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Blood and lymph transport of DDT after oral and parenteral administration to anaesthetised rats

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Summary

The distribution of DDT between blood and lymph following intraduodenal (i.d.) and parenteral administration of a soybean o/w emulsion was investigated in anaesthetised rats. In addition, the effect of 'pre-loading' the animal with chylomicrons on this distribution was examined. Following i.d. administration of DDT in a soybean emulsion 19.37 ± 4.13% S.E. of the dose was transported in the lymph over 11 h. Blood levels from these lymph collected animals were low but very variable; possible explanations for this unexpected behaviour are discussed. When blood alone was sampled, following i.d. administration, the higher blood levels obtained indicate redistribution of DDT from lymph to blood. Following i.v. administration similar blood profiles were obtained with and without lymphatic cannulation. Low levels of DDT were detected in the lymph indicating poor, but not insignificant, distribution from blood to lymph. Preloading the animal with chylomicrons caused a slight, but not statistically significant, increase in the cumulative percentage dose transported in the lymph.

Introduction

Following oral administration, the lipophilic compound DDT (2,2-bis (*p*-chlorophenyl)-1,1,1,-trichloroethane; *p,p*-DDT) is transported, to a large extent, via the mesenteric lymphatic system (Charman and Stella, 1986a). The degree of lymphatic transport of DDT after oral dosing is enhanced by the co-administration of oily or lipid vehicles (Palin, 1982; Charman and Stella, 1986a). The lipid vehicles stimulate the production of chylomicrons, and the DDT is transported solubilized by, or associated with chylomicrons and other lipoproteins such as VDL (Sieber, 1976; Vost and Maclean, 1984; Charman and Stella, 1986a).

The advantages of increasing lymphatic transport of xenobiotics include: a reduction in the first-pass metabolism, direct delivery of cytotoxic and immunogenic drugs into the lymphatic system and the possibility of controlling the rate of entry of lymphatically transported drugs into the general circulation (Charman and Stella, 1986a).

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In order to optimise lymphatic targeting, consideration must be given (a) to the design or formulation of the lipid vehicle, and (b) to the factors affecting kinetics of drug distribution between lymph and blood. Previous studies which looked at DDT transport after oral or parenteral administration have focused on either systemic relative bioavailability (Palin, 1982) or mesenteric and thoracic lymphatic transport (Noguchi et al., 1985; Charman and Stella, 1986a; Charman et al., 1986a,b). In the present study, efforts were made to estimate both systemic absolute bioavailability and lymphatic transport (mass balance) of DDT after oral, intraduodenal (i.d.), and parenteral administration of a soybean o/w emulsion to anaesthetised rats. This also enabled the effect of emulsification of the lipid vehicle on lymphatic transport following i.d. administration to be determined. In addition, the distribution of DDT between blood and lymph and the effect of preloading the animal with chylomicrons on this distribution were investigated.

Materials and Methods

Chemicals

The sources of chemicals used in this study were as follows: DDT, purity 99 + % and glycerol 99.5% (Aldrich Chemicals, Milwaukee, WI), soybean oil and L-α-phosphatidylcholine (Sigma Chemicals, St. Louis, MO). The water used in all experiments was deionized and filtered through charcoal prior to distillation from an all-glass still (Corning Mega-Pure System MP-1, Corning, NY). All other chemicals were of analytical grade.

General surgical procedures

Male Sprague-Dawley rats (280–320 g) (purchased from SASCO, Omaha, NE) were fasted overnight. Continuous anaesthesia was maintained by 50 mg/kg intraperitoneal injections at 2 h intervals of pentobarbital sodium. Tracheal cannulations, using a 4 cm piece of polyethylene tubing (1.67 mm I.D., 2.42 mm O.D., Clay-Adams, Parsippany, NJ), were performed that facilitated the anaesthetized animals' breathing throughout the experiment. The right and left jugular veins were exposed. The duodenum was cannulated by insertion of polyethylene tubing (0.5 mm I.D., 0.8 mm O.D., Dural Plastics, Auburn, NSW, Australia) into the duodenum 2 cm below the pylorus and secured with instant cyanoacrylate adhesive (Loctite, Cleveland, OH). The cannula was externalized through the abdominal wall.

