Absorption and Metabolic Extraction of Diltiazem from the Perfused Rat Small Intestine

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Summary: The metabolic extraction of diltiazem was examined in conjunction with its absorption, using rat small intestine perfused in situ by the single-pass method, to clarify its intestinal metabolism. This is a topic of increasing interest which has not been fully clarified, particularly as far as the extent of metabolic extraction and the enzymes involved (cytochrome P450 (CYP) 3A and/or others) are concerned. The intestinal availability \( F_i \) of diltiazem was evaluated at steady-state by dividing the fraction absorbed into the mesenteric venous blood \( (F_{a,b}) \) by the fraction that disappeared from the intestinal lumen \( (F_a) \). The \( F_i \) of diltiazem (0.05 mM) was 0.126 and, hence, the extraction ratio \( E_i = 1 - F_i \) was 0.874, indicating that diltiazem undergoes extensive first-pass metabolism during its passage through the intestinal mucosa. The \( E_i \) was unchanged when the concentration was increased to 0.5 mM, suggesting that metabolism is linear over this concentration range. Thereafter, \( E_i \) decreased with concentration, demonstrating saturable metabolism, and reached an insignificant level at the highest concentrations of 30 and 50 mM. The decrease in \( E_i \), or increase in \( F_i \), was brought about by an increase in \( F_{a,b} \) (from about 0.02 to about 0.05) in the concentration range up to 10 mM and by a decrease in \( F_a \) (from about 0.15 to about 0.05) at concentrations higher than that. These results suggest that the extraction observed at the lower concentrations is almost solely attributable to metabolic extraction of a saturable nature. However, ketoconazole and cyclosporin A, which are specific CYP3A inhibitors, inhibited the metabolic extraction of diltiazem (0.05 mM) by only about 20% at the concentration (40 \( \mu \)M) at which they inhibited CYP3A almost completely, suggesting that the contribution of CYP3A to intestinal diltiazem metabolism is not marked. Thus, the present study demonstrates that diltiazem undergoes extensive first-pass metabolism in the rat small intestine, although the contribution of CYP3A seems to be relatively minor.

Key words: diltiazem; absorption; metabolism; CYP3A; small intestine; rat

Introduction

Although the liver is mainly responsible for the metabolic elimination of drugs from the systemic circulation, it has been suggested that the small intestine could also significantly contribute to first-pass metabolism. Intestinal first-pass metabolism has been an issue of increasing importance since CYP3A (3A4 in humans, and 3A9 and 3A18 in rats), a major drug metabolizing enzyme, has been found to be present at a significant level in the small intestine and involved in drug interactions.1-7 Drug interactions arising from intestinal metabolism include the well known inhibition of CYP3A by grapefruit juice,20 which could lead to an increase in the bioavailability of drugs that are substrates of CYP3A.

Diltiazem, a benzothiazepine calcium channel blocker widely used in the treatment of angina, hypertension and cardiac arrhythmias, is known to undergo extensive first-pass metabolism after oral administration in humans.8,9 It is also known that diltiazem is a substrate of CYP3A10 and, as suggested by a pharmacokinetic study using rats,11 it is likely that diltiazem undergoes extensive metabolism in the small intestine as well as in the liver. Nevertheless, its bioavailability is reported to be unaffected or increased only minimally by grapefruit
Unlike that of nifedipine and several other CYP3A substrate drugs. One possibility that could explain this is that CYP3A is not the major enzyme involved in the intestinal metabolism of diltiazem, taking into account the finding that hydrolytic deacetylation by esterases as well as demethylation by CYP3A are major pathways of the metabolism of diltiazem in various animals, including humans and rats. To address such issues, we examined the metabolism of diltiazem in more detail using the perfused rat small intestine, which was previously shown to be effective in evaluating metabolism more directly and specifically at the intestinal tissue level in a study involving the CYP3A-mediated metabolism of nifedipine. The bioavailability of diltiazem is reportedly greater in the former as far as its total elimination is concerned.

