International Journal of Pharmaceutics, 33 (1986) 155–164
Elsevier

IJP 01114

An experimental system designed to study the in situ intestinal lymphatic transport of lipophilic drugs in anesthetized rats

W.N.A. Charman *, T. Noguchi and V.J. Stella

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 21 October 1985)
(Modified version received 23 May 1986)
(Accepted 27 May 1986)

Key words: Intestine — Lymphatic transport — Methodology — DDT — Chylomicron — Effect of fasting

Summary

An experimental system has been developed that enables an estimate of the lymphatic transport of DDT (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane; *p,p*-DDT) by the small intestine. The system has been designed after consideration and evaluation of the effect of fasting, surgical methodology and the site of administration of drug and lipid vehicle on the lymphatic transport of DDT in the anesthetized rat. The fasting state of the animal, and the methodology used in the estimation of transport has a large effect on the lymphatic appearance of intraduodenally administered DDT. Additionally a simple spectrophotometric method has been developed to estimate lipid transport by the intestinal lymphatic system. The total lipid transported was used as an indication of the chylomicron flux in the intestinal lymph. The appearance of DDT in the intestinal lymphatics was quantitated after administration in 200 μl of either peanut oil or oleic acid. There were statistically significant differences in the amount of DDT transported into the lymph over the post-dosing period when the DDT was administered in the two vehicles. The kinetics of lymphatic DDT transport were also lipid vehicle dependent. On comparing DDT transport with chylomicron or lymph flow, chylomicron flux, and not lymph flow, was the major determinate of lymphatic DDT transport.

Introduction

Various methods have been developed for estimating the extent of intestinal lymphatic transport of lipophilic drugs and model compounds. The major variables in different literature methods are the site of cannulation of the lymphatic system, the fasting state of the animal and the gastrointestinal site of drug and lipid vehicle administration. The mesenteric intestinal lymphatic duct, rather than the traditionally utilized thoracic lymphatic duct, is the preferred site of cannulation to estimate the ability of the small intestine to lymphatically transport lipophilic molecules (Noguchi et al., 1985).

In the present study the effect of fasting and differing methodologies and techniques for assessing lymphatic drug transport were evaluated. In all transport studies, DDT was used as a lymphatically absorbed model compound because it is highly lipid-soluble, relatively metabolically stable and well absorbed by the intestinal lymphatic

* *Present address*: Sterling-Winthrop Research Institute, Rensselaer, NY 12144, U.S.A.
Correspondence: V.J. Stella, Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045, U.S.A.

system of the rat (Sieber, 1976; Pocock and Vost, 1974; Noguchi et al., 1985).

For a lipophilic drug to be preferentially absorbed by the intestinal lymphatic system, the drug is best administered with a lipid vehicle as the coadministered lipid vehicle will promote the formation of chylomicrons by the intestinal epithelial cells. Chylomicrons (a class of lipoproteins) are the triglyceride transport system of the small intestine. The relationship between DDT transport and either lymph flow or chylomicron flux was determined and the importance of chylomicron formation on intestinal lymphatic transport confirmed. The potential effect of formulating a drug for lymphatic transport in either a triglyceride (peanut oil) or fatty acid (oleic acid) lipid vehicle was observed and the differences rationalized.

Materials and Methods

Chemicals

The sources of chemicals used in this study were as follows: DDT, purity 99 + % (Aldrich Chemicals, Milwaukee, WI 53233, U.S.A.); testosterone undecanoate (Research Plus Steroid Laboratories, Denville, NJ 07834, U.S.A.); peanut oil (Sigma Chemicals, St. Louis, MO 63178, U.S.A.); oleic acid (NF, Fisher Scientific, Fair Lawn, NJ 07410, U.S.A.); Tween 80 (Aldrich Chemicals, Milwaukee, WI 53233, U.S.A.); Intralipid 10% (Cutter Laboratories, Berkeley, CA 94710, U.S.A.). Diethyl ether was used after glass distillation. All other chemicals were of analytical grade.

Animals

Male Sprague–Dawley rats purchased from Sasco (Omaha, NB 68102, U.S.A.), and were maintained on Purina lab chow and water ad libutum for at least 7 days prior to experimentation. Rats were housed, three per cage, on aspen bedding. Room temperature was maintained at 72°F with an air exchange rate of 10–12 changes per hour. The photoperiod was a 12 h light/dark cycle. Rats weighing 260–320 g were fasted for either 24 h prior to surgery, or for 24 or 48 h after surgery.

