ELSEVIER

International Journal of Pharmaceutics 119 (1995) 173–181

international journal of pharmaceutics

Effect of lipid vehicle on the intestinal lymphatic transport of isotretinoin in the rat

R. Nankervis [a], S.S. Davis [a], N.H. Day [b,1], P.N. Shaw [a,*]

[a] *Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK*
[b] *Roche Products Ltd, 40 Broadwater Road, Welwyn Garden City AL7 3AY, UK*

Received 27 May 1994; revised 7 October 1994; accepted 1 November 1994

Abstract

Many lipophilic compounds are absorbed to some degree via the lymphatics, however, the mechanisms and factors controlling this absorption process are unclear. In order to provide some information on this area we have studied the effect of lipid vehicle on the lymphatic transport of isotretinoin following oral dosing to the rat. Oils containing higher percentages of the linoleate triglyceride ester appeared to promote both enhanced lymph flow and chylomicron concentration. Long chain triglyceride oils proved to be the most effective vehicles for increasing lymphatic transport – especially cottonseed oil and peanut oil. The solubility of the drug in the oil was also shown to be a key factor in lymphatic transport.

Keywords: Isotretinoin; Lymph; Lymphatic transport; Chylomicron; Solubility; Triglyceride oil; Oral dosing

1. Introduction

It is now well established that many lipophilic compounds are absorbed, to a certain extent, via the lymphatic route following oral administration (Kamp and Neumann, 1975; Palin et al., 1982; Ueda et al., 1983; Yoshikawa et al., 1984; Noguchi et al., 1985a,b; Takada et al., 1985; Charman and Stella, 1986a,b; Gowan and Stavchansky, 1986; Yangawa et al., 1989; Lamka et al., 1990). The mechanisms whereby lipophilic molecules are absorbed from the gastrointestinal tract and transported to the general circulation are complex and poorly understood (Patton, 1981). It has been suggested that most lymphatically transported drugs are present in the chylomicron fraction of the intestinal lymph and two main factors may influence the quantity of drug carried via this intestinal transport pathway. Firstly, the quantity of lipid transported in the lymph in the form of chylomicrons and secondly, the amount of drug per chylomicron (Charman and Stella, 1986b). It is therefore clear that total chylomicron flux is an important determinant of the lymphatic transport of lipophilic drugs.

The measurements of octan-1-ol/water partition coefficient (more frequently expressed as its

* Corresponding author.
[1] Present address: Fisons RandD, Bakewell Road, Loughborough LE11 0RH, UK.

SSDI 0378-5173(94)00390-4

logarithm to base 10, log P) and oil solubility are often used as estimates of the lipophilicity of a particular molecule. The solubility of a compound in oil is an absolute measurement of the solubility of a solute in equilibrium with its pure phase. It is possible for a compound to have a large log P value but not necessarily a high solubility in a particular oil whilst another compound may be very soluble in an oil but have a relatively low log P value. In addition, a high solubility of a compound in a solvent such as octan-1-ol will not always relate to a high solubility in a poorly solvating, largely apolar vehicle such as a long chain fatty acid triglyceride (Charman and Stella, 1986b).

In order to define some of the parameters affecting the lymphatic uptake and transport of lipophilic drugs we have examined the effect of lipid vehicle on the lymphatic transport of the retinoid, isotretinoin (log P = 6.6, Nankervis, 1992) following oral dosing of the compound to the rat. The relationship between solubility of isotretinoin in the oil and its lymphatic uptake has also been investigated as has the extent of chylomicron transport (assessed as lymph turbidity) as a function of the orally dosed lipid vehicle.

2. Materials and methods

It has been well established (Brazzell and Colburn, 1982) that retinoids are sensitive to light, especially ultraviolet light. Consequently all samples were protected using aluminium foil wrapping and all procedures involving the use of retinoids (e.g., weighing) were undertaken under conditions of red/amber illumination.

2.1. Chemicals and reagents

Isotretinoin was supplied by Roche Products Ltd. Pentobarbitone and arachis (peanut) oil were purchased from Evans (Slough, UK), and Miglyol 812 (a mixed medium chain triglyceride oil) from Dynamit Nobel (Slough, UK). All other chemicals were from Sigma (Poole, UK) and were of analytical grade or better.

