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Lymphatic appearance of DDT in thoracic or mesenteric lymph duct cannulated rats

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

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

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Summary

Lymphatic absorption studies were conducted in rats using DDT (2,2-bis(*p*-chlorophenyl)1,1,1-trichloroethane; p,p-DDT) as a lymphatically absorbed model compound. The lymphatic appearance of DDT in either mesenteric or thoracic duct fistulated rats was followed after oral administration of 2 mg DDT in oleic acid (200 μl) per rat or intravenous administration of 1 mg DDT in Intralipid per rat.

For the oral studies, 2 differing protocols were followed that were concerned with evaluating the effect of the timing of lymph duct cannulations on the subsequent lymphatic transport of DDT.

The results of the study confirm that the lymphatic route is an important pathway for DDT absorption following oral dosing. Thoracic lymph was found to contain DDT absorbed directly into the lymph via the mesenteric lymph duct, as well as DDT absorbed via other routes, presumably portal blood. Significant amounts of DDT appeared in thoracic lymph following oral administration, even when the mesenteric lymph duct was occluded. Moreover, after intravenous administration, higher quantities of DDT appeared in thoracic lymph (3.6% of dose/24 h) than in mesenteric lymph (0.6% of dose/24 h).

It appears, therefore, that collection of thoracic lymph overestimates the extent of direct intestinal lymphatic absorption of DDT after oral administration. The timing of the lymph duct cannulation with respect to the fasted state of the animal appears to effect the rate and extent of lymphatic absorption of orally administered DDT.

Correspondence: V.J. Stella, School of Pharmacy, University of Kansas, Lawrence, KS 66045, U.S.A.

Introduction

The intestinal lymphatic pathway is generally considered a minor pathway of drug absorption, except for highly lipophilic molecules such as the natural lipids (Treadwell and Vahouny, 1968; Patton 1981), the lipid-soluble vitamins (Cornwell et al., 1962; Yeung and Von Saigent, 1972) and other lipoidal molecules (Kamp and Neuman, 1975; Sieber, 1976). For drug molecules that undergo significant pre-systemic metabolism, direct lymphatic absorption should in theory, bypass the liver, thereby improving the systemic delivery of these drugs.

Various protocols have been developed for estimating the extent of lymphatic absorption of drugs and other model compounds. Most studies attempting to evaluate the extent of intestinal lymphatic absorption of a particular drug have utilized thoracic duct cannulated animals (Sieber, 1976; Palin et al., 1982). However, the thoracic duct lymph drains not only the mesenteric lymph which originates solely from the small intestine, but also the hepatic and other peripheral lymph (Tilney, 1971). Therefore, the possibility exists that the collection of thoracic lymph will overestimate the intrinsic transport of a particular molecule via the intestinal lymphatic pathway. This possibility has also recently been noted by Blomhoff et al. (1984). In addition, De Marco and Levine (1969) found that a significant amount of *p*-aminosalicylic acid distributed into thoracic lymph following intravenous administration, while a similar observation has been reported with the undecapeptide, cyclosporin A (Ueda et al., 1983a and b), thus demonstrating the likelihood of a drug distributing between blood and thoracic lymph.

No reports, prior to this, have appeared in the literature that have attempted to evaluate whether thoracic or mesenteric lymph duct cannulations correctly estimate the extent of intestinal lymphatic transport of an orally administered lipophilic drug.

The timing of lymph duct cannulation has also been investigated by following two different methods. The most popular method in the literature (Method 1) involves initial lymph duct cannulation, followed by a 24-h, or longer, fasting period (McDonald et al., 1980; Green and Green, 1983) after which the drug in a suitable vehicle is orally administered. This procedure owes its popularity to the fact that lymph duct visualization and subsequent surgery is easier before the animal is fasted. The second procedure (Method 2) involves fasting the animal for 24 h prior to lymph duct cannulation. Following a recovery period of 3 h, the drug is then administered and the lymph collected (Blomhoff et al., 1984).

In the following studies, DDT was used as a model lymphatically absorbed compound as it is highly lipid-soluble and is well absorbed via the intestinal lymphatic system (Sieber, 1976; Pocock and Vost, 1974).