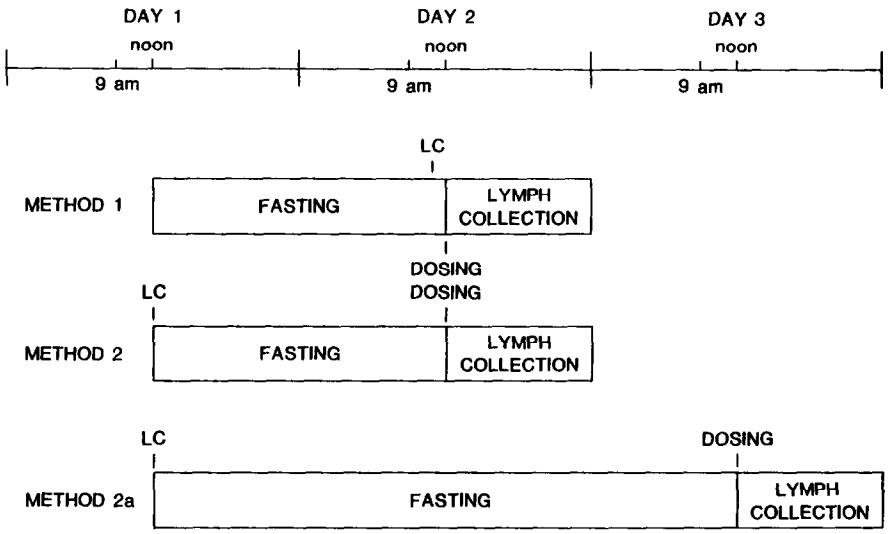
Surgical procedures

The mesenteric lymph duct of male Sprague–Dawley rats was cannulated according to a previously described method (Noguchi et al., 1985). Tracheal cannulations were performed that facilitated the anesthetized animal's breathing during the subsequent absorption studies. Continuous anesthesia, where required, was maintained by 2 hourly 50 mg/kg intraperitoneal injections of pentobarbital sodium (D-M Pharmaceuticals, Sellersville, PA 18960, U.S.A.). For the in situ lymphatic transport studies, a 5 cm J-shaped, heat-molded segment of polyethylene tubing (Intramedic PE 50, Clay Adams, Parisappany, NJ 07054, U.S.A.) was inserted into the duodenum 2 cm below the pylorus and secured with instant adhesive (Loctite, Cleveland, OH 44128, U.S.A.). The cannula was externalized through the abdominal wall. Following completion of all surgical procedures, the abdomen was closed with continuous 4.0 silk sutures.

Experimental procedures

Drug administration

Three separate protocols were followed in preparing the animals for drug administration, as depicted in Scheme 1. In method 1, the animals ($n = 4$) were maintained under constant anesthesia and underwent sequential tracheal, lymphatic and duodenal cannulation. They were immediately transferred to and secured on a heated pad (Clinical Scientific Equipment, Melrose Park, IL 60160, U.S.A.) maintained at 37°C. A continuous intraduodenal infusion of normal saline containing 0.2% Tween 80 at 1.44 ml/h via a constant infusion syringe pump (Sage Instruments, Cambridge, MA 02139, U.S.A.) was used to maintain body hydration and intestinal lymph flow. Three hours later, 2 mg of DDT dissolved in 200 μl of either peanut oil or oleic acid was infused, by a second infusion pump, over a 2 h period via a T-piece connector (Technicon Instrument Corporation, Tarrytown, NY 10591, U.S.A.) into the normal saline/Tween 80 solution flowing into the

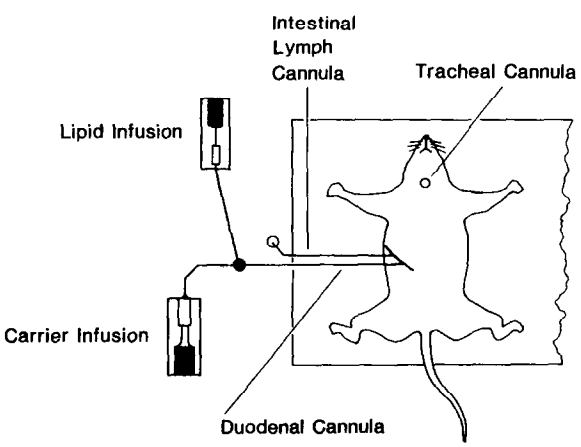


LC = LYMPHATIC CANNULATION

Scheme 1

duodenal cannula. This method of administration is represented in Scheme 2.

