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Testing potential dosage form strategies for intestinal lymphatic drug transport: studies in the rat

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Summary

The effect of some differing formulation strategies on the intestinal lymphatic transport of DDT has been evaluated. Drug–lipid vehicle combinations were administered to conscious rats by the peroral route, or to anesthetized rats by intraduodenal infusion. A 2 mg dose of DDT was dissolved in either 200 μl of peanut oil (triglyceride) or 200 μl of oleic acid (fatty acid) and administered to mesenteric lymph duct cannulated rats. During the post-dosing period, there were no significant differences in the percent dose of DDT collected in intestinal lymph when administered in peanut oil by either the peroral (24.3 ± 3.0% dose) or intraduodenal route (19.9 ± 1.8% dose). When the same dose of DDT was administered in oleic acid, greater lymphatic transport occurred when the DDT–lipid vehicle combination was administered intraduodenally (35.7 ± 2.1% dose), rather than perorally (16.6 ± 3.3% dose). It is postulated that these differences may be due to differing effects of the fatty acid and triglyceride lipids on gastric emptying and/or intestinal motility. When considering that a drug product can release a drug–lipid combination at different sites within the gastrointestinal tract (stomach or small intestine), these results may have a bearing on potential formulation strategies and dosage form design for the enhancement of the lymphatic transport of lipophilic drugs. Simulated sustained release preparations using a 200 μl/8 h infusion of peanut oil containing 2 mg of DDT did not afford significantly enhanced lymphatic transport of DDT when compared to a 200 μl/2 h infusion of the same drug–lipid combination. There was a large amount of variation in the lymphatic transport of DDT administered at the slower rate of infusion. When animals were pre-dosed with 75 μl of peanut oil prior to infusion of 2 mg DDT in 200 μl over 8 h, there was much less variability in the lymphatic transport of DDT. It appears that there may be an optimal rate of lipid input into the small intestine to trigger, or initiate, significant lymphatic transport of lipophilic molecules.

Introduction

The experimentally observed rate and extent of intestinal lymphatic transport of suitably lipophilic drugs in the rat is dependent upon various factors. These include: (i) the fasting state of the animal and the experimental procedure utilized to estimate transport (Charman et al., 1986a); (ii) the site of cannulation of the lymphatic system (Noguchi et al., 1985); (iii) the volume of administered lipid vehicle (Yamihara et al., 1978; Laher et al., 1984); and (iv) the class of lipid in which the drug was administered (Charman and Stella, 1986b).

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Another potentially significant factor is the site of gastrointestinal administration of a drug–lipid vehicle combination. Studies have been conducted in rats to evaluate the effect of drug–lipid vehicle administration at the level of either the stomach, by peroral dosing, or the small intestine, via intraduodenal administration, on the lymphatic transport of a model compound, DDT. DDT was used in these studies due to its high degree of lipophilicity and relative metabolic stability in the rat (Palin et al., 1982; Noguchi et al., 1985). Administration of drug–lipid vehicle combinations at differing sites within the gastrointestinal tract is analogous to the rupture of a dosage form at different sites within the gastrointestinal tract.

If a lipophilic drug were to be formulated for intestinal lymphatic transport, the dosage form may well contain a lipid composed of long chain fatty acids. The basis for the inclusion of this type of lipid is that long chain fatty acid vehicles are required for the synthesis and formation of chylomicrons within the intestinal cells. The chylomicrons appear to be intimately related to the lymphatic transport process for lipophilic compounds from the small intestine (Sieber, 1976; Vost and Maclean, 1984; Charman et al., 1986a, 1986b). Two potential choices of a lipid are either a long chain fatty acid such as oleic acid, or a triglyceride such as peanut oil.

As the drug–lipid vehicle combination was administered into either the stomach or small intestine, the potential effect of gastric emptying on the intestinal lymphatic transport of the model compound could be indirectly appraised. It is well known that lipids can affect gastric emptying and intestinal motility (Cooke, 1975; Malagelada, 1981) although the mechanism by which these effects occur is not fully understood.

An additional dosage form strategy investigated in these studies was a sustained release drug–lipid vehicle system. This type of dosage form was simulated in the rat by an 8 h infusion period of the lipid vehicle (containing DDT) and measurement of the resulting lymphatic transport of DDT. For these simulated sustained release studies, peanut oil was utilized as the lipid vehicle and was infused into the duodenum of anesthetized rats.

