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A comparison of intestinal lymphatic transport and systemic bioavailability of saquinavir from three lipid-based formulations in the anaesthetised rat model

Brendan T. Griffin and Caitriona M. O'Driscoll

Abstract

Saquinavir is a lipophilic, poorly water-soluble HIV protease inhibitor that undergoes extensive first-pass metabolism and exhibits poor oral bioavailability. Redirection of the absorption pathway of anti-HIV compounds from the portal blood to the HIV-rich intestinal lymphatics may enhance therapeutic efficacy and reduce the extent of the first-pass effect. This study investigates the potential of targeted intestinal lymphatic transport of saquinavir via a lipid formulation approach. Three formulations containing oleic acid were examined: cremophor–oleic acid mixed micelles, D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)–oleic acid mixed micelles and an oleic acid microemulsion. The mesenteric lymph duct cannulated anaesthetised rat model was employed. Plasma and lymph samples were analysed by HPLC. Lymph triglyceride was measured using an enzymatic colorimetric technique. The extent of lymphatic transport from the lipid vehicles was 0.025–0.05% of the dose administered. The microemulsion produced higher and more prolonged mesenteric lymph concentrations than the micellar formulations. A strong correlation existed between the concentration of saquinavir in intestinal lymph and lymph triglyceride levels. The systemic bioavailability was estimated to be 8.5% and 4.8% for the cremophor mixed micelle and the microemulsion, respectively. The cremophor mixed micelles produced higher bioavailability than TPGS mixed micelles, implying that the nature of the surfactant can influence the distribution of drug between lymph and plasma.

Introduction

Gastrointestinal drug transport to the systemic circulation may occur via either the hepatic portal blood or mesenteric lymphatic routes. The lymphatic uptake route is of particular interest because, in addition to facilitating absorption of highly lipophilic drugs and avoiding hepatic first-pass metabolism (as the lymphatics drain directly into the internal jugular vein), this route offers the potential to selectively target compounds to the lymphoid tissue (O'Driscoll 1992; Porter & Charman 2001). While the exact mechanisms by which a drug is transported by the intestinal lymphatic system are not fully elucidated, it is widely reported that lipophilic drugs with cLog P > 4.7 and a lipid solubility > 50 $mg\,mL^{-1}$ are potentially good candidates (Charman & Stella 1986a). It is generally believed that high lipophilicity facilitates the association of the drug with triglyceride-rich lipoproteins, which are synthesized by intestinal enterocytes and secreted into lymph lacteals as part of the triglyceride absorption process. Lipid-based formulations have been widely reported to enhance systemic bioavailability of poorly water-soluble drugs; it is suggested that the lipid excipients present may enhance drug solubility or increase membrane permeability, resulting in increased drug uptake via the portal route (Pouton 2000; Gursoy & Benita 2004). The potential merits of lipid-based formulations as the drug delivery vehicles of choice in targeting drugs to the intestinal lymphatics, however, are less well reported (O'Driscoll 2003).

Saquinavir is a highly lipophilic HIV protease inhibitor that, when formulated as a mesilate salt in a hard-gel capsule (Invirase), was the first drug of its class to

School of Pharmacy, University College Cork, Ireland

Brendan T. Griffin, Caitriona M. O'Driscoll

Correspondence: B. T. Griffin, School of Pharmacy, University College Cork, Ireland. E-mail: brendan.griffin@ucc.ie

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become available for the treatment of patients with HIV infection in 1995. However, the bioavailability of saquinavir mesilate is low (4%), variable and increases postprandially (Perry & Noble 1998). Subsequently, a new formulation of saquinavir was introduced (Fortovase), which contained the more lipophilic free-base form of the drug in a lipid formulation consisting of medium-chain mono- and diglycerides, povidone and alpha tocopherol, with a reported 3-fold increase in bioavailability relative to Invirase (Perry & Noble 1998). Fortovase is a self-emulsifying drug delivery system (SEDDS) that upon administration in the gastrointestinal fluids can form a fine oil-in-water emulsion or micro-emulsion. The design rationale behind the lipid-based formulation is an enhanced solubilisation of saquinavir in the gastrointestinal lumen and/or improved membrane permeability. Saquinavir is classified as a Class II compound, in accordance with the terms of the Biopharmaceutical Drug Classification Scheme (Amidon et al 1995). Saquinavir is also a substrate for efflux by P-glycoprotein (Pgp) and metabolism by cytochrome P450, although the contribution of intestinal efflux and first-pass metabolism on the oral bioavailability of saquinavir in man has yet to be established (Mouly et al 2004; Sinko et al 2004).

Saquinavir is an ideal candidate for lymphatic targeting for the following reasons: firstly, its high lipophilicity (clog P of the freebase is 4.5); secondly, it undergoes extensive first-pass metabolism; thirdly, its increased bioavailability following co-administration of a fatty meal; and, finally, the potential therapeutic benefit of targeting this potent HIV protease inhibitor to the lymphatic system. There are numerous reports indicating that the lymphoid tissue is the major storage and replication site of HIV in-vivo and modes of enhancing distribution into the lymph have been explored (Pantaleo et al 1993). The degree of intestinal lymphatic transport of saquinavir in rats has, to our knowledge, not been published previously.

