Species Differences in Stereoselective Hydrolase Activity in Intestinal Mucosa

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Purpose. The aim of this study is to investigate species differences in the stereoselective hydrolysis for propranolol ester prodrugs in mammalian intestinal mucosa and Caco-2 cells.

Methods. Hydrolase activities for propranolol prodrugs and *p*-nitrophenylacetate in man (age: 51–71 years), the beagle dog (age: 4 years) and Wistar rat (age: 8 weeks) intestinal mucosa, and also in Caco-2 cells (passage between 60–70) were estimated by determining the rate of production of propranolol and *p*-nitrophenol, respectively.

Results. The hydrolase activities for both propranolol prodrugs and *p*-nitrophenylacetate were in the order of man > rat >> Caco-2 cells > dog for intestinal microsomes, and rat > Caco-2 cells ≒ man > dog for intestinal cytosol. Dog microsomes showed stereoselective hydrolysis for propranolol prodrugs, but not those from human or rat. Interestingly, both subcellular fractions of Caco-2 cells showed remarkable R-enantioselectivity except acetyl propranolol. Enzyme kinetic experiments for each enantiomer of butyryl propranolol in microsomes revealed that dog possesses both low and high affinity hydrolases. Both Km and Vmax values in rat were largest among examined microsomes, while Vmax/Km was largest in man. Finally, it was shown that the carboxylesterases might contribute to the hydrolysis of propranolol prodrug in all species by inhibition experiments.

Conclusions. The hydrolase activities for propranolol prodrugs and *p*-nitrophenylacetate in intestinal mucosa showed great species differences and those in human intestine were closer to those of rat intestine than dog intestine or Caco-2 cells.

KEY WORDS: *O*-acyl-propranolol; stereoselective hydrolysis; intestinal mucosa; carboxylesterase.

INTRODUCTION

Recently, considerable attention has been focused on drug metabolism in the small intestine, after the oral administration of drugs, since various types of metabolic enzymes, such as oxidases, hydrolases, and related enzymes are located in the small intestinal mucosa (1,2). While the levels of intestinal drug metabolizing enzymes might be lower than liver, the intestinal mucosa might play an important role as the first drug metabolizing organ (3,4). This is due to the fact that drugs must be transported across the intestinal membrane during absorption and that the transit time for this process is lengthy (5). The long transit time for this process might lead to an increased opportunity for contact with enzymes in enterocytes, as compared with hepatocytes. The hydrolases, including carboxylesterases (EC 3.1.1.1), which contribute to the hydrolysis of ester functional groups in drugs in several tissues are in the small intestinal mucosa of several species, including human (6). Indeed, it has been clearly shown that the small intestine contributes to first-pass metabolism, after oral administration of some ester drugs in *in vivo* (7).

In general, esterase activities show great species-differences in liver and plasma, and they possess high stereoselectivity, a very important process for the stereoselective metabolism of racemic ester drugs. Therefore, in animal scale up for the evaluation of pharmacokinetics and pharmacological effects, it is important to understand the difference in esterase activities in tissues of humans, *vis-a-vis* experimental animals. Few reports, however, are available concerning species differences for activity and stereoselectivity of esterases in the small intestine. Moreover, little is known about esterase activity in Caco-2 cell monolayers, which is the most widely used membrane model for the intestinal absorption of drug (8,9).

It has been reported that ester derivatives of propranolol (PL) enhanced the oral bioavailability of PL by inhibiting presystemic metabolism via masking the glucuronidation-site of PL and/or causing saturation of hepatic metabolism of the PL in rat and dog (10–12). In addition, it has been reported that enzymatic regeneration of PL ester prodrugs occurs stereoselectively in liver and plasma (13) and that the stereoselective hydrolysis of these compounds is different between rat and dog, and dependent on structures of substituents as well (14).

The purpose of this paper is to compare the stereoselective hydrolase activity in human intestinal mucosa with those in dog and rat intestinal mucosa and in Caco-2 cells using PL ester prodrugs as substrates. The hydrolase activities of six *O*-acyl PL prodrugs with nonbranched acyl groups, ranging from acetyl to enantyl in several species were compared with that of *p*-nitrophenylacetate (PNPA) which is often used as a substrate for esterases. Moreover, the nature of intestinal hydrolase were examined by enzyme-kinetics and inhibition experiments of hydrolysis for butyryl PL enantiomers in each species and in Caco-2 cells as well.

