Structured Triglyceride Vehicles for Oral Delivery of Halofantrine: Examination of Intestinal Lymphatic Transport and Bioavailability in Conscious Rats

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Purpose. To compare the influence of triglyceride vehicle intramolecular structure on the intestinal lymphatic transport and systemic absorption of halofantrine in conscious rats.

Methods. Conscious, lymph cannulated and nonlymph cannulated rats were dosed orally with three structurally different triglycerides; sunflower oil, and two structured triglycerides containing different proportion and position of medium–(M) and long-chain (L) fatty acids on the glycerol backbone. The two structured triglycerides were abbreviated MLM and LML to reflect the structural position on the glycerol. The concentration of halofantrine in blood and lymph samples was analyzed by HPLC.

Results. Both the lymphatic transport and the total absorption of halofantrine were enhanced by the use the MLM triglyceride. The estimated total absorption of halofantrine in the lymph cannulated animals was higher than in the nonlymph cannulated animals, and this was most pronounced for the animals dosed with the structured triglycerides.

Conclusions. Using MLM as vehicle increases the portal absorption of halofantrine and results in similar lymphatic transport levels when compared to sunflower oil. Total absorption when assessed as absorption in the blood plus lymphatic transport for halofantrine after administration in the MLM triglyceride was higher than after administration in sunflower oil.

KEY WORDS: halofantrine; structured triglyceride; lymphatic transport; bioavailability; lipid solubility.

INTRODUCTION

The intestinal lymphatic transport of xenobiotics following oral administration has been the subject of many studies (1–4). Generally, compounds processed by the intestinal lymph are transported to the systemic circulation in association with the lipid core of lipoproteins (5), and as such require co-administered lipid to stimulate lipoprotein formation. Short- and medium-chain fatty acids (with a carbon chain length shorter than 12 carbon atoms) are transported to the systemic circulation by the portal blood and are not incorporated to a great extent in chylomicrons (6). In contrast, long-chain fatty acids and monoglycerides are re-esterified to triglycerides within the intestinal cell, incorporated into chylomicrons and secreted from the intestinal cell by exocytosis into the lymph vessels. Long-chain triglycerides (LLL), such as sunflower and soybean oil, are therefore most suitable for enhancement of lymphatic drug transport (7,8). In addition to the stimulation of lymphatic transport, administration of lipophilic drugs with lipids may typically enhance drug absorption into the blood when compared to nonlipid formulations, although the relative bioavailability enhancement introduced by long- and medium-chain triglycerides (MMM) is not well understood and appears to be drug specific (9).

The physicochemical properties of candidate drug molecules appear to be a critical determinant of the total extent of lymphatic transport. Charman and Stella (10) have suggested that the compound must have a high partition coefficient (log $p > 5$) and a high solubility in triglyceride lipid (solubility >50 mg/ml) for intestinal lymphatic drug transport to be significant. For this reason halofantrine (log $p = 8.5$, solubility in triglycerides >50 mg/ml) has been used in this study as a model compound, because intestinal lymphatic transport has previously been found to play a major role in the gastrointestinal absorption of halofantrine, especially when co-administered with a lipid formulation (9,11,12).

In a previous study, Hauss *et al.* (13) described differences in the extent of absorption of a lipophilic, lymphatic transported drug, when assessed in lymph cannulated and non-cannulated animals, which could not be explained by the extent of lymphatic transport. The authors suggested that lymphatically transported drug was preferentially cleared from the blood into adipose tissue and therefore did not contribute to a proportional increase in plasma area under the curve (AUC). Caliph *et al.* (9) similarly examined the mass balance of halofantrine between lymph cannulated and noncannulated animals after dosing in triglycerides with different fatty acid chain length. Significant differences in the extent of overall absorption, in lymph cannulated vs. noncannulated animals, were found after administration of halofantrine in a long-chain triglyceride vehicle, where the percent of lymphatic transport was high. In contrast, when halofantrine was co-administered with a medium- or short-chain triglyceride, the degree of lymphatic transport was low, and there was no difference in the overall extent of absorption between the lymph cannulated and noncannulated animals. These data are consistent with the large body of work, which suggests that association of lipophilic drugs with lipoproteins can markedly affect drug pharmacokinetics and distribution patterns (14,15).

