A Quantitative Concept of the Mechanism of Intestinal Lymphatic Transfer of Lipophilic Molecules

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The partition of mepitiostane, testosterone, and some structurally related compounds between lymph and blood in rat jejunum (lymph–blood partition ratio; LBPR) was determined, and the quantitative relationship between LBPR and lipophilicity was examined. When the ΔR_m values (hydrophobic parameter derived from the mobility) relative to testosterone were <0.2, their logLBPRs remained approximately constant in the range of −2 to −3. When the ΔR_m values of the compounds were >0.2, a linear correlation (r = 0.986, n = 8) was observed between these values and the logLBPRs. The LBPR, but not the extent of lymphatic absorption, of lipophilic molecules was determined strictly by the superlipophilicity, and for high partitioning into the lymph (>50% of the absorbed amount), the ΔR_m value had to be >0.50 (5.65 as the log$P$ value). The relationship between LBPR and superlipophilicity could be explained on the basis of the theoretical equations derived from absorption kinetics based on a dynamic partitioning model.

KEY WORDS: mepitiostane; superlipophilicity; lymph–blood partition ratio; dynamic partitioning model; chylomicron.

INTRODUCTION

In general, compounds absorbed from the intestine by the lymphatic system are extremely lipophilic (1–4). Thus, lipophilicity has been considered an important factor in the lymphatic absorption of low molecular weight compounds. However, some studies (5–11) have suggested that lipophilicity does not strictly parallel the extent of lymphatic absorption. These different views are considered to arise from the complex absorption process of drugs from the intestine. The lymphatic transfer mechanism of the lipophilic molecules from the intestine has not been clearly defined.

We previously established a method for direct measurement of the partition ratio of drugs between the lymph and the blood in the rat intestine. Using this method, we found that after passage through mucosal cells, 99.6% of epitiostanol (EP) is partitioned into the blood and 0.4% into the lymph, while 92.4% of mepitiostane (MP) is partitioned into the lymph (12). This led us to propose a hypothesis in which the difference in lymphotropic properties between EP and MP can be explained by differences in the release velocity of the drug from the lymph containing chylomicrons and very low-density lipoproteins (VLDL) to the plasma (13). The present study was designed to establish whether the partition between blood and lymph of a lipophilic molecule absorbed from the upper small intestine is determined strictly by its extremely high lipophilicity (superlipophilicity), which enables drug retention in the core lipid of chylomicrons and VLDL during the transfer of these lipoproteins into the lymph (13).

THEORETICAL CONSIDERATIONS

The dynamic partitioning model for the partition between blood and lymph of a low molecular weight drug absorbed from the upper small intestine is shown in Fig. 1. Drugs that penetrate from the lumen of the intestine into the epithelial cells are metabolized to some extent during absorption. The unchanged drug appears to be incorporated into the core lipids of the chylomicrons and VLDL in the epithelial cells. When the chylomicrons and VLDL are transferred from the epithelial cells to the lamina propria of the villi and then into the central lacteal, the drug is also transferred into the lymphatics, with partitioning between the core lipids of chylomicrons and VLDL and the aqueous environment of the villi. It is assumed that the partition equilibrium of the drug is rapidly set up between the core lipids of the chylomicrons and VLDL and the aqueous environment of the lamina propria of the villi, and occurs with apparent first-order kinetics. In Fig. 1, k_1 is the apparent first-order transfer rate constant of the free drug from the lamina propria of the absorption site into the blood capillaries, k_2 is the apparent first-order transfer rate constant of the free drug from the lamina propria of the absorption site into the lymphatics, and k_3 is the apparent first-order transfer rate constant of the drug in the chylomicrons and VLDL from the lamina propria of the absorption site into the lymphatics. For simplification, it is further assumed that the transfer to the lymphatics of the drug that is bound to the protein (d > 1.006) is negligible because in the large intestine, where there is no chylomicron secretion, MP is absorbed via the portal system, but not the lymphatic system (14), and phenylbutazone, which is highly bound to the plasma proteins, is absorbed via the portal system (15).

Based on the model in Fig. 1 and the above assumptions, the ratio of the transfer rate of intact drug from the lamina propria of the absorption site into the lymphatics to the rate into the blood capillaries is expressed as follows

$$\frac{dX_L/dt}{dX_B/dt} = \frac{k_2 X_F + k_3 X_{CH}}{k_1 X_F} \quad (1)$$

where X_B is the amount of drug transferred into the blood capillaries; X_L, the amount of drug transferred into the lymphatics; X_F, the amount of free drug in the lamina propria of the absorption site; and X_{CH}, the amount of drug in the chylomicrons and VLDL in the lamina propria of the absorption site.

