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

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We evaluated the dose-dependent (saturable) gastrointestinal absorption of cefatrizine, an aminopenicillin 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 (μmol/5 ml/kg) from 5 (low) to 50 (high), the intestinal absorption rate constant (kₐ), which was estimated by analysis of gastrointestinal disposion, 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 kₐ. However, the apparent absorption rate constant (kₐ'), 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, kₐ' was far smaller than kₐ and comparable with kₑ (gastric emptying rate constant), suggesting gastric emptying-limited absorption. At the high dose, where intestinal cefatrizine absorption was less efficient, kₐ' was closer to kₑ than kₐ.

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, aminopenicillins and angiotensin converting enzyme (ACE) inhibitors. For aminopenicillins, 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 aminopenicillin transported by peptide carriers, 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. This approach enables us to evaluate the intestinal absorption process, which has not been hydridized 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.). [¹⁴C]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 μg/ml) of [¹⁴C]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 μmol/5 ml/kg (low dose) or 50 μmol/5 ml/kg (high dose) of cefatrizine with 38.5 μg/5 ml/kg of [¹⁴C]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 [¹⁴C]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). The gastrointestinal contents were obtained by washing the segments with 3 ml, for the stomach, or 2 ml, for intestinal segments, of citrate

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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 (\(^{14}C\)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.

Cefazitine 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, μBondapak C18 (3.9 mm i.d. ×300 mm; Waters, Milford, MA, U.S.A.); mobile phase, 7% CH3CN in 20 mM NaH2PO4 (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 μg (r²=0.99) and the coefficient of variation (C.V.) value was less than 5% for every point.

**Analysis of Gastrointestinal Disposition**

The fraction of cefazitine 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 cefazitine 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\(_g\)) and small intestine (FR\(_a\)) are described as a function of time (t) as follows:\(^3\)

\[ FR_g = e^{-k_g t} \]

\[ FR_a = (1 - e^{-k_a t})/(1 - k_a/k_g) \]

Equations 1 and 2 were simultaneously fitted to FR\(_g\) and FR\(_a\) data for cefazitine to estimate k\(_g\) and k\(_a\) using a nonlinear regression program, Penonlin (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,\(^1\) assuming that the apparent intestinal membrane permeability clearance for unit length (CL\(_{app}\)) and the average intestinal lumen volume for unit length (V\(_{int}\)) can be assigned as constant values along the small intestine, and that k\(_a\) represents the ratio of CL\(_{app}\) to V\(_{int}\), 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 μmol/5 ml/kg (low dose) or 50 μmol/5 ml/kg (high dose) of cefazitine, 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 cefazitine 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 1 μg (r²=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 cefazitine 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.\(^3\)

\[ C = A\cdot e^{-\alpha t} + B\cdot e^{-\beta t} \]  
\[ C = A\cdot e^{-\alpha t} + M\cdot e^{-\beta t} - N\cdot e^{-\gamma t} \]  
\[ \text{where} \]
\[ L = \frac{A\cdot F\cdot k'_g}{(k'_a - \alpha)} \]  
\[ M = \frac{B\cdot F\cdot k'_a}{(k'_g - \beta)} \]  
\[ N = L + M \]  

and where A, B, α and β are constants, and k\(_g\)' 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, Penonlin. With the values of A, B, α and β fixed, the values of k\(_g\)' 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\(_{1/2}\)β), the distribution volume of the central compartment (V\(_{d\beta}\)), the steady-state distribution volume (V\(_{d\alpha}\)) and the total body clearance (CL\(_{tot}\)) were calculated from A, B, α and β.\(^3\)
Intestinal Perfusion Experiments Perfusion solutions were prepared in phosphate buffer (20.1 mM Na₂HPO₄, 47.0 mM KH₂PO₄, 101.0 mM NaCl, pH 6.4), and contained ceftarizine (0.01 or 10 mM) and a trace amount of [³¹C]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. 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 after ether anesthesia and perfusion was initiated immediately 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 ([³¹C]PEG 4000) by liquid scintillation counting. For the assay of ceftarizine, 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 (Fₚ) ceftarizine 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 (CLₘ) was estimated to be one of the basis of the concentration at the intestinal surface, using the following equations:

\[
\frac{1}{CL_{app}} = \frac{1}{CL_m} + \frac{1}{CL_{as}}
\]

\[
CL_{app} = \frac{Q}{L} \ln(1 - F_p)
\]

\[
CL_m = 2\pi R \cdot P_{as} = 2\pi R \cdot \frac{D}{\delta}
\]

where CLₘ and CLₚ 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 Pₚ 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/CLₚ) consists of the resistances of the intestinal membrane (1/CLₘ) and UWL (1/CLₚ) in a series. Equation 9 gives, assuming linearity in absorption and based on a tube model, CLₚ 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, where CLₚ can be approximated by CLₚ as 1/CLₘ is negligible in Eq. 8. The D of ceftarizine was calculated to be 4.39 × 10⁻⁶ cm²/s from that of D-glucose (7.04 × 10⁻⁶ cm²/s), assuming that D is inversely proportional to the square root of molecular weight. Using these D and δ values and R of 0.234 cm, the CLₘ of ceftarizine was calculated by Eq. 10, and, with the estimate of CLₚ, by Eq. 9, CLₚ was calculated by Eq. 8.

Gastric Absorption The rats were anesthetized with urethane (1.25 g/kg, i.p.), and a low dose (5 µmol/5 ml/kg) of ceftarizine was administered to the stomach, which was ligated at the cardia and the pylorus. Cefarizine remaining in the antral contents was determined 60 min after administration in the same way as that in the gastrointestinal contents to evaluate ceftarizine 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 ceftarizine solution (10 mM), which was prepared in phosphate buffer (pH 6.4) to which was added [³¹C]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 ceftarizine absorption (disappearance) from the loop.