When required, the mesenteric lymph duct was cannulated according to a previously described method (Noguchi et al., 1985). Following completion of all surgical procedures, the abdomen was closed with continuous silk sutures.

Drug administration

A recovery period of at least 1 h was included in all experiments to help the animals recover from the surgical trauma and to aid the return to normal intestinal motility. To maintain body hydration and intestinal lymph flow during this period, the duodenum was continuously perfused with normal saline 1–1.5 ml/h via a constant infusion syringe pump (Sage Instruments, model 341A, Cambridge, MA). After the recovery period, three different protocols were followed:

(1) The drug was administered by intraduodenal infusion over 2 h by a Sage infusion syringe pump through the duodenal cannula. The intraduodenal infusion of normal saline was stopped during drug administration and resumed immediately afterwards.
(2) The drug was administered by i.v. bolus injection into the jugular vein.
(3) Soybean emulsion (2 g) without drug was administered intraduodenally over 2 h by a Sage infusion syringe pump through the duodenal cannula; saline infusion was stopped during this period and resumed immediately afterwards. Following administration of the blank emulsion, the drug was administered by i.v. bolus injection into the jugular vein.

Sample collection

Following i.v. administration, blood samples (approx. 200 μl) were collected at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 6, 8, 10, and 11 h directly from the jugular vein by venous puncture. When required,

all mesenteric lymph was collected at 1 h intervals for the duration of the experiment in 2 ml tubes containing 0.3 mg EDTA and 0.2 ml of normal saline.

Following intraduodenal administration, blood samples were collected, as above, at 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8, 10 and 11 h; this included the 2 h dosing phase. When required, lymph was collected at 1 h intervals, as described above, for a total of 10–11 h, 2 h during the dosing phase and 8–9 h post dosing.

Preparation of the dosage form

The drug was administered as an o/w emulsion containing 10% w/w soybean oil, 1.2% w/w phosphatidylcholine, 4.5% w/w glycerol and water to 100%. The emulsion was prepared by dissolving the drug in the oil, mixing this solution with a suitable volume of the lecithin/glycerol/water and emulsifying the system by sonicating for three 3-min intervals (Heat Systems Ultrasonics, Model W-385); 2 g of the emulsion was administered in each experiment. The particle size of the emulsion was assessed using a submicron particle size analyser (Nicomp Model 370). In each case, the mean particle diameter was within 0.2–0.4 μm.

Sample analysis

An HPLC procedure, similar to that previously described (Charman et al., 1986a), was used to analyse DDT in blood and lymph. The DDT and internal standard, penclomedine, were co-extracted using redistilled ether. After centrifugation, the aqueous phase was frozen in dry ice/acetone, the supernatant was decanted into a centrifuge tube, and the ether evaporated under nitrogen. The samples were then reconstituted with acetonitrile, vortexed and subjected to HPLC analysis: column, 15 cm C-8 column (plus pre-column); mobile phase, acetonitrile 75% in water UV detector ($\lambda = 254$ nm) flow rate, 2 ml/min.

Statistical analysis

Statistical analysis was carried out by a Student's *t*-test.

Results and Discussion

Following intraduodenal administration of DDT (10 mg) in a soybean oil emulsion, both lymph and blood samples were taken. Fig. 1 shows that 19.37 ± 4.13% (S.E.) of the dose of DDT was transported in the lymph over 11 h. Charman et al. (1986b), using a similar procedure, reported that 35.7 ± 2.1% and 19.9 ± 1.8% of the dose of DDT was transported in the lymph over 12 h, following administration of the compound in oleic acid and peanut oil, respectively. Prior to digestion and absorption, ingested fats undergo emulsification in the gastrointestinal tract facilitated by factors such as bile salts and fatty acids (Shiau, 1987). The formation of such an emulsion increases the surface area of the oil-water interface at which pancreatic lipase hydrolyses the triglyceride (Alvarez and Stella, 1989). There is considerable evidence in the literature to show that lipophilic drug absorption is influenced by both food and the co-administration of lipid vehicles (Muranishi, 1985; Palin, 1985). Lipid foodstuffs are themselves poorly soluble, but are very well absorbed. It seems likely, therefore, that the pro-