MATERIALS AND METHODS

Materials

Racemic PL hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and R- and S-PL hydrochloride (Sigma Chemical Co., St Louis, MO, purity: 99.8%, respectively) were obtained as commercial samples. A Caco-2 cell line was obtained from the American Type-Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), nonessential amino acid (NEAA) were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Cytosystems PTY (Castle Hill, Australia). The racemic *O*-acyl PL hydrochlorides and each enantiomer of butyryl PL hydrochloride were synthesized from racemic or enantiomeric PL hydrochloride and fatty acid chlorides (Tokyo Kasei, Tokyo, Japan) according to previously reported methods (11,13). The identity and purity of the synthesized prodrugs were established by infra-red, elemental analysis,

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^{1}H-nuclear magnetic resonance and high performance liquid chromatography (HPLC, purity by HPLC: >99.0%) (11). All other reagents used were of analytical grade.

Cell Culture

Caco-2 cells between passage 60 to 70 were routinely cultured in DMEM (pH 7.4) which was supplemented 10% heated-inactivated FBS, 1% NEAA, 100U/ml penicillin and 100μg/ml streptomycin at 37°C under an atmosphere of 95% air and 5% CO_2. Cells were harvested by treatment with 0.05% trypsin 0.53mM/ethylenediamine tetraacetate (EDTA) for 5 min at 37°C prior to reaching confluence. Cells were washed twice with DMEM to remove trypsin and EDTA and were used for preparations of microsomes and cytosol.

Preparation of Microsomes and Cytosol from Intestinal Mucosa and Caco-2 Cells

A portion of the jejunum from five, nonsmoking male patients (age: 51, 61, 63, 67 and 71 years) were obtained as the result of a carcinectomy of stomach in Japanese Red Cross Kumamoto Hospital. Pathological tests showed that the obtained small intestines were free of carcinoma. After resection of intestine, the intestines were immediately frozen in dry-ice and the mucosa removed within 1 hr. The study was approved by the local ethics committee. Male beagle dogs (9–10kg, 4–5 years, n = 4) and male Wistar rats (230–270g, 8 weeks, n = 6) were used after overnight fasting with free access to water. Dogs and rats were sacrificed by exsanguination under ether anesthesia and decapitation, respectively. The intestine was removed and washed with ICE-cold 0.15M KCl. The stripped intestinal mucosa and Caco-2 cells were homogenized with 3 volumes of 10mM phosphate buffer (pH 7.4) containing 0.15 M KCl in a Potter-Elvehjem glass homogenizer equipped with a Teflon pestle under ice-cold conditions. The following method of preparation for microsomes and cytosol was according to a previous report (14). The homogenates (25%wet w/v) were centrifuged at 10,000g for 20 min at 0°C, to give the supernatant fraction (S10). The S10 fraction was further centrifuged at 105,000 × *g* for 1 hr at 4°C, and the resulting supernatant was used as the cytosolic fraction. The pellets were washed and resuspended by homogenization in phosphate buffer and were used as microsomes. Protein contents were determined by the method of Lowry *et al.* with bovine serum albumin as a standard (15). The microsomal and cytosolic fractions were stored at −80°C until use.

Hydrolysis Experiments for PL Prodrugs and PNPA

Hydrolysis experiments of PL prodrugs and PNPA were performed in the subcellular fractions of rat, dog and human intestine and Caco-2 cells according to methodology described in previous reports (1,14). The reactions were initiated by the addition of PL prodrugs and PNPA (final concentration: 100μM). The subcellular fractions were diluted with pH7.4 Tris-HCl (50mM) buffer at the specified concentrations. An HPLC assay for each enantiomer of PL was performed, as described in an earlier report (14). The hydrolysis of PNPA to *p*-nitrophenol was determined spectrophotometrically (U best-35, Jusco, Tokyo, Japan) from the increase in absorbance at 400nm (1).

