improved dose linearity of cyclosporin pharmacokinetics from a microemulsion formulation." *Pharmaceutical Research* 11(2): 301-4.

Muller, C., J. D. Bailly, F. Goubin, J. Laredo, J. P. Jaffrezou, C. Bordier, and G. Laurent. 1994. "Verapamil decreases P-glycoprotein expression in multidrug-resistant human leukemic-cell lines." *International Journal of Cancer* 56(5): 749-54.

Muranishi, S. 1990. "Absorption Enhancers." *Critical Reviews in Therapeutic Drug Carrier System* 7: 1-35.

Muranishi, S., T. Fujita, M. Murakami, and a. Yamamoto. 1997. "Potential for lymphatic targeting of peptides." *Journal of Controlled Release* 46(1-2): 157-64.

Muranushi, N., Y. Nakajima, M. Kinugawa, S. Muranishi, and H. Sezaki. 1980. "Mechanism for inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles. II. Effect of the incorporation of various lipids on the permeability of liposomal membranes." *International Journal of Pharmaceutics* 4: 281-90.

Myers, R. A. and V. J. Stella. 1992. "Factors affecting the lymphatic transport of penclomedine (Nsc-338720), a lipophilic cytotoxic drug - comparison to DDT and hexachlorobenzene." *International Journal of Pharmaceutics* 80(1): 51-62.

Myers, R.A. 1990. "Systemic bioavailability and lymphatic transport of penclomidine and other polychlorinated compounds". *PhD Thesis* University of Kansas.

Naito, M. and T. Tsuruo. 1989. "Competitive inhibition of ATP dependent high affinity vincristine binding the plasma membrane of multidrug resistant K562 cells without calcium ion involvement." *Cancer Research.* 49(6): 1452-5, Mar 15

Nakamura, T., Y. Aoyama, and G. Katsui. 1975. "Studies of tocopherol derivatives: V. intestinal absorption of several D-1,3,4,H2-Alpha Tocopheryl esters in the rat." *Lipids* 10: 627-33.

Nankervis, R., S. S. Davis, N. H. Day, and P. N. Shaw. 1996. "Intestinal lymphatic transport of three retinoids in the rat after oral administration: effect of lipophilicity and lipid vehicle." *International Journal of Pharmaceutics* 130(1): 57-64.

Naylor, L. J., V. Bakatselou, and J. B. Dressman. 1993. "Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems." *Pharmaceutical Research* 10(6): 865-70.

Naylor, L. J., V. Bakatselou, N. Rodriguez-Hornedo, N.D. Weiner and J. B. Dressman. 1995. Dissolution of steroids in bile salt solutions is modified by the presence of lecithin. *Eur. J. Pharm. Biopharm.* 41: 346-353.

Nerurkar, M. M., N. F. H. Ho, P. S. Burton, T. J. Vidmar, and R. T. Borchardt. 1997. "Mechanistic roles of neutral surfactants on concurrent polarized and passive membrane transport of a model peptide in caco-2 cells." *Journal of Pharmaceutical Sciences* 86(7): 813-21.

Nerurkar, M. M., P. S. Burton, and R. T. Borchardt. 1996. "The use of surfactants to enhance the permeability of peptides through Caco-2 Cells by inhibition of an apically polarized efflux system." *Pharmaceutical Research* 13(4): 528-34.

Nishigaki, R., S. Awazu, M. Hanano, and E. Fuwa. 1976. "The effect of dosage form on absorption of vitamin A into lymph." *Chem. Pharm. Bull* 24: 3207-11.

Noble, S. and D. Faulds. 1996. "Saquinavir. A review of its pharmacology and clinical potential in the management of HIV infection." *Drugs* 52(1): 93-112.

Noguchi, T., W. N. Charman, and V. J. Stella. 1985a. "The effect of drug lipophilicity and lipid vehicles on the lymphatic absorption of various testosterone esters." *International Journal of Pharmaceutics* 24: 173-84.

Noguchi, T., W. N. Charman, and V. J. Stella. 1985b. "Lymphatic appearance of DDT in thoracic or mesenteric lymph duct cannulated Rats." *International Journal of Pharmaceutics* 24: 185-92.

O' Driscoll, C. M. 1992. "Anatomy and physiology of the lymphatics." In: Lymphatic transport of Drugs, Charman, W. N., and Stella, V.J. Eds., CRC Press, Boca Raton, 1.

O' Driscoll, C. M. 1996. "Micellar solubilization/self emulsifying systems for improved absorption of drugs." In: P. Couvreur, D. Duchene and I. Kalles (Eds.)., Formulation of Poorly Available Drugs for Oral Administration, Edition De Sante, Paris. 126-29.

O' Driscoll, C. M., J. R. O' Reilly and O. I. Corrigan. 1991. A comparison of the effect of synthetic and naturally occurring surfactants on the solubility and absorption of Clofazimine (B663). Proceedings of the 4th European Congress on Biopharmaceutics and Pharmacokinetics, special issue III, 1991, Pg 116-119

O' Driscoll, C. M. 1998. "The use of the Caco-2 model to investigate biochemical and metabolic aspects of lipid absorption." *Bull. Tech. Gattefosse* 9: 141-46.

O' Driscoll, C. M., R. A. Myers, and V. J. Stella. 1991. "Blood and lymph transport of DDT after oral and parenteral administration to anaesthetized rats." *International Journal of Pharmaceutics* 73(2): 177-83.

O' Morchoe, C. C., K. H. Albertine, and P. J. O' Morchoe. 1982. "The rate of translymphatic endothelial fluid movement in canine kidney." *Microvas. Res* 23(180).

O' Reilly, J. R. 1991. "Physiochemical aspects of the absorption of clofazimine from micellar systems in the rat". *Ph.D. Thesis*, University of Dublin, Trinity College Dublin.