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 ceftarizine 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 ceftarizine is represented by that of PEG 4000 and that there was no significant biliary excretion. The fractional recovery of ceftarizine 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 ceftarizine was close to unity for both doses, suggesting that ceftarizine does not undergo significant degradation in the gastrointestinal tract. Therefore, the lower recovery of ceftarizine, compared with PEG 4000, in the small intestine is attributable to the disappearance of ceftarizine 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 ceftarizine as well as PEG 4000 was assumed to be restricted within the region of the gastrointestinal tract.

Insufficient gastric absorption and biliary excretion of ceftarizine were confirmed in rats anesthetized with urethane as follows. The gastric absorption from the closed stomach was 6.9 ± 4.7% (mean ± S.E.; n = 3) in 60 min, giving an absorption rate constant of 0.0012 ± 0.0009 min⁻¹, which was negli-
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 cefazolin) 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 cefazolin 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 cefazolin recovered was corrected by dividing the total recovery of PEG 4000, and was named remaining fraction ($FR$) for model analysis. The remaining fraction versus time profiles of cefazolin 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 cefazolin 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 ($24 \mu l/cm \times 90 cm$), 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 ($k_e$) was not affected by dose, the intestinal absorption rate constant ($k_a$) was markedly reduced with dose. Although the gastrointestinal disposition of cefazolin 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, $k_e$ was about 4 times larger than $k_a$, suggesting gastric emptying-limited absorption. However, this was not the case at the high dose, where the $k_a$ value was comparable with the $k_e$ value. In the following section, the $k_e$ and $k_a$ are compared with the apparent absorption rate constant ($k_{app}$) estimated by the analysis of plasma concentrations.
Table 1. Dose Dependency in Gastrointestinal Disposition Parameters of Cefatrizine in Rats

<table>
<thead>
<tr>
<th>Dose (μmol/kg)</th>
<th>$k_e$ (min$^{-1}$)</th>
<th>$k_i$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.077±0.012</td>
<td>0.301±0.038</td>
</tr>
<tr>
<td>50</td>
<td>0.063±0.007</td>
<td>0.056±0.006</td>
</tr>
</tbody>
</table>

Values of $k_e$ and $k_i$ 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 $FR_e$ and $FR_i$ versus time (Fig. 2), weighed according to the reciprocal of the variance.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (μmol/kg)</th>
<th>5</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ (% of dose/ml)</td>
<td>1.98±0.14</td>
<td>1.26±0.31</td>
<td></td>
</tr>
<tr>
<td>$B$ (% of dose/ml)</td>
<td>1.92±0.02</td>
<td>0.93±0.15$^{a}$</td>
<td></td>
</tr>
<tr>
<td>$\alpha$ (min$^{-1}$)</td>
<td>0.267±0.025</td>
<td>0.218±0.066</td>
<td></td>
</tr>
<tr>
<td>$\beta$ (min$^{-1}$)</td>
<td>0.0065±0.0000</td>
<td>0.0118±0.0037</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>107±6</td>
<td>79±34</td>
<td></td>
</tr>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>99.4</td>
<td>183±25$^{a}$</td>
<td></td>
</tr>
<tr>
<td>$CL_{tot}$ (ml/min/kg)</td>
<td>191±2</td>
<td>381±60$^{a}$</td>
<td></td>
</tr>
<tr>
<td>$CL_{app}$ (ml/min/kg)</td>
<td>1.27±0.06</td>
<td>4.32±0.14$^{a}$</td>
<td></td>
</tr>
<tr>
<td>$k'$ (min$^{-1}$)</td>
<td>0.037±0.004</td>
<td>0.023±0.001$^{a}$</td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>0.988±0.012</td>
<td>0.914±0.086</td>
<td></td>
</tr>
</tbody>
</table>

Data are represented as the mean±S.E. (n=3). Level of statistical significance compared with the value for the low dose (5 μmol/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 ($V_d$ and $V_d$) with dose may be due to a decrease in protein binding. The increase in $CL_{tot}$ may be due to decreases in protein binding and renal reabsorption.

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

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 ($F_{oral}$) predicted to be as much as 0.988 from the combination of $k_i$ of 0.056 min$^{-1}$, observed at the high dose, and small intestinal transit time ($T_{int}$) of 10 min from our previous study, assuming that $F_{oral}$ is, at each dose, defined by linear absorption in the small intestine ($F_{oral}=1-e^{-t/T_{int}}$).

Thus, it was found that dose dependency in intestinal cefatrizine absorption represented by $k_i$ is apparently attenuated in its overall gastrointestinal absorption represented by $k'$ 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. 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 μmol/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 μl/cm×27 cm) for the high dose (15 μmol/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 μl/cm×27 cm). Therefore, carrier-mediated transport would have been predominant at 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 ($CL_{m,in}$) 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 $CL_{m,in}$ are listed in Table 3 with the values of $CL_{app,in}$ as the product of $k_i$ and $V_d$ (24 μl/cm from our previous report). The $CL_{m,in}$ values in anesthetized rats were comparable with those reported by others, and the extent of concentration depen-
dency in CL_{m,situ} 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). 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, 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 ceftrazinie was successfully characterized in rats by a physiological mechanism-based approach. Secondly, it was found that saturability in the intestinal transport of ceftrazinie 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 ceftrazinie absorption in humans.

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 ceftrazinie transport as well as for 5-FU transport, 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.

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