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Short Communication

Bioavailability of Seocalcitol IV: Evaluation of Lymphatic Transport in Conscious Rats

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Purpose. To study the use of long chain triglycerides (LCT) as a lymphotropic carrier of ^{3}H-seocalcitol by comparing the lymphatic transport and the portal absorption of ^{3}H-seocalcitol when dissolved in a (1) LCT solution or a (2) reference solution without lipid containing propylene glycol (PG).
Materials and Methods. A lymph cannulated conscious rat model was dosed orally with ^{3}H-seocalcitol dissolved in either LCT or PG. Lymph was collected continuously, and blood was sampled over 9 h. ^{3}H-seocalcitol in blood and lymph and triglycerides in lymph were analysed.
Results. A statistically significantly ($p < 0.05$) higher recovery of the dosed ^{3}H-seocalcitol was found in the intestinal lymph upon administration of the LCT solution (1.3 ± 0.6%) compared to the PG solution (0.5 ± 0.4%). The portal absorption of ^{3}H-seocalcitol was significantly ($p < 0.05$) higher from the LCT solution (16.2 ± 2.2%) than from the PG solution (10.8 ± 0.8%).
Conclusions. The LCT solution resulted in a statistical significantly higher level of lymphatic and portal transport of ^{3}H-seocalcitol compared with the PG solution. However, even though LCT facilitates the formation of chylomicrons, ^{3}H-seocalcitol favours absorption directly to the portal blood probably due to the moderate lipophilicity of the molecule.

KEY WORDS: long chain triglycerides; lymphatic transport; propylene glycol; seocalcitol.

INTRODUCTION

Orally administered drug substances are generally absorbed via the portal blood to the systemic circulation. Triglycerides (TG) containing long chain fatty acids, lipid soluble vitamins, cholesterol and some highly lipophilic drug substances are, however, typically lymphatically transported (1). Transport of orally administered drug substances to the systemic circulation via the intestinal lymph is likely to lead to higher serum concentrations and increased bioavailability via reduced first pass metabolism.

For many poorly soluble drug substances, the limiting step for absorption in the gastro-intestinal tract (GIT) is the dissolution process. In order to circumvent the dissolution process, poorly soluble drug substances can be dissolved in the formulation before entering the GIT, e.g., in water miscible systems or lipid vehicles. The advantage of applying a lipid vehicle rather than a simple water miscible system, is that the formed lipid degradation products may increase drug absorption via enhanced solubilisation and dispersion of the drug substance in the intestine (2,3). Lipid-based formulations containing long chain triglycerides (LCT) are likely to enhance the lymphatic transport of lipophilic drug substances (4). Lipolysis products of LCT are absorbed by the enterocyte and re-esterified in the smooth endoplasmic reticulum into TG and incorporated into chylomicrons before released into the intestinal lymphatics (5). Charman and Stella (6) have proposed that lipophilic drug substances having a log *P* value above 4.7 and a lipid solubility above 50 mg/ml may be potential candidates for lymphatic transport. However, many drugs do not apply to this; penclomedine (log $P = 5.36$, $S_{LCT} = 177$ mg/ml) and CI-976 (log $P = 5.83$, $S_{LCT} > 100$ mg/ml), are not transported significantly in the lymphatics, even though they both fulfil the stated criteria (7,8). The above examples illustrate that log *P* and TG solubility cannot alone estimate the lymphatic transport of a lipophilic drug substance. Recently, Holm and Hoest (9) used the VolSurf software to correlate nine molecular descriptors with lymphatic transport and found a better correlation compared to using only log *P* and TG solubility.

Seocalcitol (Fig. 1), is a synthetic vitamin D analogue, that has shown pronounced antiproliferative effects on cancer cells, both *in vitro* and *in vivo* (10). Selected physicochemical properties of seocalcitol are shown in Table I. The pathway (portal *versus* lymphatic) by which seocalcitol is transported *in vivo* after oral administration is not known, however, several studies have investigated the lymphatic transport of vitamin D_3 suggesting that this vitamin is

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ABBREVIATIONS: AUC, area under the serum concentration–time curve; LCT, long chain triglycerides; PG, propylene glycol; TG, triglycerides.

