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Factors affecting the lymphatic transport of penclomedine (NSC-338720), a lipophilic cytotoxic drug: Comparison to DDT and hexachlorobenzene

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Summary

Penclomedine (NSC-338720) is a low melting point, poorly water soluble cytotoxic agent with good solubility in triglycerides and a high octanol/water partition coefficient. Because of these physical properties, an attempt was made to assess the role that intestinal lymphatic transport plays in the systemic absorption of penclomedine and to compare its behavior to those of DDT and hexachlorobenzene, two other xenobiotics with somewhat similar properties. After intraduodenal administration to anesthetized rats as either a 10% o/w emulsion prepared from tributyrin, trioctanoin, triolein, soybean oil or mineral oil, or as a suspension, the rank order of lymphatic transport for penclomedine was soybean oil ≈ triolein > trioctanoin ≈ mineral oil ≈ tributyrin ≈ suspension. Correcting for bioavailability, approx. 3% of the absorbed dose was lymphatically transported for both soybean oil and triolein emulsions. Less than 0.75% of the absorbed dose was transported after administration in the other vehicles. The low percent of the absorbed dose transported in the intestinal lymphatics may be related to the high extent of plasma protein and red blood cell binding of penclomedine and lower partition coefficient compared to DDT, whereas the low degree of lymphatic transport of hexachlorobenzene may be related to its more limited water and lipid solubility combined with considerable plasma protein and red blood cell binding. A high degree of plasma protein and red blood cell binding is proposed to compete with chylomicron affinity in the dynamics of intestinal lymph versus portal blood transport.

Introduction

The lymphatic contribution to the overall systemic transport and bioavailability following oral administration of a compound has been of interest for many years (Bollman et al., 1948; Bloom et al., 1950; Greenberger et al., 1966; Sylven and Borgstrom, 1969; Gallo-Torres, 1970; Sieber and Cohen, 1974; Chu and Hegsted, 1980; Deak and Csaky, 1984; Muranishi, 1989; Noguchi et al., 1985; Palin, 1985; Charman and Stella, 1986a,b; Charman et al., 1986). The physicochemical properties of the compound and the type and quantity of co-administered vehicle have been shown to

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affect the total extent of lymphatic delivery (Sylven and Borgstrom, 1969; Chu and Hegsted, 1980; Palin et al., 1982; Deak and Csaky, 1984; Noguchi et al., 1985; Palin, 1985; Charman and Stella, 1986a,b). Most previous studies of lymphatic transport have not addressed the question of whether an increase in mesenteric or thoracic lymph transport by the manipulation of a suspected variable was due to a selective delivery to the intestinal lymphatics or an overall increase in bioavailability. The goals of this paper, therefore, were to determine the contribution of mesenteric lymph transport versus portal blood transport of the lipophilic drug, penclomedine (Myers and Stella, 1992), to observe any selective delivery to the mesenteric lymphatic system by various vehicles, and to determine the effects of physicochemical and biological properties on the lymphatic transport of a series of polychlorinated compounds (penclomedine, DDT and hexachlorobenzene).

Fig. 1 illustrates the various pathways whereby compounds incorporated into lipids may be handled by the body. For the short- or medium-chain triglycerides, the primary digestion products, fatty acids and monoglycerides, are transported in systemic circulation via the portal blood. They are not thought to be incorporated, to a great extent, in chylomicrons as re-esterified triglycerides (Castro, 1985). The longer chain fatty acids and monoglycerides from long-chain triglyceride digestion, after absorption, may be resynthesized into triglycerides via at least two pathways (Brindley, 1974; Johnston, 1978; Castro, 1985).

The re-formed triglycerides are incorporated into lipid microspheres, chylomicrons, having a core consisting of triglycerides, esterified cholesterol, and other fat-soluble components, are coated with apoprotein, lipoprotein, and free cholesterol. The chylomicrons are then secreted from the intestinal cell by an exocytosis process and transverse the basement membrane to enter the interstitial space and subsequently the lymph vessels (Mansbach and Arnold, 1985). The lymphatics are able to transport the chylomicrons due to the larger gaps between the cells of the lacteals preferentially to portal blood transport (Tso and Weidman, 1987). Lipid-soluble vitamins and other lipophilic compounds are generally transported in the lacteals of the mesenteric lymphatic system (Shiau, 1987) in association with the chylomicrons. Materials transported in this manner reach systemic circulation without passing through the liver (Fig. 2).