2.2. Determination of the solubility of isotretinoin in a series of oils

The solubility of isotretinoin was determined in each of the six oils used for oral dosing studies, cottonseed, soybean, peanut, oleic acid, linoleic acid and Miglyol 812 using the following procedure. Duplicate measured volumes of each oil (1.0 ml) were transferred by positive displacement pipette into microcentrifuge tubes (1.5 ml). Small additions (5–10 mg) of isotretinoin were accurately weighed into each tube, to give a known concentration in the oil. The oil samples were placed in an ultrasonic water bath for 30 min (during which time the temperature of the samples increased from 24°C to 55–60°C). The samples were allowed to equilibrate at 24°C and each oil sample was visually examined for signs of precipitation. After centrifugation ($3000 \times g$, 10 min), a small sample of oil (approx. 20 mg) was removed from each tube for analysis. In oil samples where no visible signs of precipitation was observed, further additions of isotretinoin and subsequent sample sonication were made until visible precipitation was observed. Quantitative analysis of the retinoid in each sample of oil was performed using the HPLC method described below.

2.3. In vivo studies

Male Wistar rats weighing 200–250 g were used for the studies. They were fed standard rat chow with water ad libitum and maintained on a 12 h cycle of day and night. Animals were fasted for 16 h prior to the experimental procedure.

2.3.1. Oral dosing of isotretinoin in a series of oily vehicles and sample collection

Six oily solutions were prepared. Each solution contained isotretinoin (3.0 mg ml^{-1}) in one of six oils, cottonseed, peanut, soybean, Miglyol 812, oleic acid or linoleic acid for oral dosing to rats. The prepared solutions were transferred into screw-capped vials, stored at room temperature (20°C) and were found to remain stable, with respect to their isotretinoin content, for several weeks. 12 groups of six rats (two groups [A and B]

for each oil under study) were used. Blood samples were collected from animals in group A at approx. 15 min intervals and lymph samples were taken from animals in group B when sufficient sample for analysis was collected in the cannula (20–50 mg).

The oil vehicles (0.5 ml oil, 6 mg kg^{-1} isotretinoin) were orally administered by gavage over a period not exceeding 30 s. After a period of 30 min, anaesthesia was induced with pentobarbitone (72 mg kg^{-1}) administered intraperitoneally. Additional pentobarbitone was administered as and when required to maintain anaesthesia. Animals from group A underwent tracheotomy and jugular vein cannulation for the collection of blood samples (0.2 ml). Plasma was separated and stored at −20°C. Animals from group B underwent intestinal lymphatic duct cannulation based on the method of Bollman et al. (1948), the cannula being glued into position using a drop of cyanoacrylate cement. The cannula was exteriorised, cut short (approx. 3 cm) and immobilised using solid adhesive. A further length of saline filled cannula (approx. 50 cm) and containing a small air space (5 μl), was attached to the lymphatic cannula using a snapped off hypodermic syringe needle. The second cannula was allowed to hang vertically to provide a slight negative pressure on the lymph duct and assist the flow of lymph. The air space in the cannula was used to monitor the flow of lymph. The second cannula and was replaced periodically and lymph was drained into pre-weighed microcentrifuge tubes (1.5 ml) and stored at −20°C. The time of collection of each lymph sample and its size (mass) were recorded. The density of lymph was assumed to be 1.0 g ml^{-1}, thus allowing flow to be represented in terms of the traditional units of ml h^{-1}.

2.3.2. Collection of lymph samples for turbidity estimation

Two groups of six animals were orally dosed (0.5 ml) with one of the following vehicles, cottonseed, soybean, peanut, linoleic acid, oleic acid, Miglyol 812. Control animals were dosed with isotonic saline (0.5 ml). Mesenteric lymph duct cannulations were performed on each animal using the method described above (anaesthesia was induced immediately after dosing) and lymph samples were collected for turbidity estimation.

2.4. Lymph turbidity estimation

Aliquots (20–50 mg) in duplicate from each lymph sample were diluted in isotonic saline (2 ml) in order to give a spectroscopic absorbance ($\lambda = 400$ nm) value within the range 0.20–1.00. The wavelength of 400 nm was chosen for these absorbance measurements since at lower wavelengths non-specific absorbance was observed, possibly due to other chromophores in the lymph samples (proteins, vitamins, etc.), whilst at higher wavelengths the absorbance decreased rapidly and may have presented difficulties in obtaining sufficient volumes of lymph for analysis. The product of the absorbance for each diluted lymph sample and its dilution factor was used to determine the absorbance of undiluted lymph.