Given the therapeutic goal of maximising drug concentrations to prevent viral proliferation in lymphoid tissue, and the advantage of decreasing first-pass metabolism via lymphatic transport, the objectives of this study are as follows: firstly, to investigate the potential of three different lipid-based formulations to target intestinal lymphatic delivery of saquinavir, and secondly, to compare the relative effects of these formulations on both the lymphatic transport and total systemic (plasma) bioavailability.

The lipid formulations used consisted of two mixed micellar formulations and a microemulsion formulation. Oleic acid was chosen as the oil phase in all three lipid formulations as it is the principal long-chain fatty acid that occurs in human mixed intestinal lipids (Staggers et al 1990) and in recognition of the potential merits of long-chain unsaturated fatty acids, particularly oleic acid, in the design of a lymphotropic lipid delivery systems (Palin & Wilson 1984; Charman & Stella 1986b).

Two synthetic mixed micellar systems were examined: cremophor–oleic acid mixed micelles, and D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)–oleic acid mixed micelles. The potential advantages of cremophor EL versus naturally occurring bile salts include a higher solubilisation capacity and enhanced intestinal permeability (O'Reilly et al 1994). TPGS is a water-soluble form of vitamin E that is increasingly used as an oral absorption enhancer for poorly water-soluble drugs such as ciclosporin (Chang et al 1996) and, indeed, is a component of the new Fortovase formulation. In addition, both TPGS and cremophor have been reported to inhibit presystemic drug metabolism and intestinal efflux mediated by Pgp (Nerurkar et al 1996; Yu et al 1999). Finally, a novel SEDDS formulation for saquinavir using the surfactants Labrasol and Plurol Oleique, that forms a micro-emulsion upon dilution, was also investigated.

Materials and Methods

Materials

Saquinavir base (Ro-31-8959/000), saquinavir mesilate (Ro 31-8959/003) and internal standard (Ro 31-9564) were kindly donated by Roche Products UK (Herts, UK). Labrasol (saturated polyglycolysed C8–C10 glycerides, 50–80% C8; 20–50% C10) and Plurol Oleique (polyglyceryl oleate) were kindly donated by Gattefosse (Saint-Priest, France) and cremophor EL (polyoxyl 35 castor oil) was donated by BASF (Ludwigshafen, Germany). TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate) was donated by Eastman (USA) and oleic acid (cis-9-octadecenoic acid) and Tri-olein were obtained from Sigma (UK). All solvent were of HPLC grade.

Lipid formulations

Mixed micelle preparation

The mixed micelle formulations contained the synthetic surfactants cremophor EL and TPGS, and oleic acid. To facilitate the dissolution of TPGS, a 6:1 blend of TPGS–PEG was used throughout. The oleic acid was added drop-wise (at 37°C with continuous stirring) over 30 min to a simple micellar solution of either cremophor EL (2% w/v) or TPGS (2% w/v) dissolved in a volume of phosphate buffer (pH 7.2). All solutions were viewed in front of a strong light source to check that the solution was transparent, and allowed to equilibrate at room temperature for 12–24 h.

Microemulsion preparation

The microemulsion formulation was designed by establishing a pseudo ternary phase diagram with systems comprising four components: a long-chain free fatty acid (oleic acid), a low-HLB (hydrophilic–lipophilic balance) co-surfactant (Plurol Oleique, HLB 10), a high-HLB surfactant (Labrasol HLB 14) and an aqueous phase (water). An oleic acid–Labrasol–Plurol Oleique blend of 7:54:9, which formed an isotropically clear microemulsion upon infinite dilution with water, was selected. Microemulsion droplet sizes, following 1:200 dilution, were measured by photon correlation spectroscopy (Malvern Lo-C model no. MAN 0055), giving a Zave value of 130.64 ± 2.41 nm and polydispersity of 0.315 ± 0.002.

Saquinavir formulations

Saquinavir ($1.667\,mg\,mL^{-1}$) was added to the lipid solutions 2 h before intraduodenal dosing. A lipid formulation containing saquinavir mesilate was similarly prepared by addition of the mesilate salt ($1.667\,mg\,mL^{-1}$) to a mixed micelle formulation of 2% cremophor–oleic acid. The intravenous formulation consisted of saquinavir ($3.33\,mg\,mL^{-1}$) dissolved in a 5% cremophor EL (in phosphate-buffered saline, pH 7.2) solution. Drug concentrations in all lipid formulations were verified on the day of dosing using a validated HPLC assay.