O' Reilly, J. R., O. I. Corrigan, and C. M. O' Driscoll. 1994a "The effect of simple micellar systems on the solubility and intestinal-absorption of clofazimine (B663) in the anaesthetized rat." *International Journal of Pharmaceutics* 105(2): 137-46.

O' Reilly, J. R., O. I. Corrigan, and C. M. O Driscoll. 1994b "The effect of mixed micellar systems, bile-salt fatty-acids, on the solubility and intestinal-absorption of clofazimine (B663) in the anaesthetized rat." *International Journal of Pharmaceutics* 109(2):147-54.

Obodozie, O. G. 1997. "Absorption and distribution kinetics of DDT following oral administration in micellar systems to the rat." *Ph. D. Thesis* University of Dublin, Trinity College Dublin.

Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. "Very low density lipoproteins in intestinal lymph: role of triglyceride and cholesterol transport during fat absorption." *Journal of Clinical Investigation* 2: 367-73.

Ockner, R. K., R. M. Kaikaus, and N. M. Bass. 1992. "Fatty acid binding proteins: recent concepts of regulation and function." *Prog. Clin. Biol. Res.* 37: 5189-204.

Order, S. E. 1977. "Immunospecific radionuclide immunoglobin lymphography" In: Clinical Lymphography Vol 7, Clouse, M.E., Ed., Williams and Witkins, Baltimore 316.

Order, S. E., W. D. Bloomer, A. G. Jones, W. D. Kaplan, M. A. Davis, S. J. Adelstein, and S. Hellman. 1975. "Radionuclide immunoglobulin lympography: a case report." *Cancer* 35(1487).

Orrego, H., I Mena, E. Baraona, and R. Palma. 1965. "Modifications in hepatic blood flow and portal pressure produced by different diet" *Am. J. Dig. Dis.* 10: 239-248.

Paine, M. F., D. D. Shen, K. L. Kunze, J. D. Perkins, C. L. Marsh, J. P. Mcvicar, D. M. Barr, B. S. Gillies, and K. E. Thummel. 1996. "First-pass metabolism of midazolam by the human intestine." *Clinical Pharmacology & Therapeutics* 60(1): 14-24.

Palin, K. J. and C. J. Wilson. 1984. "The effect of different oils on the absorption of probucol in the rat." *Journal of Pharmacy and Pharmacology* 36: 641-43.

Palin, K. J., C. G. Wilson, S. S. Davis, and A. J. Phillips. 1982. "The effect of oils on the lymphatic absorption of DDT." *J Pharm Pharmacol* 34(11): 707-10.

Pang, S., Y. Koyanagi, S. Miles, C. Wiley, H. V. Vinters, and I. S. Y. Chen. 1990. "High-levels of unintegrated Hiv-1 Dna in brain-tissue of Aids dementia patients." *Nature* 343(6253):85-89.

Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and a. S. Fauci. 1993. "HIV-Infection is active and progressive in lymphoid-tissue during the clinically latent stage of disease." *Nature* 362(6418): 355-58.

Pantaleo, G., O. J. Cohen, T. Schacker, M. Vaccarezza, C. Graziosi, G. P. Rizzardi, J. Kahn, C. H. Fox, S. M. Schnittman, D. H. Schwartz, L. Corey, and a. S. Fauci. 1998. "Evolutionary pattern of human immunodeficiency virus (Hiv) replication and distribution in lymph nodes following primary infection: implications for antiviral therapy." *Nature Medicine* 4(3): 341-45.

Papisov, M. I., R. Weissleder, B. Schaffer, a. A. Bogdanov, and T. J. Brady. 1994. "Intravenous carriers for drug-delivery to lymph-nodes." *Journal of Controlled Release* 28(1-3): 293-94.

Patel, M.J., D.Q.M. Craig and M. Ashford. (1999) "Factors influencing the absorption of drugs from self-emulsifying drug delivery systems." 18th Pharmaceutical Technology conference and exhibition, 4: 63-74

Patton, J. S., B. Stone, C. Papa, R. Abramowitz, and S. H. Yalkowsky. 1984. "Solubility of fatty acids and other hydrophobic molecules in liquid trioleoylglycerol." *Journal of Lipid Research* 25(2):189-97.

Peeters, T. L., G. Vantrappen, and J. Janssens. 1980. "Bile acid output and the interdigestive migrating motor complex in normals and cholecysectomy patients." *Gastroent.* 79: 678-681.

Perry, C. M. and S. Noble. 1998. "Saquinavir soft-gel capsule formulation - a review of its use in patients with HIV infection." *Drugs* 55(3): 461-86.

Phillips, W. T., R. Klipper, and B. Goins. 2000. "Novel method of greatly enhanced delivery of liposomes to lymph nodes." *Journal of Pharmacology and Experimental Therapeutics* 295(1): 309-13.

Pichard, L., I. Fabre, G. Fabre, J. Domergue, B. Saintaubert, G. Mourad, and P. Maurel. 1990. "Cyclosporine-a drug-interactions - screening for inducers and inhibitors of cytochrome-P-450 (Cyclosporine-a Oxidase) in primary cultures of human hepatocytes and in liver-microsomes." *Drug Metabolism and Disposition* 18(5): 595-606.

Pocock, D. M. E. and A. Vost. 1970. "DDT absorption and chylomicron transport in rat." *Lipids* 9: 374-81.

Poelma, F. G. J., J. J. Tukker, and D. J. A. Crommelin. 1990. "The role of bile-salts in the intestinal-absorption of drugs." *Acta Pharmaceutica Technologica* 36(2): 43-52.

Poelma, F. G., J. J. Tukker, and D. J. Crommelin. 1989. "Intestinal absorption of drugs. I: the influence of taurocholate on the absorption of dantrolene in the small intestine of the rat." *Journal of Pharmaceutical Sciences* 78(4): 285-9.

Poirier, H., I. Niot, P. Degrace, M. C. Monnot, a. Bernard, and P. Besnard. 1997. "Fatty acid regulation of fatty acid-binding protein expression in the small intestine." *American Journal of Physiology-Gastrointestinal and Liver Physiology* 36(2): G289-G295.