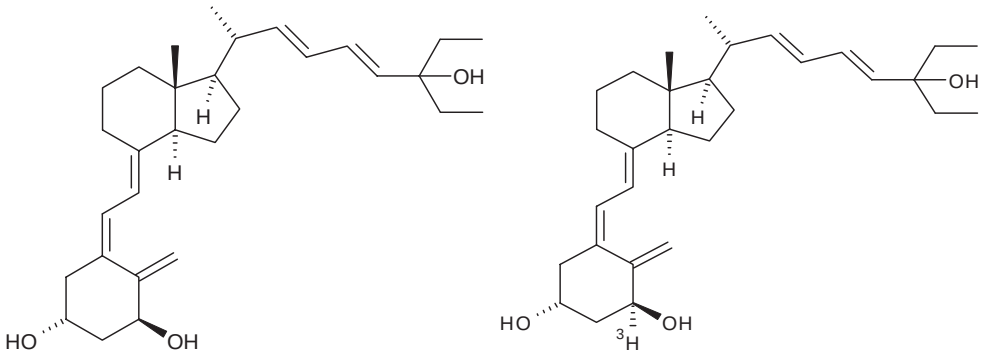

Fig. 1. Chemical structure of seocalcitol and ^{3}H-seocalcitol.

primarily transported via the lymphatics (11–13). A direct comparison of the studies with vitamin D_3 is difficult as these studies were conducted in different animal models (anaesthetised/conscious animals), with different formulations (non-dispersed/dispersed), with different amounts of lipid and with different experimental sampling periods. However, the percentage of the administered vitamin D_3 dose recovered in the lymph varied from 6.5 to 33.2% in mesenteric lymph cannulated rats (11–13). The highest recovery was found upon administration of a peanut oil solution to conscious mesenteric lymph cannulated rats where lymph was sampled for 48 h (11). The hydroxylated metabolites of vitamin D_3; 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3, have been dosed to mesenteric lymph cannulated rats administered in propylene glycol mixed with intralipid 10% (50/50 v/v%). A recovery of 5.1 and 1.6% was found for the metabolites, respectively, which may be ascribed the more hydrophilic character of the molecules (13).

Since vitamin D_3 has shown lymphatic transportability, it is interesting to study whether the synthetic vitamin D analogue seocalcitol is transported via this pathway. The objective of the present study is to: (a) Determine the *in vivo* lymphatic and portal transport of ^{3}H-seocalcitol administered in either a propylene glycol (PG) solution or a LCT solution, using the conscious mesenteric lymph cannulated rat model and (b) relate the *in vivo* data to theoretical considerations with regard to prediction of lymphatic absorption.

MATERIALS AND METHODS

Materials

Seocalcitol and tritium labelled seocalcitol (^{3}H-seocalcitol, specific activity of 0.844 MBq/μg and radiochemical purity of 97%) were synthesized at LEO Pharma A/S. Propylene Glycol was purchased from Lyondell Chem. Comp., France, and Sesame oil (LCT) from Henry Lamotte GmbH, Germany. The fatty acid composition of the triglycerides in LCT is as specified in Ph.Eur. Hypnorm® (fentanyl 0.2 mg/ml and fluanisone 10 mg/ml) was purchased from Janssen, Belgium, and Dormicum (Midazolam 5 mg/ml) from Roche, Switzerland. Heparin 10,000 IU/ml was produced at LEO Pharma A/S. Isotonic sterile NaCl was purchased at Dilab, Sweden. Water was obtained from a Milli-Q-water purification system (Millipore, MA, USA). Pico-Aqua™ and Pico Flour 40™ were purchased from Packard. All other chemicals were of analytical grade.

Male Spraque–Dawley rats (280–320 g) were purchased from Møllegaard Breeding Center, Lille Skensved, Denmark and maintained on standard food (Altromin, Gesellschaft für Tierernährung mbH, Deutschland) containing 4% lipid and water *ad libitum* in our laboratory for at least 1 week prior to entering the experiment. During the acclimatization, the housing conditions were two rats per cage maintained at 22 ± 2°C with a 50% relative humidity, an air change of 15 changes per hour and a 12-h light–dark cycle.

Preparation of Oral ^{3}H-Seocalcitol Formulations

Two formulations, one LCT solution (sesame oil) and one reference solution (propylene glycol) were prepared by dissolving the ^{3}H-seocalcitol in the vehicle, resulting in a concentration of seocalcitol of 57 μg/ml equal to 48 MBq/ml (radiochemical purity of 97%).

Animal Surgery

All surgical and experimental procedures were reviewed and approved by the Danish Animal Experimentation Ethics

Table I. Physicochemical Properties of Seocalcitol

Physicochemical Parameter	
Log *P*	4.8
S_{LCT}[a] (mg/g)	1.7
S_{PG}[b] (mg/g)	25
S_{water}[c] (ng/g)	20
$S_{fasted\ state}$[d] (μg/ml)	78
$S_{fasted\ state\ +\ LP}$[e] (μg/ml)	127
$S_{fed\ state}$[f] (μg/ml)	160