Inhibition Experiments

One ml of microsomal and cytosolic fraction of intestinal mucosa and Caco-2 cells were preincubated for 5 min with different concentrations of BNPP (bis-nitrophenyl phosphate, final concentration: 10^{-8}–10^{-3} M) dissolved in 5 μl of dimethyl sulfoxide (DMSO) and without esterase inhibitor (5 μl of DMSO) as the control. The protein contents were equivalent to the corresponding concentration employed in the hydrolysis experiments. The enantiomers of butyryl PL, dissolved in 5 μl of DMSO, were then added to both fractions resulting in a final concentration of 50 μM. The final concentration of DMSO was 1% which had no effect on hydrolase activity. Incubation was carried out for 5 min and the PL formed was determined by HPLC.

Statistical Analysis

Data are represented as the mean with standard deviation (mean±s.d.). Statistical significance of difference in hydrolase activity between R- and S- isomer was evaluated by paired Student's t-test.

RESULTS

Hydrolysis of Racemic PL Prodrug in Microsomes

The stereoselective hydrolyses of racemic PL prodrugs in the microsomal fraction of rat, dog and human intestinal mucosa and Caco-2 cells are shown in Table I. The human microsomal hydrolase activities for the PL prodrugs were closer to rat than to either dog or Caco-2 cells. The microsomal hydrolases in rat and human intestines showed a relatively smaller stereoselectivity, and their hydrolase activities increased with the number of carbon atoms in the substituent group up to valeryl PL. In addition, the hydrolase activities in dog intestine and Caco-2 cells were markedly low, but showed stereoselectivity except enantyl PL and acetyl PL, respectively. Prodrugs with small numbers of carbon atoms in the substituent group, especially propionyl PL, showed high activities in dogs. Interestingly, in dogs, an S-preference for hydrolysis was found for acetyl, propionyl and butyryl PL, and the opposite selectivity was found in other prodrugs with longer acyl chains. The hydrolyses of prodrugs by Caco-2 cell preparations showed a little dependency on substituent groups, and an R-preference for hydrolysis except acetyl PL. Furthermore, a large individual variation was observed in human samples, compared with rat and dog. The hydrolase activities for the donor who showed the highest activities among patients were 3-fold greater than that of donors who showed the lowest activities. The intestine from all donors showed non-stereoselective hydrolysis and the same substituent specificities.

Hydrolysis of Racemic PL Prodrug in Cytosol

Table II shows comparative data on the hydrolase activities in the cytosol of several species. The cytosolic hydrolase activities of human intestine for PL prodrugs were ten-fold lower than the microsomal activities and were similar to those in Caco-2 cells, in comparison with those in rat and dog intestine. A relatively smaller stereoselectivity and a substituent-specificity were very similar between microsomes and cytosol in man.

Table I. Hydrolysis of PL Prodrugs in Microsomes of Intestinal Mucosa and Caco-2 Cells

Prodrugs		Activity (nmol/min/mg protein)			
		Rat	Dog	Man	Caco-2 cells
Acetyl PL	R	6.4 ± 1.6	0.87 ± 0.19*	34.8 ± 18.6	2.4 ± 0.5
	S	9.6 ± 1.7	4.77 ± 1.12	60.5 ± 35.0	2.8 ± 0.5
Propionyl PL	R	31.2 ± 3.8	2.74 ± 0.84*	129.9 ± 76.8	2.9 ± 0.3*
	S	42.9 ± 5.1	10.30 ± 2.15	159.1 ± 95.2	1.9 ± 0.1
Butyryl PL	R	97.1 ± 5.3	0.61 ± 0.23*	140.9 ± 69.7	3.4 ± 1.2*
	S	77.4 ± 3.5	2.01 ± 0.50	154.0 ± 77.7	1.2 ± 0.6
Valeryl PL	R	123.6 ± 27.5	0.79 ± 0.20*	216.5 ± 114.3	4.8 ± 1.7
	S	102.0 ± 20.9	0.41 ± 0.04	242.3 ± 123.6	1.6 ± 0.5
Caproyl PL	R	128.7 ± 34.6	0.94 ± 0.32*	166.9 ± 97.5	4.4 ± 0.4*
	S	108.4 ± 37.7	0.38 ± 0.05	206.4 ± 114.4	1.5 ± 0.1
Enantyl PL	R	112.2 ± 26.4	1.09 ± 0.54	141.5 ± 80.5	3.0 ± 0.2*
	S	105.9 ± 35.0	0.44 ± 0.17	183.0 ± 97.4	1.3 ± 0.1