Several orally administered lipophilic compounds including DDT (Sieber et al., 1974; Palin

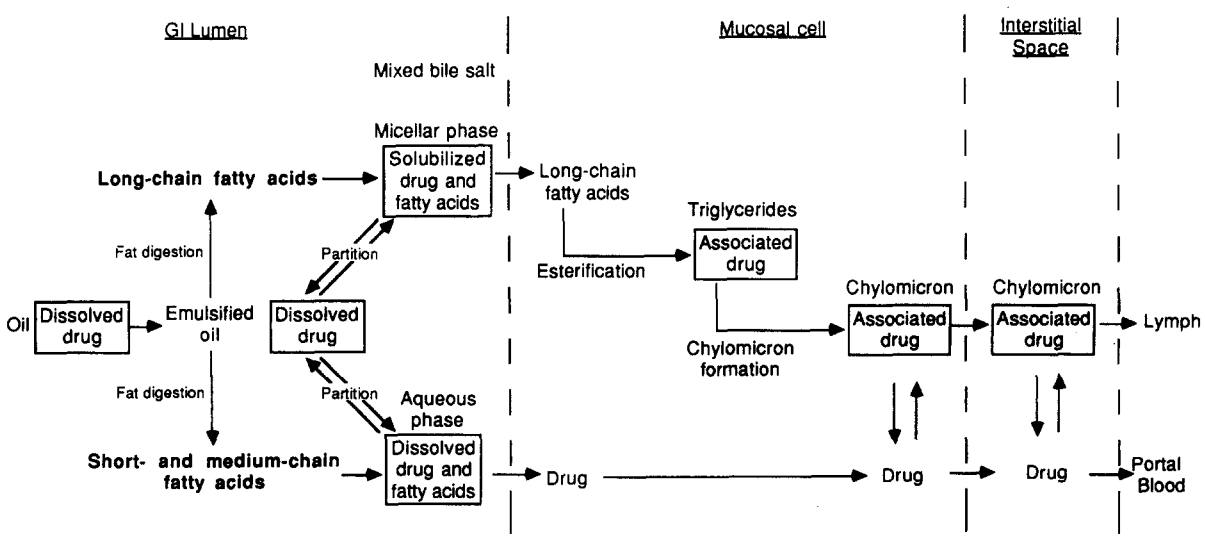

Fig. 1. Luminal and mucosal processing of an administered triglyceride oil containing dissolved drug.

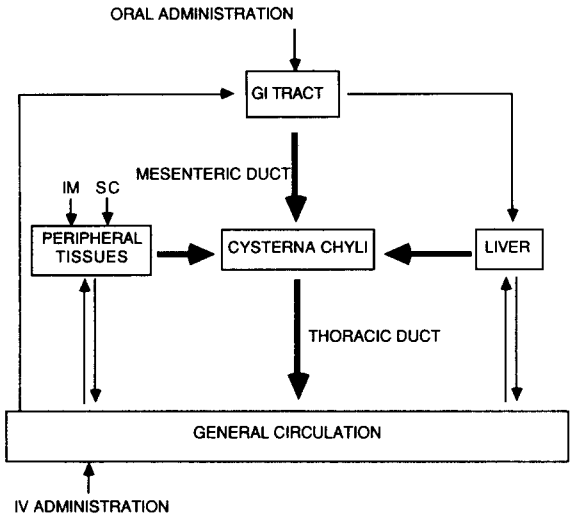

Fig. 2. Major circulation pathways. Narrow and bold lines represent blood and lymph pathways, respectively.

et al., 1982), and probucol (Palin and Wilson, 1984) have been shown to be selectively transported via the intestinal lymphatics. In the present study, DDT [high partition coefficient, good lipid solubility and good lymph transport (Palin et al., 1982; Patton et al. 1984; Charman and Stella, 1986a; Charman et al., 1986)] and hexachlorobenzene [high partition coefficient, low lipid solubility (Chiou et al., 1981; Patton et al., 1984; Leahy, 1986)] were studied along with penclomedine [lower partition coefficient, very good lipid solubility (Myers and Stella, 1992)] in an attempt to relate the importance of certain physicochemical properties to lymphatic delivery.

Experimental

Materials

All materials were identical to those defined earlier (Myers and Stella, 1992).

Methods

Solubility and partition coefficient A 2 ml vial with a teflon-lined cap containing 200 mg penclomedine and 0.5 ml oil [soybean oil, triolein (95%), trioctanoin (97–98%), tributyrin (99%) and light mineral oil] was placed into a shaking water bath maintained at 25 °C for 7 days. Each vial was then centrifuged and a 100 μl aliquot was diluted to 100 ml with acetonitrile and analyzed by HPLC. The evaluations of penclomedine solubility in each of the five oils were performed in triplicate.

An estimation for the log of the partition coefficient, log P, between octanol and water was accomplished with the use of an HPLC retention time method. In this method, structurally similar compounds with known partition coefficients were injected onto an HPLC system (see later discussion) and the retention times recorded. A plot of the log of the retention time vs the log of the partition coefficient yielded a linear relationship. The retention time of penclomedine was noted and compared to the standards. Benzene, naphthalene (both from Fisher Scientific, Fair Lawn, NJ), 2,3-dimethylnaphthalene (Columbia Organic Chemicals Co. Inc., Camden, SC), and hexachlorobenzene (Aldrich Chemical Co., Milwaukee, WI) were used as standards. Literature values for the partition coefficients of DDT and the other standards were obtained from Patton et al. (1984).

