Hepatic Transport of an Antiallergic Agent, Emedastine Difumarate: Interspecies Difference in Rats and Guinea Pigs

Takanori SAKAI,*,[a] Norio AWATA[a] and Jun WATANABE[b]

Pharmaceuticals Research Center, Kanebo, Ltd.,[a] 5–90 Tomobuchi-cho 1-chome, Miyakojima-ku, Osaka 534, Japan and Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University,[b] 3–1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan. *Received October 23, 1989*

Interspecies differences in the hepatic transport of an antiallergic agent, emedastine difumarate (KG-2413), were investigated in rats and guinea pigs, taking notice of the influx, efflux and metabolic processes. When the concentration of emedastine in the injection was varied from 2 μM to 10 mM, the extraction ratio of total radioactive compound to the liver at 18 s in rats and 25 s in guinea pigs after the rapid portal injection was more than 90% of the dose. This suggested that the influx velocity of emedastine into the liver was extremely fast, and that there was no interspecies difference in the influx process. The disappearance of unchanged emedastine from the liver was described by biexponential curve in rats and monoexponential curve in guinea pigs after rapid portal injection of 100 μM solution. This difference might be related to the interspecies difference in the binding of emedastine to the liver tissue. The time courses of disappearance of total radioactive compound and unchanged emedastine from the liver were analyzed using a compartment model for the examination of the efflux and metabolic processes. Emedastine was observed to have a pronounced interspecies difference in metabolic rate constant, but there was no pronounced interspecies difference in its efflux rate constant.

Keywords emedastine difumarate (KG-2413); antiallergic agent; interspecies difference; hepatic transport; hepatic metabolism; rat; guinea pig

Introduction

Emedastine difumarate, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazole difumarate (KG-2413), has strong antiallergic activity and less toxicity in animals than other antiallergics such as ketotifen and chlorpheniramine.[1]

The orally administered radiolabelled compound was almost completely excreted into the bile (88.5% of the dose) and urine (10.9% of the dose) by 24 h in rats.[2] This suggested that emedastine was well absorbed from the intestinal tract of experimental animals.

Urinary excretion of unchanged emedastine was negligible (less than 4% of the dose) in rats and ginea pigs, and biliary excretion of unchanged emedastine was not detectable in rats.[3] Therefore, emedastine might be mainly eliminated by metabolism in these animals.

After oral administration of emedastine difumarate, the extent of bioavailability in guinea pigs, 0.495, was 13-times greater than that in rats, 0.036.[4] These results suggested that there is interspecies difference in the behavior of emedastine during the initial passage through the liver. There are three important factors which determine the behavior of a drug in the liver, *i.e.*, influx, efflux and sequestration (metabolism and/or excretion) processes.[5]

The purpose of this study was to clarify the process of hepatic transport of emedastine, which causes the species difference between rats and guinea pigs, using an *in vivo* tissue sampling single injection technique.[6]

Experimental

Drug and Chemicals Emedastine difumarate and ^{14}C-labelled emedastine difumarate (Fig. 1) were identical to those used previously.[7] Benzene for pesticide analysis and the other chemicals of special grade were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Animals Male Wistar rats weighing 200—260 g and male Hartley guinea pigs weighing 290—370 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) maintained on a normal laboratory diet were used throughout.

Liver Uptake Study The technique used in the present study was basically according to Pardridge *et al.*[8] The animals were anesthetized with intraperitoneal pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, IL, U.S.A.) at doses of 65 mg/kg in rats and 20 mg/kg in guinea pigs, and a midline abdominal incision was made. Immediately after ligation of the hepatic artery, the portal vein was cannulated with a 25-gauge needle. This size needle did not occlude the vessel, and the free flow of portal blood past the needle was uninterrupted throughout the procedure. Then, 200 and 300 μl of injection solution were rapidly injected into rats and guinea pigs, respectively. The injection solution was made by dissolving ^{14}C-labelled and non-labelled emedastine difumarate in HEPES-buffered Ringer's solution (pH 7.4; 5 mM HEPES, *N*-hydroxyethylpiperazine-*N*-ethanesulfonic acid). The injection was sufficiently rapid so that the test solution entered the liver as a bolus, thereby minimizing mixing of the injection solution with blood during the initial passage through the hepatic vasculature. At particular times after the portal injection, the portal vein was rapidly cut off and the whole liver was excised. Immediately after excision, liver was cooled in ice-cold water and thereafter kept at −20 °C until assayed.