Note: Values represent the mean ± s.d. (n = 4–6 for animals, three experiments for Caco-2 cells).
* $P < 0.05$ versus S-isomer.

Table II. Hydrolysis of PL Prodrugs in Cytosol of Intestinal Mucosa and Caco-2 cells

Prodrugs		Activity (nmol/min/mg protein)			
		Rat	Dog	Man	Caco-2 cells
Acetyl PL	R	10.0 ± 1.8*	0.45 ± 0.07	2.6 ± 1.3	6.7 ± 0.8
	S	17.3 ± 2.7	0.60 ± 0.12	4.6 ± 3.1	7.2 ± 2.1
Propionyl PL	R	34.8 ± 4.9*	0.49 ± 0.04	8.7 ± 6.9	9.2 ± 0.9*
	S	55.8 ± 4.5	0.63 ± 0.09	11.7 ± 8.8	3.6 ± 0.3
Butyryl PL	R	171.7 ± 22.9*	0.38 ± 0.08*	10.4 ± 5.4	22.2 ± 1.6*
	S	117.4 ± 15.4	0.71 ± 0.08	12.0 ± 5.9	4.9 ± 0.9
Valeryl PL	R	260.7 ± 69.3	0.53 ± 0.12	17.2 ± 12.9	30.1 ± 4.0*
	S	201.1 ± 22.5	0.54 ± 0.16	19.7 ± 13.8	6.3 ± 0.8
Caproyl PL	R	233.6 ± 46.4	0.51 ± 0.07	13.5 ± 9.7	33.2 ± 3.3*
	S	230.1 ± 46.3	0.44 ± 0.09	17.0 ± 10.6	6.8 ± 0.5
Enantyl PL	R	217.0 ± 41.9	0.46 ± 0.21	11.2 ± 8.4	28.1 ± 3.4*
	S	227.9 ± 52.0	0.24 ± 0.09	14.7 ± 10.0	6.7 ± 0.6

Note: Values represent the mean ± s.d. (n = 4–6 for animals, three experiments for Caco-2 cells).
* $P < 0.05$ versus S-isomer.

In addition, the cytosolic activities of the Caco-2 cells were significantly R-stereospecific except acetyl PL and were 2–9-fold greater than microsomes. Furthermore, the hydrolysis activities of the R- and S-isomers in Caco-2 cells increased with substituent carbon number up to caproyl PL and were nearly constant with the substituent carbon number, respectively. The hydrolase activities in rat were extensively higher than other species, and their stereoselectivity and substituent-specificity were same as those in microsomes. The hydrolase activities in dog were nearly the same among the six prodrugs and lower than those in microsomes. Only butyryl PL showed stereoselectivity in dog cytosol in contrast to microsomes.

Hydrolysis of PNPA

The hydrolase activity of PNPA, which is frequently employed as substrate for purification of carboxylesterase, was examined in rat, dog and human intestines and Caco-2 cells (Table III). The activities were found to be in the following order, man > rat > dog ≒ Caco-2 cells in microsomes and rat>man > Caco-2 cells>dog in cytosol. The hydrolase activities for PNPA in dog, human and Caco-2 cells were similar to those reported by Prueksaritanont *et al.* (1). PNPA was hydrolyzed more rapidly than the PL prodrugs in both subcellular fractions, however, the species difference for distribution of subcellular hydrolase activity was nearly the same between PNPA and PL prodrugs.