These experiments represent biopharmaceutical investigations performed in an attempt to evaluate various potential dosage form strategies designed to promote the intestinal lymphatic transport of a lipophilic drug.

Materials and Methods

Chemicals and animals

The sources of chemicals and animals used in this study were identical to those described by Charman et al. (1986a).

Surgical procedures

The mesenteric lymph duct of male Sprague–Dawley rats was cannulated according to our previously described procedure (Noguchi et al., 1985). Tracheal cannulations were performed to facilitate the breathing of anesthetized rats during the intraduodenal administration of drug and lipid. Anesthesia was maintained with 2 hourly 50 mg/kg intraperitoneal injections of pentobarbital sodium (D-M Pharmaceuticals, Sellersville, PA 18960, U.S.A.). Intraduodenal administration of drug and lipid was performed via a J-shaped, heat-molded segment of polyethylene tubing (Intramedic PE 50, Clay Admas, Parisappany, NJ 07054, U.S.A.) 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

Site of administration

Two separate procedures were used in preparing the animals for drug administration. For intraduodenal administration of drug and lipid, the animals were maintained under constant anesthesia and underwent sequential tracheal, lymphatic and duodenal cannulation. After completion of the surgical procedures, the animals were transferred to, and secured on, a heated pad maintained at 37°C (Clinical Scientific Equip-

ment, Melrose Park, IL 60160, U.S.A.). A continuous intraduodenal infusion of normal saline containing 0.2% Tween 80 at 1.44 $ml \cdot h^{-1}$, via a syringe pump (Sage Instruments, Cambridge, MA 02139, U.S.A.) was begun that maintained body hydration and intestinal lymph flow. Three hours later, 2 mg DDT dissolved in 200 μl of either peanut oil or oleic acid was infused, by another infusion pump, over a 2 h period through a T-piece connector (Technicon Instrument Corporation, Tarryton, NY 10591, U.S.A.) into the saline/Tween 80 solution flowing into the duodenal cannula. Intestinal lymph was collected for 12 h from the time of dosing initiation. For a detailed description of these procedures, refer to Charman et al. (1986a).

When drug and lipid (200 μl) were to be administered perorally by gastric intubation, the intestinal lymphatics were cannulated under pentobarbital anesthesia. Just before arousal from the anesthetic, the rat was placed in a jacket which held a collection bottle for the externalized cannula (Noguchi, 1977). The jacket was secured by ties along the back of the rat and allowed free unrestricted movement. Lymph was collected in the bottle which also contained a 1 ml aliquot of heparin–saline solution (200 units/ml saline). The collection bottle was changed every 4 h after drug administration and lymph was collected for 24 h post-dosing. During this time, the animals had free access to drinking water.

Simulated sustained release

Animals were maintained under constant pentobarbital anesthesia and underwent tracheal, lymphatic and duodenal cannulation as previously described. Three hours after completion of these surgical procedures, an 8 h infusion of 2 mg DDT dissolved in 200 μl of peanut oil was begun and the lymph was collected for 12 h post-dosing.

In some experiments, the animals were initially pre-dosed with 75 μl of peanut oil over a 2 h period which was administered 3 h after completion of the described surgical procedures. Four hours subsequent to the beginning of the pre-dosing schedule, an 8 h infusion of 200 μl of peanut oil containing 2 mg of DDT was begun and lymph was collected for the following 12 h.

DDT analysis from lymph

Quantitation of DDT in hourly lymph samples was performed as previously described (Charman et al., 1986a).

Results and Discussion

The current study was designed to test potential dosage form strategies for facilitation of the intestinal lymphatic transport of lipophilic drugs. The strategies investigated were: (i) whether the site of administration of a drug–lipid combination in the gastrointestinal tract affected the lymphatic transport of a model compound; and (ii) whether simulated sustained release preparations of a drug–lipid combination promoted lymphatic transport of the drug.

Site of administration

DDT was dissolved in either a fatty acid (oleic acid) or a triglyceride (peanut oil) and administered by either peroral or intraduodenal routes. Oleic acid is the major fatty acid constituent of peanut oil (Windholz and Budavari, 1983). Bolus peroral dosing of the rats was designed to represent the release of DDT and lipid from a dosage form which emptied its contents in the stomach. Perorally administered drug, and lipid, would potentially be subject to gastric emptying effects of the stomach, whereas the intraduodenally administered drug and lipid would not be subjected to these physiological effects. The intraduodenal administration, of the same lipid and dose of DDT, represented the release of the contents of a dosage form in the small intestine.

