Dose Dependency in the Gastrointestinal Absorption of Cefatrizine: Correlation between *in Vivo* and *in Situ*

Kenji MATSUDA, Hiroaki YUASA,* and Jun WATANABE

Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-0027, Japan.

Received November 10, 1997; accepted February 10, 1998

We evaluated the dose-dependent (saturable) gastrointestinal absorption of cefatrizine, an aminocephalosporin transported by peptide carriers, in rats by a physiological mechanism-based approach to clarify its absorption characteristics and to examine the *in vitro* (*in situ*)–*in vivo* correlation in intestinal transport. With an increase in oral dose ($\mu\text{mol}/5\text{ ml/kg}$) from 5 (low) to 50 (high), the intestinal absorption rate constant (k_a), which was estimated by analysis of gastrointestinal disposition, decreased markedly, from 0.301 to 0.056 min^{-1} . This decrease was ascribable to the saturability of intestinal membrane transport, of which the concentration dependency in the perfused intestine was similar in extent to the dose dependency in k_a . However, the apparent absorption rate constant (k_a'), which was estimated by analysis of plasma concentrations after oral administration, decreased only modestly from 0.037 to 0.023 min^{-1} . This was associated with the result that, at the low dose, k_a' was far smaller than k_a and comparable with k_g (gastric emptying rate constant), suggesting gastric emptying-limited absorption. At the high dose, where intestinal cefatrizine absorption was less efficient, k_a' was closer to k_a than k_g . It was also observed that the bioavailability was close to unity, independent of dose, suggesting that the intestinal transit time is long enough to achieve complete absorption, even at the high dose, where intestinal cefatrizine absorption is less efficient. Thus, it was found that the effect of saturability in the intestinal transport of cefatrizine is apparently attenuated in its overall gastrointestinal absorption because of the involvement of gastric emptying and intestinal transit time as additional physiological factors to define absorption. It was also found that a scaling factor is required to correlate the intestinal membrane transport between *in vitro* (*in situ*) and *in vivo*, though this remains to be verified to be utilized for developing oral drug delivery strategies and optimizing oral drug therapy.

Key words intestinal absorption; carrier-mediated transport; cefatrizine; gastrointestinal disposition; rat

The intestinal peptide carrier-mediated transport system has been suggested to have broad substrate specificity and to transport several peptide-like drugs that belong to, for example, aminocephalosporins and angiotensin converting enzyme (ACE) inhibitors.¹⁾ For aminocephalosporins, it has been suggested that being a substrate of the peptide carriers is one of the major factors that allow it to achieve efficient absorption and to be orally available. For these reasons, and in an effort to exploit the carriers for oral drug delivery by clarifying the transport mechanism, the peptide transport system has been subjected to extensive investigation, mainly *in vitro* (*in situ*). However, there have been few attempts to characterize dose-dependent (saturable) absorption by peptide carriers *in vivo*, or to verify accumulating knowledge about the transport mechanism in terms of a quantitative *in vitro* (*in situ*)–*in vivo* correlation.

We, therefore, chose cefatrizine as a model aminocephalosporin transported by peptide carriers,²⁾ and evaluated its dose-dependent gastrointestinal absorption *in vivo* by a physiological mechanism-based approach, featuring our developed analysis of gastrointestinal drug disposition after oral administration.³⁾ This approach enables us to evaluate the intestinal absorption process, which has not been hybridized by the gastric emptying process, for direct comparison with intestinal transport *in vitro* (*in situ*), and it facilitates establishing a quantitative *in vitro* (*in situ*)–*in vivo* correlation in intestinal transport and clarifying gastrointestinal absorption characteristics. This should be helpful for developing oral drug delivery strategies and optimizing oral drug therapy.

MATERIALS AND METHODS

Chemicals Cefatrizine propylene glycol was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{14}C]Polyethylene glycol (PEG) 4000 (481.0 MBq/g) and Scintisol, a scintillation cocktail, were purchased from DuPont-NEN Co. (Boston, MA, U.S.A.). Soluene-350, a tissue solubilizer, was purchased from Packard Instrument Co., Inc. (Meriden, CT, U.S.A.). All other reagents were of analytical grade and commercially obtained.