[a]Solubility in sesame oil (S_{LCT}) at 25°C, $n = 2$.
[b]Solubility in propylene glycol (S_{PG}) at 25°C, $n = 2$.
[c]Solubility in Milli-Q-water (S_{water}) at 25°C, $n = 3$.
[d]Solubility in simulated intestinal media simulating the fasted state ($S_{fasted\ state}$) containing 5 mM taurocholic acid and 1.25 mM phospholipids, $n = 3$.
[e]Solubility in simulated intestinal media simulating the fasted state and lipolytic products ($S_{fasted\ state\ +\ LP}$). 5 mM taurocholic acid, 1.25 mM phospholipids, 5 mM long chain fatty acids and 2.5 mM long chain monoglycerides, $n = 3$.
[f]Solubility in simulated intestinal media simulating the fed state ($S_{fed\ state}$) containing 20 mM taurocholic acid and 5 mM phospholipids, $n = 3$.
[a–c]Data from internal report, LEO Pharma A/S. Seocalcitol concentration determined by HPLC.
[d–f](15)

Committee and adhered to the Guide for the Care and Use of Laboratory Animals (NIH publication, 1996). The animals were anesthetized for the duration of the experiment by subcutaneous injection of 2.7 ml/kg of a solution consisting of Hypnorm®, Dormicum and water (1:1:2).

The mesenteric lymph duct was cannulated using a slight modified method as previously described by Bollman *et al.* (14) with a tygon catheter (0.40 mm ID, 0.79 mm OD, Dilab, Sweden) and exteriorized through the right flank. Any auxiliary lymph ducts to the right of the mesenteric artery were cut to ensure that all lymph flows into the main mesenteric duct. The lymph cannula was secured with ethyl-2-cyanoakrylate (Casco expresspipetter, Nacka, Sweden) adhesive and the auxiliary lymph ducts were sealed with ethyl-2-cyanoakrylate adhesive. The vials to collect the lymph were fixed with an adhesive on the right flank. The right carotid artery was cannulated with a tygon catheter (0.40 mm ID, 0.79 mm OD, Dilab, Sweden). The catheter was exteriorized at the back of the neck and connected to a swivel apparatus designed to allow free animal movement and computerized blood sampling (Dilab, Sweden). Post surgery the rats were subcutaneously given 0.1 ml/kg of Rimadyl Vet (50 mg/ml carprofen; Pfizer, New York, NY, USA) as analgesia.

Experimental Procedures

Post surgery and during the experiment the rats were allowed free access to food and water in order to allow optimal recovery conditions after the surgeries. Furthermore, during recovery and during the experiment, the artery catheter was flushed with 25 U/ml heparin (LEO Pharma, Ballerup, Denmark) administered in isotonic saline to prevent the catheter from clotting and to stabilize the liquid balance of the animal. Two formulations were tested [one LCT solution (sesame oil) and one reference solution (propylene glycol)] and each formulation were dosed to five rats. Each rat received 700 µl formulation/kg corresponding to 40 µg ^{3}H-seocalcitol/kg (equal to 34 MBq/kg). The lymph was collected in tarred 2 ml dark borosilicate vials with a cap containing 20 µl 200 IE/ml heparin saline and the vials were changed after 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7 and 9 h. Blood samples of 250 µl were collected at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 5, 7 and 9 h after dosing. The tubes with blood were centrifuged for 10 min at 4°C at 4,000 rpm (Jouan BR4i) and the resultant serum as well as the lymph was stored at −80°C until analysis.

Radio-HPLC Analysis of ^{3}H-Seocalcitol in Serum and Lymph

Tritium labelled seocalcitol was used in the study due to analytical considerations. The concentration of ^{3}H-seocalcitol in serum and lymph was determined by the validated radio-HPLC method described earlier (15). In brief, a reversed phase HPLC system with cooling on the autosampler (8°C), online vacuum degassing, column oven (40°C; all Waters Associates, Milford, MA, USA), was added to a Packard Radiomatic Flow scintillation Analyzer using Pico-Aqua™ (Packard) as scintillator. The analytical column was a Symmetri C8, 50 × 2.1 mm ID 3.5 µm (Waters) and the absorbance was measured at 264 nm. The quantification of ^{3}H-seocalcitol was performed using a calibration curve of spiked blank serum and lymph, where the lower limit of quantification was 0.5 ng/ml.

Analysis of Triglycerides (TG) in Lymph Samples

The total amount of triglycerides was measured using an enzyme-based colorimetric assay, using a commercially available kit (Triglycerides GPO-PAP, Roche, Switzerland). Samples were analysed in a validated automatic analyser (Hitachi 911, Roche, Switzerland). The analysis was done at 37°C. The assay is based on enzymatic hydrolysis of triglycerides with subsequent determination of the liberated glycerol by colorimetry according to the reaction schemes below (the enzyme is written in brackets):

(1) Triglycerides + 3 H_2O → (Lipase) Glycerol + 3 RCOOH

(2) Glycerol + ATP → (Glycerol kinase) Glycerol-3-phosphate + ADP

(3) Glycerol-3-phosphate + O_2 → (Glycerol phosphate oxidase) Dihydroxyacetone phosphate + H_2O_2

(4) H_2O_2 + 4-aminophenazone + 4-chlorophenol → (Peroxidase) 4-(p-benzoquinon-monoimino)-phenazone + 2 H_2O + HCl.