2.5. Analysis of isotretinoin in plasma and lymph samples

The HPLC system consisted of a pump and variable-wavelength UV detector (LKB Models 2150 and 2151, respectively, LKB-Produkter, Bromma, Sweden), a Gilson auto sampling-injector (Model 231-401, Gilson International, Villiers-le-Bel, France) and a Spectra-Physics data integrator (Model SP4290, Spectra-Physics, San José, CA, USA). Plasma and lymph samples containing isotretinoin were analysed under the following conditions: the HPLC column was a Spherisorb (Phase Separations, Queensferry, UK) ODS2 (15 cm × 4.6 mm i.d.) with a 5 μm particle size; the mobile phase consisted of acetonitrile (70% v/v) and 0.1 M ammonium acetate adjusted to pH 6.0 (30% v/v). The flow rate was 1.0 ml min^{-1}, the injection volume was 20 μl and the compounds were detected by their UV absorbance at 350 nm. Plasma and lymph samples containing isotretinoin were extracted using a direct protein precipitation method involving the addition of acetonitrile (200 μl), acetonitrile (100 μl) containing the retinoid internal standard (Ro 11-5036, 7.5 ng) to plasma or lymph (100 μl). The

mixture was, after thorough mixing, centrifuged (13 000 × g, 10 min) and the supernatant removed by aspiration. The assay method was linear over the range used in these studies ($r^2 > 0.995$) and demonstrated good recovery, precision (variability < 10%) and accuracy.

2.6. Analysis of isotretinoin in oil solutions

A volumetric solution of isotretinoin (6.88 mg ml^{-1}) was prepared by accurately weighing the pure compound into a volumetric flask and diluting with tetrahydrofuran, (THF, HPLC grade).

An aliquot (approx. 150 μl) of each oil sample was transferred by positive displacement pipette into a Millipore Ultrafree^{TM} filter unit (0.1 μm pore size) and centrifuged (10 000 × g, 20 min) to remove undissolved retinoid. A small accurately weighed sample (10–50 mg) of the filtered oil was dispensed into a volumetric flask (10 ml) and adjusted to volume using THF. THF was found to disperse and solubilise the oil samples completely and was miscible with the HPLC mobile phase. A sample (25 μl) of isotretinoin in THF was injected onto the HPLC system using a fixed volume loop injection system. Peak areas were calculated using a computing integrator and retinoid concentrations were calculated by comparison of the peak areas of the oil samples with the standard retinoid solution. Isotretinoin concentrations measured in oil samples to which accurately weighed additions of the retinoid had been made, but in which isotretinoin was completely soluble, were used to assess the recovery of isotretinoin from the oils.

2.7. Analysis of data

The plasma and lymph concentration data from each group of rats were treated as follows. The concentration of isotretinoin determined in each sample was corrected for sample volume and administered dose (mg kg^{-1}) to give a dose adjusted plasma or lymph concentration (ng ml^{-1}). These data were plotted and subjected to linear regression analysis. The plasma and lymph concentrations at 90 min after dosing the animal were determined by reference to this regression line. The mean intestinal lymph flow rate (ml min^{-1}) for each dose group was calculated from the volume of each lymph sample and the time over which the sample was collected, for each oil group. This product of the mean lymph flow rate and the lymphatic isotretinoin concentration at 90 min gave a figure for the mean lymphatic uptake rate (ng h^{-1}). Plasma isotretinoin uptake was estimated from the product of the volume of distribution of isotretinoin at steady state (V_{ss}, Liu et al., 1990; Nankervis et al., 1993) and the plasma isotretinoin concentration at 90 min.

3. Results

The lymph flow rates measured in intestinal lymph duct cannulae varied between 0.8 and 3.3

Table 1
Lymphatic flow rate and turbidity after oral administration of six oil vehicles or saline (0.5 ml)

Dosing vehicle	Lymph flow rate (ml h^{-1} kg^{-1})	Lymph turbidity (corrected absorbance at 400 nm)	Lymph turbidity relative to saline dosed animals
Saline	1.61 ± 0.69	1.07 ± 0.35	1.0
Cottonseed	3.14 ± 0.82 [a]	38.0 [b]	35.2
Soybean	3.31 ± 1.76 [a]	51.3 ± 9.59	48.2
Peanut	1.73 ± 0.37	26.6 [b]	25.0
Miglyol 812	2.05 ± 0.70	4.70 ± 0.91	4.4
Oleic acid	1.94 ± 0.87	12.9 ± 1.92	12.1
Linoleic acid	0.80 ± 0.23 [a]	10.4 [b]	9.8

[a] Significantly different ($p > 0.01$) from lymph flow rate in animals dosed with saline. [b] Insufficient data for statistical analysis.

ml h^{-1} (Table 1) depending upon the oil used for dosing. The mean intrinsic lymph flow rate, measured in fasted animals which had been orally dosed with isotonic saline (0.5 ml), was 1.61 ± 0.69 ml h^{-1}. There was considerable inter-subject variation in the lymph flow rate data and only the flow rates measured after dosing isotretinoin in cottonseed oil (3.1 ml h^{-1}) or soybean oil (3.3 ml h^{-1}) were significantly elevated ($p < 0.01$) whilst the lymph flow rate after dosing isotretinoin in linoleic acid (0.8 ml h^{-1}) was significantly decreased ($p < 0.01$).