Solutions The dosing solutions were prepared in a citrate buffer (52 mM HCl, 65.6 mM citric acid, 120 mM NaOH, 35.9 mM NaCl; pH 4.0), and contained 1 mM, for low dose, or 10 mM, for high dose, of cefatrizine and a trace amount (3.7 kBq/7.7 $\mu\text{g/ml}$) of [^{14}C]PEG 4000 as a nonabsorbable marker.

Animals Male Wistar rats, weighing about 300 g, were used after fasting overnight unless otherwise indicated.

Gastrointestinal Disposition Experiments The rats were given an oral dose of 5 $\mu\text{mol}/5\text{ ml/kg}$ (low dose) or 50 $\mu\text{mol}/5\text{ ml/kg}$ (high dose) of cefatrizine with 38.5 $\mu\text{g}/5\text{ ml/kg}$ of [^{14}C]PEG 4000, using a gastric tube. The rats were then left free in a metabolic cage at an ambient temperature of 23 °C, and sacrificed at 10, 20, 40, or 60 min after administration to determine the distribution of cefatrizine and [^{14}C]PEG 4000 in the gastrointestinal tract, where the small intestine was divided into the duodenum, representing about 1/10th the length, and three equal lengths of segments (jejunum, midgut and ileum).³⁾ The gastrointestinal contents were obtained by washing the segments with 3 ml, for the stomach, or 2 ml, for intestinal segments, of citrate

* To whom correspondence should be addressed.

buffer (pH 4.0).

After adding an appropriate amount of citrate buffer (pH 4.0), the gastrointestinal contents and tissues were homogenized, and a portion of each homogenized sample was solubilized for the determination of radioactivity ($[^{14}\text{C}]\text{PEG 4000}$) by liquid scintillation counting, as described previously,³⁾ using Soluene-350 (1 ml) as a tissue solubilizer and Scintisol EX-H (5 ml) as a scintillation cocktail.

Cefatrizine in the gastrointestinal contents was analyzed by HPLC with UV detection after deproteinization, as follows.⁴⁾ Ammonium sulfate solution (50% w/v, 0.5 ml) was added to 0.5 ml of each homogenized sample, and the mixture was allowed to stand at 4 °C for 30 min (shaken at 10 min intervals). The deproteinized supernatant was obtained by centrifugation at 4 °C and 15000 *g* for 10 min with a MRX-150 centrifuge (Tomy Seiko Co., Tokyo, Japan), and filtered with a disposable filter (Dismic-25CS 0.45 μm ; Advantec Co., Tokyo, Japan) before being injected (150 μl) into a HPLC system: column, $\mu\text{Bondapak C}_{18}$ (3.9 mm i.d. $\times$ 300 mm; Waters, Milford, MA, U.S.A.); mobile phase, 7% CH_3CN in 20 mM NaH_2PO_4 (pH 5.0)⁵⁾; flow rate, 1.6 ml/min; detector, UV 270 nm (SPD-10A; Shimadzu Co., Kyoto, Japan).⁶⁾ The linearity of the calibration curve was confirmed over the range of 0.005 to 0.1 mM ($r^2=0.99$) and the coefficient of variation (C.V.) value was less than 5% for every point.

Analysis of Gastrointestinal Disposition The fraction of cefatrizine recovered from each segment of the gastrointestinal tract was estimated as the sum of that in the content sample added to that in the fluid adhering to the tissue. The volume of adherent fluid was estimated by dividing the amount of PEG 4000, a nonabsorbable marker associated with the tissue sample, by its concentration in the content sample.

The fraction of cefatrizine recovered was normalized by the total fraction of PEG 4000 dose recovered from the gastrointestinal tract to correct for minor fluctuations in sampling, and the corrected values, represented as fraction remaining (*FR*), were subjected to the following model analysis.