Conscious rats were utilized for the experiments involving peroral administration of DDT and lipid, and anesthetized rats were used for the collection of lymph from animals which underwent intraduodenal administration of DDT and lipid. It is not ideal to compare and contrast data collected from both conscious and anesthetized animals. However, unless the described protocols were followed, it would not have been possible to otherwise readily perform these experiments since the effects of anesthesia on gastric emptying rates are unknown. The following interpretation of the

reported data must be tempered with this restriction in mind.

When the drug–lipid vehicle combination was administered intraduodenally to anesthetized rats, the appearance of DDT in the intestinal lymph was followed for 12 h post-dosing. At this time the cumulative amount of DDT transported was approaching a maximal value and it was not deemed necessary to prolong the collection of lymph.

Subsequent to the peroral administration of drug and lipid to conscious rats, lymph was collected for 24 h, although the major fraction of the DDT transported in the intestinal lymph occurred over the first 12 h. Samples of lymph were collected at four hourly intervals as it was not practical to collect hourly lymph samples from conscious unrestrained rats.

Fig. 1 describes the time course of appearance of DDT in intestinal lymph when administered by peroral or intraduodenal routes. Most notable is the similar lymphatic transport of DDT when

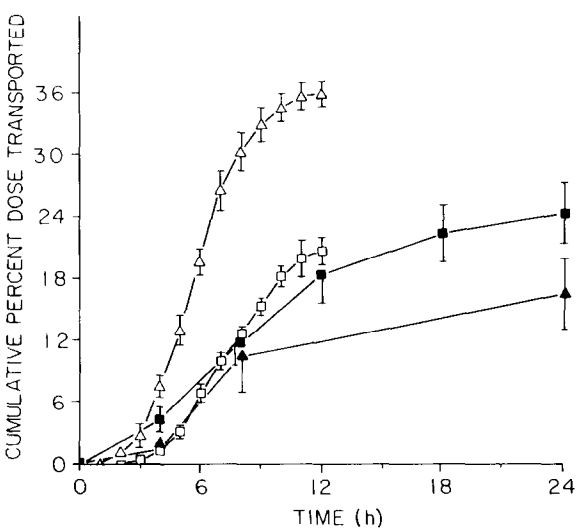

Fig. 1. Cumulative percent dose of DDT (mean ± S.E., $n \geq 4$ animals) collected in intestinal lymph as a function of time post dosing initiation. Dose was 2 mg DDT dissolved in 200 μl of lipid. Perorally administered DDT–lipid was administered by gavage to conscious rats, and intraduodenally administered DDT–lipid was by infusion to anesthetized rats. ▲———▲, oleic acid vehicle (perorally); ■———■, peanut oil vehicle (perorally); △———△, oleic acid vehicle (intraduodenally); □———□, peanut oil vehicle (intraduodenally).

administered in peanut oil by either the peroral (24.3 ± 3.0% dose, 24 h) or intraduodenal route (19.9 ± 1.8% dose, 12 h). In contrast, when DDT was administered in oleic acid by either of the two routes used in the present study, the quantities of DDT that were recovered in the intestinal lymph were markedly different. There was 16.6 ± 3.3% (24 h) of the administered DDT dose collected in intestinal lymph when administered by the peroral route as opposed to 35.7 ± 2.1% (12 h) of the dose recovered when the DDT was administered intraduodenally.

The intraduodenal administration of DDT, in either of the lipid vehicles, circumvented potential gastric emptying effects on lymphatic transport. Consequently, this data probably best reflects the intrinsic effects of the differing lipids on lymphatic transport from the small intestine. The potential basis for the observed differences in the lymphatic transport of DDT when administered intraduodenally in either of the two lipids may be due to the necessity for preabsorptive hydrolysis of the triglyceride. This point has been discussed in greater detail elsewhere (Charman and Stella, 1986b).

Interpretation of the differences in the lymphatic transport data when administered at the different sites within the gastrointestinal system could be approached from a number of perspectives. The simplest is that differences in gastric emptying of the fatty acid and peanut oil vehicles were responsible for the observed differences. Lipids, irrespective of their chemical nature, will delay gastric emptying (Malagelada, 1981; Cortot et al., 1981). The basis for this may be viscosity induced (Ehrlein and Prove, 1982), receptor mediated (Hunt and Knox, 1967; Keine and Ehrlein, 1983), a neurogenic control mechanism (Cooke, 1975) or a combination of any of these factors. However, fatty acids, rather than triglycerides, appear to specifically stimulate receptors in the upper portion of the duodenum that induce an overall decrease in the rate of evacuation of the stomach contents (Keine and Ehrlein, 1983). Since the kinetics and dynamics of the lymphatic absorption and transport process appear to be critical factors governing the overall lymphatic transport of a particular compound (Charman and

Stella, 1986b), the delayed appearance of drug and lipid in the duodenum could account for the observed differences.