However, as long as it is likely that both CYP3A and esterases are significantly involved in the intestinal extraction of diltiazem, as well as in its total elimination in both humans and rats, knowledge gained from the perfused rat intestine model could be useful in clarifying its intestinal metabolism in humans.

Materials and Methods

Chemicals: Diltiazem, theophylline, ketoconazole and cyclosporin A were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), imipramine and 7-(β-hydroxypropyl)theophylline were from Sigma Chemical Co. (St. Louis, MO, USA), bis(4-nitrophenyl)phosphate (BNPP) was from Aldrich Chemical Co. (Milwaukee, WI, USA), POE(60) hydrogenated castor oil (HCO-60) was from Nihon Surfactant Kogyo K.K., and [1,2-3H]polyethylene glycol (PEG) 4000 (55.5 MBq/g) was from PerkinElmer Life Sciences, Inc. (Boston, MA, USA). All other reagents were of analytical or HPLC grade and commercially available.

Animals: Male Wistar rats, weighing about 300 g, were purchased from Nihon SLG (Hamamatsu, Japan) and fasted overnight with free access to water before starting the experiments.

In situ intestinal perfusion: In situ single-pass perfusion was conducted in rats anesthetized with sodium pentobarbital (50 mg/mL/kg, i.p.) as described previously, using a 10-cm midgut segment and a perfusion rate of 0.15 mL/min (IC3100 microsyringe pump, KD Scientific, Inc., Boston, MA, USA) and collecting mesenteric venous blood while infusing fresh blood via the femoral vein (0.4 mL/min; Minipulse III peristaltic pump, Gilson, Inc., Middleton, WI, USA). The blood for infusion was collected in advance from several other rats. Perfusion solutions consisted of 20.1 mM Na2HPO4•12H2O, 47.0 mM KH2PO4, 101.0 mM NaCl, 1% ethanol and 0.01% HCO-60 (pH 6.4), and contained diltiazem and a trace amount of [3H]PEG 4000 (1.85 kBq/0.033 mg/mL) as a nonabsorbable marker. In inhibition experiments, ketoconazole, cyclosporin A or BNPP was added to the perfusion solution. The outflow solution and the total mesenteric venous blood draining the perfused segment were collected at 5-min intervals.

A series of experiments were also conducted using theophylline, as a reference compound known not to undergo significant first-pass metabolism after oral administration.

We confirmed that the adsorption of diltiazem and theophylline to the perfusion tubing (Naflon® PTFE tubing, Nichias Co., Tokyo, Japan) and the blood drainage tubing (Silascon® silicone tubing, Kaneka Medix Co., Osaka, Japan) was negligible.

Analytical methods: Blood samples (500 μL aliquots) were subjected to hemolysis by addition of 100 μL distilled water. Diltiazem in the samples of perfusate and the hemolysate of mesenteric venous blood was analyzed by HPLC, according to the method of Dube et al. Briefly, imipramine as the internal standard (3 μM in 100 μL methanol), 0.5 M NaHCO3 (200 μL) and t-butyldimethyl ether (5 mL) as the extraction solvent were added to each sample (100 μL perfusate or 600 μL hemolysate). After shaking for 10 min at a rate of 300 strokes/min (SR-2s reciprocating shaker, Tai Tec Co., Ltd., Nagoya, Japan) and subsequently centrifuging (1,600 g, 10 min, 4°C), 4 mL extraction solvent (the upper, organic layer) was transferred to a fresh sample tube containing 0.017 M phosphoric acid (200 μL). After shaking for 1 min at a rate of 300 strokes/min and centrifuging (900 g, 5 min, 4°C), the organic layer was discarded by aspiration under vacuum and the aqueous layer was injected (100 μL) into the HPLC system: column, μBondapak® C18 3.9 mm × 300 mm (Waters Co., Milford, M.A., USA); mobile phase, 30% acetonitrile in 10 mM ammonium phosphate buffer with 0.06% triethylamine (pH 3.75); flow rate, 1.0 mL/min; detection wavelength, 237 nm (SPD-10A, Shimadzu Co., Kyoto, Japan). Theophylline in the samples of perfusate and mesenteric venous blood was analyzed by HPLC, according to the method of Chow et al. A mixture (200 μL) of 10% perchloric acid and acetonitrile (1:1) including 7-(β-hydroxypropyl)theophylline as the internal standard (0.63 mM) was added to each sample (200 μL perfusate or blood). The sample was vortex mixed for 10 s and then centrifuged (10,000 g, 3 min, 4°C). After adding 200 μL mobile phase used in the HPLC analysis described below to the supernatant (90 μL), the mixture was injected (100 μL) into the HPLC system: column, TSK-
GEL ODS-80TM, 4.6 mm × 150 mm (Tosoh Co., Tokyo, Japan); mobile phase, 7% 2-propanol in 10 mM sodium acetate buffer (pH 4.0); flow rate, 1.0 mL/min; detection wave length, 273 nm.