Pharmacokinetic and Statistical Analysis

The area under the serum concentration *versus* time curve ($AUC_{0\rightarrow 9}$) in individual rats was calculated by non-compartmental estimations using WinNonLin Professional software version 3.3 (Pharsight Corp., Mountain View, CA, USA). The linear trapezoidal rule was used up to C_{max}, and the logarithmic trapezoidal rule was used after C_{max}. The logarithmic trapezoidal rule was used, as the serum concentration after C_{max} decreased exponentially. In order to calculate the absolute bioavailability (F) of ^{3}H-seocalcitol, the AUC obtained following an intravenous (IV) administration of ^{3}H-seocalcitol determined in a previous study, was used (15). The use of IV data from another study is not ideal due to cross-experimental conditions. However, in order to obtain an approximate bioavailability and limit the number of animals used in the present study, IV data from another study were used in the bioavailability calculations. The intravenously dosed animals were not cannulated in the mesenteric lymph duct, as results with the highly lipophilic drug substance halofantrine have shown that a negligible amount of drug substance was transferred from the blood to the lymph (16).

The percentage of the dosed amount of ^{3}H-seocalcitol recovered in the lymph, was calculated using the concentration of ^{3}H-seocalcitol found in each lymph sample, multiplied by the volume of lymph produced per hour. The value was expressed as a cumulative percentage of the dose.

Using StatGraphics, statistical analysis were performed by a two-sample t-test. The data were logarithmically transformed in order to normalise variations. The Tukey method was used to adjust for multiplicity in the pairwise comparison. The results were considered significant when $p < 0.05$.

VolSurf

A method for modelling and prediction of pharmacokinetic properties based on computed molecular interaction fields and multivariate statistics has been investigated (17). The program VolSurf has been used to correlate 3D molecular structures with physico-chemical and pharmacokinetic properties (17). VolSurf calculates quantitative numerical descriptors extracted from the information present in the 3D molecular structures. Previously, a quantitative relationship between molecular structure and lymphatic transfer of lipophilic drug substances co-administered with LCT has been established using the VolSurf software (9). A number of descriptors were found to contribute to the prediction leading to a complex model with a better correlation compared with the frequently used method relating log *P* values with lymphatic transfer. Nine VolSurf descriptors were used for the prediction: Cw5 and Cw6, which are the fraction of the hydrophilic surface area; HL1, the hydrophilic–lipophilic ratio of the molecule; *G*, the globularity, which is 1.00 for a perfect sphere; *W*5 and *W*6, the size of the hydrophilic surface; Emin3, a local interaction minima; IW8, the unbalance between the centre of mass and the centre of the hydrophilic regions of the molecule; ID3, the unbalance between the centre of the mass and the lipophilic regions of the molecule. Nineteen reference drug substances were used in the correlation; the relationship between experimentally reported values with regard to lymphatic transfer and the nine selected Volsurf descriptors were obtained using partial least squares (9). In the preparation of the model, the lymphatic transfer data were transformed into log scale prior to the data analysis. The nine VolSurf descriptors for seocalcitol, 1,25-hydroxyvitamin D_3, 25-dihydroxyvitamin D_3 and vitamin D_3 were calculated using the VolSurf software (version 4.1.3) (17,18).

RESULTS AND DISCUSSION

Seocalcitol is a poorly soluble drug substance with lipophilic character intended for oral administration. Seocalcitol is classified as a class II drug substance, indicating that the dissolution process in the GIT is likely to be the limiting step in drug absorption. Previous results have shown that the oral bioavailability of seocalcitol can be significantly enhanced upon administration in a TG vehicle (15). In the present study ^{3}H-seocalcitol was dosed in solution in order to reduce the impact of differences in dissolution rate on the extent of absorption. The advantage of applying a lipid vehicle in comparison to a simple water miscible system is that the formed lipid degradation products may increase the drug absorption via generation of colloid phases in the GIT leading to increased solubilisation and dispersion of the drug substance. The fate of ^{3}H-seocalcitol in the GIT is very much dependent on dispersion of the drug substance, as well as the solubilising capacity of the formed colloid phases. The presence of lipid degradation products in the GIT may minimize or eliminate precipitation of drug substance due to increased solubilising capacity in the mixed micelles compared with the solubilising capacity in the simple bile salt micelles being present upon administration of a water miscible system. Thus, the absorption from the LCT solution is likely to be different from the PG solution since the likelihood of precipitation is reduced and since LCT facilitates lymphatic transport which may increase the bioavailability due to reduced first pas metabolism.