Portal blood flow acts as an absorption sink, competing with the intestinal lymph, for the absorption of DDT. The portal blood flow is approximately 500 times greater than the flow of intestinal lymph (Bollman et al., 1948; Reininger and Saperstein, 1957). Therefore, the longer that lipid, and associated drug, remain in the stomach and the lumen of the small intestine, the less will be the relative concentration of drug per unit volume of lipid. This is because long chain fatty acid constituents of the administered lipids can only be transported by the lymphatic system in the form of resynthesized triglycerides, whereas the administered DDT could be absorbed into either the lymphatic system or the portal blood. The phenomenon may then lead to a decrease in the quantity of drug transported in the intestinal lymphatic system. The lack of effect of the differing sites of administration of the peanut oil on the eventual lymphatic transport of DDT could be explained if the 100 μl/h intraduodenal infusion rate was similar to the actual gastric emptying of triglyceride from the stomach. The fatty acid receptor mediated inhibition of gastric emptying (Hunt and Knox, 1968; Keine and Ehrlein, 1983) would only be apparent with a triglyceride vehicle when fatty acids resulting from the lumenal hydrolysis of the triglyceride were present at some particular or critical concentration.

Potential dosage from strategies regarding lymphatic transport of a lipophilic drug can be gained from these data. Assuming that maximal lymphatic transport of a particular lipophilic drug was desired, and the drug was formulated in a fatty acid vehicle, the current data suggests that the dosage form should rupture and release the lipid and drug in the small intestine. If the lipid vehicle was a triglyceride, then the site of release of the drug and lipid from the dosage form is not critical with respect to optimal lymphatic transport of the drug.

Simulated sustained release studies

The effect of a simulated sustained release preparation on the lymphatic transport of DDT was investigated with a view to evaluating its effect on intestinal lymphatic drug transport. The experiments involved prolongation of the 2 h infusion period to 8 h.

Fig. 2 describes the cumulative percent of the DDT dose transported in lymph as a function of time. The dashed line represents the lymphatic transport of DDT when dissolved in 200 μl of peanut oil and infused intraduodenally over a 2 h period (reproduced from Fig. 1) and is included as a reference point. Eight rats were infused with DDT in 200 μl of peanut oil over an 8 h period. There were apparently two groups of data in the cumulative transport of DDT, and these are shown separately in Fig. 2. Five of the 8 rats in the study transported only small quantities of the DDT in the 12 h post-dosing initiation period ($8.2 \pm 1.5\%$ dose), and three demonstrated much greater drug transport ($35.2 \pm 0.6\%$ dose). The integrity of implanted cannula in each animal was checked at the termination of each experiment, and lymph flow was consistent for all rats (cumulative 12 h lymph

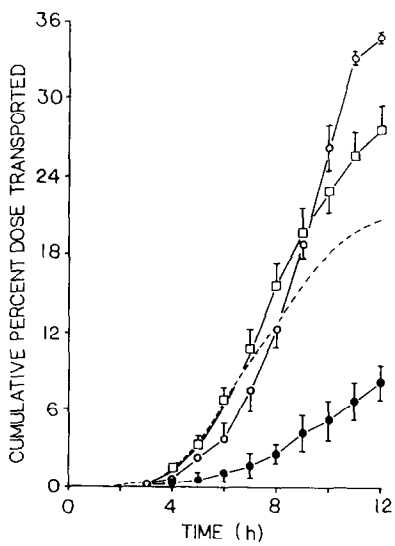

Fig. 2. Cumulative percent dose of DDT (mean $\pm$ S.E., $n \geq 4$ animals) collected in intestinal lymph as a function of time post-dosing initiation. Dose was 2 mg DDT dissolved in 200 μl of peanut oil infused intraduodenally. - - - - - -, 2 h infusion data included as a reference point; ○———○, 8 h infusion period to a group of high transport rats ($n = 3$) and, ●———●, to a group of low transport rats ($n = 5$); and □———□, pre-dosing of animal with 75 μl of peanut oil prior to administration of the 8 h infusion of DDT and peanut oil.

flow, mean ± S.E., high transporters: 8.63 ± 0.69; low transporters: 7.41 ± 0.70). The reproducibility of the % dose of DDT transported within the high and low groups, combined with the consistent lymph flow for the two groups of rats suggests that these results are probably not artifactual.