There were no interfering peaks in the HPLC chromatograms of both diltiazem and theophylline extracted from the perfusate and blood. The detectable diltiazem concentrations were as low as 0.5 μM and 0.1 μM, respectively, in the perfusate and blood, and 2 μM in both cases for theophylline, which is at least about 3-fold lower than the lowest concentration observed in each of these types of sample.

For the determination of radioactivity ([3H]PEG 4000) by liquid scintillation counting, 5 mL scintillation fluid (Scintisol EX-H, Dojindo Laboratories, Kumamoto, Japan) was added to 100 μL aliquots of perfusate samples.

Data analysis: The fraction absorbed (Fa), which represents the fraction that disappeared from the intestinal lumen, was estimated by correcting for minor volume changes, based on changes in PEG 4000 (nonabsorbable marker) concentrations:

\[
F_a = 1 - \frac{C_{\text{out}}}{C_{\text{in}}} \cdot \frac{C_{\text{in}}}{C_{\text{out}}} 
\]

where \( C_{\text{in}} \) and \( C_{\text{out}} \) are the concentrations of diltiazem in the inflow and outflow solutions, respectively; and \( C'_{\text{in}} \) and \( C'_{\text{out}} \) are the corresponding concentrations of PEG 4000.

The fraction of diltiazem absorbed into (appeared in) mesenteric venous blood (\( F_{a,b} \)) was estimated as follows:

\[
F_{a,b} = \frac{C_b \cdot Q_b}{C_{\text{in}} \cdot Q} \quad (2)
\]

where \( C_b \) is the concentration of diltiazem in mesenteric venous blood and \( Q_b \) is the flow rate of mesenteric venous blood draining the perfused segment (0.42 mL/min on average). The \( Q_b \) was estimated by dividing the volume of total mesenteric venous blood collected by the time of blood collection, where the blood volume was estimated from the blood weight, using unity as the approximate specific gravity of blood. \( Q \) is the perfusion rate (0.15 mL/min), at which \( F_a \) was significant for both diltiazem and theophylline. The multiplication of \( F_a \) by the intestinal availability (\( F_i \)) during passage through the mucosa gives \( F_{a,b} \). Therefore, \( F_i \) was estimated by dividing \( F_{a,b} \) by \( F_a \).

\[
F_i = \frac{F_{a,b}}{F_a} \quad (3)
\]

The values of \( F_a \), \( F_{a,b} \), and \( F_i \) were, after examining their changes with time, determined as the average of three 5-min sampling periods at steady-state in each rat and then averaged for 3 animals. The extraction ratio (\( E_i \)) was estimated by subtracting \( F_i \) from unity.

\[
E_i = 1 - F_i \quad (4)
\]