In a recent preliminary study Nordskog *et al.* (16) demonstrated that the intestinal lymphatic transport of vitamins A and E was higher when co-administered with a structured triglyceride (a triglyceride where the glycerol backbone is esterified with different fatty acid chains (Fig. 1)), when compared to a constituent physical mixture with similar fatty acid composition. However, there is limited additional information in the literature detailing the positional benefits in terms of drug delivery of these unique triglyceride vehicles. The objective of this study was therefore to investigate the impact of structured triglycerides with varying intramolecular struc-

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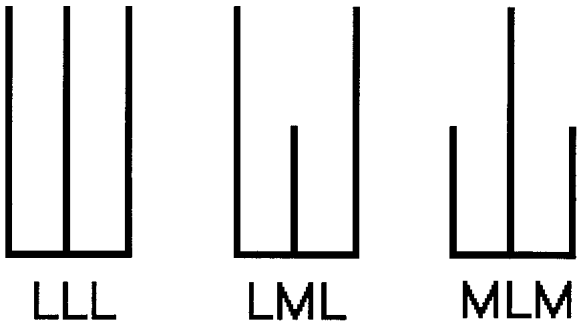

Fig. 1. Schematic presentation of the structured lipids used in this study. M represents a group with a medium-chain fatty acid (C_{8-10}), and L represents a group with a long chain fatty acid (C_{18}).

tures and chain lengths, on the intestinal lymphatic transport and absorption into the blood of halofantrine, in lymph cannulated conscious rats. Subsequent absolute bioavailability estimations in noncannulated animals have also been performed to examine the potential lack of mass balance between cannulated and noncannulated animals, and thereby to address whether the possible altered deposition profiles for lymphatically transported drugs is dependent on the triglyceride structure of a co-administered vehicle.

MATERIALS AND METHODS

Chemicals and Reagents

Crystalline halofantrine free base and the internal standard (2,4-dichloro-6-trifluromethyl-9{1-[2-dibutylamino) ethyl]}phenathrenemethanol hydrochloride) were donated by SmithKline Beecham Pharmaceuticals (Mysore, India). Sunflower oil was obtained from Róco (Copenhagen, Denmark), Captex 355 was a gift from Abitec Corporation (Janesville, WI, USA). Tricaprylin, caprylic and linoleic acid were all obtained from Sigma Chemicals (St.Louis, MO, USA). The structured triglycerides, 1,3-dioctanoyl-2-linoleyl-s*n*-glycerol (C8:0-C18:2-C8:0) (MLM) and 1,3-dilinoyl-2-octanoyl-*sn*-glycerol (C18:2-C8:0-C18:2) (LML), were manufactured at the Department of Biotechnology at the Technical University of Denmark.

Acetonitrile and tert-butylmethylether were HPLC grade and sodium dodecyl sulphate was electrophoresis grade. The water used in all experiments was obtained from a Milli-Q-water purification system (Millipore, Milford, MA, USA). All other chemicals were analytical reagent grade.

Triglyceride Synthesis and Analysis

The structured triglycerides were produced by a 1,3-specific lipase, catalyzing the interesterification of triglyceride and free fatty acid, as described by Mu *et al.* (17). The lipase hydrolyzes the esterbonds in the 1 and 3 position of the triglycerides and consecutively esterifies the position with a free fatty acid.

In the case of MLM, sunflower seed oil was used as triglyceride and caprylic acid as free fatty acid, whereas in the case of LML, tricaprylin was used as triglyceride and linoleic acid as free fatty acid. At the end of the reaction the structured triglyceride was purified by high-pressure distillation.

The total fatty acid composition of the triglycerides was determined by gas-liquid chromatography (GLC) after methylation with KOH in methanol (18). The fatty acid in the 2-position on the glycerol was determined by Grignard degradation with allyl magnesium bromide followed by isolation (19) and analysis by GLC as described by Porsgaard and Høy (20).

Halofantrine Solubility in Formulation Lipids

Excess halofantrine free base was weighed into a glass centrifuge tube with a teflon-lined cap containing triglyceride. The tubes were then flooded with nitrogen and placed in a shaking water bath, maintained at 37°C for 3 days. At specific time intervals each tube was centrifuged at 1000 *g* for 10 min, a 100-mg sample was taken and diluted with acetonitrile and analyzed by high-performance liquid chromatography (HPLC) with UV detection (21). Equilibrium solubility was assumed attained when the measured concentration in subsequent samples varies by less than 10%. The evaluation of the solubility of halofantrine in each of the triglycerides was performed in triplicate.

Halofantrine Formulations

Lipid-based Oral Formulations

Halofantrine crystalline free base was dissolved in the triglycerides, to produce a solution containing 20 mg halofantrine base in 1 g of triglyceride.

Intravenous Formulation

Halofantrine was incorporated into an intravenous emulsion using the method of El-Sayed and Repta (22). Briefly halofantrine free base was dissolved in dimethylformamide (125 mg/ml) and 0.25 ml of this solution was slowly added, under aseptic conditions, to 20 ml of a rapidly stirred Intralipid® 10% emulsion (containing 10% soybean oil, 1.2% lecithin and 2.25% glycerol in water). The formulation was then filtered sequentially though 0.45 and 0.22 μm filters, and filled into a sterilized glass bottle with a rubber membrane and a crimp lid.

The concentration of halofantrine in the formulations was determined prior to administration by high-performance liquid chromatography (HPLC), as described by Humberstone *et al.* (21). Over the period of the study, the formulations were visually inspected for physical changes.