Red blood cell and protein binding A sample of 2 ml whole blood was spiked with the compound of interest and vortexed for 15 s. A 150 μl aliquot of the blood was taken and analyzed for total drug. The remaining blood was centrifuged at 2000 rpm for 15 min to separate the plasma from the red blood cells. A 150 μl aliquot of the plasma was taken and analyzed for total drug. The remaining plasma was placed in an ultrafiltration device (model MPS-1 with YMT membrane, Amicon, Danvers, MA) and centrifuged at 2000 rpm for 60 min. A 200 μl aliquot of the filtrate was taken and analyzed for total drug.

Dosage form preparation All emulsions and the suspension used in the present study have been defined (Myers and Stella, 1992). In this study, the concentrations of penclomedine, DDT and hexachlorobenzene in the final 10% emulsions were 10, 5 and 0.5 mg/g, respectively.

Animal experimental protocol Lymphatic experiments were conducted with anesthetized rats weighing approx. 300 g after receiving an intraduodenal drug administration of 0.5 g of emul-

sion at a rate of 0.85 ml/h (Syringe pump, Sage Instruments, model 341A, Cambridge, MA). After the start of an infusion of the drug formulation, lymph was collected at intervals of 1 h from the mesenteric lymph duct and analyzed for drug content.

Lymph levels attained after the administration of the penclomedine formulations were used to determine the extent of delivery to the mesenteric lymph from these formulations. These experiments were also designed to show possible selectivity or optimal delivery to the lymphatics from the various formulations.

Blood sampling from the jugular vein was performed simultaneously with lymph sampling. Sampling times and blood analysis for intact penclomedine were the same as those described earlier (Myers and Stella, 1992).

Lymph levels obtained after the administration of the DDT and hexachlorobenzene formulations were used to determine the extent of delivery to the mesenteric lymph from these formulations. This information was used in conjunction with the red blood cell/plasma binding data to determine the effect of the physicochemical properties of the administered drug on lymphatic delivery.

Specific procedures All rats used in the experiments were fasted for 24 h prior to each experiment with free access to water. The animals were anesthetized for the duration of the experiment using 50 mg/kg sodium pentobarbital given by an intraperitoneal injection. Additional injections were given as needed (approx. every 2 h).

The animals were shaved from ventral midline to dorsal midline on the animal's right side and also on the ventral side of the neck. With the rat on its back, both of the front legs were taped to the table on the animal's left side. A 7–8 cm lateral incision was made 1 cm below the ribs from the animal's right side to the ventral midline cutting through both skin and muscle layers. The intestines were gently pushed towards the animal's left side (into the body cavity). A $2 \times 2 \times 7$ cm piece of cotton was placed into the body cavity to hold the relocated intestines in place. A pair of 4 inch pointed forceps were then placed into the fat pad beneath the right kidney. The forceps, with tips together, were gently pushed through the fat, under the vena cava, raising the peritoneal membrane on the opposite side of the vena cava. Once the membrane had been raised, it was cut to leave the mesenteric artery and the mesenteric lymph duct exposed. While the forceps are under the vena cava the lymphatic cannula can be pulled through. A syringe, filled with heparinized saline (200 U/ml), was used to flush the cannula to help prevent clotting of the lymph. Carefully, only the top of the lymph duct was cut. (The mesenteric lymphatic duct is usually located to the left, anterior, of the mesenteric artery.) The polyethylene end of the cannula was inserted into the lymph duct approx. 3–4 mm. At this time, the syringe can be removed temporarily to check for lymph flow. Any auxiliary lymph ducts to the right of the artery were cut to ensure all lymph flow was into the main mesenteric duct. One drop of superglue was placed over the area to hold the cannula and to seal the auxiliary ducts. A 5×5 mm piece of muscle tissue, which had been cut from the abdominal wall, was placed over the superglue area to help secure the cannula and to prevent adhesions of the intestines. At this point, the cotton plug was removed from the abdominal cavity and the intestines were gently brought back to their original position.

The duodenal cannula was attached to a trocar and externalized through the abdominal wall. It was then inserted into a small hole made in the duodenum approx. 1 cm from the pylorus. The cannula was secured with one drop of superglue. The intraperitoneal cannula was inserted between sutures after the closing of the animal's abdominal muscle layers. The application of superglue during the skin layer closure secured the cannula.

A tracheotomy was performed by cutting a 5×5 mm hole in the skin above the trachea. The muscle layers were separated with two pairs of 4 inch tissue forceps to expose the trachea. A pair of 4 inch curved tip forceps were inserted under the trachea, a small incision was made on the top of the trachea and the cannula was inserted about 1.5 cm. Surgical suture was tied around the trachea to secure the cannula. The trachea was realigned to its original position and the skin was closed with superglue.

Drug administration In all cases, a period of at least 1 h was allowed prior to the administration of the drug formulations. This time was allowed to aid the recovery of the animals from the surgical procedures and to permit the intestinal motility to return to normal. The intraduodenal administration of 0.5 g of emulsion was performed by a Sage infusion pump at a rate of 0.85 ml/h through the duodenal cannula.