The apparent membrane permeability clearance for the unit length of intestinal segment (\( CL_{\text{m,app}} \)) was estimated by the following equation, using a tube model,19,26,27

\[
CL_{\text{m,app}} = - \frac{Q}{L} \cdot \ln (1 - F_a) \quad (5)
\]

where \( L \) is the length of the perfused segment (10 cm). For highly permeable solutes, the diffusional resistance in the luminal aqueous phase is significant and affects the estimates of \( CL_{\text{m,app}} \). This resistance can be taken into account by assuming an unstirred water layer (UWL) adjacent to the intestinal surface. In the UWL model, \( CL_{\text{m,app}} \) is related to the membrane permeability clearance (\( CL_m \)) and the permeability clearance of UWL (\( CL_{\text{aq}} \)) as follows,26

\[
\frac{1}{CL_{\text{m,app}}} = \frac{1}{CL_m} + \frac{1}{CL_{\text{aq}}} \quad (6)
\]

The \( CL_{\text{aq}} \) for a given solute is expressed by the following equation.

\[
CL_{\text{aq}} = CL_{\text{aq,glc}} \cdot \sqrt{\frac{M_{\text{glc}}}{M}} \quad (7)
\]

where \( CL_{\text{aq,glc}} \) is the \( CL_{\text{aq}} \) for D-glucose, a highly permeable marker. \( M \) and \( M_{\text{glc}} \) are the molecular weights of the given solute and D-glucose, respectively. It is assumed that the diffusion coefficient is inversely proportional to the square root of the molecular weight20 and so is \( CL_{\text{aq,glc}} \), which is proportional to the diffusion coefficient. At a perfusion rate of 0.15 mL/min, the \( CL_{\text{aq,glc}} \) can be substituted by \( CL_{\text{m,app}} \) for UWL-limited absorption of D-glucose at 1 mM, the concentration at which its active membrane transport is most efficient. The \( CL_{\text{aq,glc}} \), which can be varied depending on the anesthetic procedure used, was previously determined to be 11.9 μL/min/cm for pentobarbital anesthesia.26 Therefore, the \( CL_{\text{aq}} \) for diltiazem (\( M, 414 \)) and theophylline (\( M, 180 \)) is estimated to be 7.8 and 11.9 μL/min/cm, respectively. Using the \( CL_{\text{aq}} \) and \( CL_{\text{m,app}} \) from experimentally determined \( F_a \) values (Eq. 5), the \( CL_m \) was estimated using Eq. 6.

Statistical analysis: Levels of statistical significance were assessed using Student’s \( t \)-test or, when multiple comparisons were needed, analysis of variance (ANOVA) followed by Dunnett’s test.

Results

Absorption and extraction of diltiazem: As shown in Fig. 1, the absorption of diltiazem (0.05 mM) reached steady-state 30 min after the initiation of perfusion in terms of both disappearance from the
intestinal lumen ($F_a$) and appearance into the mesenteric venous blood ($F_{a,b}$), demonstrating its intestinal availability ($F_i = F_{a,b}/F_a$). Therefore, the $F_a$, $F_{a,b}$, and $F_i$ for steady-state were determined as the average of three 5-min sampling periods after 30 min in subsequent analyses.