Visual examination of lymph samples suggested varying degrees of turbidity depending upon the vehicle used for oral dosing; all vehicles increasing lymphatic turbidity compared with control animals dosed with saline. These observations were supported by the turbidity data obtained after measuring the spectroscopic absorbance of lymph (Table 1). The three mixed long chain triglyceride oils, soybean, cottonseed and peanut gave the highest turbidities (48.2-, 35.7- and 25.0-times the absorbance obtained in control animals). Oleic acid and linoleic acid (12.1- and 9.8-times the absorbance obtained in control animals) demonstrated similar lymph turbidities. Lymph collected from animals dosed with Miglyol 812 was the least turbid but was still 4.4-times greater than that of lymph from control animals.

A typical plasma and lymphatic concentration-time plot obtained after dosing isotretinoin in an oily vehicle is shown in Fig. 1. The data demonstrate that no clear absorption or elimination phase can be discerned indicating that isotretinoin

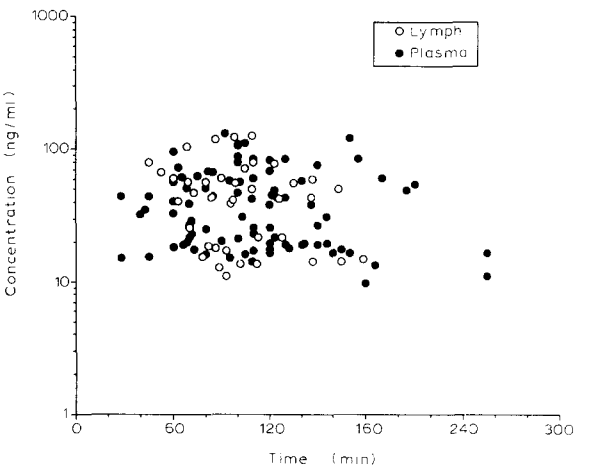

Fig. 1. Plasma and lymph concentrations of isotretinoin in the rat after oral dosing (6 mg/kg) in peanut oil (0.5 ml).

absorption from peanut oil is proceeding continuously over the experimental period and that absorption is a complex process. These data proved difficult to interpret in a traditional pharmacokinetic manner and were therefore summarised by calculating a plasma and a lymphatic concentration 90 min after the start of the experiment. The isotretinoin plasma concentrations (Table 2) were greatest when using Miglyol 812 (54.1 ng ml^{-1}) and the other mixed triglyceride oils – peanut, soybean and cottonseed gave plasma concentrations of a similar order of magnitude (28.8, 49.9 and 38.3 ng ml^{-1}, respectively). The plasma concentration obtained when using oleic acid as a vehicle (14.7 ng ml^{-1}) was approximately one-quarter of that obtained when using Miglyol and that for linoleic acid (0.8 ng ml^{-1}) around one-

Table 2
Lymphatic and plasma concentration and uptake data for isotretinoin in the rat ($n = 6$) after oral administration in each of six oils (3 mg ml^{-1})

Oil	Concentration		Uptake rate (ng h^{-1} kg^{-1})		Lymph/plasma uptake ratio (×100)
	Plasma	Lymph	Total	Lymph	
Cottonseed	38.3	48.0	7540	151	2.00
Soybean	49.9	37.5	9840	124	1.26
Peanut	28.8	41.5	5680	72	1.27
Miglyol 812	54.1	26.6	10650	55	0.52
Oleic acid	14.7	13.5	2890	26	0.90
Linoleic acid	0.8	1.4	154	1.1	0.72

seventieth. The lymphatic concentrations of isotretinoin were not dissimilar to those obtained for plasma but the rank order was different with the highest concentration being obtained when using cottonseed oil as the vehicle (48.0 ng ml^{-1}).