Surgical procedures

All animal experiments were performed in accordance with EU directive 86/609 (as implemented in Ireland by Statutory Instrument 17/9) in association with BioResources unit, Trinity College, Dublin, which is registered with the Department of Health and Children. Male Wistar rats, 280–320 g, were fasted for 24 h with free access to water. The rats were anaesthetised for the duration of the experiment using $50\,mg\,kg^{-1}$ sodium pentobarbital given by intraperitoneal injection. The duodenum and mesenteric lymph duct were cannulated as previously described (O'Driscoll et al 1991). Test lipid solutions or saline control were administered intraduodenally at $1.5\,mL\,h^{-1}$ over 2 h. Lymph was collected hourly for 8 h after commencement of drug administration in preweighed cooled glass tubes containing anticoagulant (heparin). Blood samples were taken by cardiac puncture at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 h after commencement of drug administration. To maintain body hydration and intestinal lymph flow, the duodenum was continuously perfused (except during the 2-h drug administration of the test formulations) with normal saline at $1.5\,mL\,h^{-1}$ via a constant infusion syringe pump. Non-lymph cannulated rats were sham operated in terms of the mesenteric lymph duct cannulation and had an intraduodenal cannula (for rehydration) inserted, as described above. For intravenous tests, 0.3 mL of the intravenous formulation was administered by intravenous bolus injection into the jugular vein over a period of less than 10 s. These rats also received a $1.5\,mL\,h^{-1}$ intraduodenal infusion of normal saline throughout the course of the experiment. Serial blood samples (0.2 mL) were collected at −5 min, and at 5, 10, 15, 20, 30, 45 and 60 min, and 0.5-mL blood samples were taken at 3, 4, 6 and 8 h.

Analysis of lymph triglyceride

Lymph triglyceride (expressed as mg equivalents of C18 long-chain triglyceride) was determined using a Triglceride Enzymatique PAP 150 assay (bioMeirieux). 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 rats administered lipid-based formulations. Endogenous lipid transport, which was zero order, was calculated to be $1.91 \pm 0.09\,mg\,h^{-1}$ and was determined from the slope of the regression of mean cumulative lymphatic triglyceride transport over 8 h ($r^2 = 0.99$). This compares well with previously reported levels (Porter et al 1996; Caliph et al 2000).

Assay of saquinavir in plasma and lymph

The HPLC assay for saquinavir in lymph and plasma was similar to that previously described (Wiltshire et al 2000). Saquinavir detection was performed at $\lambda = 238$ nm. The stationary phase was a 5-μm Megellen C8 column (250 × 4.6 mm, Phenomenex), equipped with a guard column of the same material. The mobile phase consisted of a 63:37 mix of acetonitrile–ammonium acetate (10 mM) and the flow rate was $1\,mL\,min^{-1}$. The retention time for saquinavir and internal standard (Ro 31-9564) was 9 and 21 min, respectively. Twenty microlitres of a $5\,\mu g\,mL^{-1}$ solution of internal standard, Ro 31-9564, in mobile phase was added to either 200 μL of plasma or lymph sample. The lymph/plasma samples were then made basic by the addition of 50 μL of 10 M K_2CO_3 and extracted with diethyl ether (4 mL). Following centrifugation of the mixture for 5 min at $3000\,rev\,min^{-1}$, the tubes were placed into a dry ice/acetone bath. The organic phase was decanted and evaporated to dryness under a stream of nitrogen. The residue was dissolved in HPLC mobile phase (0.15 mL), vortexed for 1 min, and 50 μL was injected onto the column.

The validity of the HPLC assay for both saquinavir and internal standard were established through careful examination of the linearity of response, reproducibility of standard curve and extraction recovery. The analysis of saquinavir in plasma and lymph exhibited excellent linearity ($r^2 \geq 0.99$) over the concentration range of 25–400 $ng\,mL^{-1}$ for plasma and 40–800 $ng\,mL^{-1}$ for lymph. The limit of quantitation was $25\,ng\,mL^{-1}$ for plasma and $40\,ng\,mL^{-1}$ for lymph ($CV \leq 20\%$). The extraction recoveries of saquinavir from both lymph and plasma were all in excess of 93%, whereas the recovery of internal standard was > 97% for both lymph and plasma.

Pharmacokinetic data analysis

Plasma concentrations versus time data for saquinavir in individual rats were tested using the nonlinear curve fitting and model development program, Micromath R Scientist for Windows Version 1.05 (Micromath R Scientific Software). Plasma concentrations from intravenous data were fitted to a triexponential equation using values of A, B, C, alpha, beta and gamma, and the values obtained were used to calculate the area under the curve ($AUC^{i.v.}{}_{0\rightarrow\infty h.}$). The AUC for saquinavir after intraduodenal administration was obtained using the linear trapezoidal rule from time zero to the last measured time point, followed by the addition of the extrapolated tail area, calculated by dividing the last measured plasma concentration by the terminal rate constant, as determined from intravenous data. The bioavailability in

non-lymph-cannulated rats was estimated from the ratio of the doses normalized AUCs after oral and intravenous administration as follows:

$$F = (AUC^{oral}{}_{0\rightarrow\infty h}/D_{oral})/(AUC^{i.v.}{}_{0\rightarrow\infty h}/D_{i.v.}) \quad (1)$$

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.

Statistical analysis

One-way analysis of variance (for multiple comparisons) and Student's *t*-test were used to determine the statistical significance ($P < 0.05$) of calculated results between the experimental groups.

Results and Discussion

A comparison of the various lipid formulations used is given in Table 1. The concentration of surfactants in all formulations was approximately 2% w/v and reflects a conservative range of surfactant concentration relative to similar rat intestinal lymphatic transport studies previously published (Charman & Stella 1986b; Porter et al 1996; Holm et al 2001). The 2% TPGS and 2% cremophor micellar systems displayed a greater solubilisation capacity for oleic acid, facilitating the incorporation of up to 40 mM oleic acid, to form stable mixed micellar systems. For the oleic acid microemulsion, it was not possible to use an equivalent oleic acid content. Oil phase concentrations greater than 7.1 mM oleic acid (i.e. 10%) in the SEDDS pre-concentrate resulted in unstable emulsions on dilution with aqueous phase.