In this study the impact of the formulation (LCT *versus* PG solution) on the lymphatic transport of ^{3}H-seocalcitol was studied. Co-administration with LCT provides information about whether or not LCT facilitates lymphatic transport of ^{3}H-seocalcitol. The level of lymphatic transport upon administration of the PG solution accounts for the lymphatic transport due to the endogenous production of chylomicrons. Furthermore, the absorption pathway(s) for ^{3}H-seocalcitol can be identified.

Portal Transport of ^{3}H-Seocalcitol

The concentration of ^{3}H-seocalcitol in serum as a function of time after oral administration of the two formulations is seen in Fig. 2. A statistically significant difference ($p < 0.05$) was found between the two formulations where the LCT solution proved to give the highest bioavailability of 16.2 ± 2.2% compared with 10.8 ± 0.8% for the PG solution (Table II). In non-lymph cannulated rats, a bioavailability of 22 ± 6% was found for the LCT solution and 10 ± 5% for the PG solution (15). In the concentration range 0.6 to 50 μg/kg, seocalcitol display linear kinetics in rats (Internal report, LEO Pharma A/S), which justifies the use of IV data from another study, as well as comparison of bioavailabilities between studies. As lymph containing ^{3}H-seocalcitol has been removed in the present study, the bioavailability of ^{3}H-seocalcitol is expected to be lower compared with the bioavailability found in non-lymph cannulated rats. The higher bioavailability seen for the LCT solution probably reflects a better solubilisation of ^{3}H-seocalcitol in the intestine and protection against precipitation of ^{3}H-seocalcitol. This is supported by data obtained in simulated intestinal media, where the solubility of seocalcitol increased significantly when adding lipolysis products to the media simulating the fasted state (5 mM bile salt and 1.25 mM phospholipids) (15). The solubility of seocalcitol in the pure bile salt media was 78 μg/ml compared with 127 μg/ml in the media containing 5 mM long chain fatty acids and 2.5 mM long chain monoglycerides (Table I). These data show the difference in the solubilising capacity between pure bile salt micelles and mixed micelles containing lipolysis products and explains the *in vivo* performance of the two different delivery systems. The much higher solubilising capacity in the mixed micelles results in a higher bioavailability of seocalcitol from the LCT solution. During dilution of the PG solution in the GIT in the gastro-intestinal fluid some of the drug substance may precipitate as the formulation does not contain any surface-active substances to aid keeping the drug in solution. This is also illustrated in the absorption data, where an absorption of 29% was seen from the PG solution compared with 45% from the LCT solution (15).

Transport of Triglycerides in Rat Intestinal Lymphatics

The cumulative lymphatic transport of TG after 9 h was 167 ± 29 mg upon administration of the LCT solution and 60 ± 31 mg upon administration of the PG solution (Fig. 3).

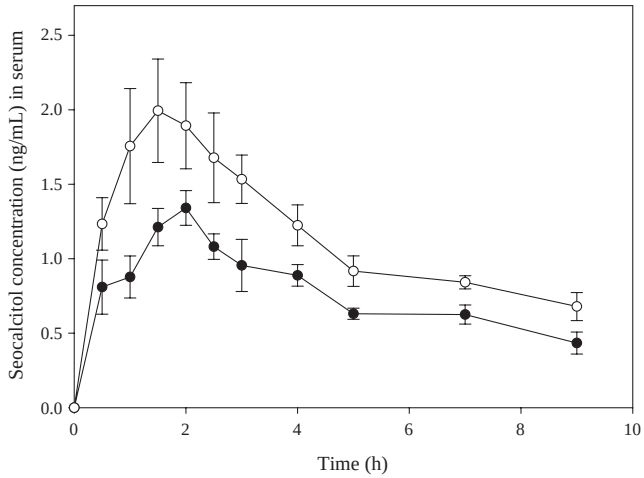

Fig. 2. Serum concentration–time profiles (mean values ± SE, $n = 5$) following oral administration of 40 μg/kg of ^{3}H-seocalcitol (equal to 34 MBq/kg) to male rats. Two formulations were tested: PG solution (*filled circle*) and LCT solution (*open circle*). $AUC_{0\rightarrow 9}$ for the reference formulation is significantly different from the LCT solution ($p < 0.05$).