Surgical Procedures

All surgical and experimental procedures were reviewed and approved by the local Animal Experimentation Ethics Committee, and the study complied with the NIH Guide for the Care and Use of Laboratory Animals. The animals were anesthetized for the duration of the surgery by intraperitoneal injection of 3 ml/kg of a solution consisting of Hypnorm® (fentanyl 0.2 mg/ml, fluanisone 10 mg/ml, Janssen, Belgium), Hypnovel® (midazolam 5 mg/ml, Roche, Switzerland) and water (1:1:2). Additional injections of the anesthetic were given as needed.

The mesenteric lymph duct was cannulated, using a slight modification of the method previously described by Noguchi *et al.* (23), and using a three part cannula consisting of a 5 mm

section polyethylene tubing (0.50 mm ID, 0.80 mm OD, Critchley Electrical Products, Australia), followed by 5 cm of flexible Silastic® tubing (0.51 mm ID, 0.94 mm OD, Dow Corning, MI, USA) and a further 5 cm section of polyethylene tubing (0.50 mm ID, 0.80 mm OD, Critchley Electrical Products, Australia). The short section of polyethylene tube was inserted into the mesenteric lymph duct. Any auxiliary lymph ducts were disrupted and sealed to ensure that all lymph flow was into the main mesenteric duct. The lymph cannula was secured in the mesenteric duct with cyanoacrylate adhesive and the auxiliary lymph duct was blocked and sealed with cyanoacrylate adhesive. The cannula was externalized through the abdominal wall, and the rats were placed in a jacket that held a collection bottle for the lymph (23).

The right carotid artery was cannulated with a piece of polyethylene tube (0.50 mm ID, 0.80 mm OD, Critchley Electrical Products, Australia). The duodenum was cannulated 2 cm below the pylorus with PE 50 tubing and secured with instant cyanoacrylate adhesive. Both the carotid and the duodenal cannula were exteriorized at the back of the neck.

The animals that received the intravenous formulation and the animals used to examine mass balance were sham operated in terms of the mesenteric lymph duct cannulation. The rats used for determination of endogenous lipid production were sham operated in terms of the carotid cannula.

Experimental Procedures

After completion of the surgical procedures, the animals were transferred to wire bottom cages and stabilized over night (16–24 h) during which the animals were fasted but received an intraduodenal infusion of lactated Ringer's solution at 1.5 ml/h via a constant rate infusion pump to assist rehydration. Rehydration was terminated two hours before dosing. Free access to 5% sucrose in a lactated Ringer's solution was permitted postoperatively and throughout the study. The animals were randomly assigned to receive one of the formulations.

Parallel groups of animals were administered 0.100 g of the structured lipids containing 2 mg halofantrine free base by oral gavage. The lymph collection tubes all contained 100 μl of a 1.0% EDTA solution. The lymph collection tubes were changed immediately prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h after dosing. The lymph was stored at 4°C, and analyzed a maximum of 4 h after sampling.

Blood samples (0.30 ml) were taken at 5 min and 1, 2, 4, 6, 8, 10, 12, 24, and 30 h after drug administration and placed into Eppendorf tubes containing 20 μl of 200 units/ml heparin solution as an anticoagulant. Plasma was immediately harvested by centrifugation (at 1800 g for 10 min), and stored at –20°C until analysis.

In the intravenous group, the rats received halofantrine (0.52 mg in 0.3 ml emulsion) via the left external jugular vein. Blood samples (0.30 ml) were taken from the right carotid artery at –5, 0.5, 2, 5, 10, 20, 60,min, and 2, 4, 6, 8, 10, 12, 24, and 30 h postdosing. Endogenous lymph triglyceride output over 12 h was determined from rats receiving 0.1 Ml of normal saline by oral gavage.

At the end of the experiment, the animals were sacrificed by an overdose of sodium pentobarbitone (0.5 ml, 200 mg/ml) administered slowly via the carotid catheter.

Analysis of Halofantrine in Blood and Lymph

From the lymph samples, 100 μl of lymph was added to 8-ml acetonitrile. The sample was vortexed for 1 min. The insoluble protein-based components were removed by centrifugation and the supernatant analyzed by HPLC as previously described by Porter *et al.* (11). Recovery of spiked halofantrine from blank lymph was found to be greater than 99%.

Blood samples were analyzed using a validated method previously described by Humberstone *et al.* (21), by adding 100 μl plasma and 100 μl internal standard (2 μg/ml) to 0.5 ml acetonitrile in a 12 ml polypropylene centrifuge tube. Samples were subsequently vortexed for 2 min to precipitate plasma proteins then centrifuged, and a 4-ml aliquot of tert-butyl methyl ether was added. The tube was vortexed and centrifuged and 4 ml of the upper organic layer was removed into a polypropylene centrifuge tube containing 100 μl of 0.005 M HCl (in acetonitrile). The organic layer was then evaporated to dryness under nitrogen at 35°C. The residue was reconstituted with 100 μl acetonitrile and 25 μl was injected onto the LC column. The limit of quantification by this procedure was 40 ng/ml. The assay was linear between 40 ng/ml and 4000 ng/ml, and the extraction efficiency for halofantrine was greater than 90% across the concentration range.