Table III. Hydrolysis of PNPA in Intestinal Mucosa and Caco-2 Cells

Species	Activity (nmol/min/mg protein)	
	Microsomes	Cytosol
Man	1818 ± 234.5	98.94 ± 60.06
Dog	22.05 ± 1.123	8.911 ± 2.892
Rat	211.1 ± 7.646	369.3 ± 9.612
Caco-2 cells	20.91 ± 1.632	34.64 ± 1.361

Note: Values represent the mean ± s.d. (n = 4–6 for animals, three experiments for Caco-2 cells).

Enzyme Kinetics of Each Enantiomer of Butyryl PL

Enzyme kinetic parameters were estimated by Lineweaver-Burk plots for the hydrolysis of the R- and S-isomers of butyryl

PL (Table IV). Human intestine showed the highest values of Vmax/Km indicative of hydrolytic clearance. The differences of Km or Vmax between each enantiomer were not observed for human and rat. The Vmax in rat was higher than that of man, however, the Vmax/Km of rat was smaller than that of man, reflecting a low affinity. Interestingly, biphasic kinetics with low and high affinity components were observed in dog intestine. The Vmax values of both affinity phases were very low compared to human and rat, and were greater for the S-isomer. However, the stereoselectivity of Vmax/Km was found only in the case of the low affinity hydrolase owing to small Km value for R- isomer in the case of the high affinity hydrolase. In Caco-2 cells, the affinity and turnover for the R-isomer were higher than for the S-isomer at about four- and two-fold, respectively, and hydrolytic clearance was extremely different for the enantiomers.

Inhibition of Hydrolysis in Microsomes for Each Enantiomer of Butyryl PL by BNPP

BNPP, a selective inhibitor for carboxylesterase, was employed as an inhibitor (10 nM–1mM) for the hydrolysis of each enantiomer of butyryl PL. The relative activities in intestinal microsomes against logarithm of mol of BNPP per g protein of microsomes are shown in Fig. 1. BNPP inhibited the hydrolysis of each enantiomer of butyryl PL in microsomes of all species. Similar inhibition curves by BNPP were obtained for cytosol (data not shown), indicating that carboxylesterases might contribute to the hydrolysis of PL prodrugs in the intestinal microsomes and cytosol of all species. The sensitivities for esterase inhibitor in the microsomes of human intestinal mucosa were relatively less than those in rat and dog. Interestingly, stereoselective inhibition was not observed in man, rat and dog, while Caco-2 cells showed a different sensitivity between each enantiomer. In Caco-2 cells, the sensitivity of each enantiomer was different, and about 30% of the activity of the S-isomer remained at examined maximum concentrations of BNPP.

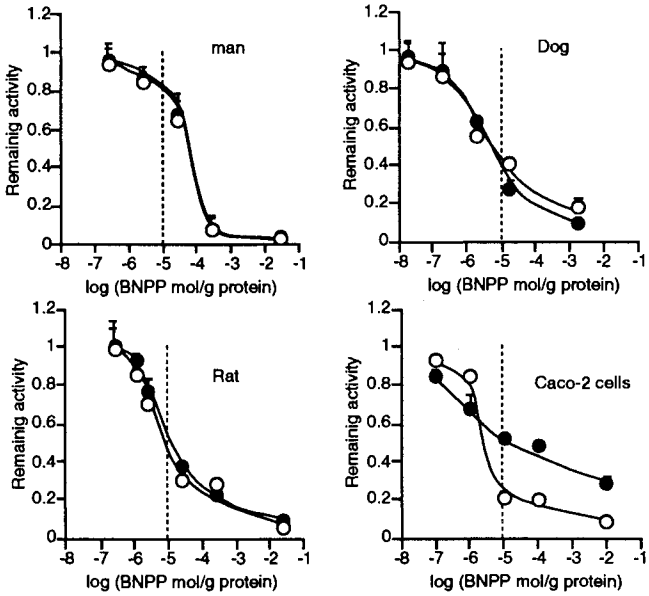

Fig. 1. The inhibition of BNPP for the hydrolysis of each enantiomer of butyryl PL in human, dog and rat intestinal and Caco-2 cell microsomes. BNPP concentration were 10 nM–1 mM. Remaining activities (○: R-isomer, ●: S-isomer) were plotted against logarithm of mol of BNPP per g protein of microsomes. Values represent the mean ± s.d. (n = 4-6 for animals and three experiments for Caco-2 cells).