Hydration Since a substantial amount of body fluid is removed during the lymphatic experiments, the animals were administered a hydrating fluid. Normal saline was infused at a rate of 0.8 ml/h through the intraperitoneal cannula for the duration of the experiment.

Sampling, sample handling and analysis The lymph from the mesenteric lymphatic duct cannula was collected in 2 ml tubes containing 0.3 mg EDTA and 0.2 ml of normal saline with heparin (200 U/ml). The tubes were changed at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12 h after starting the drug infusion. From the lymph samples, a 150 μl aliquot was taken and placed into a 15 ml glass centrifuge tube. An appropriate internal standard spike in acetonitrile was added to each sample. DDT was used as the internal standard for penclomedine. Penclomedine was used as the internal standard for both DDT and hexachlorobenzene. After the internal standard spike, 1 ml of normal saline and 5 ml distilled diethyl ether were added. The tubes were then vortexed for 1 min. To separate the aqueous and organic layers, the tubes were centrifuged for 3 min at 2000 rpm. The tubes were then placed into a dry ice/acetone bath to freeze the aqueous layer. After a 30 s centrifugation, the ether layer was decanted into a clean 5 ml centrifuge tube and evaporated to dryness with a nitrogen stream. The samples were reconstituted with 100 μl of 75% acetonitrile in water and analyzed by HPLC. Blood samples, from the jugular vein, were handled and analyzed as described earlier (Myers and Stella, 1992).

A 20 μl aliquot of the reconstituted sample was injected onto a modular HPLC system. The HPLC system used for analysis included a Beckman 110B pump, a Perkin-Elmer ISS-100 Auto injector, and a Kratos 757 variable-wavelength detector operated at 243 nm. The separations of penclomedine, DDT and hexachlorobenzene from each other and the biological matrix were accomplished by using a reversed-phase C_8 column (15 cm $\times$ 4.6 mm, 5 μm particle size) with a mobile phase of acetonitrile : water (75 : 25) at a flow rate of 2.0 ml/min. Peak area measurements were performed by a Shimadzu CR-6A integrator. The peak area ratio of the compound of interest to the internal standard was determined and compared to the standard curve to determine the concentration of the sample.

Data handling The area under the curve for the blood sampling data was calculated from 0 to 12 h using the trapezoidal method. The absolute bioavailability of penclomedine with and without lymph drainage was approximated from the ratio of the average area under the curve from 0 to 12 h for an intraduodenal formulation to that of an intravenous formulation. Details of the specific protocols have been described previously (Myers and Stella, 1992).

Statistical analysis for experimental results where more than two sets of data are compared was performed by the use of the GT2 method (Hochberg, 1974). This method is used to compare means of multiple data sets of unequal sample sizes. In all cases, except where noted, statistical significance was determined at the 95% confidence limit ($p = 0.05$).

Statistical analysis for experimental results where only two sets of data were compared was performed by the use of the F-distribution method (Sokal and Rohlf, 1981). This method is used to compare means of two data sets of unequal sample sizes. In all cases, except where noted, statistical significance was determined at the 95% confidence limit ($p = 0.05$).

Results and Discussion

Solubility and partition coefficient

The solubility of penclomedine in water, water-saturated *n*-octanol and 95% ethanol has been reported to be < 0.5 μg/ml, 91 ± 1 mg/ml and 20–25 mg/ml, respectively, by Prankerd et al. (1988). The values for penclomedine solubility in various solvents used in the emulsions in this

study were found to be 177 ± 4 mg/ml (soybean oil), 175 ± 3 mg/ml (triolein), 232 ± 4 mg/ml (trioctanoin), 280 ± 4 mg/ml (tributyrin) and 108 ± 5 mg/ml (light mineral oil).

The log partition coefficient (log P), as reported here, is the logarithm of the relative affinity for the agent between octanol and water. Since the aqueous solubility of penclomedine is extremely low, measurement of the partition coefficient by conventional means becomes quite difficult if not impossible. The log P value calculated from the ratio of the solubility in water-saturated octanol to the aqueous solubility was found to be approx. 5.36. A plot of the logarithm of the retention time vs the logarithm of the octanol/water partition coefficient (log P), of a series of structurally similar compounds, specifically benzene (1.927 min, 2.13), naphthalene (2.507 min, 3.30), 2,3-dimethylnaphthalene (3.328 min, 4.40), and hexachlorobenzene (5.607 min, 6.53), was found to be described by Eqn 1.

log retention time

$$= 0.106 \log P + 4.80 \times 10^{-2} \quad (r^2 = 1.00) \qquad (1)$$

The retention time of penclomedine was determined to be 4.327 min resulting in an estimate of the log P of 5.48. Both of the methods (solubility ratios and HPLC retention properties) resulted in similar values.