Analysis of Lymph Triglyceride

The triglyceride concentration in lymph was measured using a Roche Cobas Mira clinical chemistry analyzer (Basle, Switzerland) and commercial enzyme-based colorimetric assay (Boehringer Mannheim, Germany).

Lymphatic triglyceride transport due to exogenously administered lipid was determined by subtracting the endogenous lipid component from the mass of triglyceride lipid determined in each collected lymph sample. Endogenous lymphatic transport (mean ± S.E., $n = 4$), was found to be 3.6 ± 1.2 mg/h as determined from the amount of triglyceride appearing in the lymph over 12 h in four rats dosed with 0.1 ml saline.

Lymph Distribution of Halofantrine

The lymph was pooled into 0–4, 4–8 and 8–12 h fractions and chylomicrons were separated as described by Raub *et al.* (24), using a Beckman SW-60 rotor. Tubes were centrifuged at 44100 rpm (262000 *g*) for 1 h and 20 min at 15°C, and the brake was used for deceleration. After centrifugation, the bottom of the tube was pierced with a needle to enable the remaining lymph to be removed, leaving only the chylomicron fraction that forms a white semi-solid plug at the top of the tube. After fractionation, the chylomicrons fraction was dissolved in acetonitrile and the amount of halofantrine associated with the chylomicrons was determined by HPLC.

Pharmacokinetic Analysis

Plasma concentrations vs. time data for halofantrine in individual rats were analyzed using WinNonlin version 2.1. After intravenous administration, the AUC for halofantrine was determined using a two-compartment model, and the extrapolated area from the last measured point to infinity was calculated using the linear trapezoidal rule.

The total bioavailability (0–30 h) in lymph cannulated

animals, was calculated by addition of the cumulative percent of halofantrine transported in the lymph (determined as volume lymph collected multiplied by the concentration of halofantrine in each lymph sample) with the percent of halofantrine found in the plasma (9). The percent of halofantrine in the plasma was estimated by normalizing the AUC after oral and intravenous administration with the dose administered.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance using Statgraphics version 7.0, and the Student-Newman-Keuls multiple comparison test was applied for analyzing differences between the formulations. The results were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Fatty Acid Composition of Triglycerides

The total fatty acid compositions, and the specific fatty acids present in the 2 position of glycerol backbone in the structured triglycerides are presented in Table I. The data confirm the accuracy of the proposed structure presented in Fig. 1 and indicate a high degree of structural homology with respect to the presence of medium- and long-chain fatty acids in the 2 position on the glycerol backbone.

Solubility

The solubility (mean ± SD, n = 3) of halofantrine at 37°C was 45.0 ± 0.8 mg/g for sunflower oil, 71.1 ± 2.6 mg/g MLM and 72.5 ± 7.6 for LML. The previously reported solubility of halofantrine in a medium-chain triglyceride, 88.5 ± 5.9 mg/g (9) was higher than the solubility in the long-chain triglyceride reported in the work. The solubility of halofantrine in the structured triglycerides, containing both medium- and long-chain fatty acids, was intermediate to the pure long and medium-chain triglycerides. Previously, Anderson and Marra (25) found the ester concentration in various lipids to correlate linearly with the solubility of benzyl alcohol and an investigational anti-HIV agent (NSC 675186). The ester concentration in triglycerides is directly correlated to the molar mass, and based on Anderson and Marra's prediction, the following rank of halofantrine solubility was expected: MMM > MLM > LML > LLL. However, the solubility of halofantrine in LML and MLM was similar in spite of very different ester concentrations in the two structured triglycerides.

Table I. Total Fatty Acid Profile in Triglycerides (TAG) and the Fatty Acid Presented in the 2 Position on the Glycerol Backbone (*sn*-2) of Sunflower Oil and the Two Structured Triglycerides Examined (mol%)

Fatty acid	Sunflower oil TAG	MLM		LML	
		TAG	*Sn*-2	TAG	*Sn*-2
8:0	0.0	48.7	1.8	36.9	88.5
10:0	0.0	0.2	0.2	0.0	0.0
12:0	0.0	0.0	0.2	0.0	0.0
14:0	0.1	0.1	0.7	0.1	0.0
16:0	7.2	2.4	0.8	2.0	0.4
18:0	4.3	0.7	0.3	0.8	0.2
18:1	23.1	7.0	12.7	17.9	3.4
18:2	63.6	40.0	78.4	37.3	6.5
18:3	1.2	0.1	0.0	4.7	0.8
20:0	0.3	0.3	0.5	0.0	0
Others	0.2	0.5	4.4	0.3	0

Note: Results are average of three determinations.