Intestinal lymphatic transport of saquinavir from lipid-based formulations

The extent of lymphatic transport of saquinavir (free base) was determined after intraduodenal administration of 5 mg over 2 h to mesenteric-lymph-duct-cannulated rats. Figure 1 compares the cumulative extent (% dose administered) of lymphatic transport versus time for the three lipid formulations. The total amount of the administered dose collected in the mesenteric lymph after 8 h for the cremophor–oleic acid, TPGS–oleic acid and oleic acid microemulsion systems was $1.34 \pm 0.3\,\mu g$, $1.26 \pm 0.38\,\mu g$ and $2.59 \pm 0.66\,\mu g$, respectively. The extent of transport after 8 h was greatest for the oleic acid microemulsion formulation, although the differences were not statistically significant ($P = 0.09$).

Table 1 Composition of three lipid formulations

Formulation	Oleic acid content	Surfactant content
2% Cremophor–40 mM oleic acid mixed micelles	33.85 mg	60 mg Cremophor
2% TPGS–40 mM oleic acid mixed micelles	33.85 mg	51.5 mg TPGS 8.5 mg PEG
Oleic acid microemulsion (formed from a 1:50 dilution of SEDDS blend)	6 mg	46.3 mg Labrasol 7.7 mg Plurol Oleique

The lipid formulations were infused intraduodenally at a rate of $1.5\,mL\,h^{-1}$ for 2 h, containing a total dose of 5 mg saquinavir in 3 mL of lipid formulation.

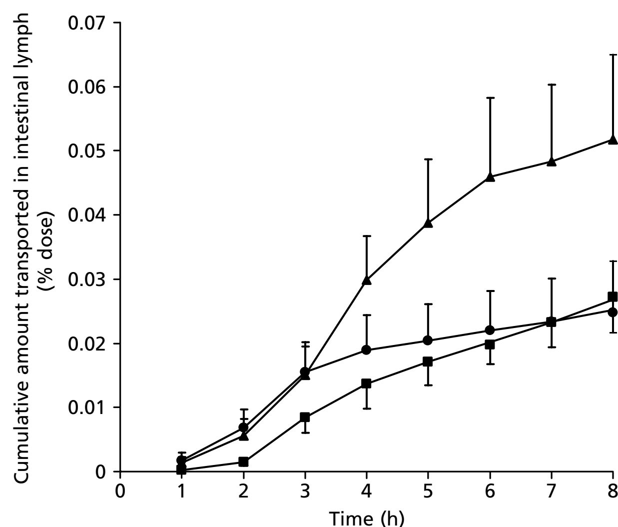

Figure 1 Cumulative percent of dose administered of saquinavir (mean ± s.e., n ≥ 5) collected in mesenteric intestinal lymph as a function of time. Saquinavir (5 mg) was administered intraduodenally to anaesthetised rats in a cremophor–oleic acid mixed micellar formulation (■), a TPGS–oleic acid mixed micellar formulation (●) or as an oleic acid microemulsion (▲).

The lymph concentration versus time profiles of the mesenteric lymphatic transport of saquinavir for the three lipid vehicles are presented in Figure 2. The microemulsion formulation appeared to prolong the period of intestinal lymphatic transport (longer Tmax). The concentrations of saquinavir in intestinal lymph at the 3–4, 4–5 and 5–6 h points were significantly higher ($P < 0.05$) for the microemulsion than for the mixed micellar formulations. Formulation as a microemulsion may therefore confer therapeutic advantages in terms of prolonged and higher concentrations in the intestinal lymphatics.

The microemulsion had a peak rate at 3–4 h compared with a peak rate of 2–3 h for the micellar systems (Figure 2). Porter et al (1996) reported a delayed peak transport rate for lipid solution (50 μL of a 2:1 w/w oleic acid–glycerol monooleate) of approximately 4 h compared with a micellar vehicle (4% Tween, 50 μL of 2:1 w/w oleic acid–glycerol monooleate) and proposed that this reflected the lag phase for conversion from the lipid solution to a pre-absorptive micellar state. The apparent delay of approximately 1 h found in this study most likely reflects the need for conversion of the microemulsion phase, as administered, to a pre-absorptive mixed micellar phase.