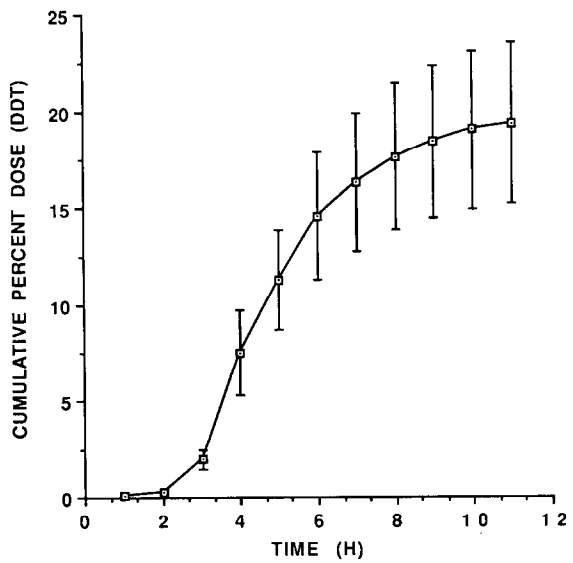

Fig. 1. Cumulative percent dose of DDT (mean ± S.E., $n = 7$) collected in mesenteric intestinal lymph vs time, following intraduodenal administration of 10 mg DDT in an o/w soybean oil emulsion to lymph and blood sampled animals.

cesses involved in the absorption of fats may also play a role in the absorption of lipophilic drugs. The results of the current study suggest that pre-emulsification of the triglyceride soybean oil, in a lecithin stabilised emulsion, did not significantly enhance lymphatic transport of DDT relative to the results of Charman et al. (1986b), where the triglyceride was not fully emulsified but was dispersed. Similar lag times of 2 h were seen for both the emulsion and the dispersed systems. This implies that emulsification may not be the major rate-limiting step in overall lymphatic transport, providing the oil is at least dispersed. This is an area which requires further exploration. The lymphatic transport rate is shown in Fig. 2. The maximum rate of appearance in the lymph occurs 3–4 h post initiation of DDT dosing. Post peak, the appearance rate in the lymph seems to follow exponential behaviour with a $t_{1/2}$ of 1.9–2 h.

Blood levels of DDT from the same experiment (lymph collected animals) were very low; an average $AUC_{0\rightarrow 11\,h}$ of 18.2 ± 8.8 (S.E., $n = 7$) was obtained. Table 1 summarises the data. It can be seen that blood levels are highly variable, since DDT was detected in only three out of seven rats tested. Possible explanations for this unexpected behaviour will be discussed later.

In a second experiment, following intraduodenal administration of the same formulation and dose of DDT, blood samples alone were taken, and the results are shown in Fig. 3. The higher mean values obtained, when blood alone was sampled, are due to redistribution of absorbed drug from the lymph into the blood, an average $AUC_{0\rightarrow 11\,h}$ of 28.1 ± 5.8 (S.E., $n = 5$) was obtained (Table 2). Although this redistribution reached a maximum at 6 h, it is still incomplete at 12 h, thus indicating that absorption and transport are slow and prolonged processes. Blood samples beyond 12 h would be required for a complete profile.

Following i.v. administration of DDT (5 mg) in a soybean emulsion, blood samples alone were taken and the blood profile is shown in Fig. 4; an $AUC_{0\rightarrow 11\,h}$ of 66.4 ± 2.9 (S.E., $n = 5$, trapezoidal method) was obtained. In a separate experiment following i.v. administration of the same dose and formulation, both blood and lymph samples were collected and an $AUC_{0\rightarrow 11\,h}$ of 73.4 ± 17.3 (S.E., $n = 7$, trapezoidal method) was calculated. Therefore, similar blood levels were achieved in both

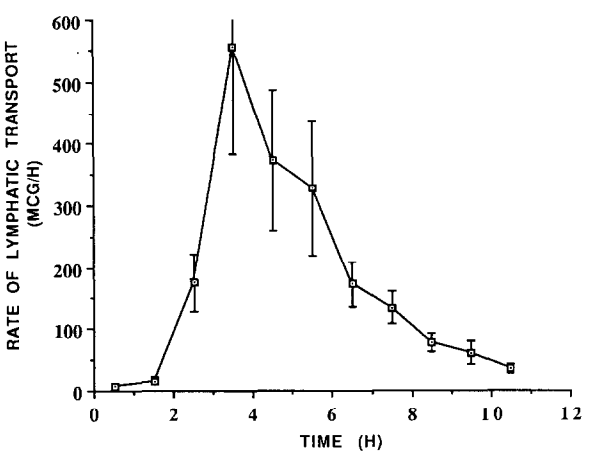

Fig. 2. Rate of mesenteric lymphatic transport of DDT (mean ±S.E., $n = 7$) following intraduodenal administration of 10 mg DDT in an o/w soybean oil emulsion to lymph and blood sampled animals.