The amounts of total radioactive compound and unchanged emedastine taken up into the liver were determined as follows. The whole liver was homogenized in an equal volume of ice-cold water with a Physcotron (Niti-On Medical and Physical Instruments Mfg. Co., Ltd., Chiba, Japan). A two hundred microliter aliquot of the homogenate was solubilized in 1.5 ml of Soluene-350® (Packard Instrument Co., Downers Grove, IL U.S.A.) by shaking at 50 °C for 2 h, then 12 ml of Scintisol® EX-H (Wako Pure Chemical Industries Ltd., Osaka, Japan) was added before liquid scintillation counting. A one milliliter aliquot of the homogenate was mixed with 1 ml of 0.2 N sodium hydroxide and 6 ml of benzene in a centrifuge tube. The mixture was vigorously shaken for 10 min and centrifuged for 10 min at 3000 rpm. The aqueous layer was re-extracted with benzene in the same manner as described above, the organic layers were pooled and were made up to a constant volume with benzene. A preliminary study showed that emedastine was almost completely extracted into the organic layer in this manner. Then, a 1 ml aliquot of organic layer was transferred into counting vials for scintillation counting, and the other organic layer was evaporated to dryness under a stream of nitrogen at about 40 °C. The residue was redissolved in 50 μl of methanol–triethylamine (100 : 1) and a 10 μl aliquot was applied onto a thin layer chromatography (TLC)-plate (Silica gel 60 F_{254}, Merck Co., Inc., Rahway, NJ, U.S.A.). The plates were developed with a solvent system of dichloromethane : methanol : ammonia water = 40 : 9 : 1. Radioactive zones were located by autoradiography,

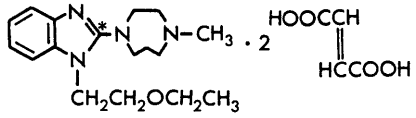

Fig. 1. Chemical Structure of Emedastine Difumarate

* labelled with ^{14}C.

removed by scraping, dissolved in methanol, and the zones quantified by determination of radioactivity. The radioactivity of each sample was determined with a liquid scintillation counter (Model LSC-1050, Aloka Co., Ltd., Tokyo, Japan).

Tissue Binding of Emedastine to Liver Homogenate Livers obtained from rats and guinea pigs were homogenized (33% w/v) in phosphate buffered isotonic saline (pH 7.0), and the homogenate was dialyzed against the same buffer solution for 16 h at 4 °C. The dialyzed homogenate was diluted at a final concentration of 25% (v/v) with the buffer solution. One milliliter of the liver homogenate containing ^{14}C-emedastine difumarate and unlabelled emedastine difumarate in concentrations between 0.1 and 20 μM was dialyzed against an equal volume of the buffer solution in a two-chambered apparatus of 2 ml capacity (Sanplatec Co., Osaka, Japan), separated by a cellulose dialysis membrane (Type 20/32, Sanko Junyaku Co., Tokyo, Japan). After 32 h of agitation at 4 °C, 0.1 ml of the protein solution in one chamber was solubilized in 1 ml of Soluene-350® for scintillation counting, and 0.1 ml of the buffer solution in the other chamber was treated similarly. Preliminary studies indicated that equilibration between the protein and buffer solutions occurred within 32 h under these conditions, and that the formation of metabolites was negligible during equilibrium dialysis.

Data Analysis Kinetics and binding data were fitted using a nonlinear least-squares analysis program MULTI.[9]

Results

Liver Uptake Study The amounts of total radioactive compound in the liver (as fraction of dose) at 18 s in rats and 25 s in guinea pigs after rapid portal injection of ^{14}C-emedastine difumarate at various injection concentrations are shown in Fig. 2. In the concentration range between 2 μM and 10 mM, the amounts of total radioactive compound in the liver were more than 90% of the doses in both species.

Subsequently, the time courses of the amounts of total radioactive compound and unchanged emedastine in the liver after rapid portal injection of 100 μM solution in rats and guinea pigs are shown in Fig. 3. The amount of unchanged emedastine in the liver decreased biexponentially and monoexponentially in rats and guinea pigs, respectively.