In methods 2 and 2a, the intestinal lymphatics were cannulated under pentobarbital anesthesia after which the animals ($n = 4$) were aroused to consciousness and blank lymph was continuously collected in collection jackets (Noguchi et al., 1985) for 24 h or 48 h after initial surgery. During this time the animals were fasted but had free access to drinking water. After this period the animals were reanesthetized, tracheal and duodenal cannulations implanted, and then transferred to the heated pad as described above. The same protocol, including the 3 h recovery period prior to drug administration (2 mg DDT per 200 μl peanut oil per 2 h infusion), was followed. It was assumed that flow of pancreatic juice and biliary secretion was normal in all animals.



Scheme 2

Lymph was collected hourly for a total of 11–12 h, 2 h during the dosing phase and 9–10 h post-dosing, into tared 10.25 mm × 50 mm collection tubes (Terumo Medical Corp., Elkton, MD 21921, U.S.A.) containing 3 mg EDTA dissolved in 200 μl of normal saline solution. The EDTA solution inhibited clot formation in the collected lymph. Following this 11–12 h experimental period, the animals were sacrificed by sodium pentobarbital overdose, the abdomen opened, and the integrity of all cannula verified.

Sample analysis

DDT analysis in lymph. The high performance liquid chromatographic procedure was identical to that previously described (Noguchi et al., 1985), although the extraction procedure was slightly different. A 100 μl aliquot of acetonitrile containing

2 μg of testosterone undecanoate, as internal standard, was added to 150 μl of lymph and diluted with 1 ml of saline. The DDT and internal standard were co-extracted with 5 ml of redistilled ether. After centrifuging, the aqueous phase was frozen in dry-ice-acetone, the supernatant was decanted into a centrifuge tube, and the ether evaporated under a gentle stream of nitrogen gas. The residue was then suspended in 0.2 ml of 5% (w/v) sodium chloride solution and the DDT and internal standard were extracted with 100 μl of cyclopentanone. An aliquot of the cyclopentanone phase was then subjected to HPLC analysis.

Lymph lipid quantitation. The optical density of each lymph sample was used as a relative index of chylomicron lipid content. The optical density of 150 μl of lymph diluted with 2 ml of saline was measured at 560 nm in a Cary 118 spectrophotometer (Varian Instruments, Palo Alto, CA 94303, U.S.A.). Optical density readings were found to be linearly related to lipid concentration in diluted lymph samples, between 0.0 and 1.6 absorbance units. Correlation of lymph turbidity with lipid content was confirmed by taking lymph samples of varying turbidity and extracting total lipids. Extraction was accomplished by following the methanol/chloroform method of Thompson et al. (1971). Extractable lipid was gravimetrically quantified by taking the lipid residues (after solvent evaporation) from the extraction procedure and drying over phosphorus pentoxide desiccant to constant weight. The extraction and quantitation procedure was verified by using Intralipid 10% as a standard lipid emulsion. Hourly chylomicron (CM) flux is reported, in units of optical density per hour (OD/h), which essentially represents an extrapolated absorbance value. These values were calculated by multiplying the collected lymph volume by the optical density of the diluted lymph sample by the appropriate dilution factor, i.e. CM transport (OD/h) = OD_{560} × lymph volume × dilution factor.

Statistical analysis

Statistical equivalence of group mean values was tested by analysis of variance. If differences were evident at the 5% level of significance, Tukey's pairwise comparison of means was performed.

Results and Discussion

Methodology development

An in situ experimental system was developed for estimating the ability of the small intestine to lymphatically transport lipophilic drugs in anesthetized rats. DDT was used as a model compound, and Scheme 2 describes the experimental system used in these studies. Rats were anesthetized for the duration of the lymphatic transport experiments which allowed hourly collection of lymph samples, and therefore delineation of the transport kinetics of the administered drug. Some workers often use conscious animals, maintained in restraining cages, for the collection of lymph and other biological fluids. Major drawbacks with the use of these restraining cages include the inability of the investigator to evaluate the effect of induced stress on the experimental animal, which is especially crucial when studying the gastrointestinal absorption profile of a particular drug, and to easily sample other body fluids and tissues. The current anesthetized animal system was developed, in part, to overcome some of these concerns. Anesthesia may well have an effect on the lymphatic transport of the model compound; however, it does not appear to be substantial in this particular system (Charman et al., 1986a). It would at least be expected that the same trends exhibited in data collected from the cannulated anesthetized animal, would apply to the conscious intact animal.