Intestinal Lymphatic Transport of Halofantrine and Triglyceride

The lymphatic transport of halofantrine, expressed as the cumulative percentage of administered dose, is presented in Fig. 2 and Table II. The rank order of lymphatic transport of halofantrine after 12 h (mean %dose ± SE) was LML > sunflower oil > MLM. Examination of the transport data from the animals dosed with MLM revealed two distinct and significantly different ($p < 0.05$) groups and these have been designated as either MLM-high or MLM-low. The appearance of two separate groups for the animals dosed with MLM is consistent with previous reports (10,11,26), where the appearance of low- and high-lymphatic transport groups was seen after administration of low (50 μl) lipid doses. Charman *et al.* (10,26) suggested that a threshold level of lipid absorption was required to stimulate or initiate the lymphatic lipoprotein synthesis and thereby recruit the lymphatic transport process for the dosed drug. The data described here suggest that the MLM formulation contained insufficient quantities of long-chain fatty acids to stimulate significant lipoprotein synthesis in some of the rats, leading to the lower lymphatic transport of halofantrine in these animals. Statistically, the MLM-low group was significantly different from the three other groups, whereas no significant difference was seen between the LML, LLL and MLM-high dosed animals. These data suggest that the structured triglycerides may be advantageous in that they retain the lymphatic transport stimulation properties of LLL and possess solubilizing properties closer to the MMM triglyceride lipids.

The cumulative transport of C_{18} triglyceride into the mesenteric lymph, after correction for the endogenous contribution, is presented in Fig. 3. Exogenous lipid recovery in the mesenteric lymph in the 12 h after dosing (expressed as

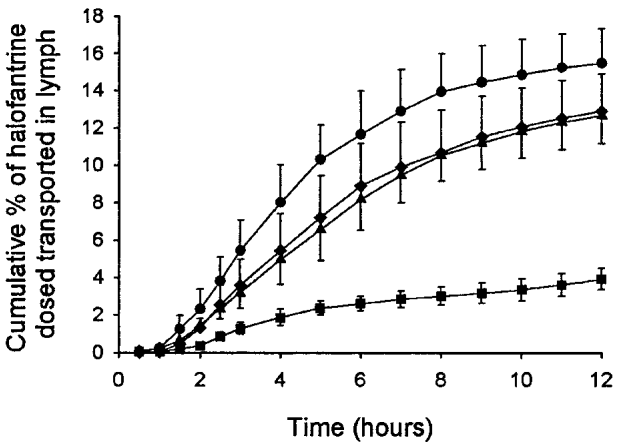

Fig. 2. Cumulative percentage dose of halofantrine (mean ± SE) collected in the intestinal lymph as a function of time after oral administration of 2 mg halofantrine dissolved in three different triglycerides to conscious rats. Sunflower oil (n = 5, ♦), MLM, low (n = 4, ■), MLM, high (n = 4, ▲) and LML (n = 5, ●).

Table II. Halofantrine Bioavailability in Lymph-Cannulated Rats after Oral Administration in Different Structured Triglycerides

Lipid	Lymphatic transport[a,b]	Plasma availability[c]	Total bioavailability[d]	% of adsorbed halofantrine in lymph
LLL	12.9 ± 2.0	5.5 ± 0.7	19.1 ± 2.2	67.5 ± 6.8
MLM, low	3.9 ± 0.6[e]	7.1 ± 1.2	11.3 ± 1.4[e]	34.5 ± 3.8
MLM, high	12.7 ± 1.5	12.6 ± 2.3[f]	25.3 ± 3.9[f]	50.2 ± 7.0
LML	15.5 ± 1.8	7.4 ± 1.5	23.4 ± 2.3	66.2 ± 5.5

Note: Mean % dose ± SE, n = 4–5

[a] Cumulative amount of lymph recovered over 12 hours in the mesenteric lymph was 8.9 ± 0.9 g for animals dosed with LLL, 9.9 ± 3.0 g for LML-low, 7.7 ± 0.6 g for MLM-high and 8.8 ± 0.5 g for LML.

[b] Cumulative percentage of dose halofantrine administered recovered over 12 h in the mesenteric lymph.

[c] The percentage of halofantrine absorbed into the blood was calculated based on the AUC_{0-30h} relative to the $AUC_{0\rightarrow\infty}$ obtained after intravenous administration of 0.52 mg halofantrine to nonlymph-cannulated animals (AUC was 15.51 μg · h/ml).

[d] Total bioavailability was calculated as percentage transported by the lymph plus percentage absorbed directly into the blood.

[e] Lymphatic transported halofantrine and total bioavailability for MLM-low were statistically significantly different ($p < 0.05$) when compared to the three other groups.