When $k_2 X_F \gg k_3 X_{CH}$

$$\frac{dX_L/dt}{dX_B/dt} = \frac{k_2}{k_1} = K_I = \text{LBPR} \quad (2)$$

where $K_I = k_2/k_1$, the lymph–blood partition ration (LBPR),

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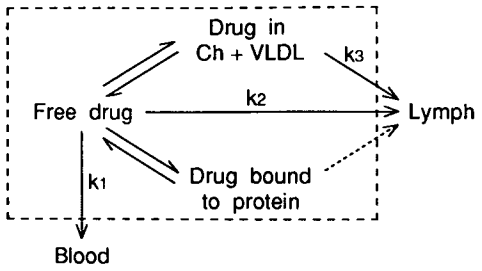

Fig. 1. Dynamic partitioning model for lymphoblood partitioning of drugs in the upper small intestine. Ch, chylomicron; VLDL, very low-density lipoprotein.

the ratio of the transferred amount of unchanged drug in lymph to that in blood during the time interval 0 to t.

In logarithmic form,

$$\log\text{LBPR} = C_I \tag{3}$$

where $C_I = \log K_I$.

When $k_2 X_F \ll k_3 X_{CH}$

$$\frac{dX_L/dt}{dX_B/dt} = \frac{k_3 X_{CH}}{k_1 X_F} = K_{II} \cdot f \cdot p' \tag{4}$$

where $K_{II} = k_3/k_1$, $f = V_{CH}/V_w$, and $p' = (X_{CH}/V_{CH})/(X_F/V_w)$. V_{CH} is the volume of the core lipids of chylomicrons and VLDL, and V_w is the volume of the aqueous phase in the lamina propria of absorption site. It is assumed that V_w and V_{CH} remain approximately constant because the experimental conditions are fixed, and thus, f is a constant.

In logarithmic form,

$$\log\text{LBPR} = \log p' + \log(K_{II} \cdot f) \tag{5}$$

Since the determination of the physiological partition coefficient, p', of the compounds investigated is difficult because of their extremely low water solubility and high lipid solubility, the R_m value, $\log(1/R_f - 1)$, i.e., the thin-layer chromatographic parameter (16), was introduced as a hydrophobic parameter.

The $\log p'$ value is linearly related to the R_m value (17).

$$\log p' = aR_m + b \tag{6}$$

In the reference compound

$$\log p'_o = aR_{m_o} + b \tag{7}$$

From Eqs. (5)–(7); Eq. (8) was obtained.

$$\log\text{LBPR} = a\Delta R_m + \log(K_{II} \cdot f \cdot p'_o) \tag{8}$$

and

$$\log\text{LBPR} = a\Delta R_m + C_{II} \tag{9}$$

where $\Delta R_m = R_m - R_{m_o}$ and $C_{II} = \log(K_{II} \cdot f \cdot p'_o)$.

EXPERIMENTAL

Materials

[4-^{14}C]Epitiostanol (^{14}C-EP; 2.13 MBq/mg), [4-^{14}C]mepitiostane (^{14}C-MP; 392 kBq/mg), [4-^{14}C]mepitiostane-olefin (^{14}C-MPO; 466 kBq/mg), [4-^{14}C]testosterone-17β-methoxycyclopentyl ether (^{14}C-TE; 551 kBq/mg), and [4-^{14}C]Δ^4-androstan-17β-methoxycyclopentyl ether (^{14}C-Δ^4-AE; 577 kBq/mg) were synthesized at Shionogi Research Laboratories. *N*-[*methyl*-^{14}C]Antipyrine (^{14}C-AP; 11.2 MBq/mg), [4-^{14}C]testosterone (^{14}C-T; 7.4 MBq/mg), [1-^{14}C]oleic acid (^{14}C-OA; 7.4 MBq/mg), and [1-^{14}C]stearic acid (^{14}C-SA; 7.4 MBq/mg) were purchased from Amersham International plc. The radiochemical purity of the radioactive compounds was confirmed by thin-layer chromatography (TLC) to be higher than 98%. Progesterone (PR; Nacalai Tesque, Co., Ltd.) and other chemicals were of analytical or reagent grade. The test solution of ^{14}C-AP was prepared by dissolving it in saline solution at 2.5 mg/mL. Test solutions of other compounds were prepared by dissolving them in sesame oil at 10 mg/mL.