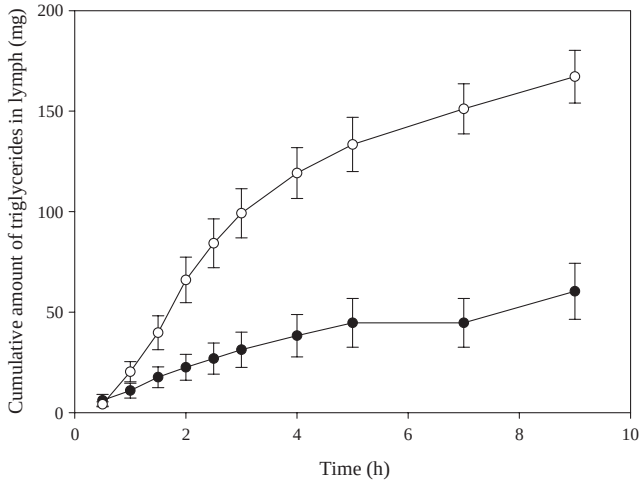

Fig. 3. The cumulative amount of triglycerides in lymph (mean values ± SE, $n = 5$) as a function of time. Two formulations were tested: PG solution (*filled circle*) and LCT solution (*open circle*). The PG solution represents the endogenous level of triglycerides. The rats receiving the lipid formulation were dosed 0.66 g TG/kg. After 9 h, the cumulative amount of triglycerides in lymph is statistically significantly different between the two formulations ($p < 0.05$).

The PG solution did not contain TG, thus TG found in the lymph originates from endogenous TG. Subtracting the endogenous production of TG from the amount of TG found in the mesenteric lymph after administration of the LCT solution, a recovery of 58 ± 14% of the dosed amount of TG is found (Table III). This is in agreement with previous findings where a recovery of 67–121% of the administered TG was found in the lymph of rats (19). Hussain (5) has proposed that secretion, rather than synthesis of TG, is the rate limiting step in chylomicron output from the enterocyte, which may explain why not all the dosed TG appears in the lymph within the duration of the study. As expected, the administration of LCT facilitated a statistically significantly ($p < 0.05$) increased amount of TG in the lymph, compared with the PG solution (Table III).

In the present study it was decided to allow free access to food and water in order to allow optimal recovery conditions after surgery. The rats were offered a standard rat feed containing only 4% fat. The endogenous level of TG in the lymph in the fasted state has been determined in several studies to be within the range of 1.1–9.3 mg/h (20–27). The endogenous level found in the present study is 6.7 mg/h, which is within this range. The TG output in the lymph upon administration of the LCT solution was 18.5 mg/h. This illustrate that the presence of food has not affected and overshadowed the effect of the LCT solution as the concentration of TG in the lymph is three times higher after administration of the LCT solution compared to the PG solution.

Table II. Portal Absorption, F (Mean Values ± SD, $n = 5$) of ^{3}H-Seocalcitol after Administration in a LCT Solution and a PG Solution

	Portal Blood F (%)[a]	Cumulative Percentage of Dose Appearing in the Mesenteric Lymph (%)
LCT solution*	16.2 ± 2.2	1.3 ± 0.6
PG solution	10.8 ± 0.8	0.5 ± 0.4

Forty micrograms of ^{3}H-seocalcitol per kilogram was dosed equal to 34 MBq/kg.
*Statistically significantly different compared with the PG solution ($p < 0.05$).
[a]After administration of the LCT and the PG solution the $AUC_{0\text{-}9}$ was 10.6 ± 1.9 and 7.0 ± 0.7 h ng ml^{-1}, respectively. The absolute bioavailability was calculated using $AUC_{0\text{-}9}$: 13 ± 2 h ng/ml for an intravenous solution with correction of the actual dose: 8 μg ^{3}H-seocalcitol/kg (equal to 7 MBq/kg) (15).

Transport of ^{3}H-Seocalcitol in Rat Intestinal Lymphatics

The lymphatic transport of ^{3}H-seocalcitol in lymph cannulated rats, plotted as the cumulative percentage of the administered dose as a function of time, is shown in Fig. 4. The LCT formulation induced a statistically significantly ($p < 0.05$) higher intestinal lymphatic transport of ^{3}H-seocalcitol (1.3 ± 0.6%) compared with the PG solution (0.5 ± 0.4%). As seen in Fig. 4 the majority of the lymphatic transport of ^{3}H-seocalcitol takes place within the first 3 h after dosing.

A total of approximately 17.5% (16.2% via the portal blood and 1.3% via the lymph) of the dosed ^{3}H-seocalcitol was found to be available after LCT dosing. Calculating the percentage of lymphatically transported ^{3}H-seocalcitol based on the total percentage of available ^{3}H-seocalcitol, 7.4% of the absorbed dose is transported via the lymph for the LCT solution and 4.4% for the PG solution.