The $F_a$, $F_{a,b}$, and $F_i$ for 0.05 mM diltiazem were 0.142, 0.017 and 0.126, respectively (Table 1), indicating that diltiazem undergoes extensive first-pass metabolism with an extraction ratio ($E_i = 1 - F_i$) of 0.874 during its passage through the intestinal mucosa. As shown in Fig. 2A, the $F_{a,b}$ was unchanged at about 0.02 when the diltiazem concentration was increased to 0.5 mM, increased with the concentration up to 10 mM, and then remained unchanged at about 0.05. The $F_a$ was unchanged at about 0.15 up to 10 mM, then fell to about 0.05 at 30 mM, and remained at this level up to 50 mM. As a consequence (Fig. 2B), the $F_i$ was unchanged over the concentration range of 0.05 to 0.5 mM, indicating linear metabolic kinetics, and increased with the concentration up to 30 mM, reaching about 0.8 at 30 and 50 mM. The $F_i$ of about 0.8 translates into an $E_i$ as low as about 0.2. This nominal extraction is negligible because the $F_{a,b}$ was close, and statistically not significantly different from the $F_a$ at the highest concentrations. The extensive reduction in $E_i$, with an increase in concentration, to an insignificant level suggests that the extraction observed at lower concentrations is almost solely attributable to metabolic extraction of a saturable nature. It was intriguing to find a reduction in $F_a$ with an increase in concentration (Fig. 2A). This is an indication that a saturable (or carrier-mediated) mechanism may be involved in the mucosal uptake of diltiazem, although more detailed investigations are required to confirm this.

**Effect of inhibitors on absorption and extraction of diltiazem:** The effect of CYP3A inhibitors, ketoconazole and cyclosporin A,

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### Table 1. Absorption and extraction of diltiazem at steady-state during single-pass perfusion in the rat small intestine: effect of inhibitors.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$F_a$</th>
<th>$F_{a,b}$</th>
<th>$F_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>0.142 ± 0.023</td>
<td>0.017 ± 0.002</td>
<td>0.126 ± 0.026</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.133 ± 0.019</td>
<td>0.045 ± 0.007*</td>
<td>0.349 ± 0.020*</td>
</tr>
<tr>
<td>(40 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>0.116 ± 0.002</td>
<td>0.027 ± 0.003*</td>
<td>0.241 ± 0.026</td>
</tr>
<tr>
<td>(40 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNPP</td>
<td>0.095 ± 0.008</td>
<td>0.016 ± 0.001</td>
<td>0.182 ± 0.029</td>
</tr>
<tr>
<td>(10 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. (n = 3). The concentration of diltiazem was 0.05 mM. *Significantly different from the control at p < 0.05. $F_a$, $F_{a,b}$, and $F_i$ are noted in the legend of Fig. 1.
Fig. 2. Concentration-dependence of the absorption and extraction of diltiazem at steady-state during single-pass perfusion in the rat small intestine. Data are represented as the mean ± S.E. (n = 3). F_a and F_{a,b} are shown by closed circles and open circles, respectively, in panel A, and F_i (= F_{a,b} - F_a) is shown in panel B. F_a, F_{a,b} and F_i are noted in the legend to Fig. 1. *Significantly different from the value for 0.05 mM at p < 0.05.

Table 2. Absorption and extraction of theophylline at steady-state during single-pass perfusion in the rat small intestine.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>F_a</th>
<th>F_{a,b}</th>
<th>F_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.188 ± 0.009</td>
<td>0.199 ± 0.009</td>
<td>1.085 ± 0.099</td>
</tr>
<tr>
<td>0.5</td>
<td>0.237 ± 0.004**</td>
<td>0.213 ± 0.019</td>
<td>0.905 ± 0.075</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. (n = 3). **Significantly different from the value for 0.1 mM at p < 0.01. F_a, F_{a,b} and F_i are noted in the legend to Fig. 1.

Absorption and extraction of theophylline: The F_a, F_{a,b} and F_i of theophylline were determined as the average of three 5-min sampling periods beginning 15 min after the initiation of perfusion, when steady-state had been achieved. At a theophylline concentration of 0.1 mM, the F_a and F_{a,b} were almost identical at about 0.2, giving an F_i of about unity (Table 2). At a higher concentration of 0.5 mM, although F_a was slightly increased, F_{a,b} was unchanged at about 0.2 and F_i was still close to unity. Thus, theophylline was shown not to undergo metabolism in the small intestine over the low concentration range at which diltiazem was shown to undergo extensive metabolism. This is consistent with the fact that theophylline is known not to undergo first-pass metabolism after oral administration. This also confirms that the perfused rat intestine model is relevant to the in vivo situation and that the extensive intestinal extraction found for diltiazem is specific for that drug.