The time courses of the disappearance from liver were found to be described by a three-compartment model and five transfer constants (with dimensions of min^{-1}) for rats, and a two-compartment model and three transfer constants (with dimensions of min^{-1}) for guinea pigs, as shown in Fig. 4, where X_1 and X_2 represent the fraction of dose for the amount of unchanged emedastine in the central and peripheral compartments of the liver, respectively, and M_1 represents the fraction of dose for the amount of some metabolites of emedastine formed in the liver at time t (min) after injection. For the sake of simplicity, two assumptions were made as follows: the influx velocity of emedastine into the liver is very much faster than those of the other processes, and recirculation of labelled compound is negligible.

The pharmacokinetic parameters were estimated by a non-linear least-squares method using the obtained equations (see Appendix) and are listed in Table I. There was a

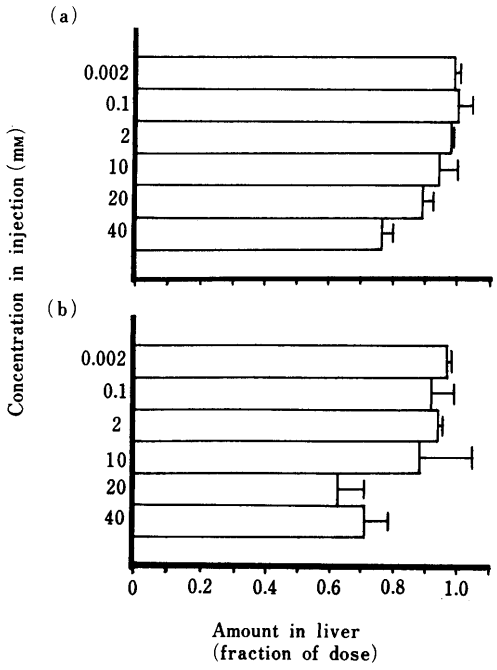

Fig. 2. Amounts of Total Radioactive Compound in Liver at 18 s in Rats (a) and 25 s in Guinea Pigs (b) after Rapid Portal Injection of ^{14}C-Emedastine Difumarate

Concentration in the injection was varied from 2 μM to 40 mM. Each bar represents the mean ± S.D. of 3 animals, or the mean ± range of 2 animals.

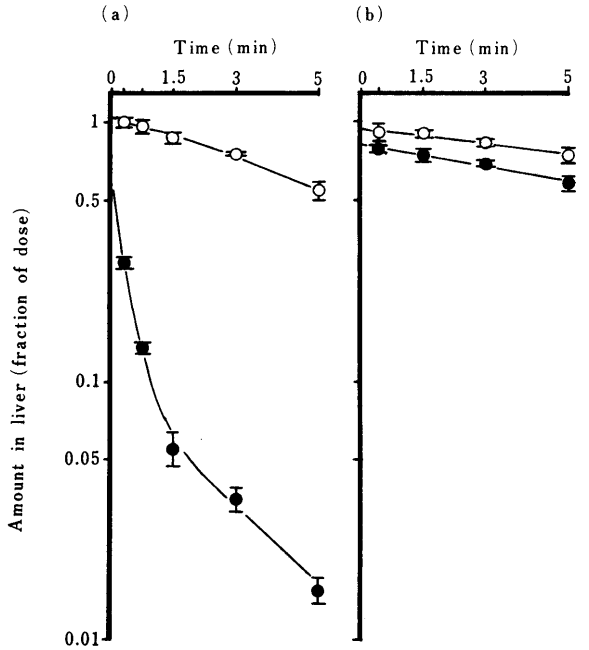

Fig. 3. Disappearance of Total Radioactive Compound (○) and Unchanged Emedastine (●) from Liver after Rapid Portal Injection of ^{14}C-Emedastine Difumarate at Concentration of 100 μM in Rats (a) and Guinea Pigs (b)

Each point represents the mean ± S.D. of 3 or 4 animals. The lines represent the least-squares fit of the data.

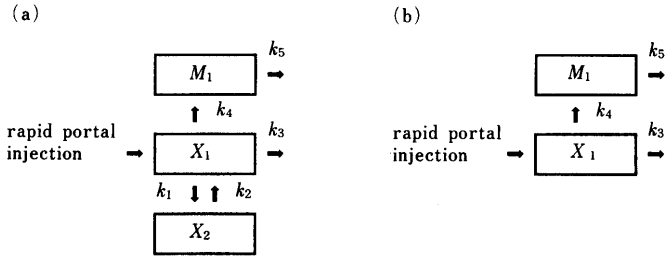

Fig. 4. Pharmacokinetic Model for the Hepatic Transport of Emedastine in Rats (a) and Guinea Pigs (b)

X_1 and X_2 represent the fraction of dose for the amount of unchanged emedastine in the central and peripheral compartment of the liver, respectively, and M_1 represents the fraction of dose for the amount of some metabolites of emedastine formed in the liver at time t after injection. k's are the first-order rate constants.