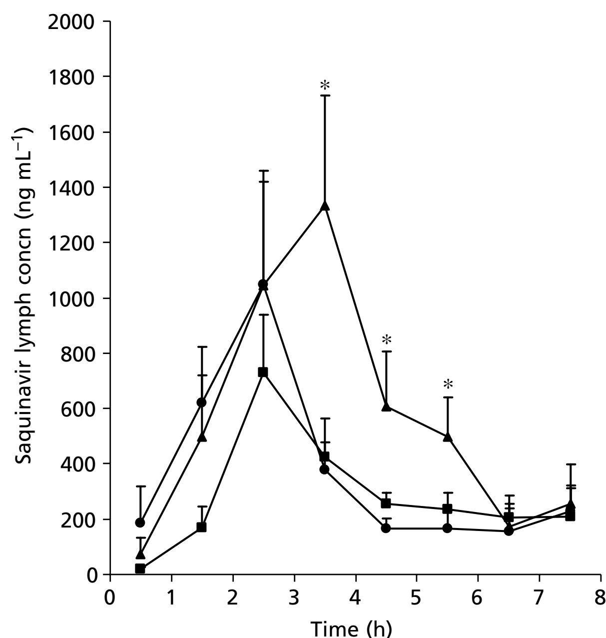

Figure 2 Concentration of saquinavir in intestinal lymph versus time (mean ± s.e., n ≥ 5). Saquinavir (5 mg) was administered intraduodenally to anaesthetised rats in a cremophor–oleic acid mixed micellar formulation (■), a TPGS–oleic acid mixed micellar formulation (●) or as an oleic acid microemulsion (▲). $*P < 0.05$, compared with micellar formations.

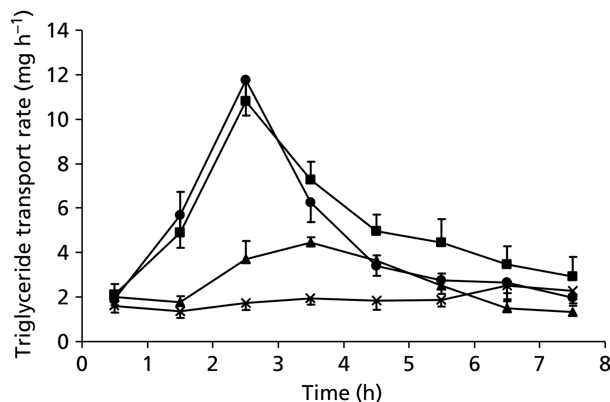

Figure 3 Rate of transport of triglyceride in intestinal lymph versus time (mean ± s.e., n ≥ 4). The formulations administered to anaesthetised rats were a cremophor–oleic acid mixed micellar formulation (■), a TPGS–oleic acid mixed micellar formulation (●), as an oleic acid microemulsion (▲) or a saline control (×).

A comparison of the intestinal lymphatic transport of saquinavir free base and mesilate salt was performed. The mesilate salt is a more water-soluble form of saquinavir (aqueous solubility 2.2 mg mL^{-1}) and has a lower cLog P (2.12) (Perry & Noble 1998). The cumulative extent of lymphatic transport following administration of the mesilate salt of saquinavir in the cremophor mixed micellar formulation was $1.175 \pm 0.419\,\mu$g, equivalent to 0.024% of the administered dose, which was similar to that of the free base. The rate profiles for saquinavir mesilate and free base were also similar (data not shown). This suggests the partial conversion of the saquinavir mesilate to the saquinavir free base ($pK_a \sim 7.1$) following administration in the gastrointestinal tract, similar to the acid–base conversion that has previously been reported for halofantrine hydrochloride and free base (Khoo et al 2002).

Intestinal lymphatic transport of triglycerides

As lipophilic compounds are believed to be transported in association with triglyceride-rich lipoproteins, such as chylomicrons, the triglyceride levels in the intestinal lymph samples were evaluated. Figure 3 presents the transport rate of C_{18} triglyceride in intestinal lymph following administration in either cremophor or TPGS mixed micelles, a microemulsion or as a saline solution (control). The maximal rate of triglyceride transport following administration of the microemulsion formulation occurred at 3–4 h compared with 2–3 h for the micellar vehicles. In all cases, the tri-glyceride levels returned to endogenous levels after 8 h. The cumulative C_{18} triglyceride transport data after intraduodenal dosing in the lipid formulations examined in this study is summarised in Table 2.

The cremophor 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 (33.85 mg) recovered as re-synthesized long-chain triglyceride in intestinal lymph after 8 h was $82.75 \pm 13.23\%$ and $62.92 \pm 7.00\%$ for the cremophor and TPGS mixed micellar systems, respectively. These figures are comparable with those previously reported by Porter et al (1996), where the percentage of exogenous lipid recovered in intestinal lymph after administration of a Tween (4%)–oleic acid–glycerol monooleate (50 μL) micellar formulation was 79.7%. Seeballuck et al (2003) reported inhibition of chylomicron secretion by cremophor in the Caco-2 model and proposed that the effect may be related to inhibition of Pgp-like biochemical processes. This effect on the in-vitro cell line was reversed following co-addition of a digestible long-chain lipid surfactant (Seeballuck et al 2004). In our study, the high recovery of exogenous lipid (i.e. 82.75%) suggested that any possible inhibitory effect on lipoprotein processing was masked or reversed by the oleic acid present in the formulation.