The drug, dissolved in an appropriate lipid, was infused with the saline solution directly into the duodenum of the anesthetized animal. Intraduodenal administration of drug and lipid was desired as it circumvented any potential effect that differing lipid vehicles may have on gastric emptying, and potentially on drug absorption, following the oral or intragastric administration of a particular dose of drug dissolved in different chemical classes of lipids (Roman and Gonalla, 1981). Intraduodenal infusion of drug and lipid is best performed in the anesthetized rat as it is not a practical means of administration in the conscious restrained (or unrestrained) animal. The saline carrier solution helped maintain the state of hydration of the animal, as well as aiding in the disper-

sion of the lipid and drug once in the intestinal lumen. Tween 80 was used to help the oil–normal saline mixing. Bile acid salts could have been used in place of the Tween 80.

The dual infusion of lipid (containing dissolved drug) and carrier solution into the duodenum of the anesthetized rat allowed for the evaluation of the effects of varying infusion rate, dosage volume and lipid class on the lymphatic transport of the co-administered drug. The model readily lends itself to further modifications such as: simultaneous blood sampling (including portal as well as venous sampling) to measure the relative systemic bioavailability as well as the lymphatic transport of administered drug, the implantation of a second small intestine cannula to collect unabsorbed lipid, drug, and possible intestinal metabolites (Charman and Stella, 1986b), as well as allowing the effects of differing input forms such as pre-emulsification of the lipids, to be tested. The results of these types of studies constitute future reports.

The chylomicron appears to be an important lipoprotein with respect to the lymphatic transport of lipophilic drugs from the small intestine (Sieber, 1976; Lahrer et al., 1984; Vost and Maclean, 1984). Electron microscopy is the traditional technique used to assess intestinal lymphatic chylomicron (lipid) transport (Jones and Price, 1968; Forte and Nichols, 1972). Although this technique is extremely accurate and precise, it is labor intensive and not readily applied to multiple sample analysis. In order to readily estimate chylomicron lipid flux and its relationship to lipophilic drug transport, we have confirmed a simple indirect spectrophotometric assay to evaluate chylomicron transport (Noguchi et al., 1975). Aliquots of intestinal lymph were appropriately diluted in normal saline and the optical density at 560 nm (OD_{560}) was used as an index of the "chylomicron concentration" in the diluted lymph sample. Fig. 1 describes the relationship between OD_{560} and the corresponding concentration of extractable lipid. The linear relationship between OD_{560} and extractable lipid indicates that the OD_{560} values of appropriately diluted lymph samples can serve as an approximate, although fairly reliable indicator of their apparent lipid content. Thus, this plot serves as a standard curve for the relationship between OD_{560} and chylomicron transport, as the chylomicron is the major triglyceride transporting lipoprotein of the small intestinal mucosa [1].

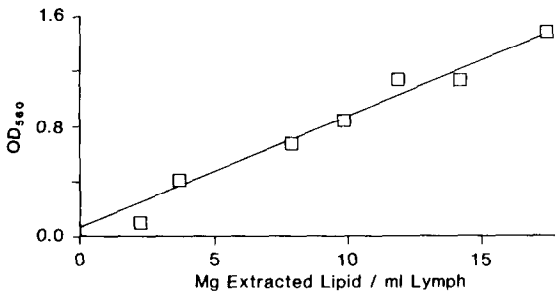


Fig. 1. Relationship between OD_{560} of diluted lymph samples and their content of extractable lymph lipid; correlation coefficient $r = 0.9778$.

Earlier, units of optical density per hour (OD/h) were used to describe hourly relative chylomicron flux. These units are readily converted, if required, into lipid fluxes by referring to the standard curve in Fig. 1. Laher et al. (1984) recently described a similar method of lymph lipid quantitation based on transmittance measurements of diluted lymph samples.

Effect of fasting

Lymphatic drug transport is affected by the fasting state of the animal and the timing of lymph duct cannulation and fistulation (Noguchi et al., 1985). However, our previous data regarding the effect of fasting was preliminary in nature. While developing the experimental methodologies described here for estimating lymphatic transport, these effects were investigated in greater detail under more controlled conditions. Lymphatic absorption studies employing protocols similar to methods 1, 2 and 2a (Scheme 1) appear throughout the literature (Wu et al., 1975; McDonald et al., 1980; Palin et al., 1982; Laher et al., 1984; Blomhoff et al., 1984). Before being able to compare

[1] Although lipid transport is reported as chylomicron flux, it is possible that VLDL (very low density lipoproteins) are also significant contributors to the total lipid transport from the small intestine (Shiau, 1981; Shiau et al., 1985).

and extrapolate data from other workers, the effect of fasting and the timing of lymphatic cannulation and fistulation on lymphatic transport required evaluation. Fig. 2 describes the cumulative percent dose of DDT transported in the intestinal lymph of anesthetized rats as a function of the methodologies described in Scheme 2. The DDT was administered in peanut oil in this study. The transport of DDT is dependent upon the methodology used in its estimation as differing quantities were transported over 11 h. Lymphatic transport of DDT was significantly greater ($P < 0.05$) in rats that had been fasted for the preceding 48 h (method 2a), and to a lesser extent for the preceding 24 h (method 2) compared to method 1. However, methods 2 and 2a were not significantly different from each other. There was more scatter in the data in the DDT transport data under methods 2 and 2a than when following method 1, suggesting that method 1 was more reliable with respect to the accumulation of kinetic transport data.