Table I. Pharmacokinetic Parameters for Hepatic Transport of Emedastine and Its Metabolites after Rapid Portal Injection of ^{14}C-Emedastine Difumarate in Rats and Guinea Pigs

Parameter	Rat	Guinea pig
X_0 (Fraction of dose)	1.039	0.942
k_1 (min^{-1})	0.186	—[a]
k_2 (min^{-1})	0.520	—[a]
k_3 (min^{-1})	0.122	0.030
k_4 (min^{-1})	2.176	0.033
k_5 (min^{-1})	0.216	0.300
f_m (—)	0.467	0.126

Each parameter was estimated by the least-squares fit of the data based on the simplex method. The mean values of 3 or 4 observed values at each time point were used for the least-squares regression analysis. The standard deviation of each parameter could not be estimated because one observed value was obtained from one animal. The reasonable parameters converging could not be obtained by using the Gauss-Newton methods which are known to give standard deviations.[9] *a*) Not calculated.

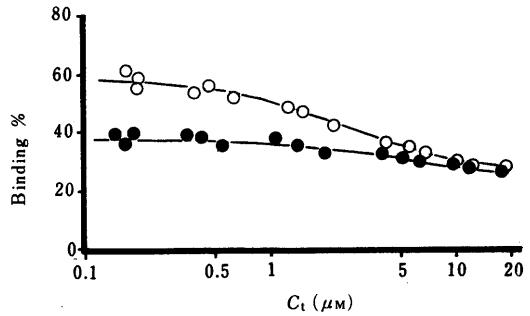

Fig. 5. Binding % of Emedastine to 25% Liver Homogenate of Rats (○) and Guinea Pigs (●)

C_t is the total (bound and unbound) concentration of emedastine in 25% liver homogenate. The lines represent the least-squares fit of the data.

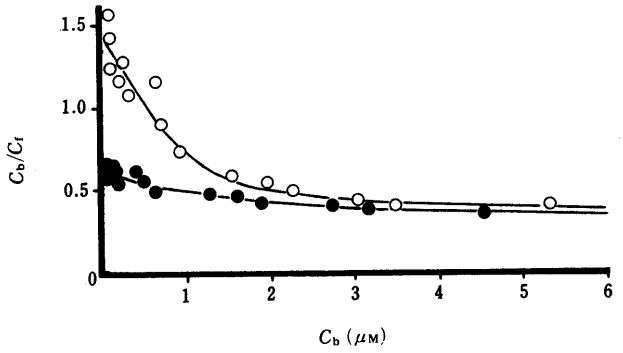

Fig. 6. Scatchard Plots of Emedastine Binding to 25% Liver Homogenate of Rats (○) and Guinea Pigs (●)

C_b and C_f are the concentration of the bound and unbound emedastine, respectively. The lines represent the least-squares fit of the data.

pronounced interspecies differences in the metabolic rate constant, k_4. And the value of f_m in rats was about 4-times larger than that for guinea pigs, where f_m is a parameter related with metabolism in the isolated liver after the excision (see Appendix). We also tried to analyze the hepatic transport using some other models, *i.e.*, a model without defining f_m or without the compartment for X_2 in rats. However, the predicted values were well fitted to the observed values only using the model shown in Fig. 4.

Tissue Binding Binding % of emedastine to 25% liver homogenate and its Scatchard plots are shown in Figs. 5 and 6, respectively. The binding of emedastine to rat liver homogenate was higher than that to guinea pig liver homogenate. Binding data were fitted to the following equation by a non-linear least-squares method, since Scatchard plots were biphasic in both species,

$$C_b = P_1 C_f/(Kd_1 + C_f) + (P_2/Kd_2)C_f \quad (1)$$

where C_b and C_f are the concentrations of the bound and unbound drug, P_1 and P_2 are the capacities of the high and low affinity sites, and Kd_1 and Kd_2 are the dissociation constants for the high and low affinity sites, respectively. The calculated binding parameters are shown in Table II. The binding affinity of emedastine to rat liver homogenate was higher than that to guinea pig liver homogenate, since Kd_1 in guinea pigs, 3.42, was 4-times greater than that in rats, 0.807. On the other hand, the binding capacity at high affinity site and the binding characteristic at low affinity site in rats were comparable to those in guinea pigs.