Poirier, H., P. Degrace, I. Niot, a. Bernard, and P. Besnard. 1996. "Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine - comparison with fatty acid-binding proteins (FABP)." *European Journal of Biochemistry* 238(2): 368-73.

Porsgaard, T. and C. E. Hoy. 2000. "Absorption by rats of tocopherols present in edible vegetable oils." *Lipids* 35: 1073-78.

Porter, C. J. H. 1997. "Drug delivery to the lymphatic system." *Critical Reviews in Therapeutic Drug Carrier Systems* 14(4): 333-93.

Porter, C. J. H. and W. N. Charman. 1997. "Uptake of drugs into the intestinal lymphatics after Oral administration." *Advanced Drug Delivery Reviews* 25(1): 71-89.

Porter, C. J. H. and W. N. Charman. 2001. "Intestinal lymphatic drug transport: an update." *Adv. Drug Del. Rev.*50: 61-80.

Porter, C. J. H., S. A. Charman, and W. N. Charman. 1996a "Lymphatic transport of halofantrine in the triple-cannulated anaesthetized rat model: effect of lipid vehicle dispersion." *Journal of Pharmaceutical Sciences* 85(4): 351-56.

Porter, C. J. H., S. A. Charman, a. J. Humberstone, and W. N. Charman. 1996b "Lymphatic transport of halofantrine in the conscious rat when administered as either the free base or the hydrochloride salt: effect of lipid class and lipid vehicle dispersion." *Journal of Pharmaceutical Sciences* 85(4): 357-61.

Pouton, C. W. 2000. "Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-micro-emulsifying' drug delivery systems." *European Journal of Pharmaceutical Sciences* 11: S93-98.

Pouton, C. W. 1985. "Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification." *International Journal of Pharmaceutics* 27: 335-348.

Pouton, C. W. 1997. "Formulation of self-emulsifying drug delivery systems." *Advanced Drug Delivery Reviews* 25(1): 47-58.

Profit, L., V. A. Eagling, and D. J. Back. 1999. "The effect of P-glycoprotein on saquinavir absorption in Caco-2cells." *British Journal of Clinical Pharmacology* 47(5): 594.

Pullinger, C. R., J. F. North, B. B. Teng, V. A. Rifici, A. F. Ronchild de Brito, and J. scott. 1989. "The Apo lipoprotein B gene is constitutively expressed in Hep G2 Cells: regulation of secretion by oleic acid, albumin and insulin and measurement of MRNA half life." *Journal of Lipid Research* 30: 1065-77.

Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, and Doran. 1985. "Complete nucleotide sequence of the Aids virus." *Nature* 313(6000): 277-84.

Rautureau, M, A. Bisalli, and J. C. Rimbaud. 1981. "Bile salts and lipids in the aqueous intraluminal phase during the digestion of a standard meal in man." *Clin. Biol.* 5: 417-425.

Raviv, Y., H. B. Pollard, E. P. Bruggemann, I. Pastan, and M. M. Gottesman. 1990. "Photosensitized labelling of a functional multidrug transporter in living drug-resistant tumor-Cells." *Journal of Biological Chemistry* 265(7): 3975-80.

Reininger, E. J. and L. A. Saperstein. 1957. "Effect of digestion on the distribution of blood flow in the rat." *Science* 126: 1176.

Reiss , H. 1975. "Entropy-induced dispersion of bulk lipids." *J. Colloids Interface Sci.* 53: 61-71.

Ritschel, W. A. 1991. "Microemulsions for improved peptide absorption from the gastrointestinal-tract." *Methods and Findings in Experimental and Clinical Pharmacology* 13(3): 205-20.

Ritschel, W. A., S. Adolph, G. B. Ritschel, and T. Schroeder. 1990. "Improvement of peroral absorption of cyclosporin-a by microemulsions." *Methods and Findings in Experimental and Clinical Pharmacology* 12(2): 127-34.

Roberts, N. A., J.A. Martin, D. Kinchington, A.V.Broadhurst, J.C. Craig et al. 1990. "Rational design of peptide based HIV proteinase inhibitors" *Science* 248: 358-61.

Robinson, J. R. 1996. " Introduction: semi-solid formulations for oral drug delivery" *Bull. Tech. Gattefosse* 89: 11.

Rothe, C.F., A.M. Mattson, R.M. Nueslein and N.J. Hayes. 1957. *Amer. Med. Assoc. Arcg. Ind. Health.* 16: 82-86.

Rozman, K., L. Ballhorn, and T. Rozman. 1983. "Mineral oil in the diet enhances faecal excretion of DDT in the rhesus monkey." *Drug Chem. Toxicol.* 6: 311-16.

Saitoh, H. and B. J. Aungst. 1995. "Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations ccross rat intestine." *Pharmaceutical Research* 12(9): 1304-10.

Sakai, A., N. Mori, S. Shuto, and T. Suzuki. 1993. "Deacylation-Reacylation cycle: a possible absorption mechanism for the novel lymphotropic antitumor agent dipalmitoylphosphatidylfluoroouridine in rats." *Journal of Pharmaceutical Sciences* 82(6): 575-78.

Salphati, L. and L. Z. Benet. 1998. "Effects of ketoconazole on digoxin absorption and disposition in rat." *Pharmacology* 56(6): 308-13.

Sandstrom, R., T. W. Knutson, L. Knutson, B. Jansson, and H. Lennernas. 1999. "The effect of ketoconazole on the jejunal permeability and cyp3a metabolism of (R/S)-verapamil in humans." *British Journal of Clinical Pharmacology* 48(2): 180-189.

Sandstrom, R., A, Karlsson, L. Knutson and H. Lennernas. 1998. "Jejunal absorption and metabolism of R/S verapamil in humans". *Pharmaceutical Research* 15 (6): 586.