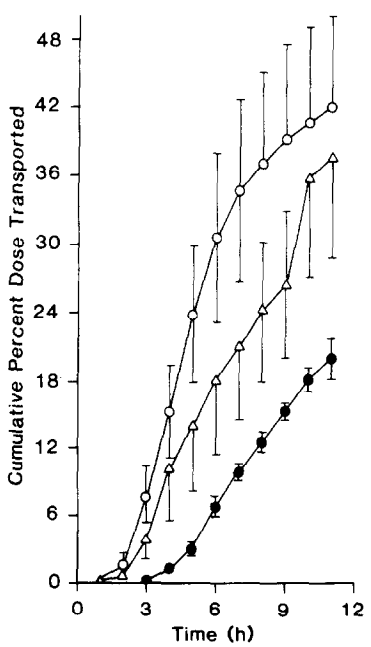


Fig. 2. Cumulative percent dose of DDT (mean ± S.E., $n = 4$) collected in intestinal lymph as a function of time and methodology. Dose was 2 mg DDT per 200 μl of peanut oil infused over 2 h. ○———○, LC/48 h fast/dosing (method 2a); △———△, LC/24 h fast/dosing (method 2); ●———●, 24 h fast/LC/dosing (method 1). Refer to scheme 1 for descriptions of methods 1, 2 and 2a.

DDT absorbed lymphatically was presumably transported via the triglyceride core of the chylomicrons (Pocock and Vost, 1974; Seiber, 1976; Vost and Maclean, 1984). Absolute lipid fluxes were not determined for methods 2 and 2a as the standard curve of optical absorbance versus lipid transport in Fig. 1 was based on lymph collected under the protocol of method 1. The direct comparison of chylomicron fluxes under the differing protocols can not be made as one must assume that the mean particle size and particle size range of the chylomicrons remain constant under the three protocols.

The significantly increased transport of DDT seen in methods 2 and 2a relative to method 1 may be due to a greater efficiency in the processing of the lipid and co-administered lipophilic drug between the point of drug–lipid co-administration in the intestinal lumen and the point of lymph fistulation. This greater efficiency in lipid processing must be a function of either the 24 or 48 h post-surgery fasting and/or concurrent fistulation of the lymph prior to lipid and drug administration.

The peanut oil vehicle, being a triglyceride, must first be hydrolyzed to its corresponding fatty acids and monoglyceride in the lumen of the small intestine prior to absorption across the intestinal membrane (Patton, 1981). The luminal hydrolysis of triglycerides is performed primarily by pancreatic lipase. The different lag times before significant lymphatic transport of DDT is observed under method 1 (4 h), and methods 2 and 2a (2 h) may possibly be due to differing rates or degrees of hydrolysis of the peanut oil vehicle in the intestinal lumen by lipase. Alternatively, as the process of chylomicron formation is complex (Sabesin, 1976) any of the sequential steps in chylomicron formation and transport could potentially be affected by fasting and lymph fistulation during the period of post-surgery fasting (methods 2 and 2a). During this period, the body is drained of vital proteins and electrolytes. Physiological and biochemical effects are potentially significant during these periods as the animals lose approximately 10% body weight (unpublished data). Shiau

et al. (1978) have demonstrated that intestinal fatty acid metabolism, for example, is dependent upon the metabolic status of the epithelial cell. The basis for the changes and differences seen in lymphatic transport of DDT under these differing protocols remains undetermined, although studies currently in progress in our laboratory are designed to evaluate any changes in the integrity of the small intestine that may occur as a result of the fasting and concurrent lymph fistulation.

It is apparent that the fasting state of an animal and the timing of lymph duct cannulation and fistulation has a significant effect on the eventual lymphatic transport of a lipophilic molecule. This makes the comparison of lymphatic transport data collected under different methodologies difficult. As lymphatic transport appears to be method dependent, ordering or ranking of data may best be performed when collected from animals subject to comparable experimental conditions.