Table II. Binding Parameters of Emedastine to Liver Homogenate of Rats and Guinea Pigs

Parameter		Rat	Guinea pig
P_1[a]	(μM)	3.70±0.69	4.72±2.20
Kd_1	(μM)	0.81±0.17	3.42±1.32
P_2/Kd_2[a]	(—)	1.26±0.11	1.10±0.16

Each value represents the computer-estimated parameter±S.D. Each parameter was estimated by the least-squares fit of the data based on the damping Gauss-Newton method. *a*) Corrected for dilution of the liver homogenate.

Discussion

Interspecies differences in the hepatic transport of emedastine were examined in rats and guinea pigs, taking notice of the influx, efflux and metabolic processes by use of an *in vivo* tissue sampling single injection technique.[6]

When the concentration of emedastine in the injection was varied in the liver uptake study (Fig. 2), the extraction ratio of total radioactive compound to the liver at 18 s in rats and 25 s in guinea pigs after the rapid portal injection was more than 90% of the dose at a concentration of less than 10 mM. These periods are considered to be sufficient for a single pass of the bolus through the liver, but short enough to minimize the efflux of labelled compound from the liver or the recirculation of labelled compound.[8] The influx velocity of emedastine into the liver appeared to be extremely rapid in both species, and there were no interspecies differences in the influx processes.

Subsequently, the time courses of disappearance of total radioactive compound and unchanged emedastine from the liver were analyzed using the compartment model (Fig. 4) for the examination of efflux and metabolic processes (Fig. 3, Table I). Two assumptions were made for the model analysis. One was that the influx velocity of emedastine into the liver is much faster than those of the other processes, and the other was that the recirculation of radiolabelled compound is negligible. The former is supported by the fact mentioned above (Fig. 2); the latter is partly supported by the fact that the distribution volumes of emedastine (rats: 7.92 l/kg, guinea pigs: 2.66 l/kg) were very large.[7] Emedastine was observed to have a pronounced interspecies difference in metabolic rate constant, k_4 (Table I). This result was in good agreement with the larger value of

f_m in rats than in guinea pigs (Table I). But there was no pronounced interspecies difference in efflux rate constant of emedastine, k_3, or metabolites, k_5 (Table I). Consequently, the interspecies difference in the behavior of emedastine in the first pass through the liver was due to the difference in the metabolic process and not in the influx and efflux processes.

Since the time courses of disappearance of unchanged emedastine from liver were biphasic in rats and monophasic in guinea pigs (Fig. 3) the compartment for X_2 existed in rats and not in guinea pigs for the model analysis (Fig. 4). In the model, emedastine in the compartment for X_2 is not available for transport from liver and hepatic metabolism. It has generally been believed that only a drug not bound to protein is available for metabolism and transport across the tissue membrane. Since the binding of emedastine to the macromolecule in the liver was relatively high in rats and low in guinea pigs (Figs. 5, 6, Table II), it might be presumed that the emedastine in the compartments for X_1 and X_2 represent the unbound and bound form, respectively. Namely, interspecies difference in tissue binding might be responsible for the difference in disappearance curves (Fig. 3) of unchanged emedastine from the liver in rats and guinea pigs.

In conclusion, when emedastine first passes through the liver of rats and guinea pigs, there is a pronounced interspecies difference in the metabolic process, but not in the influx and efflux processes.

Appendix

Model Analysis of Hepatic Disappearance The transfer processes in the compartment system presented in Fig. 4 may be described as follows.