The aims of the present study were to evaluate: (a) whether the mesenteric or thoracic duct was the physiological correct site of cannulation for estimating the intrinsic amount of DDT absorbed by the intestinal lymphatic route; and (b) to observe the effect of the timing of lymph duct cannulation, and the fasting state of the animal, on the lymphatic transport of DDT.

Materials and Methods

Chemicals

The sources of the chemicals used in this study were as follows: DDT (Aldrich Chemicals, Milwaukee, WI 53233, U.S.A.), purity was listed as 99 + %, and was confirmed by DSC analysis using a Perkin-Elmer DSC-4 Differential Scanning Calorimeter (Perkin-Elmer, Norwalk, CT 06856, U.S.A.). Testosterone undecanoate (Research Plus Steroid Laboratories, Denville, NJ 07834, U.S.A.); oleic acid (NF, Fisher Scientific, Fair Lawn, NJ 07410, U.S.A.). Diethyl ether was used after glass distillation. Other chemicals were of analytical grade.

Animals

Male Sprague–Dawley rats were purchased from Sasco (Omaha, NB 68102, U.S.A.) and maintained on Purina Lab Chow and water ad libitum in our laboratory for at least 1 week. The housing conditions were 3 rats per cage (on Aspen bedding) maintained at 72°F with an air exchange rate of 10–12 changes per hour. The photoperiod was a 12-h light–dark cycle. Rats weighing 270–370 g were fasted for one day either before or after lymph duct cannulation.

Thoracic duct cannulation

Surgical procedures were performed under sodium pentobarbital anesthesia (40 mg/kg, i.p.). The thoracic duct was cannulated by the method of Bollman et al. (1948), with some modification, using polyethylene tubing (SP31, Dural Plastics and Eng., Dural, N.S.W. 2158, Australia) rinsed with heparin–saline solution (200 units/ml). The cannula was secured in place with a drop of instant adhesive (Loctite, Cleveland, OH 44128, U.S.A.) instead of a silk ligature. Attachment of silicone tubing (0.025 inch i.d., Elkay Products, Shrewsbury, MA 01545, U.S.A.) 5 mm from the end of polyethylene tubing supported the cannula. In some experiments, the mesenteric lymph ducts were intentionally disrupted with forceps and occluded with instant adhesive, while thoracic lymph was collected. The cannula was externalized through the abdominal wall.

After the surgical procedures were completed, the abdomen was closed with continuous sutures and the animals were placed on a heated pad maintained at 37°C. 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, that was secured by ties along the back of the rat, held the collection bottle and allowed the rat 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.

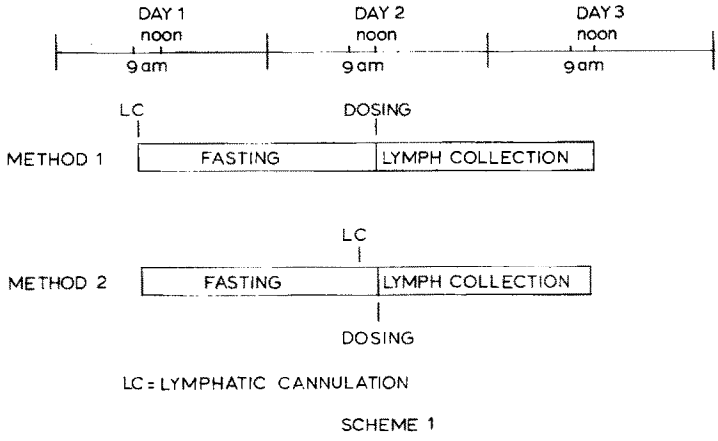
Mesenteric lymph duct cannulations

The mesenteric lymph duct was cannulated by a procedure almost identical to that described for the thoracic duct cannulation. In this case, the cannula was

introduced into the major mesenteric lymph duct. Any accessory mesenteric lymph duct(s) were intentionally disrupted with forceps and occluded with a drop of instant adhesive, in order to increase the return of lymph through the main lymphatic canal. This ensured the collection of all mesenteric lymph as it exited the small intestine (Warshaw, 1972). Two separate protocols were used in preparing the animals for drug administration. The time schedules, Methods 1 and 2 are outlined in Scheme 1.

Oral administration of DDT

2 mg DDT/200 μl oleic acid was administered to each rat by gastric intubation at the times indicated by Methods 1 and 2, as depicted in Scheme 1.