DDT lymphatic transport

The versatility of the experimental system consisting of the surgical procedures, chylomicron estimation, timing of cannulation/fasting and intraduodenal administration of drug and lipid was exemplified by investigating, in preliminary fashion, the intestinal lymphatic transport of DDT when administered in two lipid classes, a triglyceride and a fatty acid. Fig. 3 describes the cumulative lymphatic transport (% dose) of 2 mg of DDT transported in intestinal lymph after 200 μl of either peanut oil or oleic acid infused intraduodenally over a 2 h period, in otherwise identical experiments. Peanut oil served as the "triglyceride equivalent" of oleic acid in these studies (Windholz and Budavari, 1983).

The administration of DDT in an oleic acid vehicle resulted in essentially doubled lymphatic transport of DDT relative to administration of the same dose of DDT in peanut oil. Specifically, at 12 h, the quantity of DDT transported lymphatically was significantly greater ($P < 0.05$), using the Student's t-test, from the oleic acid vehicle compared to the peanut oil vehicle. The lag time for lymphatic transport was less for the oleic acid vehicle than for the peanut oil. These differences in the kinetics of DDT transport, as evidenced by

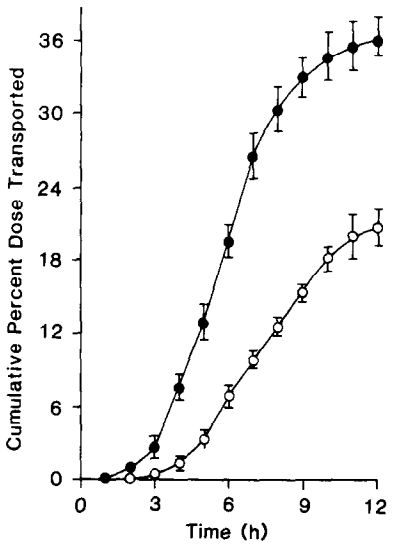


Fig. 3. Cumulative percent dose of DDT (mean ± S.E., $n = 4$) collected in intestinal lymph 12 h post-dosing after administration in either 200 μl of peanut oil or oleic acid. Dose of DDT was 2 mg and the procedure was method of Scheme 1. ●——●, oleic acid; ○——○, peanut oil.

the differences in the rate of transport (slopes of the curves) and lag times, are probably related to the initial hydrolysis of the triglyceride that must occur in the lumen of the intestine before lipid absorption can occur. The fatty acid vehicle can be absorbed directly by the intestinal mucosal cells without metabolism.

Table 1 describes the apparent quantities of total lipid transported in the intestinal lymph dur-

TABLE 1

APPARENT QUANTITIES OF LIPID TRANSPORTED IN INTESTINAL LYMPH FOLLOWING INFUSION OF 2 mg DDT DISSOLVED IN 200 μl OF EITHER PEANUT OIL OR OLEIC ACID

Lipid vehicle containing 2 mg DDT infused over 2 h	Apparent quantity of lymph lipid transport (0–11 h) [a,b]
Peanut oil	76 ± 4.4 mg [c]
Oleic acid	83 ± 5.4 mg [c]

[a] Mean ± S.E., $n = 4$.

[b] Estimated by cumulative OD_{560} measurements and related to Fig. 1.

[c] Not significantly different, Student's t-test.

ing these experiments using the two lipid vehicles. The lipid transport was estimated spectrophotometrically using the standard curve reported in Fig. 1. There was no significant difference in the quantity of lipid transported in the lymph when the two classes of lipid were administered. It appears that the differences in lymphatic transport of DDT were not related to differing degrees of total lipid transport, but were most likely a function of differences in kinetics of lipid and drug processing within the intestinal lumen or the epithelial cells of the small intestine (Vetter et al., 1985).

Lymph versus chylomicron flow

Fig. 4 describes the relationship between the hourly lymphatic transport of DDT and the corresponding lymph flow during different collection periods. These data are from experiments when peanut oil was the lipid vehicle, although the same type of relationship exists when using oleic acid or other lipids. The different symbols refer to different animals from which the data was collected. There is no obvious relationship between lymph flow and lymphatic drug transport, suggesting that the process of lymphatic DDT transport is not one of simple lymph flow related mass transfer.

Fig. 5 (oleic acid vehicle) and Fig. 6 (peanut oil vehicle) represent the relationship between DDT transport and chylomicron transport, as estimated by optical density measurements. It is evident that chylomicron transport correlates very well with drug transport. Moreover, these linear relationships were observed when the DDT was administered in the differing classes of lipid vehicles, i.e. fatty acid or triglyceride. These results confirm that a major biological event in the lymphatic transport of DDT is that of chylomicron synthesis and transport, in accord with the recent report of Vost and Mclean (1984), and others describing the chylomicron based transport of various hydrocarbons. For lipophilic drugs such as DDT, the chylomicron appears to be the major determinant of lymphatic transport. In contrast, for hydrophilic drugs, the chylomicron would not be expected to be a major determinant of its lymphatic transport as hydrophilic molecules would be transported in the aqueous fraction of intestinal lymph. In this situation, the lymphatic transport of hydrophilic drugs may be described by the principles of relative mass transfer.