Assuming that solute transfer is defined by gastric emptying and subsequent intestinal absorption with the first-order rate constants of k_g and k_a , respectively, the remaining fractions of dose in the stomach (FR_s) and small intestine (FR_{si}) are described as a function of time (*t*) as follows:³⁾

$$FR_s = e^{-k_g \cdot t} \quad (1)$$

$$FR_{si} = (e^{-k_s \cdot t} - e^{-k_a \cdot t}) / (1 - k_g/k_a) \quad (2)$$

Equations 1 and 2 were simultaneously fitted to FR_s and FR_{si} data for cefatrizine to estimate k_a and k_g using a nonlinear regression program, Pcnonlin (Scientific Consulting, Inc., Cary, NC, U.S.A.), and weighed according to the reciprocal of the variance. Although these model equations are mathematically the same as those for a linear compartment model consisting of the stomach and small intestine compartments, they are not derived from the ordinary compartment model assumption of even drug distribution in the small intestine. A drug given orally is normally distributed unevenly along the intestinal tract. However, as discussed in our previous report,³⁾ assuming that the apparent intestinal membrane per-

meability clearance for unit length (CL_{app}) and the average intestinal lumen volume for unit length (V_{av}) can be assigned as constant values along the small intestine, and that k_a represents the ratio of CL_{app} to V_{av} , the intestinal absorption rate can be expressed as the product of k_a and the total amount of the drug in the small intestine independent of the distribution of the drug in the intestinal tract, allowing for mathematical handling identical to that for compartment models.

Plasma Concentration Measurements The rats were cannulated in the right jugular vein under light ether anesthesia. After regaining consciousness and being allowed a recovery period of 1 h, each rat was orally (through a gastric tube) or intravenously (through the cannula) given a dose of 5 $\mu\text{mol}/5 \text{ ml/kg}$ (low dose) or 50 $\mu\text{mol}/5 \text{ ml/kg}$ (high dose) of cefatrizine, and left free in a metabolic cage at an ambient temperature of 23 °C; 0.25 ml of blood was taken periodically through the cannula and placed in a centrifuge tube containing 5 units of heparin and centrifuged for 3 min with a Microfuge E (Beckman Instruments, Inc., Palo Alto, CA, U.S.A.) to obtain plasma. Citrate buffer (pH 4.0, 0.2 ml) and ammonium sulfate solution (50% w/v, 0.3 ml) were added to 100 μl aliquots of plasma, and the mixture was subjected to deproteinization and subsequent HPLC assay for the determination of cefatrizine concentration as described for gastrointestinal contents, except that the supernatant was filtered with Dismic-13CP (0.45 μm) instead of Dismic-25CS (0.45 μm) before being injected into the HPLC system. The linearity of the calibration curve was confirmed over the range of 0.001 to 0.1 mM ($r^2=0.99$) and the C.V. value was less than 5% for every point.

Pharmacokinetic Analysis of Plasma Concentration

The plasma concentration (*C*) versus time (*t*) profiles of cefatrizine were analyzed by a two-compartment model with first-order absorption, where the plasma concentrations after intravenous and oral administrations are described by Eqs. 3 and 4, respectively,⁷⁾

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \quad (3)$$

$$C = L \cdot e^{-\alpha \cdot t} + M \cdot e^{-\beta \cdot t} - N \cdot e^{-k_a \cdot t} \quad (4)$$

where

$$L = \frac{A \cdot F \cdot k_a'}{(k_a' - \alpha)} \quad (5)$$

$$M = \frac{B \cdot F \cdot k_a'}{(k_a' - \beta)} \quad (6)$$

$$N = L + M \quad (7)$$

and where *A*, *B*, α and β are constants, and k_a' and *F* are the apparent absorption rate constant and the bioavailability, respectively. The values of *A*, *B*, α and β were estimated by fitting Eq. 3 to the concentration versus time profiles after intravenous administration using a nonlinear regression program, Pcnonlin. With the values of *A*, *B*, α and β fixed, the values of k_a' and *F* were estimated by fitting Eq. 4 to the concentration versus time profiles after oral administration. The half-life of the β phase ($t_{1/2\beta}$), the distribution volume of the central compartment (Vd_1), the steady-state distribution volume (Vd_{ss}) and the total body clearance (CL_{tot}) were calculated from *A*, *B*, α and β .⁷⁾

Intestinal Perfusion Experiments Perfusion solutions were prepared in phosphate buffer (20.1 mM Na_2HPO_4 , 47.0 mM KH_2PO_4 , 101.0 mM NaCl; pH 6.4), and contained cefatrizine (0.01 or 10 mM) and a trace amount of [^{14}C]PEG 4000 as a nonabsorbable marker. Intestinal single-pass perfusion was carried out in rats (without fasting) anesthetized with urethane (1.25 g/kg, i.p.) or without anesthesia as previously described,⁸⁻¹⁰ using a 10-cm midgut segment and a perfusion rate of 0.15 ml/min. In perfusion in unanesthetized rats, surgical operation to attach inflow and outflow cannulas was carried out under ether anesthesia and perfusion was initiated right after the rat regained consciousness in a Bollman cage. The outflow solution was collected for 20 min at 5-min intervals, starting 25 min after the initiation of perfusion, by which time steady state was achieved.