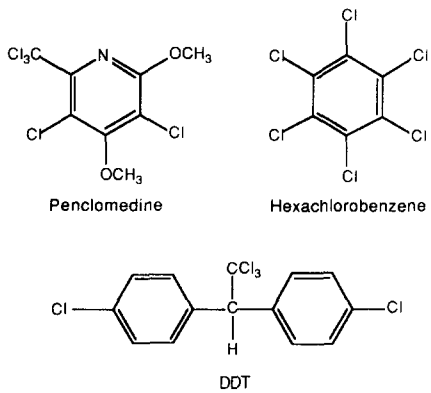

Fig. 3. Structures of penclomedine, DDT and hexachlorobenzene.

Red blood cell to plasma ratios and protein binding

The results from the binding studies for penclomedine, DDT, and hexachlorobenzene are listed in Table 1. For penclomedine and hexachlorobenzene, a higher degree of binding to the red blood cell fraction was seen when compared to DDT. In all cases, the amount of drug in the ultrafiltrate was well below the analytical detection limit. The lack of drug in the ultrafiltrate fractions indicates a high degree of binding to plasma proteins and red blood cells which may be expected from such hydrophobic, lipophilic compounds. The average hematocrit value of rat whole blood was found to be 0.448 ± 0.022 (n = 5). The concentrations of drug in whole blood

TABLE 1

Concentrations of drug found in whole blood, plasma, red blood cell fraction and the ratio of the red blood cell fraction concentration to the plasma concentration following a spike of compound to whole blood

Compound	[Whole blood] [c] (μg/ml) ± SD [f]	[Plasma] [d] (μg/ml) ± SD	[RBC] [e] (μg/ml) ± SD	[RBC]:[Plasma] ratio ± SD
PCD [a]	51.79 ± 3.21	12.42 ± 2.12	101.7 ± 17.4	8.19 ± 0.99
PCD	108.4 ± 9.62	31.02 ± 3.28	204.4 ± 21.6	6.59 ± 0.25
DDT	19.61 ± 1.78	28.43 ± 0.88	8.81 ± 0.27	0.31 ± 0.10
DDT	47.07 ± 2.76	51.18 ± 4.34	42.99 ± 3.64	0.84 ± 0.30
HCB [b]	18.84 ± 1.55	8.19 ± 0.40	32.10 ± 1.57	3.92 ± 0.51
HCB	31.21 ± 0.55	14.63 ± 1.10	51.94 ± 3.90	3.55 ± 0.32

[a] Penclomedine.
[b] Hexachlorobenzene.
[c] Whole blood concentration.
[d] Plasma concentration.
[e] Calculated red blood cell fraction concentration.
[f] Standard deviation (n = 3).

and plasma were determined experimentally while the red blood cell concentration was calculated from the whole blood and plasma concentrations and the hematocrit. These values are shown in Table 1. The ratio values were statistically different ($p < 0.05$) between compounds. The ratio values from the two different concentrations of each compound varied somewhat but were not statistically different. These values show that both penclomedine and hexachlorobenzene had higher affinity for red blood cells than plasma compared to DDT. The possible importance of these observations will be discussed later.

Penclomedine formulations

Lymph levels from intraduodenal formulations

When the penclomedine formulations were administered intraduodenally with the mesenteric lymphatic duct cannulated, the contribution of drug transport by the intestinal lymphatic system after absorption from the intestinal lumen could be determined. These experiments were intended to demonstrate whether optimal delivery to the lymphatics was specifically affected by any of the formulation variables, namely, the different oils compared to the suspension.

The results from the lymphatic cannulation experiments for all penclomedine formulations are shown as a plot of the cumulative amount of penclomedine found in the lymph vs time in Fig. 4. The points shown are the average of six determinations with standard error bars. As shown in Fig. 4, only about 80 μg, approx. 1.5%, of the administered dose was transported by the mesenteric lymphatic system over 12 h in the best cases (soybean oil and triolein).

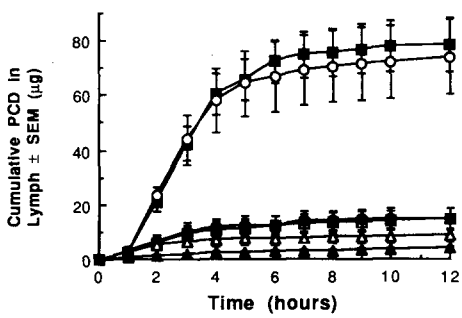

Fig. 4. Plot of the average (±SE) of cumulative amount of penclomedine (PCD) recovered from intestinal mesenteric lymph over 12 h vs time following intraduodenal administration of 5 mg penclomedine (PCD) from 10% o/w emulsions and a suspension to 300 g rats. Soybean oil ($n = 6$, ■), triolein ($n = 6$, ○), trioctanoin ($n = 6$, □), mineral oil ($n = 6$, ●), tributyrin ($n = 6$, △) and an aqueous suspension ($n = 6$, ▲).