Sandstrom, R., A, Karlsson and H. Lennernas. 1998 "The absence of stereo selective P-glycoprotein mediated transport of R/S-verapamil across the rat jejunum". *J. Pharm. Pharmacol.* 50: 729-735.

Sarciaux, J. M., L. Acar, and P. A. Sado. 1995. "Using microemulsion formulations for oral-drug delivery of therapeutic peptides." *International Journal of Pharmaceutics* 120(2): 127-36.

Sato, H., H. X. Liu, I. Adachi, M. Ueno, M. Lemaire, and I. Horikoshi. 1994. "Enhancement of the intestinal-absorption of a cyclosporine derivative by milk-fat globule-membrane." *Biological & Pharmaceutical Bulletin* 17(11): 1526-28.

Schinkel, A. H., J. J. M. Smit, O. Vantellingen, J. H. Beijnen, E. Wagenaar, L. Vandeemter, Mol Caam, M. A. Vandervalk, E. C. Robanusmaandag, H. P. J. Teriele, a. J. M. Berns, and P. Borst. 1994. "Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain-barrier and to increased sensitivity to drugs." *Cell* 77(4): 491-502.

Schmid-Schonbein, G. W. 1990. "Microlymphatics and lymph flow." *Physiological Reviews* 70(4): 987-1028.

Schmiedlin-Ren, P., D. J. Edwards, M. E. Fitzsimmons, K. He, K. S. Lown, and P. M. Woster. 1997. "Mechanisms of enhanced oral availability of Cyp3A4 substrates by grapefruit constituents." *Drug Metabolism & Disposition* 25(11): 1228-33.

Schoeller, C., M. Keelan, G. Mulvey, W. Stremmel, and a. B. R. Thomson. 1995. "Role of a brush-border membrane fatty-acid-binding protein in oleic-acid uptake into rat and rabbit jejunal brush-border membrane." *Clinical and Investigative Medicine-Medecine Clinique et Experimentale* 18(5): 380-388.

Schrager, L. K. and M. P. D'souza. 1998. "Cellular and anatomical reservoirs of Hiv-1 in patients receiving potent antiretroviral combination therapy." *Journal of the American Medical Association* 280(1): 67-71.

Schuetz, E. G., W. T. Beck, and J. D. Schuetz. 1996. "Modulators and substrates of P-glycoprotein and cytochrome P4503a co-ordinately up-regulate these proteins in human colon carcinoma cells." *Molecular Pharmacology* 49(2): 311-18.

Schurtenberger, P., N.A. Mazer, W. Kanzig and R. Preisig 1984. In: *Proc. Int. Symp. On surfactants in solution*, Mitthal, K.L. and B. Lindman. (Eds.) Plenum Press, N.Y. 841-855

Senior, J. R. and K. J. Isselbacher. 1962. "Direct esterification of monoglycerides with palmityl coenzyme A by intestinal epithelial subcellular fractions." *Journal of Biological Chemistry* 237: 1454-59.

Seymour, L. W. 1992. "Passive tumor targeting of soluble macromolecules and drug conjugates." *Critical Reviews in Therapeutic Drug Carrier Systems* 9(2): 135-87.

Shah, N. H., M. T. Carvajal, C. I. Patel, M. H. Infeld, and A. W. Malick. 1994. "Self-emulsifying drug-delivery systems (SEDDS) with polyglycolyzed glycerides for improving in-vitro dissolution and oral absorption of lipophilic drugs." *International Journal of Pharmaceutics* 106(1): 15-23.

Shankland, W. 1970. "The equilibrium and structure of lecithin-cholate mixed micelles." *Chem. Phys. Lipids.* 4(2): 109-130.

Shapiro, A. B. and V. Ling. 1995. "Reconstitution of drug transport by purified P-glycoprotein." *Journal of Biological Chemistry* 270(27): 16167-75.

Shapiro, J.M., MA Winters, F Stewart, B Efron, J. Norris, MJ. Kozal et al. 1996. "The effect of high dose saquinavir on viral load and CD4+ T cell counts in HIV infected patients. " *Ann. Intern Med.* 124: 1039-50

Shaw, D.J. 1980.*Introduction to Colloid and surface chemistry*, 3rd Eds. Butterworth London, 1980.

Shiau, Y. F. 1990. "Mechanism of intestinal fatty-acid uptake in the rat - the role of an acidic microclimate." *Journal of Physiology-London* 421: 463-74.

Shiau, Y. F., D. A. Popper, M. Reed, C. Umstetter, D. Capuzzi, and G. M. Levine. 1985. "Intestinal triglycerides are derived from both endogenous and exogenous sources." *American Journal of Physiology* 248: G164-69.

Shiau, Y. F., P. Fernandez, M. J. Jackson, and S. McMonagle. 1985. "Mechanisms maintaining a low pH microclimate in the intestine." *American Journal of Physiology* G608-G619.

Shimizu, M., M. Tsunogai, and S. Arai. 1997. "Transepithelial transport of oligopeptides in the human intestinal cell, caco-2." *Peptides* 18(5):681-687 .

Sieber, S. M. 1974. "The entry of foreign compounds into the thoracic duct lymph of the rat." *Xenobiotica* 4(5): 265-84.

Sieber, S. M. 1976. "The lymphatic absorption of *p,p* DDT and some structurally related compounds in the rat." *Pharmacology* 14443-54.

Siegsmund, M. J., C. Cardarelli, I. Aksentijevich, Y. Sugimoto, I. Pastan, and M. M. Gottesman. 1994. "Ketoconazole effectively reverses multidrug-resistance in highly resistant Kb cells." *Journal of Urology* 151(2): 485-91.

Simmonds, W. J. 1972. "The role of micellar solubilization in lipid absorption." *Aust. J. Exp. Biol. Med. Sci.* 50: 403-21.