The basis for the differences in DDT transport between the two groups of rats which underwent the 8 h infusion appears to be due to differing degrees of lymphatic chylomicron transport. Chylomicrons are a class of intestinal lipoproteins, and are the major triglyceride transporting lipoproteins of the small intestine. Lymphatic transport of DDT has been shown to correlate with chylomicron formation and transport (Charman et al., 1986a), as the transported DDT appears to be solubilized in the triglyceride core of the chylomicrons (Sieber, 1976; Vost and Maclean, 1984). Using cumulative optical density measurements of diluted lymph samples as an indication of chylomicron flux (Charman et al., 1986a), there was 2.1 ± 0.2 (mean ± S.E.) times greater chylomicron flux in the lymph of the rats which transported 35.2% of the administered DDT dose as compared to the low transport (8.2% of dose) group of rats. The relative concentration of DDT per unit of chylomicron lipid in the lymph from the group of high transport rats, relative to the low transport group of rats, was 2.2 ± 0.2 (mean ± S.E.). The 4.3-fold difference in the lymphatic transport of DDT between the two groups of rats which underwent the 8 h infusion of DDT and lipid is consistent with the 4.6-fold difference in the chylomicron based transport of DDT, i.e. 2.1 times greater quantity of chylomicron lipid multiplied by a 2.2 times greater concentration of DDT per chylomicron. It appears that the basis for the two groups of rats exhibiting different lymphatic DDT transport is due to differing degrees of chylomicron transport and loading of DDT per chylomicron.

One possible explanation for this phenomenon is the need for some critical or necessary rate of lipid input to the small intestine in order to initiate, or trigger, chylomicron formation and transport necessary for the lymphatic transport of lipophilic drugs. The basis for this proposed triggering could be at either the initial preabsorptive triglyceride hydrolysis step or any of the many other biochemical processes involved in chylomicron formation.

This hypothesis was partially tested by pre-dosing rats with a 75 μl dose of peanut oil (not containing DDT) over 2 h, waiting for a 2 h period, and then initiating the simulated sustained release experiment with the 8 h infusion of DDT in peanut oil. As depicted in Fig. 2, the lymphatic transport of DDT was consistent for all animals tested ($n = 5$), and 27.8 ± 0.6% of the dose was transported in the 12 h post-dosing period. The fact that an initial level of lipid in the intestine was sufficient to overcome the variability exhibited in the 8 h infusion experiments supports the contention of a critical rate of lipid input, or quantity, being potentially important for lymphatic drug transport. It may be that the 25 μl/h input rate of lipid into the small intestine is at a borderline value for the initiation of significant chylomicron production and subsequent lymphatic transport of lipophilic drugs.

The observation that the pre-dosing of the rat with a quantity of lipid is necessary to trigger consistent lymphatic transport of DDT may be analogous to the need for a patient to have a meal containing some fats prior to administration of a dosage form designed to promote lymphatic drug transport.

From a dosage form standpoint, this simulation of a sustained release preparation represents the best possible conditions for increased lymphatic transport as the drug and lipid are infused into the proximal duodenum. In reality, a sustained release product may not be as effective as this simulation because the product may release a portion of the dose in the intestine beyond where triglyceride hydrolysis and subsequent chylomicron formation occurs. There is an absorption window in the small intestine for the efficient intestinal lymphatic transport of lipids and lipophilic drugs (Borgstrom et al., 1957). Therefore, it does not appear that sustained release preparations,as simulated here in the rat, are likely to be significant potential dosage forms for the lymphatic transport of lipophilic drugs.

In conclusion, potential formulation considerations for lipophilic drugs that are absorbed by the

intestinal lymphatic system have been presented. These included evaluation of the class of lipid in which a drug was formulated (fatty acid or triglyceride) and the effect of administering the different lipid–drug formulations at different sites within the gastrointestinal tract. When DDT was formulated in triglyceride, the site of administration did not affect the lymphatic transport, in contrast to the effects on the lymphatic transport of DDT when administered in a fatty acid vehicle. Simulated sustained release experiments of DDT dissolved in peanut oil did not consistently promote the lymphatic transport of DDT.

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