[f] Plasma bioavailability for MLM-high were significantly different from LLL ($p < 0.05$).

the mean ± SE, n = 4–5) was 146.8 ± 14.5 mg for animals dosed with sunflower oil, 135.7 ± 10.1 mg for animals dosed with LML, 95.0 ± 7.9 mg for animals in the MLM-high group and 49.1 ± 5.2 mg for animals in the MLM-low group. The mass of triglyceride absorbed in the animals dosed with sunflower oil and LML was not statistically different, whereas a significantly lower absorption of triglyceride was seen for both MLM-high and MLM-low. These data suggest that decreased triglyceride transport is responsible for the reduced extent of lymphatic drug transport seen in the MLM-low group. A correlation between the lymphatic transport of triglycerides and halofantrine has previously been found by Holm *et al.* (27) after administration of halofantrine in vehicles consisting of free fatty acids with different degrees of unsaturation. Correlations between the cumulative halofantrine and triglyceride transport into the mesenteric lymph are shown in Fig. 4. The figure shows a linear relationship between halofantrine and triglyceride transport into the lymph for the first 8 to 9 h post-dosing for all the groups. The mass of triglyceride necessary to transport a given mass of halofantrine was comparable for the two structured triglycerides, whereas the animals dosed with LLL seemed to require a larger mass of lymph triglyceride to transport the same halofantrine mass. The structured triglycerides therefore appear to provide a more effective vehicle for lymphatic drug transport, in term of chylomicron loading capacity. This trend likely mimics the increased solubility of halofantrine in the LML and MLM lipid when compared to LLL.

Lymph Distribution of Halofantrine

The distribution of halofantrine to chylomicrons isolated from the intestinal lymph as a function of the dosed lipid is shown in Table III. The major proportion of lymphatically transported halofantrine was associated with the chylomicron fraction, irrespective of the co-administered lipid. The low lymphatic transport of halofantrine in the MLM-low group did not affect the distribution of halofantrine in the chylomicron fraction. These observations suggest that optimal lym-

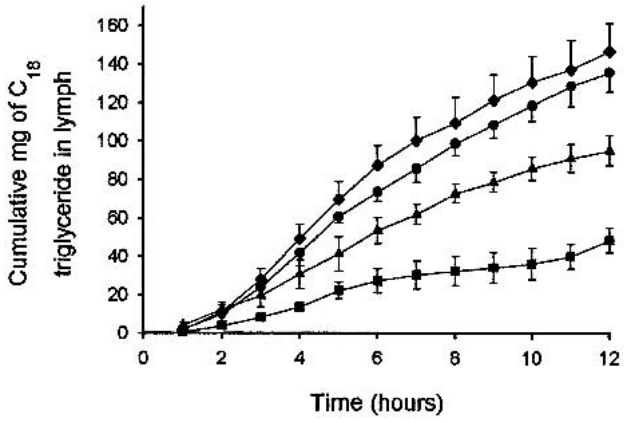

Fig. 3. Cumulative mass (mg) of triglycerides (mean ± SE) in intestinal lymph after oral administration of sunflower oil (n = 5, ♦), MLM, (n = 4, ■), MLM, high (n = 4, ▲) and LML (n = 5, ●).

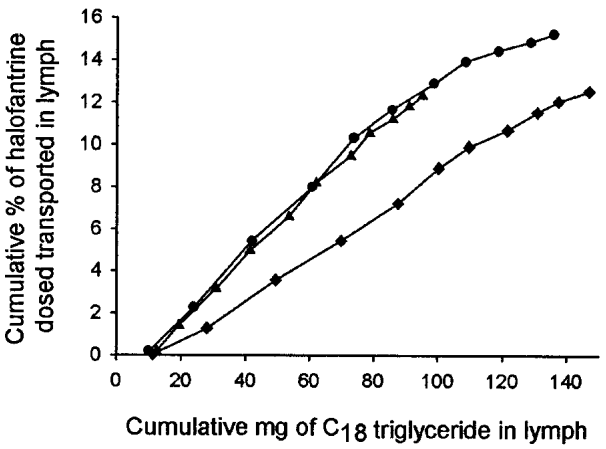

Fig. 4. Correlation between cumulative mass of triglycerides and cumulative halofantrine transport in the lymph after oral administration in sunflower oil (n = 5, ♦), MLM, high (n = 4, ▲) and LML (n = 5, ●).

Table III. Percentage Distribution (Mean ± SE, n = 4–5) of Halofantrine in Mesenteric Lymph Chylomicrons Fraction as a Function of Lipid Dosed and Time

Lipid	Percentage distribution of halofantrine in chylomicrons		
	0–4 h	4–8 h	8–12 h
Sunflower oil	64.6 ± 4.0	68.3 ± 6.3	61.3 ± 15.0
MLM, low	64.5 ± 7.1	69.4 ± 11.1	63.1 ± 3.9
MLM high	65.1 ± 5.0	68.0 ± 6.3	49.9 ± 7.5
LML	73.9 ± 3.0	56.6 ± 4.4	61.7 ± 9.4

phatic drug transport (in terms of extent) was not a function of increased transport via the chylomicron fraction but more likely reflect the mass and type of triglyceride transported in the intestinal lymph.

Absorption of Halofantrine into the Systemic Blood Circulation and Total Bioavailability

Figure 5 presents the plasma concentration vs. time profiles for lymph cannulated rats after oral administration of halofantrine in the triglyceride vehicles. The plasma levels obtained in the MLM-high group were statistically higher than that seen for the other lipid doses, suggesting that the MLM structured lipid may be capable of both maintaining optimal lymphatic transport and also stimulate absorption into the portal blood as described below.