The lymphatic uptake rate of isotretinoin varied considerably with the oil type used for oral administration. This variation followed the same general pattern as for the effect of oil on lymph flow rate but the magnitude of the effect was much greater. The mixed long chain fatty acid triglyceride oils, cottonseed, soybean and peanut oil, promoted the lymphatic absorption of isotretinoin to the greatest extent (151, 124 and 72 ng h^{-1} kg^{-1}, respectively). The long chain free fatty acids, oleic acid and linoleic acid, gave very poor lymphatic uptake (26 and 1.1 ng h^{-1} kg^{-1}) whilst the mixed medium chain triglyceride oil, Miglyol 812, gave lymphatic uptake intermediate in extent (55 ng h^{-1} kg^{-1}) between the mixed long chain triglyceride oils and the unsaturated long chain free fatty acids. The total isotretinoin uptake rate again varied considerably with the vehicle used but the most noteworthy factor was the comparison between the plasma and the lymphatic uptake rates. These are given in Table 2 and are summarised as lymph/plasma uptake ratios. The rank order obtained with these ratios is similar to that obtained from examining the lymph uptake rate alone but the plasma drug uptake rate is always very much greater than that absorbed via the lymphatics.

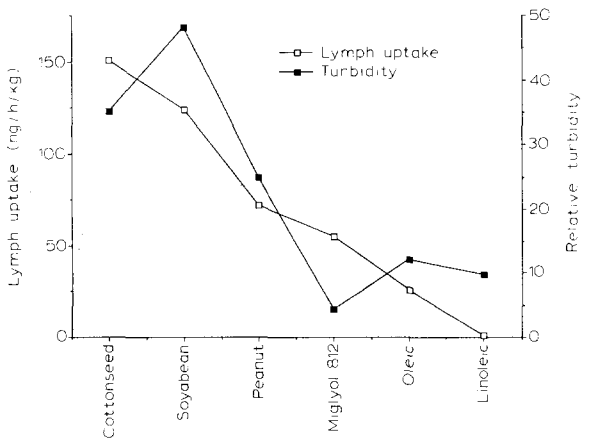

Fig. 2. Relationship between lymphatic uptake of isotretinoin and lymph turbidity for a series of oil vehicles.

Table 3
Solubility (at 24°C) of isotretinoin in a series of oily vehicles

Oil	Solubility (mg ml^{-1})
Cottonseed	5.10
Soybean	5.68
Peanut	6.10
Miglyol 812	12.0
Oleic acid	24.4
Linoleic acid	30.5

Lymphatic uptake of isotretinoin from any of the oil systems was extremely poor, with a maximum dose adjusted uptake rate after dosing in cottonseed oil, accounting for only 2.0% of overall plasma absorption and only approx. 0.015% h^{-1} of administered oral dose. Lymphatic uptake measured after administration of isotretinoin in linoleic acid, which gave the poorest uptake rate, contributed 0.71% of the total absorption and only 0.00010% h^{-1} of the administered oral dose. Total uptake data show a similar trend to the lymph uptake data resulting in reasonably comparable lymph:plasma uptake ratios for each of the six oils studied (0.5% and 2.0%). A graph illustrating the relationship between lymphatic uptake of isotretinoin and lymph turbidity is shown in Fig. 2.

The solubility of isotretinoin in various oil systems varied between 5.1 mg ml^{-1} for cottonseed oil to 30.5 mg ml^{-1} for linoleic acid (Table 3). The solubility of isotretinoin in each of the six oils used in the oral dosing studies showed an inverse relationship with the lymphatic uptake from the oil (Fig. 3).

4. Discussion

Six different oils were used as oral dosing vehicles in these studies. Peanut, cottonseed and soybean are all mixed long chain triglycerides containing primarily varying proportions of palmitate, oleate and linoleate esters. Peanut comprises 12% palmitate, 53% oleate and 26%

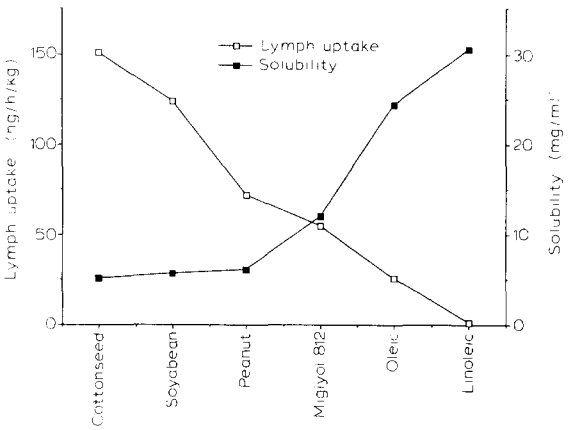

Fig. 3. Relationship between lymphatic uptake of isotretinoin and solubility of isotretinoin in a series of oil vehicles.