On analyzing the slopes of the data in Figs. 5 and 6, it appears that the loading or apparent concentrations of DDT in the chylomicrons is a function of the lipid vehicle in which the drug was

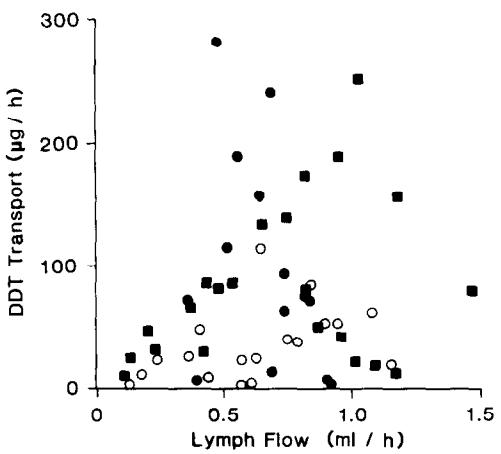


Fig. 4. Relationship between hourly lymphatic transport of DDT (μg/h) and lymph flow of corresponding lymph samples (ml/h). Dose was 2 mg of DDT administered in 200 μl of peanut oil.

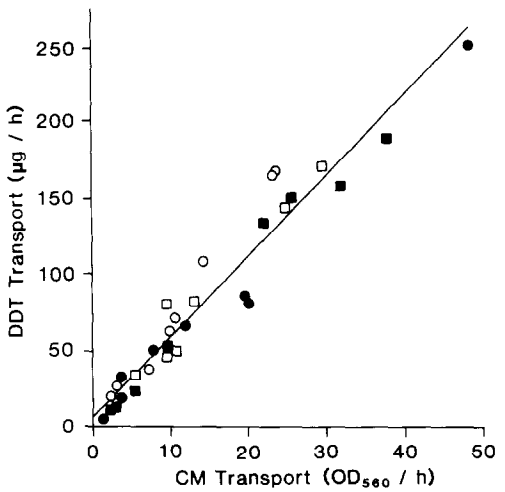


Fig. 5. Hourly intestinal lymphatic transport of DDT (μg/h) as a function of chylomicron flux (OD/h) of the corresponding lymph samples. Dose was 2 mg of DDT administered in 200 μl of oleic acid; correlation coefficient $r = 0.9727$.

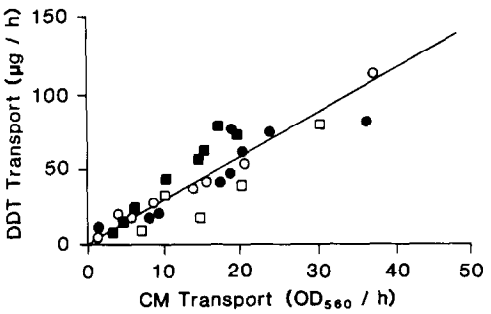


Fig. 6. Hourly intestinal lymphatic transport of DDT (μg/h) as a function of chylomicron flux (OD/h) of the corresponding lymph samples. Dose was 2 mg of DDT administered in 200 μl of peanut oil; correlation coefficient $r = 0.8892$.

administered. These differences appear to be related, in some manner, to the kinetics and dynamics of the processing of the lipid and drug within the lumen and the epithelial cells of the small intestine (Vetter et al., 1985). Therefore, in order to promote or enhance lymphatic drug transport, future efforts are best directed towards increasing drug related chylomicron transport rather than lymph flow.

In conclusion, lymphatic drug transport from the small intestine is dependent upon concurrent lipoprotein/chylomicron synthesis by the epithelial cells. The lipid vehicle in which the lipophilic drug is administered serves in large part as the required substrate and constituent for chylomicron formation. Therefore, the potential exists for lymphatic drug transport to be optimized or enhanced by the careful and judicious choice of appropriate lipid vehicles and other factors that can promote chylomicron formation.

Acknowledgement

This work was supported by R.P. Scherer Corporation.

References

Blomhoff, R., Helgerud, P., Dueland, S., Berg, T., Pederson, J.I. Norum, K.R. and Drevon, C.A., Lymphatic absorption and transport of retinol and vitamin D-3 from rat intestine. *Biochim. Biophys. Acta*, 772 (1984) 109–116.