Sjovall, J. 1959. *Acta Physiol. Scand.* 46: 339

Sokol, R. J., K. E. Johnson, F. M. Karrer, M. R. Narkewicz, D. Smith, and I. Kam. 1991. "Improvement of cyclosporin absorption in children after liver-transplantation by means of water-soluble Vitamin-E." *Lancet* 338(8761): 212-15.

Solomon, L. J., J. K. Embleton, and C. W. Pouton. 1996. "Inhibition of lipolysis of medium chain triglycerides by non-ionic surfactants, a structure/activity study. " In: P. Couvreur, D. Duchene and I. Kalles (Eds.)., Formulation of Poorly Available Drugs for Oral Administration, Edition De Sante, Paris. 437-38.

Solomon, L. J., J. K. Embleton, and C. W. Pouton. 1996. "Solubilization of steroidal compounds by mixed bile salt/lecithin micelles". In: P. Couvreur, D. Duchene and I. Kalles (Eds.)., Formulation of Poorly Available Drugs for Oral Administration, Edition De Sante, Paris. 219-20.

Sparreboom, a., J. Vanasperen, U. Mayer, a. H. Schinkel, J. W. Smit, D. K. F. Meijer, P. Borst, W. J. Nooijen, J. H. Beijnen, and O. Vantellingen. 1997. "Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine." *Proceedings of the National Academy of Sciences of the United States of America* 94(5): 2031-35.

Spencer, D. C. and R. W. Price. 1992. "Human-Immunodeficiency-virus and the central-nervous-system." *Annual Review of Microbiology* 46: 655-93.

Sprenger, R., K. M. Toellner, C. Schmetz, W. Luke, C. Stahlhennig, M. Ernst, G. Hunsmann, H. Schmitz, H. D. Flad, J. Gerdes, and J. P. Zimmer. 1995. "Follicular dendritic cells productively infected with immunodeficiency viruses transmit infection to T-Cells." *Medical Microbiology and Immunology* 184(3): 129-34.

Staggers, J. E., O. Hernell, R. J. Stafford, and M. C. Carey. 1990. "Physical-chemical behaviour of dietary and biliary lipids during and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings." *Biochemistry* 29: 2029-40.

Stremmel, W. 1988. "Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein." *Journal of Clinical Investigation* 82: 2001-2010.

Storch, J. and A.E. Thumser. 2000. "The fatty acid transport function of fatty acid binding proteins." *Biochim. Biophys. Acta 1486: 28-46*

Supersaxo, A., W. R. Hein, and H. Steffen. 1991. "Mixed micelles as a proliposomal, lymphotropic Drug carrier." *Pharmaceutical Research* 8: 1286-91.

Swenson, E.S. and W.J. Curtalo. 1992. "Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity." *Adv. Drug Delivery Reviews* 8: 39-92

Swenson, E. S., W. B. Milisen, and W. Curatolo. 1994. "Intestinal permeability enhancement, efficacy, acute local toxicity and reversibility." *Pharmaceutical Research* 11: 1132-42.

Takada, K., H. Yoshimura, N. Shibata, Y. Masuda, H. Yoshikawa, S. Muranishi, T. Yasumura, and T. Oka. 1986. "Effect of administration route on the selective lymphatic delivery of cyclosporin A by lipid-surfactant mixed micelles." *J Pharmacobiodyn* 9(2): 156-60.

Takada, K., N. Shibata, H. Yoshimura, Y. Masuda, H. Yoshikawa, S. Muranishi, and T. Oka. 1985. "Promotion of the selective lymphatic delivery of cyclosporin A by lipid-surfactant mixed micelles." *J Pharmacobiodyn.* 8(4): 320-3.

Takada, K., Y. Furuya, H. Yoshikawa, and S. Muranishi. 1988. "Biological and pharmaceutical factors affecting the absorption and lymphatic delivery of cyclosporin A from gastrointestinal tract." *J Pharmacobiodyn.* 11(2): 80-7.

Takada, K., Y. Furuya, T. Nakata, H. Yoshikawa, S. Muranishi, T. Yasumura, and T. Oka. 1987. "Development of a new carrier for cyclosporin A with selectivity for lymphatics." *Transplant Proc* 19(1 Pt 2): 1711-2.

Takada, T., H. Yoshimura, H. Yoshikawa, S. Muranishi, T. Yasumura, and S. Oka. 1986. "Enhanced lymphatic delivery of cyclosporin A by solubilisers and intensified immunosuppresive activity against mice skin allograft." *Pharmaceutical Research* 348-51.

Takahashi, Y.I. and B.A. Underwood. 1974. "Effect of long and medium chain length lipids upon aqueous solubility of alpha tocopherol" *Lipids* 9(11): 855-859.

Takakura, Y., T. Fujita, M. Hashida, and H. Sezaki. 1990. "Disposition characteristics of macromolecules in tumor-bearing mice." *Pharmaceutical Research* 7(4): 339-46.

Tam, Y. K. 1993. "Individual variation in 1st-pass metabolism." *Clinical Pharmacokinetics* 25(4): 300-328.

Tangerman, A., A. van Schaik, and E. W. van der Hoek. 1986. "Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal." *Clinica Chimica Acta* 159(2): 123-32.

Taniguchi, K., S. Muranishi, and H. Sezaki. 1980. "Enhanced intestinsal permeability to macromolecules II. Improvement of the large intestinal absorption of heparin by lipid-surfactant mixed micelles in rat." *International Journal of Pharmaceutics* 4:219-28.

Tarr, B. D. and S. H. Yalkowsky. 1989. "Enhanced intestinal absorption of cyclosporin in rats through the reduction of emulsion droplet size." *Pharmaceutical Research* 6: 40-43.

Tauber, U., K. Schroder, B. Dusterberg, and H. Matthes. 1986. "Absolute bioavailability of testosterone after oral administration of testosterone undecanoate and testosterone." *Eur. J. Drug Metab. Pharmacokinetics* 11: 145-49.

Thiebaut, F., T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan, and M. C. Willingham. 1987. "Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues." *Proc Natl Acad Sci U S A* 84(21)): 7735-8.