linoleate whereas the respective figures for cottonseed are 26, 18 and 52% and for soybean are 12, 24 and 51% (Altman and Dittmer, 1972). Oleic acid is a C_{18} free fatty acid with one C-C double bond whereas linoleic acid has two C-C double bonds in the same length fatty acid chain. Miglyol 812 is a mixture of caprylic (C_8) and caprate (C_{10}) triglyceride esters in the proportion 55 to 45%, respectively.

Raub et al. (1992) have reported that the average flow rate of lymph was 1.60 ± 0.4 ml h^{-1} when saline was infused into the duodenum and other workers (Yoffey and Courtice, 1970) have also reported values close to 1 ml h^{-1}. These value compare extremely well with our observation of a lymph flow rate of 1.61 ± 0.69 ml h^{-1} in control animals. The lymph flow rates obtained in our studies are also consistent with those previously reported in the literature, (Tso et al., 1985) after the infusion of an oleic acid/1-monoolein emulsion into the duodenum of the rat. Our data also demonstrate that not only do certain oils enhance lymph flow rate but also that other oils may suppress it. Oils such as soybean and cottonseed comprise higher percentages of the linoleate triglyceride ester than does peanut oil and these oils appear to promote enhanced lymphatic flow. This observation is confirmed by an examination of the turbidity data which also show larger values for these oils. The combination of these two sets of data suggest that total lymphatic transport is increased with these two oils – both in terms of flow rate and chylomicron concentration. Peanut oil proved to be much poorer in this respect for whilst turbidity was increased, the lymphatic flow rate was not significantly different from control values. The mixed medium chain triglyceride oil Miglyol 812 was less effective in terms of increasing chylomicron formation (turbidity) and also had little influence on lymph flow rate. The free fatty acids, linoleic acid and oleic acid, tended to increase lymph turbidity but had either no significant effect of lymph flow (oleic) or decreased flow significantly (linoleic).

It can be seen from our data relating oil type and lymph uptake that certain oils do appear to increase the lymphatic uptake rate of isotretinoin and Fig. 2 shows a clear relationship between turbidity and lymphatic uptake. This finding confirms the observations of Charman and Stella (1986b) who studied the lymphatic transport of DDT in rats after oral (intra-duodenal) administration in oleic acid. They demonstrated a good linear relationship ($r^2 = 0.9561$) with chylomicron transport rather than lymph flow and showed the importance of chylomicron flow (determined from lymph turbidity measurements) in the lymphatic absorption of DDT. It has been reported that lymph chylomicrons are almost certainly the carrier vehicle for lipophilic compounds (Sieber, 1976; Vost and Maclean, 1984).

Moreover, our results show clearly that the most effective oil vehicles for promoting lymphatic uptake are the long chain mixed triglyceride oils – especially cottonseed and soybean. Peanut oil was poorer in this respect followed by Miglyol 812. Other workers results in this area have reported similar findings. Palin et al. (1980) studied the effects of three oily vehicles on the absorption of the highly lipophilic model compound DDT ($\log P = 6.2$) in the rat. They showed that the area under the plasma concentration time curve was greater after orally dosing DDT in peanut oil compared with dosing in Miglyol 812 or liquid paraffin. These workers also reported (Palin et al., 1982) that the absorption of DDT was almost totally via the lymphatic route and that lymph flow was not important in determining lymphatic uptake rate since both peanut oil and

liquid paraffin stimulated lymph flow to the same extent. Holmberg et al. (1990) studied the absorption of vitamin D in humans after administration in gelatin capsules containing either peanut oil or Miglyol 812. In fasting subjects, the mean peak plasma concentration was approx. 3-times greater when vitamin D was administered in a mixed long chain triglyceride oil (peanut oil) than in a mixed medium chain triglyceride oil (Miglyol 812).