TABLE 1

Cumulative percentage of the dose transported in the lymph; area under the plasma concentration vs time curve (trapezoidal method) and bioavailability of DDT following intraduodenal administration of DDT:soybean emulsion in blood and lymph sampled animals

Rat	Cumulative % dose in lymph	$AUC_{0\rightarrow 11\,h}$ (μ h ml^{-1})	% Bioavailability [a]
Group A			
1	6.14	0	0
2	27.36	0	0
3	25.67	0	0
4	34.44	0	0
Mean ± S.E.	23.4 ± 6.1	0	0
Group B			
5	22.79	33.26	22.38
6	15.22	46.69	31.42
7	5.16	47.67	32.08
Mean ± S.E.	14.39 ± 5.1	42.54 ± 4.6	28.63 ± 3.1
Overall Mean ± S.E.	19.37 ± 4.1	18.23 ± 8.8	12.27 ± 5.9

[a] Bioavailability calculated as $(AUC_{oral}/D_{oral})/(AUC_{i.v.}/D_{i.v.})$.

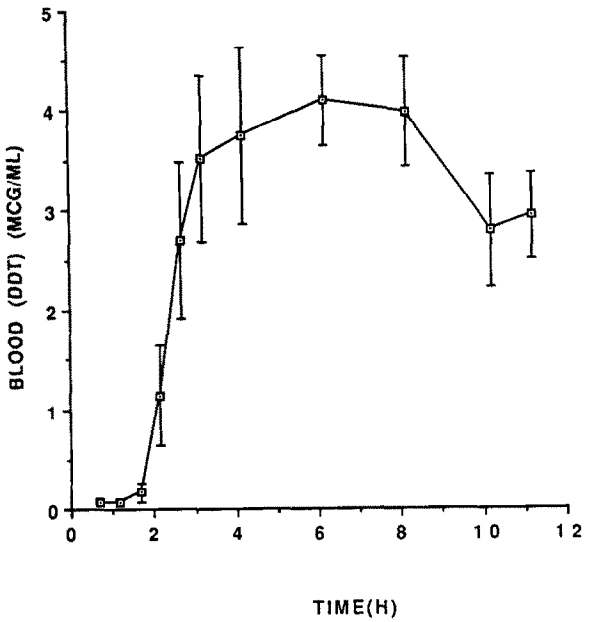

Fig. 3. Whole blood concentration (mean ± S.E., $n = 5$) vs time profile following intraduodenal administration of 10 mg DDT in an o/w soybean oil emulsion; sampling blood only.

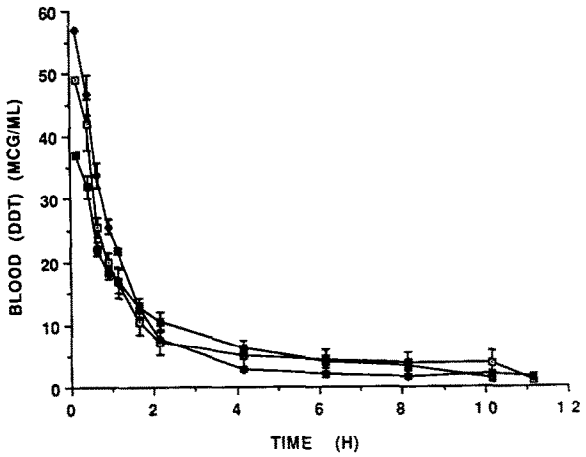

Fig. 4. Whole blood concentration (mean ± S.E.) vs time profiles following intravenous administration of 5 mg DDT in an o/w soybean oil emulsion; (◆) sampling blood only ($n = 5$); (□) sampling blood and lymph ($n = 7$); and (■) sampling blood and lymph in animals predosed with drug-free emulsion to stimulate chylomicron production ($n = 3$).

experiments, however, the AUC values were not significantly different at the 95% confidence level.