Thomson, A. B., C. Schoeller, M. Keelan, L. Smith, and M. T. Clandinin. 1993. "Lipid absorption: passing through the unstirred layers, brush border membrane, and beyond." *Can. J. Physiol. Pharmacol.* 71: 531-55.

Thummel, K. E. and G. R. Wilkinson. 1998. "In vitro and in vivo drug interactions involving human cyp3a." *Annual Review of Pharmacology and Toxicology* 38: 389-430.

Thummel, K. E., D. Oshea, M. F. Paine, D. D. Shen, K. L. Kunze, J. D. Perkins, and G. R. Wilkinson. 1996. "Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic Cyp3a-mediated metabolism." *Clinical Pharmacology & Therapeutics* 59(5): 491-502.

Thummel, K. E., K. L. Kunze, and D. D. Shen. 1997. "Enzyme-catalysed processes of first-pass hepatic and intestinal drug extraction." *Advanced Drug Delivery Reviews* 27(2-3): 99-127.

Thumser, a. E. A. and J. Storch. 2000. "Liver and intestinal fatty acid-binding proteins obtain fatty acids from phospholipid membranes by different mechanisms." *Journal of Lipid Research* 41(4): 647-56.

Tomida, H., T. Yooysuyanagi, and K. Ikelda. 1978. "Solubilization of steroid hormones by polyoxyethylene lauryl ether." *Chem. Pharm. Bull* 26: 2832-37.

Tomizawa, H., Y. Aramaki, Y. Fujii, T. Hara, N. Suzuki, K. Yachi, H. Kikuchi, and S. Tsuchiya. 1993. "Uptake of phosphatidyl-serine liposomes by rat peyers-patches following intraluminal administration." *Pharmaceutical Research* 10(4): 549-52.

Trezise, a. E. O., P. R. Romano, D. R. Gill, S. C. Hyde, F. V. Sepulveda, M. Buchwald, and C. F. Higgins. 1992. "The multidrug resistance and cystic-fibrosis genes have complementary patterns of epithelial expression." *Embo Journal* 11(12): 4291-303.

Trotter, P. J. and J. Storch. 1993. "Fatty-acid esterification during differentiation of the human intestinal-cell line caco-2." *Journal of Biological Chemistry* 268(14): 10017-23.

Trotter, P. J., S. Y. Ho, and J. Storch. 1996. "Fatty acid uptake by caco-2 human intestinal cells." *Journal of Lipid Research* 37(2): 336-46.

Trull, A. K., K. K. C. Tan, L. Tan, G. J. M. Alexander, and N. V. Jamieson. 1995. "Absorption of cyclosporin from conventional and new microemulsion oral formulations in liver-transplant recipients with external biliary diversion." *British Journal of Clinical Pharmacology* 39(6): 627-31.

Tso, P. 1994. "Intestinal lipid absorption". In: Physiology of the Gastrointestinal Tract, Johnson, L.R., Raven Press, N.Y., Pp. 1867-1908. .

Tso, P., K. L. Buch, J. A. Balint, and J. B. Rodgers. 1982. "Maximal lymphatic triglyceride transport rate from the rat small intestine." *American Journal of Physiology* 242(4): G408-15.

Tso, P., M. B. Lindström, and B. Borgström. 1987. "Factors regulating the formation of chylomicrons and very- low-density lipoproteins by the rat small intestine." *Biochim Biophys Acta* 922(3):304-13.

Tucker, G. 1993. "Drug delivery: lymphing along." *The Lancet* 341: 1314-15.

Ueda, C. T., M. Lemaire, G. Gsell, P. Misslin, and K. Nussbaumer. 1984. "Apparent dose-dependent oral absorption of cyclosporin a in rats." *Biopharmaceutics & Drug Disposition* 5(2): 141-51.

Ueda, K., Y. Taguchi, and M. Morishima. 1997. "How does P-glycoprotein recognize its substrates?" *Seminars in Cancer Biology* 8(3): 151-59.

Van den Bosch, H., N. M. Postema, G. H. De Haas, and L. L. M. Van Deenen. 1965. "On the positional specificity of phospholipase A from the pancreas." *Biochimica Et Biophysica Acta* 98: 657-59.

Van Greevenbroek, M. M. J. and T. W. A. De Bruin. 2001. "Lipid metabolism." *Current Opinion in Lipidology* 12(4): 461.

Van Greevenbroek, M. M. J., D. W. Erkelens, and T. W. A. De Bruin. 2000. "Caco-2 cells secrete two independent classes of lipoproteins with distinct density: effect of the ratio of unsaturated to saturated fatty acid." *Atherosclerosis* 149(1): 25-31.

Van Greevenbroek, M. M. J., G. van Meer, D. W. Erkelens, and T. W. A. de Bruin. 1996. "Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of Apo B containing lipoproteins by Caco-2 cells." *Atherosclerosis* 121: 139-50.

Van Greevenbroek, M. M. J., M. G. Robertus-Teunissen, D. W. Erkelens, and T. W. A. De Bruin. 1998. "Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with saturated and unsaturated dietary fatty acids." *Journal of Lipid Research* 39(1): 173-85.

Van Greevenbroek, M. M., W. F. Voorhout, D. W. Erkelens, G. van Meer, and T. W. de Bruin. 1995. "Palmitic acid and linoleic acid metabolism in Caco-2 Cells: Different triglyceride synthesis." *Journal of Lipid Research* 36(1): 13-24.

Vanhelvoort, a., a. J. Smith, H. Sprong, I. Fritzsche, a. H. Schinkel, P. Borst, and G. Vanmeer. 1996. "Mdr1 P-glycoprotein is a lipid translocase of broad specificity, while Mdr3 P-glycoprotein specifically translocates phosphatidylcholine." *Cell* 87(3): 507-17.