The total bioavailability of halofantrine for the lymph-cannulated animals is shown in Table II. The extent of lymphatic transport of halofantrine in the animals dosed with the two structured triglycerides was not statistically different to that seen in animals dosed with LLL. The plasma availability for MLM-high was similar to that seen previously in lymph cannulated rats after administration of halofantrine in short- and medium-chain triglycerides vehicle by Caliph *et al.* (9). The structured triglycerides therefore seem to combine the absorption characteristics of both the medium- and long-chain triglycerides; i.e. the presence of medium-chain-fatty acids enhances absorption into the systemic blood circulation whereas presence of long-chain fatty acids enhances lymphatic transport. The total bioavailability of halofantrine after administration in the LML vehicle was intermediate between MLM-high and LLL groups and showed a slightly higher lymphatic transport of halofantrine when compared to MLM-high and LLL. LML also produced slightly higher blood availability than LLL although levels were still lower than animals in the MLM-high group. These data suggest that the structured triglycerides enhance drug transport routes (blood vs.

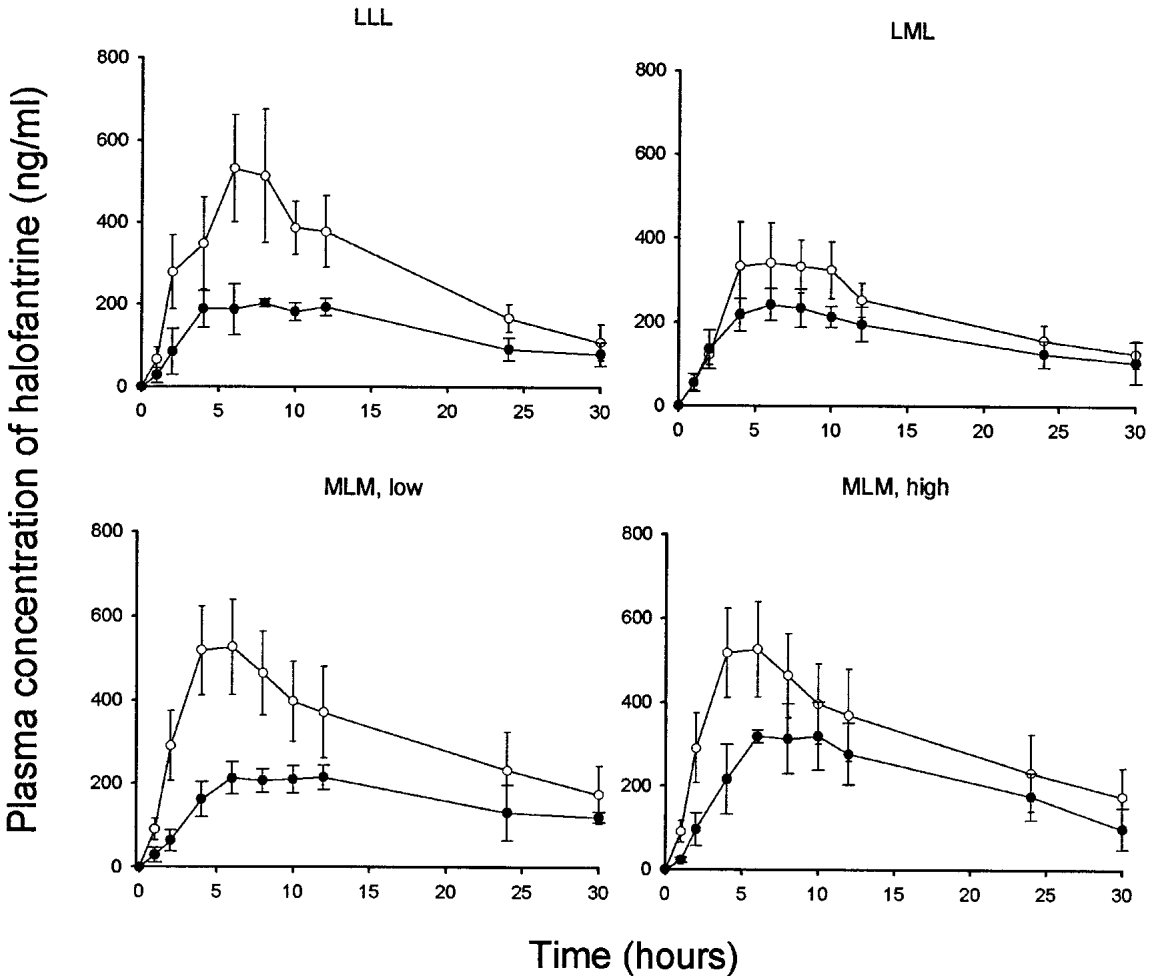

Fig. 5. Plasma concentration time profile of halofantrine (mean ± SE of 5 to 7 rats) in nonlymph-cannulated (O) and lymph-cannulated (●) animals after oral administration of 2 mg halofantrine in three different triglycerides with different molecular composition.

lymph) differently depending on the proportion, composition and/or placement of the medium- and long-chain fatty acids on the glycerol backbone.