DISCUSSION

An investigation of esterase activity of intestine is necessary for interpreting and predicting the oral disposition of drug which contains ester group. Furthermore, it is very important whether an ester prodrug is hydrolyzed to the parent drug in the intestine before reaching liver after oral administration, because the first hepatic extraction of parent drug varies, depending on the unchanged fraction of prodrug, as the result of difference in hepatic uptake clearance between the prodrug and parent drug (16). Therefore, species-difference in esterase activities in the intestine might lead to differences in *in vivo* disposition of ester prodrugs between experimental animals and man. Recently, Prueksaritanont *et al.* reported species differences in the hydrolase activity of PNPA and acetylsalicylic acid (ASP) in intestinal mucosa among man, monkey and dog (1). Interestingly, in their results, human

Table IV. Enzyme Kinetics of Each Enantiomer of Butyryl PL in Microsomes of Intestinal Mucosa and Caco-2 Cells

Parameters		Rat	Dog	Man	Caco-2 cells
Km 1[a]	R	560.3 ± 56.9	13.5 ± 3.2*	70.4 ± 27.9	39.5 ± 7.9
	S	561.2 ± 90.7	37.1 ± 9.6	99.9 ± 36.8	160.3 ± 33.4
Vmax 1[b]	R	837.2 ± 70.1	1.2 ± 0.5*	311.7 ± 124.9	3.9 ± 0.2*
	S	939.4 ± 52.5	4.0 ± 1.1	469.6 ± 124.9	1.9 ± 0.2
Vmax/Km 1[c]	R	1.51 ± 0.27	0.094 ± 0.041	4.47 ± 0.86	0.102 ± 0.012*
	S	1.69 ± 0.18	0.116 ± 0.051	4.89 ± 1.15	0.011 ± 0.003
Km 2[a]	R	N.D.[d]	216.7 ± 20.1	N.D.[d]	N.D.[d]
	S	N.D.[d]	179.5 ± 63.2	N.D.[d]	N.D.[d]
Vmax 2[b]	R	N.D.[d]	3.62 ± 1.14*	N.D.[d]	N.D.[d]
	S	N.D.[d]	9.51 ± 2.43	N.D.[d]	N.D.[d]
Vmax/Km 2[c]	R	—	0.022 ± 0.015*	—	
	S	—	0.061 ± 0.024		

Note: Values represent the mean ± s.d. (n = 4–6 for animals, three experiments for Caco-2 cells).
[a] μM.
[b] nmol/min/mg.
[c] ml/min/mg.
[d] N.D.: not detected.
* $P < 0.05$ versus S-isomer.

intestine microsomes showed markedly high hydrolase activity for PNPA but quite low hydrolase activity for ASP. The hydrolytic properties for PL prodrugs and their species difference were similar to PNPA, compared to ASP. It should be also noted that the hydrolyses of PNPA, ASP and PL prodrugs were extremely slow in dog, compared to other mammals. Moreover, substitute specificities, stereoselectivity and biphasic kinetics for the hydrolysis of PL prodrugs in dog were markedly different from rat and man. These results indicate that the oral disposition of an ester-containing drug through the small intestine in dog might differ from man. The intestinal hydrolase activity in man was closer to that of rat, in comparison with dog. However, a calculation of the volume ratio of subcellular fraction in a cell suggests that the total hydrolase activity for PL prodrugs is higher in rat than in man, due to high cytosolic activity in rat.