Animals

Female Sprague–Dawley rats (11–13 weeks) were purchased from CLEA Japan, Inc. The LBPR was determined by the *in situ* loop method reported previously (12). The oily test solution (30 mg), dispersed in fresh rat bile (0.6 mL), was instilled into the jejunal loop. In the case of antipyrine, a saline solution (0.2 mL) and sesame oil (30 mg) dispersed in bile (0.6 mL) were instilled.

Determination of Lipophilicity

TLC Method. A 2-μL sample of each compound (2 mg/mL in acetone solution) was spotted at the origin of TLC plates (silica gel 60 F_{254}, silanized; Merck) along with the reference testosterone and developed in 80% (v/v) acetone aqueous solution at 24°C over 10 cm. The R_m value, $\log(1/R_f - 1)$, was derived from the R_f (16). The ΔR_m value was determined by subtracting the R_m value of testosterone from the R_m value of the compound and was utilized directly as the hydrophobic parameter. Only the ΔR_m value of antipyrine was estimated from the ΔR_m value obtained using 50% (v/v) acetone aqueous solution as the solvent system.

Fragment Addition Method (17,18). The log*P* (*n*-octanol/water) value was calculated using the program CLOGP3 (Pomona College Medicinal Chemistry, USA, Version 3.33).

Analytical Methods

Radioactivity in various samples was measured with a liquid scintillation counter (Aloka Model LSC-673), and the radioactive compounds in lymph and blood were analyzed by the TLC method reported previously (12). PR was analyzed by HPLC [Cosmosil$_5$C$_{18}$ (4.6 × 25 cm, Nacalai Tesque; acetonitrile/water (6:4, v/v), 254 nm].

RESULTS AND DISCUSSION

Partitioning of Compounds Between Lymph and Blood

Table I shows the percentage of unchanged drug based on the dose recovered from the mesenteric blood and the lymph at 3 hr following the administration of various compounds into the jejunal loop. In such an experiment, the radioactivity absorbed via the portal and the lymphatic sys-

Table I. Partitioning of Various Compounds Between Thoracic Duct Lymph and Mesenteric Blood[a]

Compound	Dose (mg/kg)	% of dose		LBPR[b]	logLBPR
		In lymph	In blood		
Antipyrine[c]	1.7	1.1 ± 0.8	93.8 ± 1.5	0.011 ± 0.008	−2.065 ± 0.342
Testosterone	1.0	0.03 ± 0.02	26.4 ± 4.4	0.001 ± 0.001	−3.057 ± 0.405
Progesterone	1.0	0.07 ± 0.09	8.4 ± 1.6	0.009 ± 0.010	−2.465 ± 0.827
Epitiostanol[d,e]	0.5	0.03 ± 0.02	8.0 ± 2.2	0.004 ± 0.001	−2.426 ± 0.172
KEP[d,e]	—	1.5 ± 0.5	17.3 ± 3.8	0.086 ± 0.001	−1.068 ± 0.071
KO	—	0.34 ± 0.07	4.9 ± 0.6	0.069 ± 0.009	−1.166 ± 0.061
TE[f]	0.7	0.77 ± 0.57	5.4 ± 1.9	0.129 ± 0.055	−0.917 ± 0.187
DHTE[g]	—	3.6 ± 2.4	9.1 ± 4.5	0.369 ± 0.086	−0.441 ± 0.107
Mepitiostane[e]	0.6	15.0 ± 1.7	1.1 ± 0.5	12.6 ± 3.0	1.094 ± 0.111
Δ^4-AE[h]	0.9	9.0 ± 3.6	0.3 ± 0.1	29.5 ± 4.5	1.467 ± 0.066
Mepitiostane-olefin	1.1	12.6 ± 1.6	0.3 ± 0.0	45.3 ± 7.4	1.652 ± 0.075

[a] The compounds in sesame oil (10 mg/mL) were dispersed in 0.6 mL of bile and administered to the jejunal loop of rats. Thoracic duct lymph and mesenteric blood were collected for 3 hr after dosing, except for EP (2 hr). Each value represents the mean ± SD of three rats.
[b] Lymphoblood partition ratio: $[X_L/X_B]$.
[c] Administered as an aqueous solution.
[d] Oxidized metabolite of EP: KEP, 2α,3α-epithio-5α-androstan-17-one; KO, 5α-androst-2-en-17-one.
[e] Published results (10).
[f] Testosterone-17β-methoxycyclopentyl ether.
[g] Oxidized metabolite of TE, dehydrotestosterone-17β-methoxycyclopentyl ether.
[h] Δ^4-Androstan-17β-methoxycyclopentyl ether.

