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# Effect of Oily Vehicles on Absorption of Mepitiostane by the Lymphatic System in Rats

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**Abstract**—[$^{14}$C]Mepitiostane in various vehicles was administered to the small intestine of anaesthetized rats with cannulated thoracic ducts, and the effect of lipids on lymphatic absorption was examined. The extent of lymphatic absorption was greatest when administered in triolein and sesame oil, which are triglycerides of long-chain fatty acids. Absorption in the presence of other vehicles was in the order of 10% Tween 80 aqueous solution > monolein > oleic acid ≈ oleic acid/monolein (2:1 mol/mol) > aqueous suspension. Differences between the extents of lymphatic absorption of mepitiostane in the various formulations were not due to variation in the lymph flow but to the increased secretion of chylomicron and very low density lipoproteins. During absorption of mepitiostane from the small intestine, oil affected not only the penetration into epithelium cells and the metabolism in them, but also the partition between blood and lymph.

The intestinal absorption behaviour of various drugs is known to be affected by the presence of lipids. Griseofulvin (Carrigan & Bates 1973; Bates & Sequeira 1975; Grisafe & Hayton 1978), phenytoin (Chakrabarti & Belpaire 1978) and other lipophilic drugs (Wagner et al 1966; Abrams et al 1978) show enhanced bioavailability when administrered as an oil/water emulsion or an oily solution. Enhanced lymphatic absorption of *p,p'*-DDT (Sieber et al 1974; Palin et al 1982) and lipophilic steroids (Giannina et al 1966; Noguchi et al 1985) is also observed with the co-administration of lipids. Thus, the concomitant absorption of lipids appear to affect the lymphatic transport of drugs.

The present study was designed to investigate the effect of lipids on the lymphatic absorption processes (penetration through epithelium cells, metabolism in the epithelium cells, transfer into blood capillaries and the central lactal in the lamina propria) of mepitiostane from the small intestine in rats.

## Materials and Methods

### *Materials*

[4-$^{14}$C]Mepitiostane (10·6 $\mu$Ci mg$^{-1}$) was synthesized at Shionogi Research Laboratories. The radiochemical purity checked by TLC (Merck Silica Gel 60 plate, petroleum ether-ethyl ether (10:1)) was >98%. The oils used were sesame oil (Maruishi Pharmaceutical Co. Ltd, Osaka, Japan), triolein, monolein and oleic acid (Nacarai Tesque, Inc., Kyoto, Japan). Sesame oil was of JP grade and the others of reagent grade were used without further purification.

An oily solution was prepared by dissolving [$^{14}$C]mepitiostane in an oily solvent at a concentration of 1%. A micellar solution was prepared by dissolving [$^{14}$C]mepitiostane in 10% (w/v) polysorbate 80 aqueous solution at a concentration of 0·1%. An aqueous suspension was formulated by dispersing [$^{14}$C]mepitiostane at a concentration of 0·1% in aqueous vehicle containing 0·5% (w/v) carboxymethylcellulose sodium, 0·4% (w/v) polysorbate 80 and 0·9% (w/v) NaCl (saline).

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### *Animals*

Female Sprague-Dawley rats (11–13 weeks, 223–290 g) purchased from CLEA Japan, Inc., Tokyo, were anaesthetized with ethyl urethane (1·4 g kg$^{-1}$, s.c.) and the thoracic duct was cannulated by the method of Bollman et al (1948), using polyethylene tubing (PE50, Becton Dickinson and Co., Parsippany, NJ, USA) rinsed with dilute heparin. Two cannulae (PE50) were introduced into the common bile duct. Both ends of the small intestine (from the pylorus to the ileocaecal valve) were ligated and [$^{14}$C]mepitiostane (2 mg kg$^{-1}$) was instilled into the lumen as an oily solution dispersed in 30 vol of fresh rat bile (containing pancreatic juice), a micellar solution or an aqueous suspension. Following administration of the micellar solution or the aqueous suspension, 1 mL of bile was further instilled into the lumen. After drug administration, the rats were secured on a warmed plate maintained at 38°C. Lymph and bile were collected in test tubes via each cannula for 6 h after dosing. Bile (containing pancreatic juice) collected from another rat was instilled into the duodenum through a cannula inserted into the common bile duct at a rate of 0·6 mL h$^{-1}$ from 1 h after dosing. At 1, 3 and 5 h after dosing, 1·5 mL of saline was also injected into the tail vein to serve as a supply of water.