Vanhove, G. F., J. M. Schapiro, M. A. Winters, T. C. Merigan, and T. F. Blaschke. 1996. "Patient compliance and drug failure in protease inhibitor monotherapy." *Journal of the American Medical Association* 276(24): 1955-56.

Vassileva, G., L. Huwyler, K. Poirier, L. B. Agellon, and M. J. Toth. 2000. "The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice." *Faseb Journal* 14(13): 2040-2046.

Vella, S. 1995. "Clinical experience with saquinavir." *Aids* 9:S21-S25.

Vella, S. and M. Floridia. 1998. "Saquinavir - Clinical pharmacology and efficacy." *Clinical Pharmacokinetics* 34(3): 189-201.

Vella, S., M. Giuliano, P. Pezzotti, M. G. Agresti, C. Tomino, M. Floridia, D. Greco, M. Moroni, G. Visco, F. Milazzo, F. Giannelli, G. Angarano, L. Ortona, and C. Zanussi. 1992. "Survival of zidovudine-treated patients with AIDS compared with that of contemporary untreated patients." *Journal of the American Medical Association* 267(9): 1232-36.

Venkataramanan, R., K. Habucky, G.J. Burckart, R.J. Ptachcinski. 1989. "Clinical pharmacokinetics in organ transplant patients" *Clin. Pharm.* 16: 134-161

Vickers, a. E. M., V. Fischer, S. Connors, R. L. Fisher, J. P. Baldeck, G. Maurer, and K. Brendel. 1992. "Cyclosporin-A metabolism in human Liver, kidney, and intestine slices - comparison to rat and dog slices and human cell-lines." *Drug Metabolism and Disposition* 20(6): 802-9.

Volberding, P. A. 1998. "An aggressive approach to HIV antiretroviral therapy." *Hospital Practice* 33(1): 81.

Volpenhein, R. A., D. R. Webb, and R. J. Jandacek. 1980. "Effect of nonabsorable lipid, sucrose polyester, on the absorption of DDT by the rat." *J. Tox. Environm. Health* 6: 679-683.

Vonderscher, J. and a. Meinzer. 1994. "Rationale for the development of Sandimmune-Neoral." *Transplantation Proceedings* 26(5): 2925-27.

Von Moltke, L. L., D. J. Greenblatt, J. M. Grassi, B. W. Granda, S. X. Duan, S. M. Fogelman, J. P. Daily, J. S. Harmatz, and R. I. Shader. 1998. "Protease inhibitors as inhibitors of human cytochromes P450: High risk associated with ritonavir." *J Clin Pharmacol* 38(2): 106-111 .

Wacher, V. J., C. Y. Wu, and L. Z. Benet. 1995. "Overlapping substrate specificities and tissue distribution of cytochrome-P450 3A and P-glycoprotein - Implications for drug-delivery and activity in cancer-chemotherapy." *Molecular Carcinogenesis* 13(3): 129-34.

Wacher, V. J., J. A. Silverman, Y. C. Zhang, and L. Z. Benet. 1998. "Role of P-glycoprotein and cytochrome P450 3a in limiting oral absorption of peptides and peptidomimetics." *Journal of Pharmaceutical Sciences* 87(11): 1322-30.

Wakerly, M. G., C. W. Pouton, and B. J. Meakin. 1987. "Evaluation of the self-emulsifying performance of a non-ionic surfactant-vegetable oil mixture." *Journal of Pharmacy and Pharmacology* 396.

Wang, C. S. 1987. "Hydrolysis of dietary glycerides and phosphoglycerides: Fatty acid positional specificity of lipases and phospholipases. " In: Fat absorption, Kuksis, A. Boca Raton: CRC Press, pp83-118.

Warisnoicharoen, W., a. B. Lansley, and M. J. Lawrence. 2000. "Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour." *International Journal of Pharmaceutics* 198(1): 7-27.

Wasan, K. M. and S. M. Cassidy. 1998. "Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs." *Journal of Pharmaceutical Sciences* 87(4): 411-24.

Watkins, P. B. 1992. "Drug metabolism by cytochromes P450 in the liver and small bowel." *Gastroenterology Clinics of North America* 21(3): 511-26.

Watkins, P. B. 1997. "The barrier function of cyp3A4 and P-glycoprotein in the small bowel." *Advanced Drug Delivery Reviews* 27(2-3): 161-70.

Watkins, P. B., R. Schade, A. S. Mills, R. L. Carithers Jr, and D. H. Van Thiel. 1988. "Fatal hepatic necrosis associated with parenteral gold therapy." *Digestive Diseases & Sciences* 33(8) 1025-9.

Watkins, P. B., S. A. Wrighton, E. G. Schuetz, D. T. Molowa, and P. S. Guzelian. 1987. "Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats." *Journal of Clinical Investigation* 80(4): 1029-36.

Webster, L., M. Linsenmeyer, M. Millward, C. Morton, J. Bishop and D. Woodcock. 1993. Measurement of cremophor EL following taxol: plasma levels sufficient to reverse drug exclusion mediated by mdr phenotyope. *J. Natl. Cancer Inst.* 85: 1685-90.

Westergaard, H. and J. M. Dietschy. 1976. "The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the mucosal cell." *Journal of Clinical Investigation* 58: 97-108.

Westermann, J. and R. Pabst. 1990. "Lymphocyte subsets in the blood - a diagnostic window on the lymphoid system." *Immunology Today* 11(11): 406-10.

White, D. G., M. J. Story, and S. G. Barnwell. 1991. "An experimental animal model for studying the effects of a novel lymphatic drug delivery system for propranolol." *International Journal of Pharmaceutics* 69:169-74.

Wiltshire, H. R., B. G. Wiltshire, a. F. Clarke, E. Worth, K. J. Prior, and J. F. Tjia. 2000. "Chromatographic and immunochemical approaches to the analysis of the HIV protease inhibitor saquinavir in plasma." *Analytical Biochemistry* 281(1): 105-14.