The soybean oil and triolein emulsions gave a significantly higher ($p < 0.05$) cumulative amount of penclomedine transported in the intestinal lymphatics compared to other vehicles which is consistent with the observations of others (Sylven and Borgstrom, 1969; Palin et al., 1982). This is probably due to the ability of the long-chain triglycerides to form chylomicrons (Castro, 1985; Shiau, 1987). When more chylomicrons are formed, more 'carriers' are formed and thus more lipophilic material can be transported in association with the chylomicrons (Vost and Mclean, 1984). After drug absorption there is a competition for transport by the portal blood supply and the mesenteric lymph. Although there are both lymph ducts and capillaries in each villus, the blood has an advantage over the lymph since the rate of flow in the capillaries is about 500-times that of the lymph (Bollman et al., 1948; Reininger and Saperstein, 1957). Although the flow differential is great, the lymph may be able to transport more lipophilic compounds if they have a favorable affinity to chylomicrons, since the chylomicrons are preferentially transported in the lymphatic vessels (Castro, 1985; Shiau, 1987).

There is a general misconception that, once agents have become associated with chylomicrons, they stay associated with these lipoprotein particles until systemic circulation is reached. Both within the cell and within the interstitial space (that area between the mucosal cells and the various transport conduits, lacteals and capillaries), there is a dynamic or pseudo-equilibrium between chylomicron associated drug and non-chylomicron associated drug (it is probably incorrect to refer to this latter category of drug as 'free' drug because the drug may associate with other intra- and extracellular components) as illustrated in Fig. 1. Drugs and agents that are relatively weakly associated with chylomicrons may rapidly dissociate from the chylomicrons if a 'sink' is provided in the non-chylomicron phase.

The sink could be an affinity for proteins and tissue components present in this phase or the non-chylomicron associated drug is being continuously drained from this phase because of the rapid capillary flow and/or high capillary content affinity (plasma protein and red blood cell affinity). Effectively, this could be thought of as a skewing of the chylomicron to non-chylomicron pseudo-equilibrium.

The trioctanoin emulsion as well as the other formulations had significantly lower ($p < 0.05$) lymphatic transport. Trioctanoin is a medium-chain triglyceride which may not form a substantial amount of chylomicrons. Subsequently, there is very little transport by the intestinal lymphatic system. Mineral oil is a nonmetabolizable, non-absorbable oil. Its lack of lymphatic transport may be due to the inability to form any chylomicrons. The extremely low lymphatic transport seen with the tributyrin emulsion and the aqueous suspension may also be attributed to low or poor levels of chylomicron after administration of these vehicles. An alternative explanation for these results is that the low transport levels may also be due to a simple lack of absorption from the gastrointestinal tract since these formulations gave very low bioavailabilities (Myers and Stella, 1992).

Since penclomedine has a relatively high log P value of about 5.5 and very good lipid solubility, one may have expected fairly good lymphatic transport. The overall lack of substantial lymphatic transport was somewhat surprising and, therefore, was probed further.

Percent of absorbed dose in lymph One attempt to explain the low extent of lymphatic delivery of penclomedine was based on establishing the amount of the absorbed dose transported in the lymph. Such mass balance experiments have generally not been carried out by investigators, including those in previous studies from this laboratory, probing the role of variables affecting lymph transport. For example, a study of testosterone undecanoate (Noguchi et al., 1985) lymphatic delivery in rats showed that only approx. 0.5% of the administered dose was transported by the mesenteric lymphatics after oral delivery. However, the small amount transported in the lymph was the only portion of the dose that reached systemic delivery intact. Therefore, as a percent of the absorbed dose, 100% was lymphatically transported. Gallo-Torres (1970) obtained a similar result with α-tocopheryl nicotinate in the rat. Although only about 20% of the α-tocopheryl nicotinate dose was found in the lymph, this was shown to be the major route of transport. When the absolute bioavailability is used to determine the actual amount of the dose absorbed, the amount of the absorbed penclomedine transported in the lymph is about 3% for the soybean oil and triolein emulsions. A plot of the percent of absorbed dose transported in the mesenteric lymph vs time is shown in Fig. 5. Clearly, the majority of the penclomedine that is absorbed is transported to the general circulation by non-lymphatic pathways.

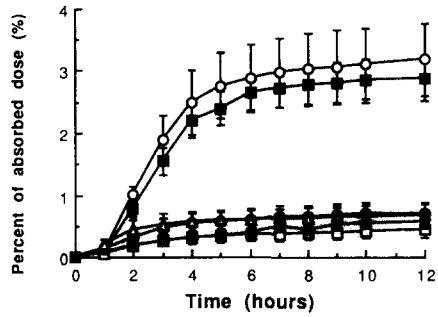

Fig. 5. Plot of the average percent (±SE) of absorbed penclomedine transported by the mesenteric lymph over 12 h vs time following intraduodenal administration of 5 mg penclomedine (PCD) from 10% o/w emulsions and a suspension to 300 g rats. Soybean oil ($n = 6$, ■), triolein ($n = 6$, ○), trioctanoin ($n = 6$, □), mineral oil ($n = 6$, ●), tributyrin ($n = 6$, △) and an aqueous suspension ($n = 6$, ▲).