For experiments to define the proportions of absorbed mepitiostane transferred by the portal and lymphatic routes, the rat was placed under ethyl urethane anaesthesia and the thoracic duct and the tail vein, respectively, were cannulated with a PE50 catheter rinsed with dilute heparin. A loop of jejunum (5 cm) was formed by ligating both ends. After injection of 0·7 mL of dilute heparin (2000 units mL$^{-1}$), a third cannula filled with dilute heparin was introduced into the mesenteric vein governing the test jejunal segments. Immediately after this, [$^{14}$C]mepitiostane test solution (0·7 mg kg$^{-1}$) dispersed in 0·7 mL of bile was instilled into this

Table 1. Effect of vehicle on the appearance of mepitiostane in 6 h lymph following administration of [$^{14}$C]mepitiostane.

| Vehicle | n | % of dose absorbed | % of dose in lymph | % of absorbed dose in lymph |
|---|---|---|---|---|
| Aqueous suspension | 3 | 32·4±4·2[b] | 7·5±1·8[b] | 23·2±4·0[b] |
| Water containing 10% polysorbate 80 | 4 | 85·9±6·1 | 40·9±5·8 | 47·5±5·4 |
| Sesame oil | 6 | 74·8±10·9 | 41·2±7·1 | 55·0±4·2 |
| Triolein | 3 | 75·4±6·8 | 45·0±5·2 | 59·6±4·1 |
| Monolein | 3 | 68·9±1·6 | 26·4±4·6[a] | 38·2±5·9[b] |
| Oleic acid | 3 | 60·5±7·4 | 18·2±2·4[b] | 30·2±1·5[b] |
| Oleic acid/monolein (2:1 mol/mol) | 3 | 61·8±5·5 | 17·6±2·5[b] | 28·4±2·2[b] |

[$^{14}$C]Mepitiostane was administered to the small intestine of rats at a dose of 1·8 mg kg$^{-1}$ in different vehicles (lipid vehicle: 0·2 mL kg$^{-1}$). [a]Significantly different from sesame oil at $P<0·05$ by Student's $t$-test. [b]Significantly different from sesame oil at $P<0·01$ by Student's $t$-test. Each value represents the mean±s.d. of 3–6 rats.

jejunal loop. The rats were placed on a warmed plate, and the lymph and mesenteric blood were collected in test tubes via each cannula for a 3 h period after dosing, under blood transfusion via the tail vein.

*Lipoprotein separation*

Lymph was collected from rats for 6 h following administration of [$^{14}$C]mepitiostane in various vehicles. The chylomicron and very low density lipoprotein (VLDL) fraction in lymph (Ch+VLDL fraction) was separated from the infranatant phase by ultracentrifugation (114000 $g$ for 16 h) at 4°C.

*Analytical procedures*

Radioactivity in various samples was measured by a liquid scintillation counter (Aloka Co., Tokyo, Japan, Model LSC-673) and mepitiostane in lymph and blood was analysed by the TLC method reported previously (Ichihashi et al 1991). Triglyceride was determined by a modification of the method of Sardesai & Manning (1968) using a Triglyceride-Test kit (Wako Chemicals, Osaka, Japan).