Scintillation cocktail (Scintisol, 5 ml) was added to 50 μl aliquots of inflow and outflow solutions for the determination of radioactivity ([^{14}C]PEG 4000) by liquid scintillation counting. For the assay of cefatrizine, 0.5 ml of perfusion solution was filtrated with a disposable filter (Dismic-13CP 0.45 μm , Advantec Co., Tokyo, Japan), and 50 μl of the filtered solution was diluted with 0.95 ml of citrate buffer (pH 4.0) before being injected (200 μl) into the HPLC system described for gastrointestinal contents.

Analysis of Intestinal Perfusion Data The fraction of absorbed (F_a) cefatrizine was estimated as the fraction which disappeared from the intestinal lumen, correcting for a minor volume change based on the change in PEG 4000 concentration.

The preepithelial diffusional resistance, which generates a concentration gradient dropping toward the intestinal surface in the water phase adjacent to the intestinal surface and may affect the permeability estimates, was taken into account by using a model incorporated with an unstirred water layer (UWL),⁸⁻¹⁰ and the intestinal membrane permeability clearance (CL_m) was estimated to be one on the basis of the concentration at the intestinal surface, using the following equations:

$$\frac{1}{CL_{app}} = \frac{1}{CL_m} + \frac{1}{CL_{aq}} \quad (8)$$

$$CL_{app} = -\frac{Q}{L} \cdot \ln(1 - F_a) \quad (9)$$

$$CL_{aq} = 2\pi R \cdot P_{aq} = 2\pi R \cdot \frac{D}{\delta} \quad (10)$$

where CL_{app} and CL_{aq} are the apparent membrane permeability clearance and the permeability clearance of UWL, respectively; Q is the flow rate; and L and R are the length and radius of the perfused segment, respectively. The P_{aq} is the permeability coefficient of UWL and equal to D/δ , where D and δ are the diffusion coefficient and the effective thickness of UWL, respectively. Equation 8 describes that the apparent (or total) resistance ($1/CL_{app}$) consists of the resistances of the intestinal membrane ($1/CL_m$) and UWL ($1/CL_{aq}$) in a series. Equation 9 gives, assuming linearity in absorption and based on a tube model, CL_{app} on the basis of the bulk concentration, which is an average of inflow and outflow concentrations. The δ values were previously estimated to be 952 and

350 μm , respectively, in rats with urethane anesthesia and without anesthesia from the UWL-limited absorption of D-glucose at 1 mM,¹⁰ where CL_{aq} can be approximated by CL_{app} , as $1/CL_m$ is negligible in Eq. 8. The D of cefatrizine was calculated to be $4.39 \times 10^{-6} \text{ cm}^2/\text{s}$ from that of D-glucose ($7.04 \times 10^{-6} \text{ cm}^2/\text{s}$),¹¹ assuming that D is inversely proportional to the square root of molecular weight.¹² Using these D and δ values and R of 0.234 cm,⁹ the CL_{aq} of cefatrizine was calculated by Eq. 10, and, with the estimate of CL_{app} by Eq. 9, CL_m was calculated by Eq. 8.

Gastric Absorption The rats were anesthetized with urethane (1.25 g/kg, i.p.), and a low dose (5 $\mu\text{mol}/5 \text{ ml/kg}$) of cefatrizine was administered to the stomach, which was ligated at the cardia and the pylorus. Cefatrizine remaining in the gastric contents was determined 60 min after administration in the same way as that in the gastrointestinal contents to evaluate cefatrizine absorption (disappearance) from the stomach.