Williams, P.E.O., G.J. Muirhead, M.J. Madigan, A.M. Mitchell and T. Shaw. 1992. "Disposition and bioavailability of the HIV proteinase inhibitor, Ro-31-8959, after single doses in healthy volunteers." *Br. J. Clinical Pharmacol.* 34: 155

Woodcock, D. M., M. E. Linsenmeyer, G. Chojnowski, a. B. Kriegler, V. Nink, L. K. Webster, and W. H. Sawyer. 1992. "Reversal of multidrug resistance by surfactants." *British Journal of Cancer* 66(1): 62-68.

Woodcock, D. M., S. Jefferson, M. E. Linsenmeyer, P. J. Crowther, G. M. Chojnowski, B. Williams, and I. Bertoncello. 1990. "Reversal of the multidrug resistance phenotype with cremophor EL, a common vehicle for water-insoluble vitamins and drugs." *Cancer Research* 50(14): 4199-203.

Wrighton, S. A. and B. J. Ring. 1994. "Inhibition of human Cyp3a Catalysed 1'-hydroxy midazolam formation by ketoconazole, nifedipine, erythromycin, cimetidine, and nizatidine." *Pharmaceutical Research* 11(6): 921-24.

Wrighton, S. A., E. G. Schuetz, K. E. Thummel, D. D. Shen, K. R. Korzekwa, and P. B. Watkins. 2000. "The human Cyp3A subfamily: practical considerations." *Drug Metabolism Reviews* 32(3-4): 339-61.

Wu, C. Y., L. Z. Benet, M. F. Hebert, S. K. Gupta, M. Rowland, D. Y. Gomez, and V. J. Wacher. 1995. "Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporin. "*Clin Pharmacol Ther* 58(5): 492-7.

Yamahira, Y., T. Noguchi, H. Takenaka, and T. Maeda. 1979. "Biopharmaceutical studies of lipid containing oral dosage forms: relationship between drug absorption rate and digestibility of vehicles." *International Journal of Pharmaceutics* 323-31.

Yu, L., A. Bridgers, J, Polli, A. Vickers, S. Long, A. Roy, R. Winnke and M. Coffin. 1999. Vitamin E TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability. *Pharmaceutical Research* 16(12): 1812-15

Yanagawa, A., T. Iwayama, T. Saotome, Y. Shoji, K. Takano, H. Oka, T. Nakagawa, and Y. Mizushima. 1989. "Selective transfer of cyclosporin to thoracic lymphatic systems by the application of lipid microspheres." *J Microencapsul.* 6(2): 161-4.

Yasumura, T., T. Oka, H. Yoshimura, H. Yoshikawa, K. Takada, and S. Muranishi. 1986. "Prolonged graft survival induced by the selective lymphatic delivery of cyclosporin in rat heart transplants." *Igakunoayumi* 136: 455-56.

Yusa, K., T. Oh-hara, S. Thalhammer, M. Reigler, E.P. Cosentini, A Ellinger, et al. 1990. Cross resistance to anti-HIV nucleoside analogues in multidrug resistance human cells. *Biochem. Biophys. Res. Commun.* 169:986-990.

Yoffey, J. M. and E. C. Courtice. 1970. "Lymphatics, lymph and the lymphomyeloid complex." *Academic Press, London* 1-63.

Yoshikawa, H, K Takada, Y Satoh, N Nauruse, and S Muranishi. 1985. "Potentiation of enteral absorption of human interferon alpha and selective transfer into the lymphatics in rats." *Pharmaceutical Research* 249-50.

Yuan, J. H., J. C. Stolzenbach, C. M. Salamon, S. S. Snook, and G. L. Schoenhard. 1997. "Improvement of bioavailability of the HIV protease inhibitorsc-52151 in the beagle dog by co-administration of the Cyp3A4inhibitor, ketoconazole." *Xenobiotica* 27(5): 489-97.

Zamora, J. M, L.H. Pearce andW.T. Beck. 1988. "Physical chemical properties shared by compounds that modulate multidrug resistance in human leukaemia cells. *Molecular Pharmacology* 33: 454-462.

Zhang, Y. C., Y. S. Hsieh, T. Izumi, E. T. Lin, and L. Z. Benet. 1998. "Effects of ketoconazole on the intestinal metabolism, transport and oral bioavailability of K02, a novel vinylsulfonepeptidomimetic cysteine protease inhibitor and a P450 3A, P-glycoprotein dual substrate, in male sprague-dawley rats." *Journal of Pharmacology and Experimental Therapeutics* 287(1): 246-52.

Zhang, Z. Q., D. W. Notermans, G. Sedgewick, W. Cavert, S. Wietgrefe, M. Zupancic, K. Gebhard, K. Henry, L. Boies, Z. M. Chen, M. Jenkins, R. Mills, H. Mcdade, C. Goodwin, C. M. Schuwirth, S. A. Danner, and a. T. Haase. 1998. "Kinetics of Cd4+ T cell repopulation of lymphoid tissues after treatment of Hiv-1 infection." *Proceedings of the National Academy of Sciences of the United States of America* 95(3): 1154-59.

Zhang, L.Q., R. Ramratnam, K. Tenner-Racz, Y.X. He et al. 1999. "Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy." *New England Journal of medicine.* 340(21): 1605-13

Zhang, H., G. Dornadula, M. Beaumont, L.J. Livorese, B. Van Uitert, K. Henning and R.J. Pomerantz. 1998. "HIV-1 in the semen of men receiving highly active antiretroviral therapy." *New Eng. J. Med.* 339: 1803-09

Zilversmit, D. B. 1965. "The composition and structure of lymph chylomicrons in dog, rat and man." *Journal of Clinical Investigation* 44: 1610-1622.

Zordon-Nudo, T., V. Ling, Z. Liu and E. Georges. 1993. "Effects of non-ionic detergents on P-glycoprotein drug binding and reversal of Multidrug resistance." *Cancer Res.* 53: 5994-6000.