## Results and Discussion

*Vehicle effects*

Table 1 shows the effect of the vehicle on the absorption of mepitiostane by the lymphatic system following administration of [$^{14}$C]mepitiostane into the small intestine of thoracic duct-cannulated rats. Significantly different extents of lymphatic absorption were observed following administration in different vehicles. When given as a solution in sesame oil or triolein, 55·0 or 59·6% of the total absorbed amount of drug was recovered in the 6 h lymph, respectively. The greatest lymphatic absorption of mepitiostane was observed with these triglyceride vehicles. Low absorption percentages were observed with monolein and oleic acid. The combination of oleic acid/monolein (2:1 mol/mol) also led to low absorption. The lowest value was observed when [$^{14}$C]mepitiostane was administered as an aqueous suspension. However, even then, 23·2% of the absorbed amount was recovered in the lymph. When [$^{14}$C]mepitiostane was administered as an aqueous solution containing 10% polysorbate 80, 47·5% of the absorbed amount was recovered in the lymph. This percentage was not significantly different from that obtained with the sesame oil vehicle.

*Vehicle volume effects*

Fig. 1 shows the effect of vehicle (sesame oil) volume administered on the appearance of mepitiostane in the lymph. The dose of sesame oil leading to the result shown in Table 1 was 0·2 mL kg$^{-1}$. At this dose, 55·0% of the absorbed amount (41·2% of the dose) was recovered in the lymph after 6 h. When the volume of sesame oil administered was increased about 4 times, 58·5% of the absorbed amount was recovered in the lymph, but this value was not significantly different from that for the 0·2 mL kg$^{-1}$ dose volume. However, the percentage of mepitiostane appearing in the lymph based on the dose, decreased to about one-half. This seems to be due to a lowering of micellar solubilization by bile, because of the excessive dose volume of sesame oil. In contrast, when the dose volume of sesame oil was decreased, although the extent of lymphatic absorption of mepitiostane from the 0·06 mL kg$^{-1}$ dose volume did not differ from that at the 0·2 mL kg$^{-1}$ dose volume, a further decrease of the sesame oil dose volume to 0·02 mL kg$^{-1}$ produced lower lymphatic absorption of mepitiostane. The volume of sesame oil in this case was about 4 μL per rat and at even this low dose volume, about 40% of the absorbed amount was recovered in the lymphatics. Although the percentage of

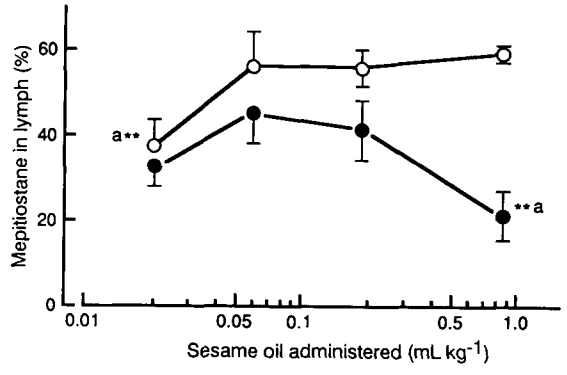


FIG. 1. Effect of sesame oil volume on mepitiostane appearance in 6 h lymph following administration of [$^{14}$C]mepitiostane. ● % of the dose, ○ % of the absorbed amount. [a]Significantly different from 0·2 mL kg$^{-1}$ at $P<0·01$ by Student's $t$-test. The dose of [$^{14}$C]mepitiostane was 1·8 mg kg$^{-1}$, except when the sesame oil volume was 0·02 mL kg$^{-1}$ (0·6 mg kg$^{-1}$). Each value represents the mean±s.d. of 3–6 rats.

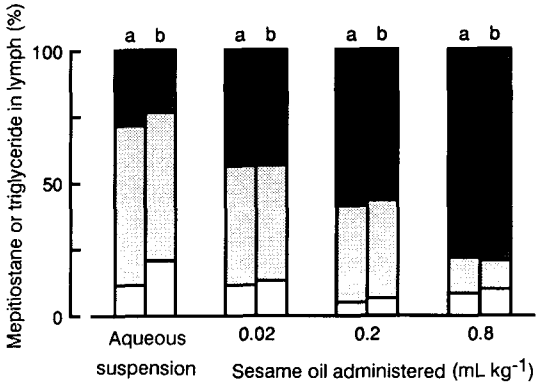


FIG. 2. Effect of vehicle volume on the distribution of mepitiostane or triglyceride among chylomicron (darkly shaded bars), VLDL (lightly shaded bars) and infranatant fraction (open bars), of 6 h thoracic duct lymph following administration of [$^{14}$C]mepitiostane. [a]Mepitiostane, [b]triglyceride. Each value represents the mean of 3 rats.