Comparison of the Mass Balance of Halofantrine in Lymph Cannulated and Noncannulated Animals

Halofantrine plasma concentration profiles after oral administration to both lymph cannulated and noncannulated animals are presented in Fig 5. The data for the lymph-cannulated animals reflect the fraction of halofantrine absorbed directly into the blood, whereas the plasma concentrations in the nonlymph-cannulated animals should effect both intestinal lymphatic transport and absorption into the portal blood.

As expected, the plasma levels obtained in noncannulated animals were higher than those seen in lymph cannulated animals (where intestinal lymph was collected) for the LLL and the MLM-low groups, consistent with results published by Hauss *et al.* (13) and Caliph *et al.* (9). Interestingly however, the difference between the plasma availability for the lymph cannulated and noncannulated animals was not as large when halofantrine was dosed with LML and in the MLM-high group. Caliph *et al.* (9) found a smaller difference between the plasma levels in lymph- and nonlymph-cannulated animals when dosed with MMM than LLL, which the authors concluded was a reflection of a reduced lymphatic drug transport when MMM was co-administered. In contrast, in these studies the animals dosed with the two structured triglycerides showed high lymphatic transport of halofantrine presumably induced by the long-chain components in the structured triglycerides combined with a relatively high plasma bioavailability induced by the medium-chain components.

Comparisons of the total bioavailability of halofantrine in lymph cannulated and noncannulated animals are presented in Table IV. For LLL, MLM-high and LML, a significantly lower bioavailability was found in noncannulated animals when compared to the lymph-cannulated animals, consistent with previous reports (9,13). These differences were most notable for the animals dosed with LML, where the bioavailability in the noncannulated animals was almost equal to the plasma availability in the lymph cannulated animals (Table II and IV) suggesting that lymphatically transported drug contribute little to the overall systemic bioavailability in noncannulated animals. This indicates that the structure of the triglyceride vehicle may have an effect on the distribution profile for lymphatically transported drugs such as halofantrine. This is consistent with data previously published by Redgrave *et al.* (28,29) who described variations in the metabolism and distribution patterns for different structured triglycerides. The possibility of altering the distribution profile of lymphatically transported drugs such as halofantrine via the structure of a co-administered triglyceride is appealing and suggests the possibility for lipid engineering as a means of providing formulations with desired pharmacokinetic and metabolic profiles, but also shows the importance of including both lymph- and nonlymph-cannulated groups in pharmacokinetic and toxicological studies of lipophilic drugs, to assure that an accurate reflection of total exposure can be made.

Table IV. Comparison of Halofantrine Bioavailability (Mean % Dose ± SE, n = 4–7) in Nonlymph-Cannulated and Lymphcannulated Animals after Oral Administration of 2 mg Halofantrine in Three Different Triglycerides

Triglyceride	Nonlymph-cannulated animals	Lymph-cannulated animals	Cannulated/ noncannulated[a]
LLL	11.08 ± 1.59	19.12 ± 2.21[c]	1.7
MLM, low	13.83 ± 3.85[b]	11.29 ± 1.37	0.82
MLM, high	13.83 ± 3.85[b]	25.16 ± 3.94[c]	1.82
LML	10.30 ± 2.22	23.42 ± 2.28[c]	2.27

[a] Calculated as the mean total bioavailability in lymph cannulated animals divided by the mean bioavailability in nonlymph cannulated animals.

[b] The low and high absorption group refers to the amount of halofantrine found in the lymph, and can therefore not be distinguished in the nonlymph-cannulated animals.

[c] Statistically significant difference ($p < 0.05$) in halofantrine bioavailability for nonlymph-cannulated and lymph-cannulated groups.

CONCLUSIONS

In conclusion, this study has demonstrated that both the lymphatic transport and absorption via the portal blood of halofantrine may be enhanced after co-administration in a structured triglyceride vehicle comprising both medium- and long-chain fatty acids on the same glycerol backbone. For the animals in the high absorption group, the MLM triglyceride resulted in substantial lymphatic transport equal to that seen after administration with long-chain triglycerides, and a similar blood bioavailability as that reported in the literature on rats dosed with medium-chain triglyceride. If total exposure is assessed as absorption into the blood plus the extent of drug absorption into the lymph, the data indicate that the MLM structured lipid may provide an absorption profile that combines the beneficial characteristics of both medium- and long-chain triglyceride, under the assumption that a sufficient amount of triglyceride is administered to trigger the lymphatic transport. The structured lipid also provide additional pharmaceutical benefits in that the solubility of lipophilic drugs such as halofantrine is typically higher in the structured triglycerides than in simple long-chain triglycerides. Further work will assess if these findings are reflected in higher species.

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