Biliary Excretion The rats (without fasting) were anesthetized with urethane (1.25 g/kg, i.p.). The common bile duct was cannulated with PE-10 tubing, and bile was collected for 60 min after the injection (1.0 ml) of cefatrizine solution (10 mM), which was prepared in phosphate buffer (pH 6.4) to which was added [^{14}C]PEG 4000 as a nonabsorbable marker, into a 10-cm midgut loop. The bile sample was processed as plasma samples, and the luminal solution obtained at the end of experiment was processed as the perfusion solution to evaluate cefatrizine absorption (disappearance) from the loop.¹³

Statistical Analysis Levels of statistical significance were assessed using Student's *t*-test.

RESULTS AND DISCUSSION

Gastrointestinal Distribution Profiles As shown in Fig. 1, the distribution profiles of cefatrizine along the gastrointestinal tract were similar in shape to those of PEG 4000 at every sampling time for both low and high doses, suggesting that the intestinal transit of cefatrizine is represented by that of PEG 4000 and that there was no significant biliary excretion. The fractional recovery of cefatrizine was comparable with that of PEG 4000 in the stomach for every sampling, suggesting insignificant gastric absorption, but lower than that of PEG 4000 in the small intestine. As described later, the bioavailability of cefatrizine was close to unity for both doses, suggesting that cefatrizine does not undergo significant degradation in the gastrointestinal tract. Therefore, the lower recovery of cefatrizine, compared with PEG 4000, in the small intestine is attributable to the disappearance of cefatrizine by intestinal absorption, and the increase in fractional recovery at the higher dose suggests saturability in absorption. Because the total recovery of PEG 4000 from the stomach and small intestine was about 100% throughout the experimental period of 60 min, the distribution of cefatrizine as well as PEG 4000 was assumed to be restricted within the region of the gastrointestinal tract.

Insignificant gastric absorption and biliary excretion of cefatrizine was confirmed in rats anesthetized with urethane as follows. The gastric absorption from the closed stomach was $6.9 \pm 4.7\%$ (mean $\pm$ S.E.; $n=3$) in 60 min, giving an absorption rate constant of $0.0012 \pm 0.0009 \text{ min}^{-1}$, which was negli-

Fig. 1. Gastrointestinal Distribution of Cefatrizine and Coadministered [^{14}C]PEG 4000 after Oral Administration in Rats

Panels 1a (cefatrizine) and 1b (PEG 4000) are for the low dose ($5\text{ }\mu\text{mol/kg}$), and panels 2a (cefatrizine) and 2b (PEG 4000) are for the high dose ($50\text{ }\mu\text{mol/kg}$). Results are represented as the average value ($n=4$). Key: S, stomach; D, duodenum; J, jejunum; M, midgut; I, ileum.

Fig. 2. Remaining Cefatrizine versus Time Profiles for the Stomach (○) and Small Intestine (●) after Oral Administration in Rats

Panels (A) and (B) are for the low ($5\text{ }\mu\text{mol/kg}$) and high ($50\text{ }\mu\text{mol/kg}$) doses, respectively. Results are represented as the mean $\pm$ S.E. ($n=4$). The solid lines represent the computer-fitted profiles.

gible compared with gastric emptying rate constants 50 to 60 times larger, as described later. The biliary excretion was only $6.17 \pm 0.28\%$ (mean $\pm$ S.E.; $n=3$) of the dose (or about 9% of absorbed cefatrizine) 60 min after administration to the closed midgut loop.

All these results meet the assumptions in the model analysis incorporated with only gastric emptying and intestinal absorption (Eqs. 1 and 2).

Kinetic Analysis of Gastrointestinal Disposition The fractions of cefatrizine dose recovered from all intestinal segments were added together for each sampling time to obtain the total fraction recovered from the small intestine. The fraction of cefatrizine recovered was corrected by dividing the total recovery of PEG 4000, and was named remaining fraction (FR) for model analysis. The remaining fraction ver-

sus time profiles of cefatrizine for the stomach and small intestine were successfully described by the model (Eqs. 1 and 2) up to 40 and 60 min, respectively, for the low and high doses (Fig. 2). For the low dose, the data at 60 min were not included in the analysis, because absorption was almost completed at about 40 min, when only about 7 and 4% of the dose were recovered from the stomach and small intestine, respectively; the remaining fractions at 60 min were unchanged, compared with those at 40 min, in both sites; and the recovery of cefatrizine from the small intestine at 60 min was statistically insignificant. The data at 40 min seemed to represent the final points in the absorption phase and, therefore, were included in the analysis. As the average recoveries at 40 and 60 min of about 4% of dose from the entire small intestine (Fig. 2), the volume of which is about 2 ml ($24\text{ }\mu\text{l/cm} \times 90\text{ cm}$),³⁾ gives the luminal concentration of about 2% of dose/ml, the luminal concentration seemed to be equilibrated with the plasma concentration at a comparable level (Fig. 3) after about 40 min.