Similar data were obtained using the even more lipophilic compound, Probucol (estimated $\log P = 11$) (Palin and Wilson, 1984., Palin et al., 1984), which confirmed peanut oil to be the oil which promoted the greatest increase in lymphatic absorption compared with Miglyol 812 or liquid paraffin. Charman and Stella (1986a) have studied the effects of peanut oil and the long chain fatty acid, oleic acid, on the oral absorption of DDT and showed that oleic acid gave even greater lymphatic absorption than peanut oil. These findings have been further supported by Gowan and Stavchansky (1986) who obtained the greatest bioavailability and also the highest lymph flow rate when radiolabelled phenytoin was administered in the salt of a long chain fatty acid, sodium oleate. Hollander (1980) has also reported some data relating to the effect of oily vehicle on lymphatic uptake. When a vitamin A-bile salt emulsion system was perfused into the duodenum of a rat, the addition of a medium chain fatty acid, caprylic acid, to the emulsion, increased the lymphatic absorption of vitamin A. In contrast, the addition of one of the unsaturated long chain fatty acids, oleic, linoleic or a saturated fatty acid, arachidonic, resulted in a decrease in lymphatic absorption. Kuksis (1987) has also reported that long chain unstaurated fatty acids may decrease the absorption of lipid soluble vitamins.

The literature view of the effect of oily vehicle on lymphatic uptake is therefore somewhat unclear. Several generalisations can be made, however. The overall conclusion being that triglyceride oils enhance lymphatic uptake; with the longer chain triglycerides having a larger effect than the medium chain triglycerides. Such a finding is in accordance with our studies reported here. We can also confirm that a clear relationship between chylomicron flow and lymph uptake exists.

A complicating feature in this view of lymph uptake is that of drug solubility in the oil. We have demonstrated in the studies reported here an inverse relationship between lymph uptake and solubility of isotretinoin in the vehicle. Isotretinoin was orally dosed (6 mg kg^{-1}) in a fixed volume of oil (0.5 ml) and it may be postulated therefore, that the results may be an effect of increasing the percentage isotretinoin saturation in the oil, thereby improving the transport process from the oil to the enterocytes.

We have demonstrated in these studies that the lymphatic uptake of isotretinoin may vary over several orders of magnitude and is clearly influenced by the dosing vehicle. It should, however, not be overlooked that the total drug uptake was some 50–150-times greater than that observed to occur via the lymphatics. Hence, although relatively high concentrations of drug may be achieved in lymph, the total amount of isotretinoin transported by this route is small.

The data presented here related to the oral dosing of isotretinoin in the rat suggest that the processes involved in the lymphatic uptake of this retinoid are very complex. There appear to be a number of interacting effects of the oily vehicle, isotretinoin solubility in the vehicle, lipid content of the lymph and lymph flow rate. In summary; the solubility of isotretinoin in an oil shows an inverse relationship with the lymphatic uptake from the oil (Fig. 3). This advantage of poorly solvating vehicles for the absorption of drugs is limited, however, by the total amount of drug which the vehicle can incorporate since this will control drug availability at the site of absorption (Armstrong and James, 1980). Certain oil types appear to enhance the lymphatic uptake of isotretinoin and the mixed long chain triglyceride oils appear to be most effective in enhancing lymphatic uptake whereas the long chain free fatty acids appear to be most ineffective.

Acknowledgements

R.N. was the recipient of an Science and Engineering Research Council Co-operative Award in

Science and Engineering with Roche Products Ltd.

References

Altman, P.L. and Dittmer D.S., Fats and oils: Properties and composition. *Biology Data Book*, Vol. 1, Federation of American Societies for Experimental Biology, MD, 1972, pp. 348–352

Armstrong, N.A. and James K.C., Drug release from lipid based dosage forms. *Int. J. Pharm.*, 6 (1980) 195–204

Bollman, J.L., Cain, J.C. and Grindlay, J.H., techniques for the collection of lymph from the liver small intestine or thoracic duct of the rat. *J. Lab. Clin. Med.*, 33 (1948) 1349–1352.

Brazzell, R.K. and Colburn W.A., pharmacokinetics of the retinoids isotretinoin and etretinate. *J. Am. Acad Dermatol.*, 6 (1982) 643–651

Charman, W.N.A. and Stella, V.J., Effect of lipid class and lipid vehicle volume on the intestinal lymphatic transport of DDT. *Int. J. Pharm.*, 33 (1986a) 165–172.

Charman, W.N.A. and Stella, V.J., Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules. *Int. J. Pharm.*, 34 (1986b) 175–178.

Gowan, W.G. and Stavchansky, S., The effect of solvent composition upon the blood and lymph levels of phenytoin in rats after gastric administration. *Int. J. Pharm.*, 28 (1986) 193–199.