In general, hydrolases possess high stereoselectivities and it has been reported that rat small intestine showed a high enantiospecificity for the hydrolysis of oxazepam 3-acetate (17) and cefuroxime axetil (18). However, the enantioselectivity of hydrolysis for PL prodrugs was relatively small in rat intestine. Unlike hydrolysis in the intestine, PL prodrugs were stereoselectively hydrolyzed in both rat plasma and liver preparations as reported previously (14). In liver, PL prodrugs were hydrolyzed by carboxylesterases (14), which also contributed to hydrolysis of PL prodrugs in intestinal microsomes (Fig. 1). Therefore, the difference in hydrolysis for stereoselectivity between rat liver and intestinal mucosa, respectively, might be derived from differences in carboxylesterase-isozymes. These differences were also supported by the following; PNPA was hydrolyzed by both low and high affinity carboxylesterases in rat liver microsomes (19), while rat intestine microsomes showed only low affinity for PNPA (19) as well as PL prodrugs (see Table IV), and antibodies of high and low-affinity hydrolases of liver failed to react with proteins in intestinal microsomes (20). In contrast to dog intestinal hydrolase, the dog hepatic hydrolase showed only a high affinity-kinetics with non-enantioselective Km value and R- enantiospecific Vmax value for the isovaleryl PL (13). These data suggest that the hydrolase in dog intestine is also different from the hepatic hydrolase. The carboxylesterase purified from human intestine showed high activity for β-naphtyl esters and low activity for ASP (6). Taking account of the fact that PL prodrugs also possess a naphtyl group, the same type of carboxylesterase might contribute to hydrolysis for PL prodrugs in human intestine. The hydrolase characteristics of human intestine, e.g. high Vmax/Km and relatively less sensitivity to BNPP inhibition, were different from other species, which might be caused by carboxylesterase-isozymes or the amount of esterases. The most similar species to human might be monkey (1). However, the prediction of the human disposition of ester prodrug based on animal experiments has been quite difficult until now. Further experiments, such as the purification of esterases and western immunoblotting techniques are required to confirm species differences of intestinal esterases, and more details of substrate specificities in terms of hydrolase activities of human intestine are also required.

In contrast to human intestinal mucosa, the hydrolases in Caco-2 cells showed a very low activity, a remarkable stereoselectivity and a distribution in cytosol. These differences of hydrolase between human intestine and Caco-2 cells might be caused by properties of Caco-2 cells originated from carcinoma of the human colon. It has been reported that the carboxylesterase was cloned from human intestine, and it was found that the carboxylesterase expressed at higher levels in the small intestine than in the colon (21). Moreover, the nature of the stereoselective hydrolysis in carcinoma was different from normal cells (22), because carcinoma expresses a different type of hydrolase, compared to normal cells (23). Caco-2 cells are frequently used as a model monolayer membrane to evaluate the absorption characteristics of drugs (8). The low activity of Caco-2 cells might be suitable to evaluate the membrane permeation of ester drug. However, caution should be exercised when the properties of esterase activity in Caco-2 cells is used to evaluate the absorption of an ester drug or a prodrug.