Charman, W.N.A., Noguchi, T. and Stella, V.J., Testing potential dosage form strategies for intestinal lymphatic drug transport: studies in the rat. *Int. J. Pharm.* (1986a) in press.

Charman, W.N.A. and Stella, V.J., Effects of lipid class and lipid vehicle volume on the intestinal lymphatic transport of DDT. *Int. J. Pharm.* (1986b) in press.

Forte, T. and Nichols, A.V., Application of electron microscopy to the study of plasma lipoprotein structure. In Paoletti and Kritchevsky (Eds.), *Advances in Lipid Research, Vol. 10*, 1972, pp. 1–41.

Green, M.H. and Green, J.B., Effects of dietary fat saturation and triglyceride and cholesterol load on lipid absorption in the rat. *Atherosclerosis*, 40 (1983) 181–194.

Jones, A.L. and Price, J.M., Some methods of electron microscopic visualization of lipoproteins in plasma and chyle. *J. Histochem. Cytochem.*, 16 (1968) 366–370.

Laher, J.M., Rigler, M.W., Vetter, R.D., Barrowman, J.A. and Patton, J.S., Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. *J. Lipid Res.*, 25 (1984) 1337–1342.

McDonald, G.B., Saunders, D.R., Weidman, M. and Fisher, L., Portal venous transport of long chain fatty acids absorbed from rat intestine. *Am. J. Physiol.*, 239 (1980) G141–G150.

Noguchi, T., Jinguyi, Y., Kimura, T. Muranishi, S. and Sezaki, H., Mechanism of the intestinal absorption of drugs from oil in water emulsions VII: Role of bile in the lymphatic transport of lipid soluble compounds from triolein emulsions. *Chem. Pharm. Bull.*, 23 (1975) 782–786.

Noguchi, T., Charman, W.N.A. and Stella, V.J., Lymphatic appearance of DDT in thoracic or mesenteric lymph duct cannulated rats. *Int. J. Pharm.*, 24 (1985) 185–192.

Palin, K.J., Wilson, S.G., Davis, S.S. and Phillips, A.J., The effect of oils on the lymphatic absorption of DDT. *J. Pharm. Pharmacol.*, 34 (1982) 707–710.

Patton, J.S., Gastrointestinal lipid digestion. In Johnson (Ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1981, pp. 1123–1146.

Pocock, D.M.E. and Vost, A., DDT absorption and chylomicron transport in rat. *Lipids*, 9 (1974) 374–381.

Roman, C. and Gonella, J., Intrinsic control of digestive tract motility. In Johnson (Ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1981, pp. 289–333.

Sabesin, S.M., Ultrastructure aspects of the intracellular assembly, transport and exocytosis of chylomicrons by rat absorptive cells. In Rommell et al. (Eds.), *Lipid Absorption: Biochemical and Clinical Aspects*, University Park Press, Baltimore, 1976, pp. 113–148.

Shiau, Y.F., Long, W.B. and Weiss, J.B., Effect of sugar and monoglyceride on fatty acid metabolism. Am. J. Physiol., 234 (1978) E236–E242.

Shiau, Y.F., Mechanisms of intestinal fat absorption. *Am. J. Physiol.*, 240 (1981) G1–G9.

Shiau, Y.F., Popper, D.A., Reed, M., Umstetter, C., Capuzzi, D. and Levine, G.M., Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am. J. Physiol.*, 148 (1985) G164–G169.

Sieber, S.M., The lymphatic absorption of *p,p*-DDT and some

structurally related compounds in the rat. *Pharmacology*, 14 (1976) 443–454.

Thompson, J.N., Erdody, P., Brien, R. and Murray, T.K., Fluorometric determination of vitamin A in human blood and liver. *Biochem. Med.*, 5 (1971) 67–89.

Vetter, R.D., Carey, M.C. and Patton, J.S., Coassimilation of dietary fat and benzo(a)pyrene in the small intestine: an absorption model using the killifish. *J. Lipid Res.*, 26 (1985) 428–434.

Vost, A. and Maclean, N., Hydrocarbon transport in chylomicrons and high-density lipoproteins in rat. *Lipids*, 19 (1984) 423–435.

Windholz, M. and Budavari, S., *The Merck Index*, 10th edn., Merck and Co, U.S.A., 1983.

Wu, A.-L., Clark, S.B. and Holt, P.R., Transmucosal triglyceride transport rates in proximal and distal rat intestine in vivo. *J. Lipid Res.*, 16 (1975) 251–257.