Similar amount (ng) of ^{3}H-seocalcitol transported in the lymph per amount (mg) of TG were found for the two formulations; 0.9 ± 0.2 ng/mg was found for the LCT solution and 0.8 ± 0.3 ng/mg was found for the PG solution (Table III). This suggests that a limit in terms of the quantity of drug substance distributing into the chylomicrons has been reached. As the level of TG in the lymph is three times higher after administration of the LCT solution, there seems to be a relation between the amount of ^{3}H-seocalcitol and the

Table III. Amount of Triglycerides (TG) Present in the Lymph After Oral Administration of the LCT and the PG Solution (Mean Values ± SD, $n = 5$)

	Cumulative Amount of TG After 9 h (mg)[a]	Cumulative Percentage of Dosed TG[b]	TG (mg) per Lymph (g)[c]	^{3}H-Seocalcitol (ng)/ Triglyceride (mg)[d]
LCT solution	167 ± 29*	58 ± 14	21 ± 7*	0.9 ± 0.2
PG solution	60 ± 31	–	9 ± 2	0.8 ± 0.3

Six hundred sixty milligrams of TG per kilogram was dosed to the rats.
*Statistically significantly different compared with the PG solution ($p < 05$).
[a]The cumulative amount of TG represents the total amount of TG (including endogenous TG). The cumulative amount seen for the PG solution represents the endogenous level of TG.
[b]Cumulative percentage of the dosed TG in the lymph.
[c]Amount (mg) of TG per amount of lymph (g) collected. The amount of lymph collected during the experiment of 9 h was 8.6 ± 2.7 g and 6.5 ± 2.9 g, after administration of the LCT solution and the PG solution, respectively. The total amount of TG is used for the calculation.
[d]The ratio between the amount (ng) of ^{3}H-seocalcitol per amount (mg) of triglyceride.

available amount of TG transported in the lymph. This verifies that lymphatic transport of drug substances is very closely related to the presence and amount of LCT.

The solubility of seocalcitol in LCT (sesame oil) is 1.7 μg/mg, which is higher than the solubility of ^{3}H-seocalcitol found in the triglycerides in the lymph. As the chylomicrons consist of more components than pure triglycerides the concentration of seocalcitol is expected to be lower. However, the understanding of incorporation of drug substances into chylomicrons is still in its infancy and the relation between solubility in pure TG compared with the solubility in TG of the chylomicrons is still not known. However, drug loading into chylomicrons is likely to be a distribution process determined by the physiochemical properties of the drug substance and the availability of TG.

Thus, increasing the dosed amount of LCT might increase the amount of seocalcitol transported in the lymph. However, as not all the dosed amount of TG appeared in the lymph during this study, it is questionable whether or not this approach will lead to increased lymphatic transport of seocalcitol.

In the literature the degree of lymphatic transport is generally expressed as a percentage of the dose administered. However, if drug targeting to the lymphatic system for increased therapeutic efficacy is the aim, the concentration of drug substance in lymph may be a relevant parameter (4). In the present study the maximal concentration of ^{3}H-seocalcitol in the mesenteric lymph was approximately 120 ng/ml with t_{max} after 1 1/2–2 h, whereas the maximal concentration of ^{3}H-seocalcitol in serum was 2 ng/ml with t_{max} after 1 1/2–2 h as well. These findings are in accordance with findings of Khoo *et al.* (28) who saw a much higher concentration of halofantrine in the lymph than in the systemic circulation. However, the therapeutic relevance of this phenomenon still remains to be clarified.

In Vivo Results *versus* Theoretically Predictive Methods

Seocalcitol has a log *P* of 4.8 and a LCT solubility of 1.7 mg/g and therefore fulfils one of the stated rule of thumb criteria suggested by Charman and Stella (6) to describe the physiochemical properties of highly lymphatically transported drug substances. Log *P* of seocalcitol fulfils the criteria of lymphatic transport, but as log *P* describes the

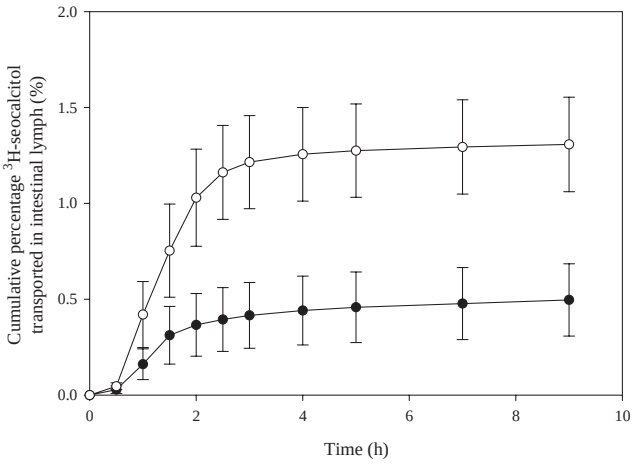

Fig. 4. Cumulative percentage of the dosed ^{3}H-seocalcitol (mean values ± SE, $n = 5$) determined in the mesenteric lymph as a function of time after oral administration of 40 μg/kg of ^{3}H-seocalcitol (equal to 34 MBq/kg) to male rats. Two formulations were tested: reference solution (*PG*; *filled circle*) and LCT solution (*open circle*). The formulations are statistically significantly different ($p < 0.05$).