REFERENCES

1. T. Prueksaritanont, L. M. Gorham, J. H. Hochman, L. O. Tran, and K. P. Vyas. Comparative studies of drug-metabolizing enzymes in dog, monkey, and human small intestines, and Caco-2 cells, *Drug Metab. Dispos.* **24**:634–642 (1996).
2. L. S. Kaminsky and M. J. Fasco. Small intestinal cytochromes P450, *Toxicology* **21**: 407–422 (1992).
3. M. Lefebvre, W. Homsy, G. Caille, and P. D. Souich. First-pass metabolism of diltiazem in anesthetized rabbit: Role of extrahepatic organs, *Pharm. Res.* **13**:124–128 (1996).
4. A. A. Raoof, P. F. Augustijns, and R. K. Verbeek. *In vivo* assessment of intestinal, hepatic, and pulmonary first pass metabolism of propofol in rat, *Pharm. Res.* **13**:891–895 (1996).
5. S. Yamashita, M. Yoshida, Y. Taki, T. Sakane, and T. Nadai. Kinetic analysis of the drug permeation process across the intestinal epithelium, *Pharm. Res.* **11**:1646–1651 (1994).
6. M. Inoue, M. Morikawa, M. Tuboi, T. Yamada, and M. Sugiura. Hydrolysis of ester-type drugs by the purified esterase from human intestinal mucosa, *Jpn. J. Pharmacol.* **29**:17–25 (1979).
7. K. Iwamoto, M. Takei, and J. Watanabe. Gastrointestinal and hepatic first pass metabolism of aspirin in rats, *J. Pharm. Pharmacol.* **34**:176–180 (1981).
8. M. D. Ribadeneira, B. J. Aungst, C. J. Eyermann, and S. M. Huang. Effects of structural modification on the intestinal permeability of angiotensin II receptor antagonists and the correlation of in vitro, in situ, and in vivo absorption, *Pharm. Res.* **13**:227–233 (1996).
9. T. S. Engelen, V. Delistrie, and Y. J. Schneider, Phase I and II biotransformations in living Caco-2 cells cultivated under serum-free conditions, *Biochem. Pharmacol.* **46**:1393–1401 (1993).
10. Y. Garuceau, I. Davis, and J. Hasegawa. Plasma propranolol levels in beagle dogs after administration of propranolol hemisuccinate ester, *J. Pharm. Sci.* **67**:1360–1363 (1978).
11. M. Shameen, T. Imai., and M. Otagiri. An in-vitro and in-vivo correlative approach to the evaluation of ester prodrugs to improve oral delivery of propranolol, *J. Pharm. Pharmacol.* **45**:246–252 (1993).
12. B. D. Anderson, W. W. Chu, and R. E. Galinsky. Reduction of first-pass metabolism of propranolol after oral administration of ester prodrugs, *Int. J. Pharm.* **43**:261–265 (1988).
13. M. Shameen, T. Imai, Y. Yoshigae, A. Sparreboom, and M. Otagiri. Stereoselective hydrolysis of O-isovaleryl propranolol and its influence on the clearance of propranolol after oral administration, *J. Pharm. Sci.* **83**:1754–1757 (1994).
14. Y. Yoshigae, T. Imai, A. Horita, and M. Otagiri, Species differences for stereoselective hydrolysis of propranolol prodrugs in plasma and liver, *Chirality* **9**:661–666 (1997).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the folin phenol reagent, *J. Biol. Chem.* **193**:264–275 (1951).
16. I. A. M. de Lannoy, F. Barker.III, and K. S. Pang. Formed and preformed metabolite excretion clearance in liver, a metabolite formation organ: Studies on enalapril and enalaprilate in the sin-

gle-pass and recirculating perfused rat liver, *J. Pharmacokin. Biopharm.* **21**:395–422 (1993).
17. S. K. Yang, Y. Y. Hsieh, W. C. Chang, and J. D. Huang. Enantioselectivity of microsomal and cytosolic esterases in rat intestinal mucosa, *Drug Metab. Dispos.* **20**:719–25 (1992).
18. G. L. Mosher, J. McBee, and D. B. Shaw. Esterase activity toward the diastereomers of cefuroxime axetil in the rat and dog, *Pharm. Res.* **9**:687–689 (1992).
19. E. W. Morgan, B. Yan, D. Greenway, and A. Parkinson. Purification and characterization of two rat liver microsomal carboxylesterases (hydrolase A and B), *Arch. Biochem. Biophys.* **315**:495–512 (1994).
20. E. W. Morgan, B. Yan, D. Greenway, and A. Parkinson, Regulation of two rat liver microsomal carboxylesterase isozymes: species differences, tissue distribution, and the effects of age, sex, and xenobiotic treatment of rats, *Arch. Biochem. Biophys.* **315**:513–26 (1994).
21. H. Schwer, T. Langmann, R. Daig, A. Becker, C. Aslanidis, and G. Schmitz, Molecular cloning and characterization of a novel putative carboxylesterase, Present in human intestine and liver, *Biochem. Biophys. Res. Comun.* **233**:117–120 (1997).
22. Y. Kageyama, Y. Yamazaki, A. S. Afify, Y. Ogawa, T. Okada, and H. Okuno, Stereoselective hydrolysis of xenobiotic esters by different cell lines from rat liver and hepatoma and its application to chiral prodrugs for designated growth suppression of cancer cells, *Chirality* **7**:297–304 (1995).
23. A. S. Afify, Y. Yamazaki, Y. Kageyama, S. Yusa, Y. Ogawa, T. Okada, and H. Okuno, Different expression of esterase variants in rat hepatic and hepatoma-derived cell lines detected by electrophoresis, *Z. Naturforsch. C.* **50**:664–8 (1995).