The microemulsion blend consisted of 10% oleic acid and 90% surfactant/co-surfactant blend, which after administration results in total lipid dosing of 6 mg oleic acid, 7.7 mg Plurol Oleique and 46.3 mg Labrasol. The total amount of exogenous lipid detected in intestinal lymph was 6.23 ± 2.71 mg. However, to assume an almost quantitative recovery of administered oleic acid is most likely incorrect, as the surfactants themselves may stimulate endogenous triglyceride turnover, as previously reported (Shiau et al 1985; Caliph et al 2000; Seeballuck et al 2003, Trevaskis et al 2005). Plurol Oleique may be digested to liberate a long-chain fatty acid, which may in turn

Table 2 Cumulative transport of triglyceride into the mesenteric lymph of anaesthetised rats and cumulative lymph flow after 8 h as a function of lipid formulation

Formulation	Cumulative mass of triglyceride appearing in mesenteric lymph (mg)[a]				Triglyceride transport (mg)[b]	Cumulative lymph flow (mL)
	0–2 h	0–4 h	0–6 h	0–8 h		
Saline control	2.93 ± 0.06	6.58 ± 0.24	10.26 ± 0.34	15.06 ± 0.85		4.45 ± 0.35
Cremophor–oleic acid 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–oleic acid 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
Oleic acid microemulsion	3.75 ± 0.38	11.92 ± 1.33	18.07 ± 1.58	21.29 ± 2.05	6.23 ± 2.71	5.29 ± 0.34
Saquinavir mesilate in cremophor–oleic acid 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

Data are means ± s.e., n ≥ 4. [a]Representing endogenous and exogenous lipid. [b]Attributable to exogenous lipid (i.e. exogenous – endogenous). *$P < 0.05$ relative to saline control.

stimulate endogenous lipoprotein production. Digestion of Labrasol may liberate C8–C10 medium-chain lipids, and the impact of medium-chain lipids on intestinal triglyceride levels is less clear. Short- and medium-chain lipids (with a chain length of less than 12 carbons) are believed to be transported to the systemic circulation by the portal blood and are not incorporated to a great extent in chylomicrons (Kiyasu et al 1952). However, Caliph et al (2000) reported that administration of medium-chain triglyceride lipids resulted in a 42% increase in the extent of endogenous long-chain triglyceride levels, possibly through stimulation of endogenous lipoprotein production.

Relationship between saquinavir lymphatic transport and triglyceride turnover

The relationship between hourly lymphatic transport of saquinavir and the corresponding hourly triglyceride transport for each lipid vehicle turnover was explored (Figure 4). The slopes of the lines describing these relationships represent the relative concentration, or apparent loading of saquinavir per mg of lymph triglyceride. For all three formulations, a positive correlation exists between saquinavir lymphatic transport and lymph triglyceride. This implies that saquinavir is transported into the lymph in association with triglyceride-rich lipoproteins. These findings are consistent with previous reports that have described a correlation between the lymph triglyceride and lipophilic drug transport (Charman & Stella 1986b; Hauss et al 1998; Holm et al 2001).

The microemulsion formulation produced a higher loading of saquinavir compared with the two mixed micellar systems, which both produced similar loadings. The reasons for the higher drug concentrations (Figure 2) and higher drug loadings (Figure 4), despite a lower overall triglyceride turnover (Table 2) from the microemulsion versus the mixed micelles are unclear. Probing aspects of

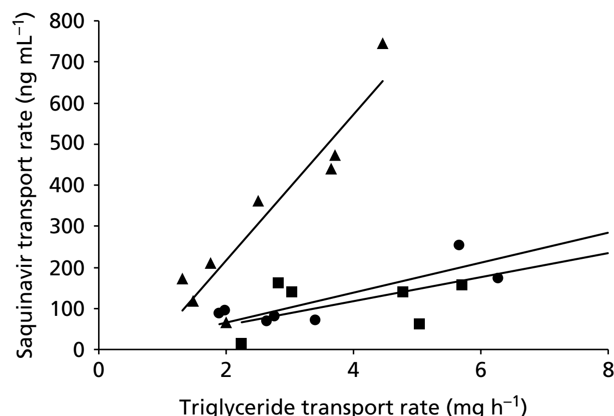

Figure 4 Intestinal saquinavir transport rate versus triglyceride turnover in intestinal lymph after intraduodenal administration of saquinavir in either the cremophor–oleic acid mixed micelles (slope = 29.32, $r^2 = 0.84$, ■), TPGS–oleic acid mixed micelles (slope = 36.66, $r^2 = 0.96$, ●) or an oleic acid microemulsion (slope = 178.31, $r^2 = 0.93$, ▲) to anaesthetised rats.

mechanistic transport studies of this kind requires an understanding of the complex interplay of both drug and lipid uptake processes. For lymphatic drug uptake, if the drug is transported in association with chylomicrons, the rate of transfer of drug to the lymphatics is determined by the drug loading within the chylomicron and the rate of processing of lipid (i.e. rate of production of chylomicrons) within the enterocytes. The most likely mechanisms whereby the microemulsion promotes a higher drug transfer rate into the intestinal lymphatics, is through improving the efficiency of drug transfer to lipoproteins within the enterocyte, as suggested by Hauss et al (1998). If it is assumed that drug uptake by lipoproteins within the enterocyte is via a simple partition process, then higher drug loadings reflect higher enterocyte drug concentrations. Hence, it may be that medium-chain lipids present

in the microemulsion are more effective at facilitating transfer of solubilised drug from the intestinal lumen, through the apical membrane and into the enterocytes. Alternatively, it may be that the cremophor and TPGS mixed micelles are less efficient at releasing saquinavir into the pre-absorptive intestinal milieu.