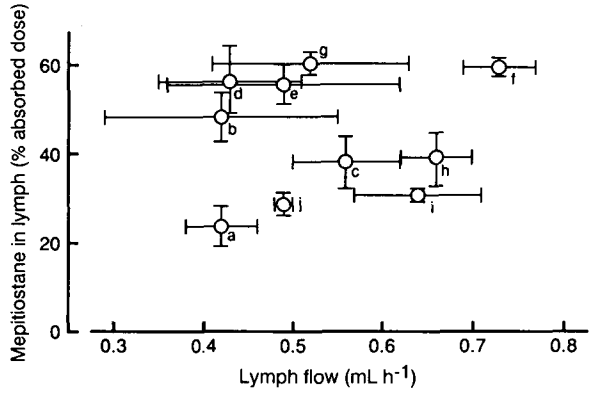


FIG. 3. Relationship between the appearance of mepitiostane in 6 h lymph and lymph flow. [a]Aqueous suspension, [b]polysorbate 80, [c]sesame oil 0·02 mL kg$^{-1}$, [d]sesame oil 0·06 mL kg$^{-1}$, [e]sesame oil 0·2 mL kg$^{-1}$, [f]sesame oil 0·8 mL kg$^{-1}$, [g]triolein, [h]monolein, [i]oleic acid, [j]oleic acid/monolein (2:1 mol/mol). Each value represents the mean ± s.d. of 3–6 rats.

mepitiostane appearing in the lymph, based on the absorbed amount tended to decrease as the sesame oil dose volume decreased, on the whole, its effect was small.

Fig. 2 shows the effect of oil volume on the distribution of mepitiostane among chylomicron, VLDL and infranatant fractions of lymph. For all formulations, most of the lymph mepitiostane was in the Ch+VLDL fraction. The proportion of mepitiostane distributed in the chylomicron fraction and the VLDL fraction changed with the dose volume of sesame oil. The lymph mepitiostane was in the VLDL fraction rather than in the chylomicron fraction after administration as an aqueous suspension. The proportion of mepitiostane distributed in the chylomicron fraction increased as the dose volume of sesame oil increased, and at 0·8 mL kg$^{-1}$ of sesame oil most of the lymph mepitiostane was in the chylomicron fraction. The distribution pattern of mepitiostane in various fractions agrees closely with that of triglyceride. Chylomicron is known to transport glycerides of dietary origin from the intestine into the lymph, and VLDL comprises a family of macromolecules rich in endogenous triglycerides (Levy et al 1971). These results indicate that mepitiostane is mainly distributed in the chylomicron fraction and transferred into the lymph when administered in a high dose volume of sesame oil, and VLDL can transfer mepitiostane from the intestine into the lymph when administered as an aqueous suspension (no lipid is given).

*Relationship between lymphatic transfer of mepitiostane and lymph flow*

Fig. 3 shows the relationship between the percentage of mepitiostane appearing in the lymph based on absorbed amount and the lymph flow following administration as various formulations. de Marco & Levine (1969) examined the lymphatic absorption of hydrophilic drugs such as *p*-aminosalicylic acid and tetracycline. These drugs were transferred to the lymph, with the quantities increasing as the lymph flow increased when lipids were coadministered. However, no correlation was obtained between the lymph flow and the percentage of mepitiostane recovered in the lymph based on absorbed amount. Mepitiostane, a lipid-soluble drug with low water solubility, appeared to be distributed in the chylomicron, VLDL and other protein fractions and little existed as free drug in the lamina propria of the intestinal villi. Hence, the lymphatic transfer of mepitiostane may not be affected by the lymph flow.