Hollander D., Retinol lymphatic and portal transport: Influence of pH bile and fatty acids. *Am. J. Physiol.*, 239 (1980) 210–214

Holmberg, I., Akones, L., Berlin, T., Lindbach, B., Zemgals, J. and Lindeke, B., Absorption of a pharmacological dose of vitamin D3 from two different lipid vehicles in man: Comparison of peanut oil and medium chain triglycerides. *Biopharm. Drug Dispos.*, 11 (1990) 807–815.

Kamp, J.D. and Neumann, H.-G., Absorption of carcinogens into the thoracic duct lymph of the rat: Aminostilbene derivatives and 3 methylcholanthrene. *Xenobiotica*, 5 (1975) 717–727.

Kuksis, A., Absorption of fat soluble vitamins. In Kuksis, A. (Ed.), *Fat Absorption*, Vol. 2, CRC Press, Boca Raton, 1987, pp. 233–245.

Lamka, J., Jindrova, O., Gallova, S., Uhrova, R. and Kvetina, J., Influence of the composition of rat central lymph on the pharmacokinetics (The steady state during infusion, bioavailability, absorption) of diazepam studied in the blood and lymph. *Physiol. Bohemslov.*, 39 (1990) 403–408.

Liu, S.S., Sandri, R. and Tang-Liu, D.D.-S., Systemic pharmacokinetics of acetylenic retinoids in rats. *Drug Metab. Dispos.*, 18 (1990) 1071–1077.

Nankervis, R., The lymphatic absorption of the retinoids. Ph.D Thesis, University of Nottingham (1992).

Nankervis, R., Davis, S.S., Day, N.H. and Shaw, P.N., Studies on the intravenous pharmacokinetics of three retinoids in the rat. *Int. J. Pharm.*, 101 (1993) 249–256.

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 (1985b) 185–192.

Noguchi, T., Charman, W.N.A. and Stella, V.J., The effect of drug lipophilicity and lipid vehicles on the lymphatic absorption of various testosterone esters. *Int. J. Pharm.*, 24 (1985a) 173–184.

Palin, K.J. and Wilson, C.G., The effects of different oils on the absorption of probucol in the rat. *J. Pharm. Pharmacol.*, 36 (1984) 641–643.

Palin, K.J., Bell, G.D. and Wilson, C.G., The effect of oil on the absorption of probucol in the rat. *J. Pharm. Pharmacol.*, 36 (1984) 85P.

Palin, K.J., Davis, S.S. and Phillips, A.J., Effect of lipid vehicles on the oral absorption of a model compound (DDT). *J. Pharm. Pharmacol.*, 32 (1980) 62P.

Palin, K.J., Wilson, C.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, L.R. (Ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1981, pp. 1123–1146.

Raub, T.J., Douglas, S.L., Melchior, G.W., Charman, W.N. and Morozowich, W., Methodologies for assessing intestinal lymphatic transport. In Charman, W.N. and Stella, V.J. (Eds), *Lymphatic Transport of Drugs*, CRC Press, Boca Raton, 1992, pp. 63–111.

Sieber S.M., The lymphatic absorption of *p,p'*-DDT and some structurally related compounds in the rat. *Pharmacology*, 14 (1976) 443–454.

Takada, K., Shibata, N., Yoshimura, H., Masuda, Y., Yoshikawa, H., Muranishi, S. and Oka, T., Promotion of the selective lymphatic delivery of cyclosporin A by lipid surfactant mixed micelles. *J. Pharmacobio-Dyn.*, 8 (1985) 320–323.

Tso, P., Pitts, V. and Granger, D.N., Role of lymph flow in intestinal chylomicron transport. *Am. J. Physiol.*, 249 (1985) G21–G28.

Ueda, C.T., Lemaire, M., Gsell, G. and Nussbaumer, K., Intestinal lymphatic absorption of cyclosporin A following oral administration in an olive oil solution in rats. *Biopharm. Drug Dispos.*, 4 (1983) 113–124.

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

Yangawa, A., Iwayama, T., Saotome, T., Shofi, Y., Takano, K., Oka, H., Nakagawa, T. and Mijushima, Y., Selective transfer of cyclosporin to thoracic lymphatic systems by the application of lipid microspheres. *J. Microencapsul.*, 6 (1989) 161–164.

Yoffey, J.M. and Courtice, F.C., *Lymphatics and the Lymphomyeloid Complex*, Academic Press, London, 1970.

Yoshikawa, H., Takada, K., Muranishi, S., Satoh, Y.I. and Naruse, N., A method to potentiate enteral absorption of interferon and selective delivery into lymphatics. *J. Pharmacobio-Dyn.*, 7 (1984) 59–62.