Pharmacokinetics of saquinavir

Saquinavir plasma concentration data following an intravenous bolus of 1 mg were analysed using a non-linear curve-fitting program as described in the Methods (Figure 5). From the intravenous blood concentration versus time data, a mean $AUC_{0\text{-}\infty h}$ of 1361.55 ng h mL^{-1} was obtained; the clearance, calculated as dose/AUC was 0.734 L h^{-1} or 2.45 L h^{-1} kg^{-1}. The apparent biological half-life was 1.71 h and is comparable with that reported by Shibata et al (2000) following oral dosing in conscious rats ($t\frac{1}{2} = 2.14$ h). Saquinavir pharmacokinetics in man are characterised by a high plasma clearance (1.41 L h^{-1} kg^{-1}), a large volume of distribution (Vd = 703 L; 10 L kg^{-1}) and substantial inter-individual variability (Williams et al 1992).

Saquinavir plasma concentration–time profiles (0–8 h) following intraduodenal administration of either cremophor mixed micelles or the oleic acid microemulsion ($n \geq 5$ for each formulation) are presented in Figure 6. The plasma concentrations for the TPGS mixed micelles were below the limit of quantitation for the assay. Plasma profiles in both lymph-cannulated rats and sham-operated non-lymph-cannulated rats are displayed. In lymph-cannulated rats, 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 (i.e. true bioavailability estimate).

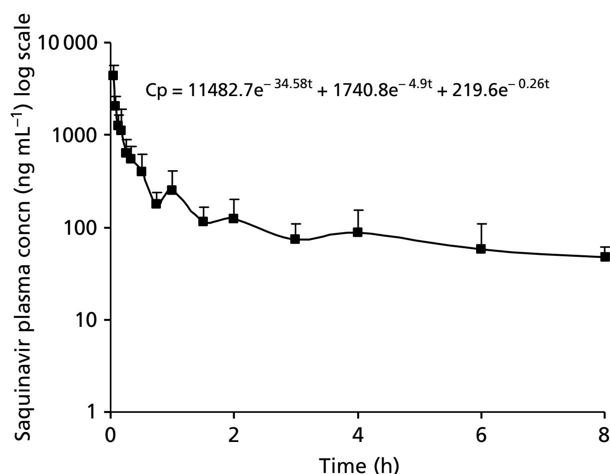

Figure 5 Saquinavir plasma concentrations versus time in rats following intravenous administration of 1 mg fitted to a triexponential equation plotted on log scales (n = 7).

The cremophor mixed micellar formulation produced significantly higher plasma levels than the TPGS mixed micellar systems. In contrast, the TPGS mixed micelle formulation produced a statistically similar extent of lymphatic transport to that of the cremophor mixed micelles. This implies that changes of surfactant type can produce differing effects on the lymph and blood distribution of saquinavir. The higher plasma (i.e. portal) levels following administration of the cremophor oleic acid mixed micelles may be related to a more pronounced effect of cremophor on intestinal Pgp compared with TPGS. Intestinal Pgp has been shown to significantly reduce intestinal uptake of saquinavir in-vitro (Alsenz et al 1998; Williams & Sinko 1999) and co-administration of the selective Pgp inhibitor GF120918 resulted in significant increases in saquinavir plasma concentrations in mice (Huisman et al 2003). Martin-Facklam et al (2002) proposed that the dose-dependent increase in saquinavir bioavailability observed following co-administration of cremophor was mediated by a Pgp-mediated effect. However, at a molecular level, the comparative effects of surfactants, such as cremophor and TPGS, on intestinal cells and Pgp-functionality is far from clear, with evidence reported of diverse functionality between surfactants on cell surface membranes or transporters (Rege et al 2001; Bogman et al 2003; Cornaire et al 2004).

A summary of the main pharmacokinetic and lymphatic transport parameters for the cremophor mixed micelles and the oleic acid microemulsion are presented in Table 3. There were no statistically significant differences between saquinavir bioavailability in lymph-cannulated and non-lymph-cannulated rats. In light of the low percentage of dosed saquinavir that was recovered in mesenteric lymph (0.027 and 0.052%), one would not expect to observe any difference in the plasma saquinavir concentration versus time profiles between lymph-cannulated and non-lymph-cannulated rats, as the apparent contribution of lymphatic transport to the overall oral bioavailability of saquinavir was negligible (< 0.3–1.3% of the bioavailable dose). While there is a trend towards higher plasma concentrations (in non-cannulated rats) for the cremophor mixed micelle formulation, saquinavir bioavailability did not differ significantly between the oleic acid microemulsion ($F = 4.83 \pm 0.73$) and cremophor mixed micelles ($F = 8.53 \pm 1.71$). Plasma levels following administration of the mesilate salt were below the limit of quantitation compared with an estimated bioavailability of 8% for the free-base formulation. Therefore, our findings confirm that formulation of the free-base form in a lipid vehicle increases the systemic bioavailability over that observed for the mesilate form.

Conclusion

In summary, this study examines the effect of lipid-based formulations on both the systemic bioavailability and intestinal lymphatic transport of saquinavir, with a view to establishing the ideal properties of a lymphotropic lipid-based formulation. The extent of lymphatic transport from a series of lipid vehicles was between 0.025 and 0.05% of the dose administered. Lymphatically transported saquinavir amounted to less than 0.3–1.3% of the bioavailable dose.