Blood levels with or without lymphatic cannulation Intraduodenal administration of the different penclomedine formulations was given to mesenteric lymphatic duct cannulated animals and non-cannulated animals. In lymph drained animals, penclomedine input is composed of transport from only the portal blood since the mesenteric lymph was collected (drained) and analyzed separately (see schematic, Fig. 2). In the non-cannulated animals (no lymph drainage), it is possible to assess, independently of lymph levels, the possible contribution to lymph transport by the various vehicles. That is, if lymph transport

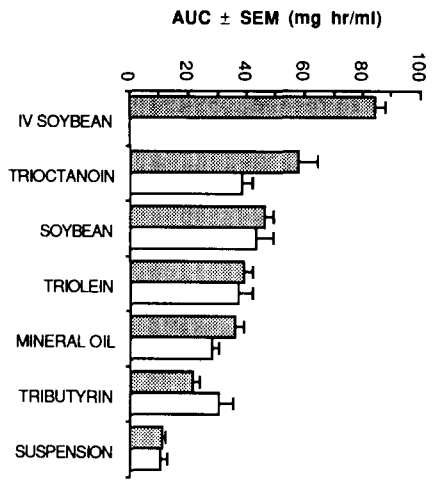

Fig. 6. Average areas (±SE) under the blood concentration vs time plots from 0 to 12 h after intraduodenal administration of 5 mg penclomedine from 10% o/w emulsions in 300 g rats with (shaded) and without (open) contribution from intestinal lymphatic transport.

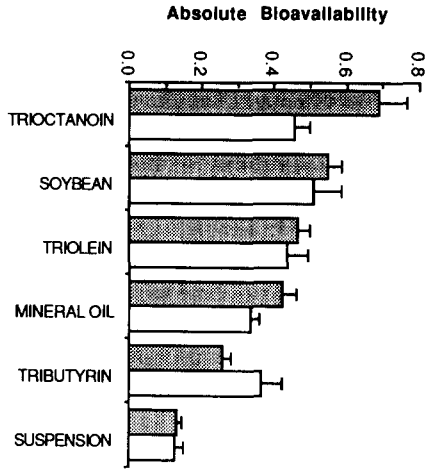

Fig. 7. Average absolute bioavailabilities (±SE) for intraduodenal administration of 5 mg penclomedine from 10% o/w emulsions in 300 g rats with (shaded) and without (open) contribution from intestinal lymphatic transport.

constitutes a significant component of the total amount of drug reaching systemic circulation, differences in the areas under the blood concentration time curves should result.

The areas under the blood concentration vs time curves in lymph drained and non-cannulated animals are shown in Fig. 6, while Fig. 7 shows the absolute bioavailability for all penclomedine preparations with and without the contribution of transport from the mesenteric lymphatics.

Except for the trioctanoin emulsion, there was no significant difference between the area under the curve values with or without the lymphatic contribution for any of the drug formulations. The etiology for the difference seen with the trioctanoin data is unknown. The absence of major differences in the area under the curves with and without lymph cannulation confirms the lack of substantial lymphatic transport seen with the lymphatic transport data.

Comparison of DDT and hexachlorobenzene with penclomedine

Physicochemical properties Some physicochemical properties of penclomedine, DDT and hexachlorobenzene are listed in Table 2. As can be seen, all of the compounds have very limited water solubility. Although hexachlorobenzene has the highest log P value, it also has the highest

TABLE 2

Selected physicochemical properties of penclomedine, DDT and hexachlorobenzene

Compound	Molecular weight	Melting point	Log P [a]	Aqueous [b] solubility	Triolein solubility
Penclomedine	325.41	80–80.5	5.48	300–500 [c]	175 [c]
DDT	354.49	109	6.19	1.2 [d]	80 [d,f]
Hexachlorobenzene	284.61	230	6.53	4.94 [d]	7.5 [d,f]

[a] Log partition coefficient, octanol/water.
[b] (ng/ml) at 25°C.
[c] Prankerd et al. (1988).
[d] Patton et al. (1984).
[e] Solubility (mg/ml) at 25°C.
[f] Solubility (mg/g) at 23°C.

melting point which indicates a relatively high degree of molecule/molecule interaction in the solid state (Valvani and Yalkowsky, 1980). This results in a low solubility in both water and the lipophilic solvent, triolein (Chiou et al., 1981; Patton et al., 1984; Leahy, 1986). This type of compound will generally be expected to behave poorly with respect to oral bioavailability. Penclomedine and DDT, on the other hand, have relatively low melting points, low water solubility (Wakita et al., 1986) and high triolein solubility (Patton et al., 1984). Generally, compounds with these properties have a greater probability of being absorbed but their bioavailability can be food sensitive (Hasegawa et al., 1981).