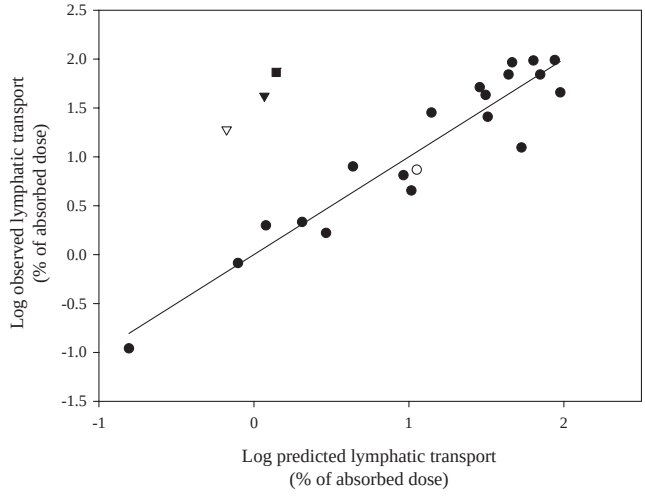

Fig. 5. Log predicted lymphatic transport *versus* log determined lymphatic transport. *Filled circle*: the correlation is based on *in vivo* data from 19 drug substances correlated with predicted values calculated by VolSurf (9); seocalcitol (*open circle*); 1,25-hydroxyvitamin D_3 (*inverted open triangle*); 25-dihydroxyvitamin D_3 (*inverted filled triangle*); and vitamin D_3 (*filled square*).

relative distribution between a hydrophobic and a hydrophilic phase, and not an absolute quantity, the lipid solubility seems to be a more critical parameter. However, ontazolast (log P = 4.0, S_{LCT} = 55 mg/ml) having high triglyceride solubility but log P value below 5 showed low lymphatic transport (<1.2% of the administered dose) even though a relatively high triglyceride solubility is seen.

Figure 5 shows the correlation between log predicted lymphatic transport (determined by VolSurf) *versus* log observed lymphatic transport (percent of absorbed dose) for 19 drug substances made by Holm and Hoest (9). When predicting lymphatic transport of seocalcitol based on VolSurf descriptors as demonstrated by Holm and Hoest, approximately 11% of the absorbed dose should be transported via the lymphatic system. In the present *in vivo* rat study, the actual lymphatic transport proved to be approximately 7%. It is therefore concluded that VolSurf is able to give a good indication of the lymphatic transport of seocalcitol.

Using VolSurf descriptors to predict lymphatic transport for vitamin D_3, 1.4% of the absorbed dose would be expected to be transported in the lymph. This is not in compliance with *in vivo* data since 73% of the absorbed dose of vitamin D_3 was recovered in the lymph after administration in LCT (11). The solubility of vitamin D_3 in LCT is 168 mg/g and log P has been determined to be 9.1 (29), which fulfil the criteria of lymphatic transport suggested by Charman and Stella (6). For the structurally related compounds; 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3, predicted values of 1.2 and 0.7% were found using VolSurf software. Related to *in vivo* data 42 and 19% of the absorbed dose were found in the lymph (13). However, these two drug substances were administered in intralipid and not dissolved in LCT which was required for the Volsurf correlation between *in vitro* and *in vivo*. For the structurally related compounds (1,25-hydroxyvitamin D_3, 25-dihydroxyvitamin D_3 and vitamin D_3) the prediction made by VolSurf does not correlate with *in vivo* observations, as a much larger amount is transported via the lymph than predicted.

The above examples illustrate that today no single in silico model can predict lymphatic transport for a given drug substance. However, using the different models available, a more differentiated picture of the potential lymphatic transport for a given compound can be achieved. The difficulties in establishing a valid theoretical model is partly due to the complex dynamic situation taking place *in vivo* and also due to the differences in studies of lymphatic transport with regard to: e.g., (1) amount of dosed lipid; (2) drug delivery systems used; (3) animals model; (4) duration of the study; and (5) Variation between laboratories. In order to obtain a predicative model of lymphatic transport a more systematic approach should be used, controlling the abovementioned factors in order to obtain satisfying results.

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