The mean i.v. blood concentration vs time profiles (with and without lymph cannulation, Fig. 4) were analysed using a curve stripping program (JANA) (Dunne, 1986). The best fit in each was obtained using two exponentials. With the mean i.v. blood concentration vs time profile, without lymph cannulation, an $AUC_{0\rightarrow\infty h}$ of 67.7 (μg h ml^{-1}) was obtained, the clearance, calculated as dose/AUC, was 65.1 ml/h and the apparent biological $t_{1/2}$ was 7.3 h. In the experiments where both blood and lymph were sampled, an $AUC_{0\rightarrow\infty h}$ of 88.8 (μg h ml^{-1}) was determined, using the mean data, the clearance was 56.3 ml/h and the apparent biological $t_{1/2}$ was 5.2 h.

TABLE 2

Areas under the plasma concentration vs time curves (trapezoidal method) following administration of DDT: soybean (SB) emulsion by various routes (intraduodenally (i.d.) or intravenously (i.v.))

Experiment (dose)	Fluid sampled (n)	AUC (μg h ml^{-1}) (± S.E.)
i.d. (10 mg)	Blood only (5)	$AUC_{0\rightarrow 11h}$ 28.1 ± 5.8
	Blood and lymph (7)	$AUC_{0\rightarrow 11h}$ 18.2 ± 8.8
i.v. (5 mg)	Blood only (5)	$AUC_{0\rightarrow 11h}$ 66.4 ± 2.9
	Blood and lymph (7)	$AUC_{0\rightarrow 11h}$ 74.3 ± 17.3
i.v. (5 mg) [a]	Blood and lymph (3)	$AUC_{0\rightarrow 8h}$ 64.1 ± 9.6

[a] Animals predosed with drug-free emulsion to stimulate chylomicron production.

The amount of drug detected in the lymph, following i.v. administration was low (Fig. 5) with approx. 1% (0–11 h) of the dose cumulatively appearing in intestinal mesenteric lymph over 10 h. This result is in agreement with the 0.6% (24 h) reported by Noguchi et al. (1985), and indicates poor but not insignificant distribution of the DDT out of the blood into the lymph following i.v. administration.

The role of chylomicrons in the lymphatic transport of lipophilic compounds has been well established (Charman and Stella, 1986a; Fukui et al., 1989). Pocock and Vost (1974) have suggested that all DDT transported in the lymph is contained within the triglyceride core of the chylomicrons. The extent of lymphatic transport is influenced by the quantity of chylomicrons in the

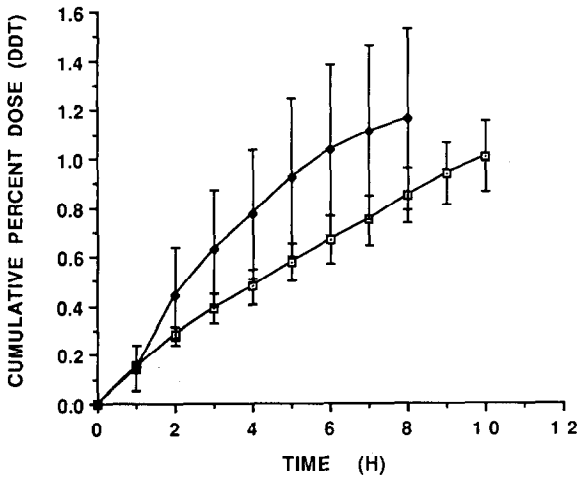

Fig. 5. Cumulative percent dose of DDT (mean ± S.E.) collected in mesenteric intestinal lymph vs time following intravenous administration of 5 mg DDT in an o/w soybean oil emulsion to lymph and blood sampled animals; (♦) with ($n = 3$); and (⊡) without ($n = 7$) predosing with drug-free emulsion to stimulate chylomicron production.