tem is collected before it is distributed to the whole body (12). Thus, the LBPR can be determined accurately; these values are also shown in Table I. When AP, T, PR, and EP were administered to mesenteric vein- and thoracic duct-cannulated rats, most of the absorbed compounds were recovered from the mesenteric blood. The LBPRs of these compounds were very low. However, in KEP and KO, which are metabolites of EP formed in the intestinal mucosa in absorption processes, about 10% of the amount absorbed appeared in the lymph. KEP, KO, TE, and DHTE seem to be somewhat lymphotropic. In contrast, most of the absorbed MP, Δ^4-AE, and MPO was recovered in the lymph. The LBPRs of MP, Δ^4-AE, and MPO were 12.6, 29.5, and 45.3, respectively, which are very large values.

Relationship Between LBPR and Lipophilicity

Figure 2 shows the relationship between the logarithm LBPRs of the compounds and their ΔR_m values. The ΔR_m value is the TLC parameter, which is utilized directly in the quantitative structure–activity relationship studies as the hydrophobic parameter (19,20). When the ΔR_m values of the compounds were <0.2 , their logLBPRs were about −2 to −3, and the results were roughly consistent with Eq. (3). In contrast, when the ΔR_m values of the compounds were >0.2, a good linear correlation was obtained between the logLBPRs of the compounds and their ΔR_m values. The relationship can be characterized by Eq. (10). The

$$\log\text{LBPR} = 7.704\,\Delta R_m - 3.872 \qquad (10)$$

correlation coefficient was $r = 0.986$ ($n = 8$). This region, for which X_{CH}/X_F is extremely high, corresponds to the case of $k_2X_F \ll k_3X_{CH}$ described under Theoretical Considerations, and the results can be explained on the basis of Eq. (9). For high partitioning into the lymph (>50% of the absorbed amount), a superlipophilicity of >0.50 as the ΔR_m value (5.65 as the log$P$) is necessary. Analysis of the relationship between the logLBPRs of the compounds and their log$P$ values showed that the correlation coefficient decreased, $r = 0.913$ ($n = 8$). Better results could be obtained by using the TLC parameter, ΔR_m, as the degree of lipophilicity of the lipophilic compounds tested.

The percentages of the compounds recovered from the lymph based on the dose are also shown in Table I. These values are not strictly correlated with the superlipophilicities of the compounds because the absorption process includes penetration through the epithelial cells, metabolism in these cells, and transfer from the epithelial cells into the blood capillaries and/or the central lacteals in the lamina propria. Specific structural requirements, in addition to superlipophilicity, contribute to determining the extent to which compounds enter the lymphatics, which can be represented by the percentage of the dose (or of the absorbed dose).

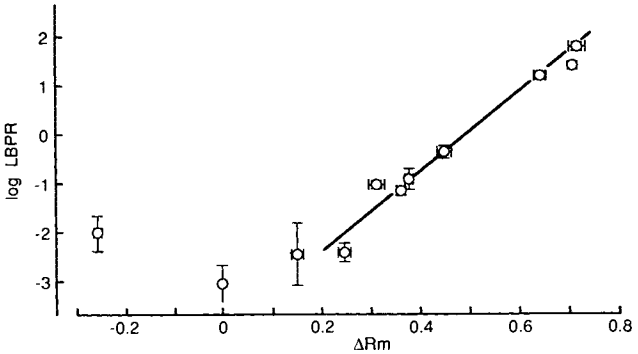

Fig. 2. Relationship between the logarithm of the LBPR and lipophilicity. LBPR = X_L/X_B. The lipophilicity of the compound is expressed as the ΔR_m value relative to testosterone. Each value represents the mean ± SD of three rats.