SCHEME 1

Intravenous administration of DDT

Intravenous administration was facilitated by initially dissolving DDT in the lipid phase of Intralipid-10% (Cutter Laboratories, Berkeley, CA 94710, U.S.A.). This was accomplished by dissolving 100 mg DDT in 4 ml of dimethylacetamide. A 400-μl aliquot of this solution was added dropwise, with adequate vortexing to 10 ml of Intralipid-10% (Repta, 1983). This formulation was used within 7 days of preparation, though neither degradation or precipitation of DDT was observed after storing this emulsion for 14 days at room temperature. One ml of this emulsion was injected into the femoral vein of rats whose lymphatic ducts had been cannulated after a 24-h fast, and the lymph subsequently collected for 24 h.

DDT analysis in lymph

An aliquot of 2 μg of testosterone undecanoate in 100 μl acetonitrile was added as an internal standard to a 1-ml lymph sample. The DDT and internal standard were co-extracted with 5 ml of ether, then centrifuged. After freezing the aqueous layer in a dry-ice–acetone bath, the supernatant was decanted into a centrifuge tube and the ether evaporated under a gentle stream of nitrogen. The residue was

suspended in 0.2 ml of 5% (w/v) NaCl solution and DDT was extracted with 0.1 ml of cyclopentanone. The extraction procedure was essentially quantitative. Recovery was 96.4 ± 2.8% (mean ± S.D.) for spiked samples analyzed in the range of 10–80 μg/ml added DDT. The coefficient of variations for samples analyzed ranged between 0.9 and 3.2%. The validity of the assay was established through a careful study of the linearity of response, reproducibility of standard curve and extraction recovery. Only DDT was analyzed in this system. An aliquot of the cyclopentanone solution was subjected to HPLC analysis under the following chromatographic conditions: pump model 110A, UV detector model 153 ($\lambda = 254$ nm) (Beckman, Berkeley, CA 94710, U.S.A.); column, Lichrosorb 10 RP18, E. Merck, New York (25 cm × 4.6 mm i.d.); mobile phase, tetrahydrofuran/acetonitrile/distilled water (30 : 45 : 25); flow rate, 2 ml/min. The retention volumes were as follows: DDT, 10 ml; internal standard, 18.8 ml.

Results and Discussion

The lymphatic appearance of DDT was measured after oral administration, (following either Method 1 or 2 in Scheme 1), of an oleic acid solution (2 mg DDT/200 μl oleic acid) to appropriately cannulated rats. These results, along with the intravenous data, are presented in Table 1.

In Method 1 and 2, 21.9 and 16.6%, respectively, of the orally administered DDT appeared in the lymph collected from mesenteric duct cannulated animals in the 24-h period post-dosing. This clearly demonstrated the direct lymphatic transport of DDT from the small intestine. Consistent with these data is the fact that only 0.6% of the intravenously administered dose of DDT was recovered after 24 h in mesenteric lymph.

Significantly greater quantities of DDT appeared in the thoracic lymph of animals as compared to the amounts of DDT recovered from mesenteric duct fistulated animals. Therefore, it would appear that significant quantities of DDT were able to reach the thoracic lymph by pathways other than the major intestinal lymphatic route. In considering the physiology of the lymphatic system in the rat, it may be postulated that the difference seen with these values after oral dosing may be due to the initial portal blood transfer of DDT from the small intestine to the systemic circulation from where it could redistribute into the thoracic lymph. Alternatively, DDT absorbed via the portal blood could gain access directly to the hepatic lymph that drains into the thoracic lymphatic system. Portal blood absorption of DDT has been demonstrated indirectly by Sieber (1976) who found that approximately 20% of an administered dose of DDT appeared in the bile of thoracic duct cannulated rats. It is hard to visualize the biliary appearance of orally administered DDT in these thoracic duct cannulated animals being due to any mechanism other than portal blood transport of DDT from the intestine to the liver. Consistent with our hypothesis of some initial portal blood transfer of DDT from the small intestine to the systemic circulation is the finding in the present study that