Lymphatic delivery When DDT and hexachlorobenzene formulations (10% o/w soybean oil emulsions identical to the penclomedine emulsion except for the concentration of drug) were administered intraduodenally with the mesenteric lymphatic duct cannulated, the contribution of drug transported by the intestinal lymphatic system after delivery to the gastrointestinal tract could be determined. Fig. 8 compares the amount of drug transported in the lymph for DDT, penclomedine, and hexachlorobenzene when administered as the soybean oil emulsion. DDT resulted in a lymphatic transport of about 15% of the administered dose whereas penclomedine and hexachlorobenzene had very limited transport.

The lack of lymphatic delivery of hexachlorobenzene can be explained by poor water

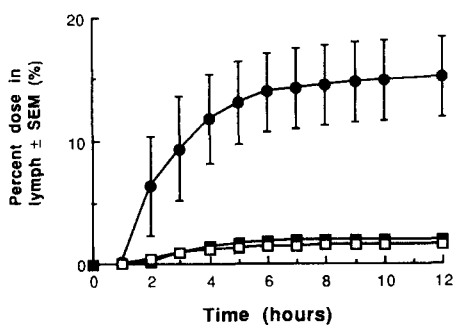

Fig. 8. Plot of the average percent of administered dose transported (±SE) by the mesenteric lymph over 12 h vs time following intraduodenal administration of 2.5 mg DDT ($n = 3$, ●), 5 mg penclomedine ($n = 6$, ■) and 0.25 mg hexachlorobenzene ($n = 3$, □) in 10% o/w soybean oil emulsions to 300 g rats.

and lipid solubility. That is, once released from any dosage form, the agent is poorly maintained in solution and probably precipitates in the intestinal tract, thus limiting absorption from the gastrointestinal lumen. These results were consistent with the earlier findings of Charman and Stella (1986b). Attempts to determine the absolute bioavailability of hexachlorobenzene from the emulsion were not successful due to the acute toxicity of intravenously administered hexachlorobenzene and the lack of measurable blood levels after oral dosing at the levels administered in this study. Ongoing studies in this laboratory and the work of Palin et al. (1982) have suggested that DDT systemic bioavailability may be limited by that fraction that is transported via the lymphatics.

The lack of lymphatic delivery for penclomedine was initially surprising. Since the bioavailability from the trioctanoin and long-chain triglyceride emulsions was found to be quite good, an explanation based on a lack of overall absorption seems invalid. Although penclomedine's lower partition coefficient is consistent with a lower extent of lymphatic transport compared to DDT, this would not seem to account for such a discrepancy. One explanation may be in the relative protein or red blood cell binding characteristics of the compounds. Penclomedine was shown to have an affinity for red blood cells 8-times greater than that for plasma. This may be important during the 'trafficking' of the penclomedine between the mucosal cells, through the interstitial fluid and eventually to the lymph versus portal blood (see earlier discussion). Although penclomedine is very lipophilic and should 'like' to associate with chylomicrons, the red blood cell affinity may cause a skewing or shift in the pseudo-equilibrium between chylomicron associated phase and non-chylomicron associated phase by providing a sink towards which the drug is drained. A quantitative way of representing this concept is to compare the relative flow of portal blood to lymph, 500:1. Assuming chylomicron flow is approx. 1% of lymph flow (Charman and Stella, 1986a), the relative chylomicron flow to blood flow would be about 50000:1 for an effective log partitioning of 4.70 based on flow consid-

erations alone. If the drug has a high affinity for red blood cells relative to plasma, not only must the agent have a high affinity for chylomicrons, but it must also overcome the added affinity for the red blood cells as well as plasma protein binding (also seen with DDT). The lower log partition coefficient of penclomedine relative to DDT (about 0.7 log units) and the higher red blood cell to plasma ratio, 8 (log 8 = 0.9) means that the effective log partition coefficient difference between DDT and penclomedine is 1.6 and not 0.7. Therefore, penclomedine acts like a compound, relative to DDT, with a log partition coefficient of 4.6 when corrected for its affinity for red blood cells. The statement obviously assumes that both DDT and penclomedine result in similar extents of plasma protein binding, a fact not verified at this point.

In conclusion, the question of whether an increase in the lymphatic transport of a compound from one vehicle compared to another was due to an overall increase in bioavailability or due to a selective delivery to the intestinal lymphatics was addressed for the experimental drug, penclomedine. The results of this study clearly point to the latter explanation as the long-chain triglycerides tend to promote lymphatic transport of penclomedine compared to trioctanoin which promotes bioavailability.

The lack of substantial transport of penclomedine in the intestinal lymphatic system indicates that factors other than partitioning and solubility may be involved in determining the degree of lymphatic transport of lipophilic compounds. The high degree of binding of penclomedine to red blood cells and/or plasma proteins could explain these results. Not only does a compound require a high partition coefficient along with good lipid solubility, but it also appears that the extent of protein and red blood cell binding must be considered when attempting to predict lymphatic transport.

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