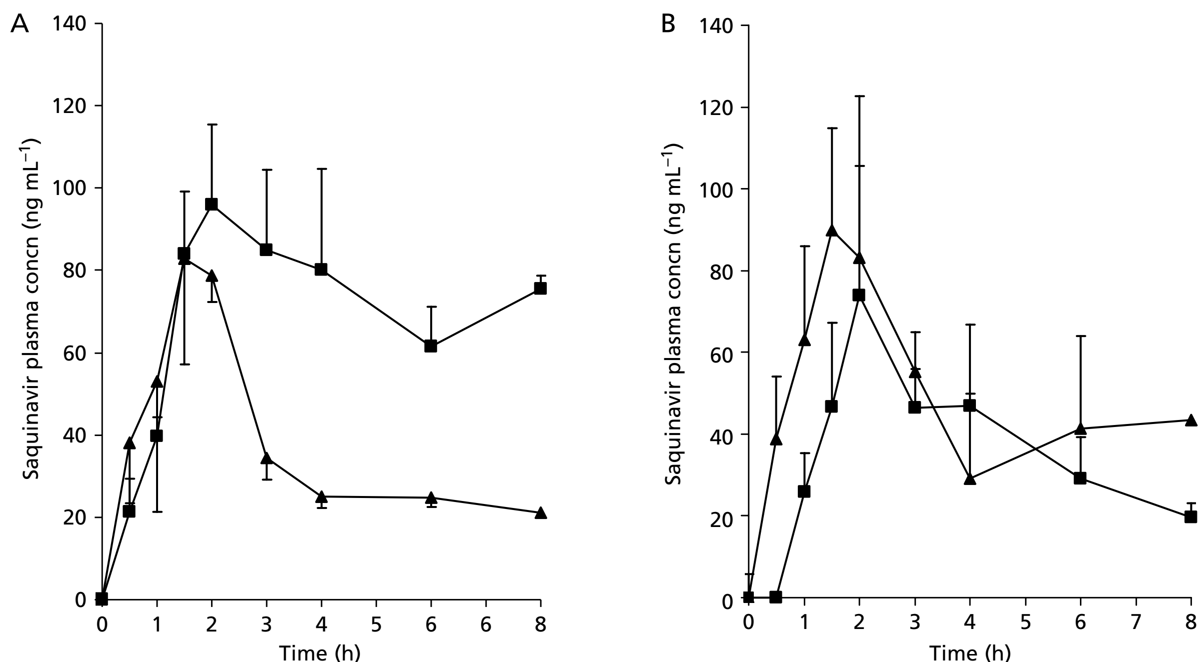

Figure 6 Plasma concentration versus time of saquinavir (mean ± s.e., n ≥ 5) in non-lymph-cannulated (A) and lymph-cannulated rats (B) after intraduodenal administration of 5 mg of saquinavir in either cremophor–oleic acid mixed micelles (■) or an oleic acid microemulsion (▲).

Table 3 Comparison of intestinal lymphatic transport and bioavailability of saquinavir in lymph-cannulated and non-lymph-cannulated rats after intraduodenal administration in a cremophor–oleic acid mixed micelle or an oleic acid microemulsion formulation

Experiment	Cremophor–oleic acid mixed micelles		Oleic acid microemulsion	
	Lymph-cannulated rats	Non-lymph-cannulated rats	Lymph-cannulated rats	Non-lymph-cannulated rats
$Cmax_{lymph}$(ng mL^{-1})	729.86 ± 209.39	N/A	1332.41 ± 979.80	N/A
Lymphatic transport[a] (% dose)	0.027 ± 0.005	N/A	0.052 ± 0.013	N/A
$Cmax_{plasma}$ (ng mL^{-1})	73.82 ± 48.88	96.04 ± 19.39	89.92 ± 22.84	82.91 ± 31.48
$AUC_{0\text{–}8}$ (ng h mL^{-1})	155.41 ± 21.22	375.48 ± 79.40	345.75 ± 100.08	263.70 ± 49.67
$AUC_{0\text{-}\infty}$ (ng h mL^{-1})	339.12 ± 89.05	580.13 ± 116.52	436.50 ± 138.37	328.40 ± 50.00
$F_{0\text{–}8}$[b]	2.50 ± 0.34	6.05 ± 1.28	5.56 ± 1.61	4.25 ± 0.80
$F_{0\text{–}\infty}$[b]	4.98 ± 1.72	8.53 ± 1.71	6.41 ± 2.03	4.83 ± 0.73

Data are mean % dose ± s.e., n ≥ 5. [a]Cumulative mass of saquinavir recovered over 8 h in mesenteric lymph calculated as a percentage of dose. [b]Bioavailability of saquinavir was estimated based on the plasma AUC relative to an intravenous control ($F = (AUC_{oral}/D_{oral})/(AUC_{i.v.}/D_{i.v.})$).

The oleic acid microemulsion formulation produced higher lymph concentrations of saquinavir than the micellar systems, and assuming a three-times-daily dosage regime, the therapeutic benefits may be considerable in terms of increased local concentrations in lymphoid tissue. The nature of the surfactant used (i.e. cremophor versus TPGS) appeared to alter the distribution of saquinavir between lymph and portal uptake.

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