The parameters are summarized in Table 1. While the gastric emptying rate constant (k_g) was not affected by dose, the intestinal absorption rate constant (k_a) was markedly reduced with dose. Although the gastrointestinal disposition of cefatrizine was approximated by a linear kinetic model at each dose, the reduction in k_a with dose suggests the involvement of saturable (nonlinear) intestinal membrane transport. At the low dose, k_a was about 4 times larger than k_g , suggesting gastric emptying-limited absorption. However, this was not the case at the high dose, where the k_a value was comparable with the k_g value. In the following section, the k_a and k_g are compared with the apparent absorption rate constant (k_a') estimated by the analysis of plasma concentrations.

Fig. 3. Plasma Concentrations of Cefatrizine after Intravenous (○) and Oral (●) Administration in Rats

Panels (A) and (B) are for the low (5 $\mu\text{mol/kg}$) and high (50 $\mu\text{mol/kg}$) doses, respectively. Results are represented as the mean $\pm$ S.E. ($n=3$). The solid lines represent the computer-fitted profiles.

Table 1. Dose Dependency in Gastrointestinal Disposition Parameters of Cefatrizine in Rats

Dose ($\mu\text{mol/kg}$)	k_g (min^{-1})	k_a (min^{-1})
5	0.077 ± 0.012	0.301 ± 0.038
50	0.063 ± 0.007	0.056 ± 0.006

Values of k_g and k_a are represented as the parameter with S.E. obtained by simultaneously fitting Eqs. 1 and 2, respectively, to the profiles of the average values of FR_s and FR_{si} versus time (Fig. 2), weighed according to the reciprocal of the variance.

Table 2. Dose Dependency in the Pharmacokinetic Parameters of Cefatrizine in Rats

Parameters	Dose ($\mu\text{mol/kg}$)	
	5	50
A (% of dose/ml)	1.98 ± 0.14	1.26 ± 0.31
B (% of dose/ml)	1.92 ± 0.02	0.93 ± 0.15^a
α (min^{-1})	0.267 ± 0.025	0.218 ± 0.066
β (min^{-1})	0.0065 ± 0.000	0.0118 ± 0.0037
$t_{1/2\beta}$ (min)	107 ± 6	79 ± 34
Vd_1 (ml/kg)	99 ± 4	183 ± 25^b
Vd_{ss} (ml/kg)	191 ± 2	381 ± 60^b
CL_{tot} (ml/min/kg)	1.27 ± 0.06	4.32 ± 0.14^b
k_a' (min^{-1})	0.037 ± 0.004	0.023 ± 0.001^b
F	0.988 ± 0.012	0.914 ± 0.086

Data are represented as the mean $\pm$ S.E. ($n=3$). Level of statistical significance compared with the value for the low dose (5 $\mu\text{mol/kg}$): a) $p < 0.01$; b) $p < 0.05$.

Pharmacokinetic Analysis of Plasma Concentrations

For both doses, the plasma concentration versus time profiles after intravenous and oral administration were successfully described by a two-compartment model and by a two-compartment model with first-order absorption, respectively (Fig. 3). The kinetic parameters are listed in Table 2. The increases in the distribution volumes (Vd_1 and Vd_{ss}) with dose may be due to a decrease in protein binding.¹⁴⁾ The increase in CL_{tot} may be due to decreases in protein binding¹⁴⁾ and renal reabsorption.¹⁵⁾

The k_a' decreased with dose only modestly by about 50% (Table 2), while k_a decreased by an order of magnitude (Table 1). This was associated with the result that, at the low dose, the k_a' of 0.037 min^{-1} was an order of magnitude smaller than the k_a of 0.301 min^{-1} and was comparable with the k_g of 0.077 min^{-1} , supporting the suggestion of gastric emptying-limited absorption. This was not the case at the high dose,