*Relationship between lymphatic transfer of mepitiostane and secretion of chylomicron and VLDL*

Chylomicron and VLDL, which transport triglycerides, have a central triglyceride core (80–95% of weight in chylomicron, 50–70% in VLDL) and a surface surrounded by an outer membrane consisting of phospholipid, cholesterol and protein (Levy et al 1971). As described above, mepitiostane was distributed in the chylomicron and VLDL and was transferred to the lymph when administered as a sesame oil solution and also as an aqueous suspension. Mepitiostane appeared to be located in the lipid core of chylomicron and

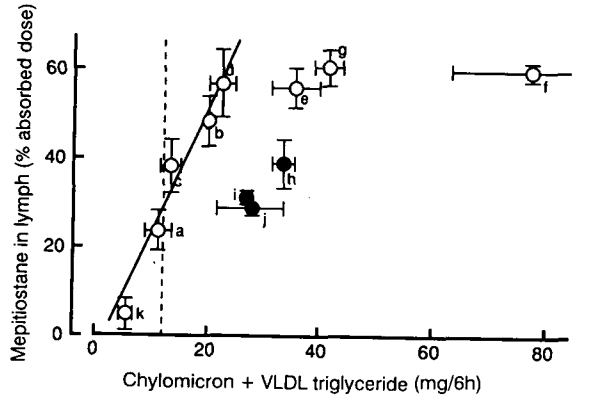


FIG. 4. Relationship between the appearance of mepitiostane and the chylomicron+VLDL triglyceride in 6 h lymph. [a]Aqueous suspension, [b]polysorbate 80, [c]sesame oil 0·02 mL kg$^{-1}$, [d]sesame oil 0·06 mL kg$^{-1}$, [e]sesame oil 0·2 mL kg$^{-1}$, [f]sesame oil 0·8 mL kg$^{-1}$, [g]triolein, [h]monolein, [i]oleic acid, [j]oleic acid/monolein (2:1 mol/mol), [k]sesame oil 0·2 mL kg$^{-1}$-bile deficient. Broken line indicates the control value of chylomicron+VLDL triglyceride. Each value represents the mean ± s.d. of 3–6 rats.

Table 2. Mepitiostane and radioactivity in blood and lymph 3 h following administration of [$^{14}$C]mepitiostane (0·7 mg kg$^{-1}$) into jejunal loop of thoracic duct- and mesenteric vein-cannulated rats.

| Sample | Sample | Blood or lymph flow (g) | % of dose | |
|---|---|---|---|---|
| | | | Radioactivity | Mepitiostane |
| Sesame oil (0·06 mL kg$^{-1}$) | Jejunal loop | — | 75·7 ± 1·4 | — |
| | Blood | 96·7 ± 14·9 | 7·4 ± 0·7 | 1·2 ± 0·4 |
| | Lymph | 2·2 ± 0·4 | 15·6 ± 1·8 | 15·0 ± 1·7 |
| Aqueous suspension | Jejunal loop | — | 75·4 ± 6·2 | — |
| | Blood | 89·3 ± 14·7 | 12·7 ± 3·5 | 2·1 ± 0·6 |
| | Lymph | 3·1 ± 0·5 | 10·3 ± 5·4 | 9·7 ± 5·1 |

Each value represents the mean ± s.d. of 3 rats.

VLDL (Ichihashi et al 1991). When bile alone was instilled into the small intestine, 12·3 ± 1·6 mg (n = 3) of triglyceride was recovered in the Ch + VLDL fraction of 6 h lymph (Fig. 4). This was termed the 'control value' shown in Fig. 4 as a broken line. Following administration of [$^{14}$C]mepitiostane as an aqueous suspension, the amount of Ch + VLDL triglyceride was approximately equal to the control value and 23·2% of the absorbed dose of mepitiostane was recovered in the 6 h lymph. When [$^{14}$C]mepitiostane was administered to bile-deficient rat as a sesame oil solution (0·2 mL kg$^{-1}$), the amount of Ch + VLDL triglyceride decreased to about one-half of the control value and under these conditions, there was very little lymphatic absorption of mepitiostane (Ichihashi et al 1992). In contrast, the extent of lymphatic absorption of mepitiostane increased with higher amounts of Ch + VLDL triglyceride, and at 22 mg, the amount reached 55%. Thus, significant differences in the extent of lymphatic absorption of mepitiostane between the formulations can be correlated to amounts of chylomicron and VLDL secreted. However, the extent of lymphatic absorption of mepitiostane remained at the same level even when the amount of Ch + VLDL triglyceride was increased to more than 20 mg, possibly because of the metabolism of mepitiostane during intestinal absorption.