TABLE 1

LYMPHATIC APPEARANCE OF DDT USING DIFFERENT CANNULATION SITES AND ADMINISTRATION PROFILES

Route of administration	Method [a]	Cannulation site	Number of animals	DDT appeared (% of dose) [b]			Lymph flow (ml/24 h)
				0–4 h	0–8 h	0–24 h	
p.o.	1	Thoracic	3	14.3 ± 3.8	27.7 ± 3.0	32.3 ± 2.6 [c]	20.2 ± 4.2
p.o.	1	Thoracic with mesenteric occl.	3	10.3 ± 4.8	14.9 ± 6.3	21.5 ± 6.4	13.1 ± 2.6
p.o.	1	Mesenteric	3	13.0 ± 2.2 [e]	17.9 ± 2.3 [f]	21.9 ± 2.3 [c,d]	7.2 ± 0.9
p.o.	2	Mesenteric	6	1.3 ± 0.4 [e]	10.5 ± 3.7 [f]	16.6 ± 3.3 [d]	4.9 ± 0.9
i.v.	2	Thoracic	3	0.8 ± 0.8	2.1 ± 1.0	3.6 ± 1.3	14.1 ± 4.1
i.v.	2	Mesenteric	3	0.1 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	4.9 ± 1.4

Levels of statistical significance for single comparisons of means were calculated using the Student's t-test, and by the Studentized Newman-Keul procedure for the multiple comparison of means.

[a] See Scheme 1.

[b] Mean ± S.E.

[c] Significant at $\alpha = 0.05$.

[d] Not significant at $\alpha = 0.05$.

[e] $P < 0.001$.

[f] $P < 0.2$.

large quantities of DDT (21.5% of dose) still appear in the thoracic lymph of animals with occluded mesenteric lymph ducts. It should be noted that the possibility cannot be ruled out that some of the DDT appearing in the thoracic lymph of animals with occluded mesenteric lymphatics was due to the incomplete occlusion of the mesenteric lymphatics. However, the contamination of thoracic lymph with significant quantities of intestinal lymph is not consistent with the fact that the 24-h lymph flow of the thoracic lymph is approximately equal to the sum of the mesenteric and thoracic (with mesenteric occlusion) lymph flows. Greater amounts of intravenously administered DDT appeared in the thoracic lymph (3.6% of dose) of rats as opposed to the intestinal lymph (0.6% of dose). The ratio of % dose DDT recovered in thoracic lymph to intestinal lymph, following intravenous administration, closely reflects the ratio of the respective lymph flows suggesting that the transfer of DDT from systemic blood to lymph is a mass transfer process. The relative quantities of DDT transferred from blood to either intestinal or thoracic lymph will depend on factors such as regional lymph flows and the relative surface area of contact between blood and lymph in the regions of the body supplying the thoracic or intestinal lymphatics. Although these values are somewhat less than the 21.5% of the DDT dose recovered in the thoracic lymph of animals with occluded mesenterics, it is the relative amounts of DDT that appear in the thoracic and mesenteric lymph that are pertinent. The absolute quantities of drug recovered in the thoracic lymph probably would have increased if oil was orally administered when the DDT was dosed intravenously. The oral administration of oil would have provided large quantities of chylomicrons in the thoracic lymph which could act as a sink thereby promoting the blood to thoracic lymph transfer of the intravenously administered DDT. In total, these results suggest that the thoracic lymph contains significant quantities of DDT that were not initially absorbed via the intestinal lymphatic system, and that the collection of thoracic lymph seems to overestimate the true extent of the intestinal lymphatic absorption of orally administered DDT.

One of the major reasons thoracic duct cannulations, as opposed to mesenteric duct cannulations, are more popular in the literature is the relative difficulty in performing the respective surgery. However, by utilizing instant adhesive and making some methodological modifications, the cannulation of the mesenteric lymph duct becomes easier than that previously described (Lambert, 1965).

The comparison of the results of the two protocols, Methods 1 and 2 (see Scheme 1 and Table 1), suggests that there was a significant difference in the rates and extent of DDT recovery in mesenteric lymph during the first 8 h post-dosing. These observations are further exacerbated when post-surgery fasting for periods greater than 48 h occurs (Charman et al., 1985). These differences may be due to varying degrees of recovery from the surgery and/or to changes in the intestinal lipid metabolism, or homeostasis of the animal, that are triggered by the loss of proteins, electrolytes, etc., that are lost due to the lymph fistulation during the 24 h fasting (after the lymph cannulation).

The basis of these initial differences between the protocols is unknown. However, this, along with the determination of the critical factors governing the intestinal lymphatic absorption of drugs, are under extensive investigation in our laboratory.

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