Table 3. Concentration Dependency in Cefatrizine Transport in the Perfused Rat Small Intestine *in Situ*

Concentration (mM)	$CL_{m,situ}$ ($\mu\text{l/min/cm}$)		$CL_{app,vivo}$ ($\mu\text{l/min/cm}$)
	With anesthesia	Without anesthesia	
0.01	3.58 ± 1.02	4.85 ± 1.08	7.22^a
10	0.46 ± 0.17	0.73 ± 0.48	1.34^b

Data are represented as the mean $\pm$ S.E. ($n=3$); $CL_{m,situ}$, membrane permeability clearance *in situ*. a) Apparent membrane permeability clearance *in vivo* ($CL_{app,vivo}$) for the low dose; b) $CL_{app,vivo}$ for the high dose.

where intestinal cefatrizine absorption was less efficient and k_a' was closer to k_g .

The bioavailability (F) was close to unity and independent of dose, suggesting that the intestinal transit time is long enough to achieve complete absorption, even at the high dose, where intestinal cefatrizine absorption was less efficient. This is in agreement with the orally absorbed fraction ($F_{a,oral}$) predicted to be as much as 0.988 from the combination of k_a of 0.056 min^{-1} , observed at the high dose, and small intestinal transit time (T_{si}) of 78 min from our previous study,¹⁶⁾ assuming that $F_{a,oral}$ is, at each dose, defined by linear absorption in the small intestine ($F_{a,oral} = 1 - e^{-k_a T_{si}}$).^{16,17)}

Thus, it was found that dose dependency in intestinal cefatrizine absorption represented by k_a is apparently attenuated in its overall gastrointestinal absorption represented by k_a' and F .

In Situ-in Vivo Correlation in Intestinal Transport

For cefatrizine absorption in the perfused rat intestine, carrier-mediated transport, with a Michaelis constant of 0.6 mM, has been suggested to be predominant at concentrations below 0.2 mM, while passive transport is predominant at concentrations above 2 mM.¹⁸⁾ In the present study, cefatrizine concentrations were 1 and 10 mM, respectively, at the low and high doses in dosing solutions, and would have been lower in the intestinal lumen after oral administration because of dilution by luminal fluid and disappearance by absorption. For the low dose (1.5 $\mu\text{mol/rat}$), the highest concentration of about 0.2 mM was observed at 10 min in the jejunum (Fig. 1), where about 5% of the dose was distributed in the jejunal volume of about 0.4 ml ($15 \mu\text{l/cm} \times 27 \text{ cm}$).³⁾ For the high dose (15 $\mu\text{mol/rat}$), the lowest concentration for the site of distribution (absorption) of about 2 mM was observed at 60 min in the ileum (Fig. 1), where about 10% of the dose was distributed in the ileal volume of about 0.8 ml ($29.5 \mu\text{l/cm} \times 27 \text{ cm}$).³⁾ Therefore, carrier-mediated transport would have been predominant at the low dose (luminal concentrations below 0.2 mM) and passive transport would have been predominant at the high dose (luminal concentrations above 2 mM).

To examine the *in situ-in vivo* correlation in intestinal cefatrizine transport, membrane permeability clearance ($CL_{m,situ}$) was evaluated in the perfused rat intestine at a low concentration of 0.01 mM, where carrier-mediated transport is predominant, and at a high concentration of 10 mM, where passive transport is predominant. The values of $CL_{m,situ}$ are listed in Table 3 with the values of $CL_{app,vivo}$ as the product of k_a and V_{av} (24 $\mu\text{l/cm}$ from our previous report³⁾). The $CL_{m,situ}$ values in anesthetized rats were comparable with those reported by others,¹⁸⁾ and the extent of concentration depen-

dependency in $CL_{m,situ}$, about a 7-fold difference between the low and high concentrations, was in excellent agreement with that of the dose dependency in $CL_{app,vivo}$ and, hence, in k_a . Thus, the saturability in k_a was suggested to be ascribable to that of intestinal membrane transport. However, the $CL_{app,vivo}$ values were about 3 times larger than the $CL_{m,situ}$ values in anesthetized rats, similar to our previous results for 5-fluorouracil (5-FU).¹⁹⁾ Although the $CL_{m,situ}$ values tended to be larger in unanesthetized rats than in anesthetized ones due to the effect of anesthesia, as previously reported,^{8,10)} it cannot fully account for the difference between $CL_{app,vivo}$ and $CL_{m,situ}$, rendering the issue a subject for future investigation.