Rat:
$$dX_1/dt = k_2X_2 - (k_1+k_3+k_4)X_1$$
$$dX_2/dt = k_1X_1 - k_2X_2$$
$$dM_1/dt = k_4X_1 - k_5M_1$$

Guinea pig:
$$dX_1/dt = -(k_3+k_4)X_1$$
$$dM_1/dt = k_4X_1 - k_5M_1$$

As initial conditions, $X_1 = X_0$ and $X_2 = M_1 = 0$ at $t = 0$. Taking the Laplace transform of these sets of differential equations, $X_1 + X_2$ and M_1 (rat) or X_1 and M_1 (guinea pig) at t are solved for,

Rat:
$$X_1 + X_2 = A\cdot\exp(-\alpha t) + B\cdot\exp(-\beta t)$$
where
$$A = X_0(\alpha - k_1 - k_2)/(\alpha - \beta)$$
$$B = X_0(k_1 + k_2 - \beta)/(\alpha - \beta)$$
$$\alpha\beta = (k_3+k_4)k_2$$
$$\alpha + \beta = k_1 + k_2 + k_3 + k_4$$
$$M_1 = [X_0k_4/\{(\alpha-\beta)(\alpha-k_5)(\beta-k_5)\}] \times \{(\alpha-\beta)(k_2-k_5)\cdot\exp(-k_5t) + (\beta-k_5)(k_2-\alpha)\cdot\exp(-\alpha t) + (k_5-\alpha)(k_2-\beta)\cdot\exp(-\beta t)\}$$

Guinea pig:
$$X_1 = X_0\cdot\exp\{(-k_3+k_4)t\}$$
$$M_1 = \{X_0k_4/(k_3+k_4-k_5)\}[\exp(-k_5t) - \exp\{(-k_3+k_4)t\}]$$

Simultaneous computer multi-line fitting of the data was done for the following equations:

Rat: fraction of dose for the amount of total radioactive compound in the liver $= (X_1+X_2)+M_1$
fraction of dose for the amount of unchanged emedastine in the liver $= (1-f_m)(X_1+X_2)$

Guinea pig: fraction of dose for the amount of total radioactive compound in the liver $= X_1 + M_1$
fraction of dose for the amount of unchanged emedastine in the liver $= (1-f_m)X_1$

where f_m is a parameter related with metabolism in the isolated liver after the excision, and is defined as follows:

Rat: $f_m = 1 - (X_1' + X_2')/(X_1 + X_2)$

Guinea pig: $f_m = 1 - X_1'/X_1$

where $X_1' + X_2'$ for rat and X_1' for guinea pig represent the fraction of dose for the amount of unchanged emedastine in the isolated liver when the metabolic reaction in the isolated liver is stopped by refrigeration after cutting off the portal vein at t.

References

1) T. Fukuda, Y. Morimoto, R. Iemura, T. Kawashima, T. Tsukamoto and K. Ito, *Arzneim.-Forsch. Drug Res.*, **34**, 801 (1984); T. Fukuda, T. Saito, S. Tajima, K. Shimohara and K. Ito, *ibid.*, **34**, 805 (1984); T. Fukuda, T. Saito and K. Ito, *ibid.*, **34**, 811 (1984); T. Fukuda, T. Saito, M. Yoshidomi and K. Ito, *ibid.*, **34**, 816 (1984).
2) T. Sakai, H. Takahashi, T. Hamada, N. Awata and J. Watanabe, *Xenobio. Metabol. Dispos.*, **2**, 123 (1987).
3) Y. Wada, H. Takahashi, T. Hamada, T. Sakai, T. Kawashima and N. Awata, *Xenobio. Metabol. Dispos.*, **4**, 459 (1989).
4) T. Sakai, T. Hamada, N. Awata and J. Watanabe, *J. Pharmacobio-Dyn.*, **12**, 530 (1989).
5) S. Miyauchi, Y. Sugiyama, Y. Sawada, K. Morita, T. Iga and M. Hanano, *J. Pharmacokinet. Biopharm.*, **15**, 25 (1987).
6) S. Miyauchi, Y. Sugiyama, Y. Sawada, T. Iga and M. Hanano, *Chem. Pharm. Bull.*, **35**, 4241 (1987).
7) T. Sakai, T. Hamada, N. Awata and J. Watanabe, *Chem. Pharm. Bull.*, **37**, 753 (1989).
8) W. M. Pardridge and L. S. Jefferson, *Am. J. Physiol.*, **228**, 1155 (1975); W. M. Pardridge, *ibid.*, **232**, E492 (1977); W. M. Pardridge and L. J. Mietus, *ibid.*, **237**, E367 (1979); W. M. Pardridge, A. J. Van Herle, R. T. Naruse, G. Flierer and A. Costin, *J. Biol. Chem.*, **258**, 990 (1983).
9) K. Yamaoka, Y. Tanigawara, T. Nakagawa and T. Uno, *J. Pharmacobio-Dyn.*, **4**, 879 (1981).