Comparison with Other Findings

Levine *et al.* (5) have shown, in studies with quaternary ammonium compounds, that the lipid solubility of these compounds did not influence the amount recovered in the lymph. Aso *et al.* (6) considered the participation of structural requirements in the lymphatic transfer of a compound after finding that vitamins K_1 and K_2 are transferred into the lymph from the intestine, while vitamin K_3 is transferred into the blood. Oliver *et al.* (7) observed that digitoxin, a highly lipid-soluble drug with a low water solubility akin to cholesterol, is absorbed into the bloodstream. Also, Deak and Csáky (8) have shown that even though dimethyl sulfoxide, diacetone glucose, and chloramphenicol are highly lipid soluble, they are not absorbed preferentially by the lymphatics from the jejunum. Thus, the particle size, and not the lipophilicity, of the absorbed compound determines whether a compound is absorbed into the blood or the lymph. Our results showed that a log*P* value >5 was necessary for a high level of partitioning into the lymph. The log*P* values of the compounds in these literature references were also examined. Among the compounds used to provide a basis for drawing conclusions in the papers mentioned above, digitoxin and vitamin K_3 have log*P* values of about 2, while those of the other compounds are <2. At this degree of lipophilicity, most of the absorbed compounds are partitioned into the blood (Fig. 2). Thus, it seemed reasonable that no linear correlation was observed between lipophilicity and lymphatic absorption.

The studies by Kamp and Neumann (9), with carcinogens, and Sieber (10), with *p,p'*-DDT and related compounds, have shown that specific structural requirements, in addition to lipophilicity, can help determine the extent to which compounds enter the lymphatics. The log*P* values calculated for the compounds tested in these studies were about 5 for carcinogens and 6–7 for *p,p'*-DDT and related compounds, except *p,p'*-DDA and 2,4D. Thus, these compounds appear to have superlipophilicities which allow them to be partitioned into the lymph. However, it should be noted that in these experiments, the extent of lymphatic transfer of the compounds was represented by the percentage of the compound based on the dose or the absorbed amount. The apparent discrepancy in the lymphatic transfer–superlipophilicity relationship may be attributed to the participation of factors related to penetration and metabolism during absorption.

Long-chain fatty acids are transferred mainly as resynthesized triglyceride in the chylomicrons of lymph. Ockner *et al.* (11) observed that the absorption of dietary unsaturated long-chain fatty acids was more complete than the absorption of saturated long-chain fatty acids. Furthermore, Ockner *et al.* (21) postulated that the difference in intestinal absorption between saturated and unsaturated long-chain fatty acids might be due to the difference in binding by fatty acid binding protein in both long-chain fatty acids. Therefore, the differences in absorption behavior between oleic acid (18:1) and stearic acid (18:0) were examined. Figure 3 shows the absorption behavior from the jejunum of oleic acid and stearic acid. The absorption at 3 hr after administration was about 25% for oleic acid and about 9% for stearic acid, and most of these penetrated fatty acids were incorporated into triglycerides in epithelial cells and transferred almost-exclusively into the lymph. As described by Ockner *et al.* (11), the lymphatic absorption of oleic acid, an unsaturated long-chain fatty acid, was about three times greater than that of stearic acid, a saturated long-chain fatty acid. However, the LBPR of the unchanged long-chain fatty acid was greater for stearic acid, which has a high log*P* value, than for oleic acid.

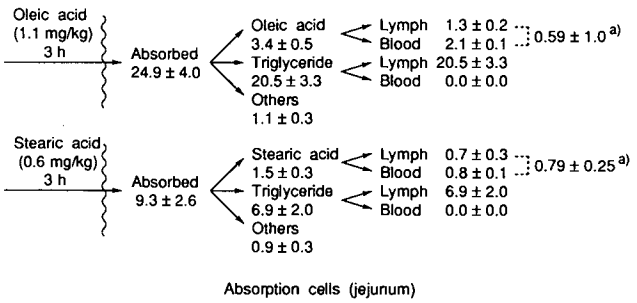

Fig. 3. Partition of fatty acids and metabolites between thoracic duct lymph and mesenteric blood. (a) LBPR. The fatty acids in sesame oil (10 mg/mL) were dispersed in 0.6 mL of bile and administered into the jejunal loop of rats. Thoracic duct lymph and mesenteric blood were collected for 3 hr after dosing. The results are the percentages of the dose (mean ± SD; $n = 3$).

Lipophilicity is an important factor in determining the extent to which drugs and nutrients enter the lymph, but it is not the only one in the overall absorption process (penetration, metabolism, partition). Another factor is the specific structural requirements related to penetration and metabolism during absorption. However, the LBPR, i.e., the partition ratio of low molecular weight compounds between lymph and blood following passage through the epithelial cells of the upper small intestine, is determined strictly by superlipophilicity.

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