Conclusions First, the dose-dependent gastrointestinal absorption of cefatrizine was successfully characterized in rats by a physiological mechanism-based approach.

Secondly, it was found that saturability in the intestinal transport of cefatrizine is apparently attenuated in its overall gastrointestinal absorption because of the involvement of gastric emptying and intestinal transit time as additional physiological factors to define absorption. This mechanism may at least partly explain the modest dose dependency in oral cefatrizine absorption in humans.¹⁵⁾

Thirdly, it was suggested that a scaling factor is required to correlate the intestinal membrane transport between *in vitro* (*in situ*) and *in vivo*. For cefatrizine transport as well as for 5-FU transport,¹⁹⁾ a factor of about 3 was suggested for the relation between $CL_{m,situ}$ (under urethane anesthesia) by a widely used perfusion technique and $CL_{app,vivo}$, though more extensive investigation is required to establish a correlation generally applicable to a variety of drugs or correlations for classified drugs. Correlations also need to be examined for various *in vitro* and *in situ* experimental systems.

Acknowledgments This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of

Education, Science, Sports and Culture, Japan.

REFERENCES

- 1) Tsuji A., Tamai I., *Pharm. Res.*, **13**, 963—977 (1996).
- 2) Sinko P. J., Amidon G. L., *J. Pharm. Sci.*, **78**, 723—727 (1989).
- 3) Yuasa H., Numata W., Ozeki S., Watanabe J., *J. Pharm. Sci.*, **84**, 476—481 (1995).
- 4) Nakashima E., Tsuji A., Mizuo H., Yamana T., *Biochem. Pharmacol.*, **33**, 3345—3352 (1984).
- 5) Yuasa H., Amidon G. L., Fleisher D., *Pharm. Res.*, **10**, 400—404 (1993).
- 6) Kimura T., Yamamoto T., Mizuno M., Suga Y., Kitade S., Sezaki H., *J. Pharmacobio-Dyn.*, **6**, 246—253 (1983).
- 7) Yuasa H., Kawanishi K., Watanabe J., *J. Pharm. Pharmacol.*, **47**, 373—378 (1995).
- 8) Yuasa H., Matsuda K., Watanabe J., *Pharm. Res.*, **10**, 884—888 (1993).
- 9) Yuasa H., Iga T., Hanano M., Watanabe J., *Biochim. Biophys. Acta*, **938**, 189—198 (1988).
- 10) Yuasa H., Matsuda K., Watanabe J., *Biol. Pharm. Bull.*, **18**, 747—752 (1995).
- 11) Yuasa H., Miyamoto Y., Iga T., Hanano M., *Biochim. Biophys. Acta*, **856**, 219—230 (1986).
- 12) Ballard B. E., Nelson E., *J. Pharmacol. Exp. Ther.*, **135**, 120—127 (1962).
- 13) Yuasa H., Kuno C., Watanabe J., *J. Pharm. Pharmacol.*, **49**, 26—29 (1996).
- 14) Deguchi Y., Nakashima E., Ishikawa F., Sato H., Tamai I., Matsushita R., Tofuku Y., Ichimura F., Tsuji A., *J. Pharm. Sci.*, **77**, 559—564 (1988).
- 15) Pfeffer M., Gaver R. C., Ximenez J., *Antimicrob. Agents Chemother.*, **24**, 915—920 (1983).
- 16) Yuasa H., Kawanishi K., Watanabe J., *J. Pharm. Pharmacol.*, **47**, 576—580 (1995).
- 17) Yuasa H., Kuno C., Watanabe J., *Biol. Pharm. Bull.*, **19**, 604—607 (1996).
- 18) Sinko P. J., Amidon G. L., *Pharm. Res.*, **5**, 645—650 (1988).
- 19) Yuasa H., Matsuda K., Gu J., Suzuki E., Yokouchi I., Watanabe J., *Biol. Pharm. Bull.*, **19**, 1494—1498 (1996).