For the oleic acid, monolein and oleic acid/monolein (2:1 v/v) vehicles indicated by the closed circles in Fig. 4, the extent of lymphatic absorption of mepitiostane was lower than with the triglyceride vehicles, although the amount of Ch + VLDL triglyceride increased. To determine the reason for this discrepancy, 10 mg of oleic acid or sesame oil containing [$^{14}$C]mepitiostane (1%) was dispersed in 0·3 mL of fresh rat bile and incubated at 37°C, and the amount of mepitiostane was determined. Degradation of mepitiostane was observed in oleic acid solution dispersed in bile (% residual of mepitiostane after 1 h: 94% in sesame oil, 54% in oleic acid). Thus, the lower lymphatic absorption of mepitiostane observed after administration of these formulations seems to be due to its degradation in the intestinal lumen.

When [$^{14}$C]mepitiostane was administered as 10% polysorbate 80 aqueous solution, the amount of Ch + VLDL triglyceride increased, though no oil was given. This seems to indicate that oleic acid is liberated from the polysorbate 80 molecule by hydrolysis in the intestinal lumen, and triglyceride is synthesized following penetration into the epithelial cells, since the formation of free fatty acid was observed when 10% polysorbate 80 aqueous solution was added to fresh rat bile (containing pancreatic juice) and incubated at 37°C.

*Effect of oil on intrinsic partition of mepitiostane between lymph and blood*

Table 2 shows the percentage of the dose of radioactivity recovered in the mesenteric blood and the thoracic duct lymph at 3 h following administration of [$^{14}$C]mepitiostane as a sesame oil solution or an aqueous suspension. Also, Fig. 5 shows the absorption behaviour from the jejunum of mepitiostane obtained from intrajejunal data (Table 2). In both formulations, the total radioactivity recovered in the jejunal loop, mesenteric blood and lymph was more than 98% of the dose. Thus, almost all of the radioactivity absorbed from the jejunal loop can be recovered in the mesenteric blood and lymph. As shown in Fig. 5, 23% of the dose of radioactivity was absorbed in the mesenteric blood and lymph at 3 h following administration using either formulation. The percentage of mepitiostane in the absorbed radioactivity was 70% when administered as the sesame oil solution and 50% for the aqueous suspension. The proportion of mepitiostane that was not metabolized or degraded during absorption increased with concomitant lipid absorption. Unchanged mepitiostane was transferred into the blood and lymph, and the proportion of mepitiostane distributed into the lymph was 92% when administered as the sesame oil solution and 81% for the aqueous suspension. Thus, the oil affected not only the penetration (see Table 1) and metabolism of mepitiostane during absorption, but also significantly increased ($P < 0·05$) the partition (lymph/blood) of mepitiostane.

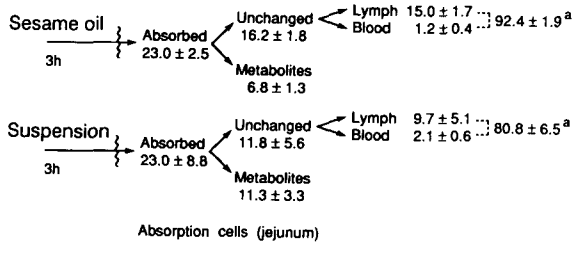


FIG. 5. Partition of mepitiostane (MP) between thoracic duct lymph and mesenteric blood. $^{a}[MP_{lymph}/(MP_{lymph} + MP_{blood})] \times 100$ (%). The results are the percentages of the dose (mean ± s.d., n = 3).

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