lymph, and, secondly, by the amount of drug per chylomicron (Charman and Stella, 1986b). In order to see if 'pre-loading' the animal with chylomicrons would act as a sink, thereby promoting blood to lymph transfer of DDT following i.v. administration, rats were dosed intraduodenally with soybean emulsion (drug free) for 2 h to stimulate chylomicron production, then DDT: soybean emulsion was administered intravenously, and blood and lymph samples were collected. The blood level profile ($AUC_{0 \rightarrow 8h}$ of 64.1 ± 9.6, S.E., $n = 3$) obtained (Fig. 4) was not significantly different (at the 95% confidence level) from those obtained with previous i.v. experiments (Fig. 4). The cumulative percentage of the dose transported in the lymph after 8 h (Fig. 5) is approx. 1.2% compared to 0.85% (0–8 h) previously obtained without intestinal lymph chylomicron loading. Although the increased lymphatic transport observed was not significantly different (at the 95% confidence level) the results imply that the transport is a dynamic process and that the distribution of lipophilic compounds such as DDT may be altered in the presence of elevated levels of lipoproteins including chylomicrons. This is a little more obvious when the rate of DDT lymphatically transported is compared (Fig. 6), with and without chylomicron loading. At the maximum rate there is a 2-fold increase with chylomicron loading. These results are in agreement with the findings of Lamka and Kvetina (1990) who reported an increased lymphatic transport for diazepam following i.v. administration to animals fed on a high lipid diet compared to fasted animals.

From the oral and i.v. blood concentration time data, and correcting for dose, a bioavailability of 21.14% was calculated by comparing the truncated $AUC_{0 \rightarrow 11h}$ oral with the corresponding i.v. data. A comparison of this value to the 19.4% transported via the lymph following oral administration indicates that the greater proportion of drug absorbed from the gut is transported in the lymph. This is not totally consistent with the mean blood level time data for lymphatically cannulated animals, described earlier in Table 1. If only that fraction of the dose transported in the lymph reached systemic circulation, then the bioavailability of DDT in the lymph cannulated animals should be negligible. Referring to Table 1, it can be seen that the animals appear to be classifiable into two groups. Group A (0% bioavailability) appear to follow the expected behaviour; group B animals show high bioavailabil-

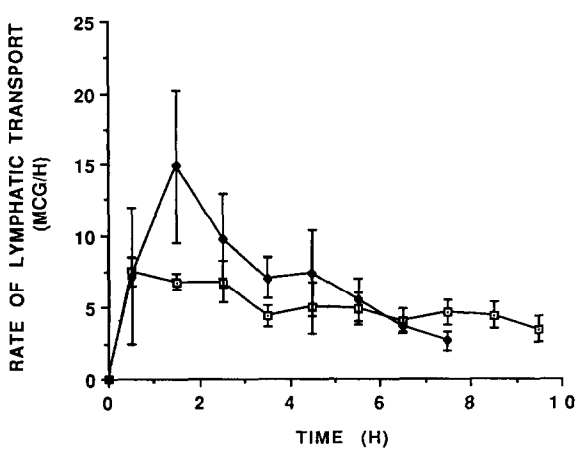

Fig. 6. Rate of lymphatic transport of DDT (mean ± S.E.) following intravenous administration of 5 mg DDT in an o/w soybean oil emulsion to lymph and blood sampled animals; (♦) with ($n = 3$); and (⊡) without ($n = 7$) predosing with drug-free emulsion to stimulate chylomicron production.

ity which compromised lymph transport (group B, 14.4 ± 5.1% S.E., vs group A, 23.4 ± 6.1% S.E.). This unexpected behaviour may be explained by assuming that mesenteric lymph cannulation was incomplete; this may be due to the presence of lymphatic shunt pathways in certain animals. It is also feasible that in these animals the overall bioavailability of DDT was elevated and that considerable absorption occurred from the lower intestinal tract or colon where chylomicron production is diminished or non-existent.

In conclusion, the results of the current study are not inconsistent with previously published data (Palin et al., 1982) indicating that lipophilic compounds such as d-α-tocopherol and DDT (Palin et al., 1982; Fukui et al., 1989) absorbed are transported via the mesenteric lymph. However, some portal transport may have occurred in certain animals (Table 1). These results suggest that differences in lymphatic transport caused by various vehicles may be via a combination of lymph transport effects and bioavailability considerations. Therefore, it is important that discussions of vehicle effects on lymphatic transport adequately address whether the results are specific to lymphatic transport or are partially due to alterations in systemic bioavailability.

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