Discussion

It is well known that in the case of drugs that are substrates of CYP3A, such as nifedipine, the co-administration of grapefruit juice leads to an increase in bioavailability due to the inhibition of CYP3A-mediated...
ed metabolism in the small intestine. However, the effect of grapefruit juice on the bioavailability of diltiazem is reportedly insignificant or minimal. This may be, in part, due to the fact that the contribution of CYP3A to intestinal diltiazem metabolism is only minor, as suggested in the present study. In the case of nifedipine, the bioavailability of which is significantly increased by co-ingestion of grapefruit juice in rats as well as in humans, its intestinal extraction is almost totally accounted for by CYP3A-mediated metabolism, as demonstrated in our previous study using rats. As far as CYP3A isoforms in the rat small intestine are concerned, it has recently been suggested that CYP3A9 as demonstrated in our previous study using rats. As demonstrated in our previous study using rats, CYP3A2 was previously presumed to play a key role. However, it has recently been suggested that CYP3A9 as demonstrated in our previous study using rats, CYP3A2 was previously presumed to play a key role.

The moderate contribution of CYP3A alone, however, cannot fully explain the minimal effect of grapefruit juice, or intestinal CYP3A inhibition, on the bioavailability of diltiazem. Even if the contribution of CYP3A to the intestinal metabolism is small, a fractional reduction in extraction by inhibition of CYP3A could result in a significant increase in $F_i$, as shown for diltiazem in the present study (Table 1), if the intestinal availability is relatively low. This could also lead to a significant increase in bioavailability. However, the effect of such a fractional reduction in extraction on $F_i$ is less evident when $F_i$ is higher, or $E_i$ is smaller. This could be the case for diltiazem in humans. Because the bioavailability of diltiazem is reportedly greater in humans (0.42) than in rats (0.06), it is likely that the intestinal availability of diltiazem is also greater in humans, leading to the less evident or minimal change following CYP3A inhibition.

In a pharmacokinetic study by Lee et al., the intestinal availability of diltiazem was estimated to be 0.15 after oral administration of a 25 mM solution to rats. Although the $F_i$ for 25 mM is in the range of 0.4 to 0.8 in the present study, it may be that the diltiazem concentration in the gastrointestinal tract was lower due to dilution by gastrointestinal fluid. Assuming a dilution of several-fold, the diltiazem concentration after oral administration could have been well below 10 mM, where the $F_i$ values estimated in the present study were comparable with that after oral administration. In humans, assuming that a typical dose of 60 mg would be taken with 200 mL water, the diltiazem concentration would be 0.3 mg/mL (0.72 mM) initially and then lower after dilution by gastrointestinal fluid. Thus, it would be in the lower concentration range where, as suggested in rats, both absorption and extraction are most efficient.

The $CL_{m}$ of diltiazem was about 4 $\mu$L/min/cm at concentrations below 10 mM and about 1 $\mu$L/min/cm at the higher concentrations of 30 and 50 mM, where the $F_i$ was reduced. Shown in Table 3 are the $CL_{m}$ values for the lowest concentration of 0.05 mM and the highest concentration of 50 mM. We previously reported that D-xylose (1 mM) and cefatrizine (10 mM), which have $CL_{m}$ values of about 0.5 $\mu$L/min/cm under urethane anesthesia, were 70 to 90% absorbed after oral administration to rats. Therefore, although the $CL_{m}$ could be as much as 50% lower under urethane anesthesia than that under the pentobarbital anesthesia used in the present study, almost complete oral absorption (mucosal uptake) of diltiazem could be expected even for the lower $CL_{m}$ at the higher concentrations. The $CL_{m}$ values of theophylline were comparable with or greater than the higher $CL_{m}$ of diltiazem at 0.05 mM, suggesting that it is highly absorbed after oral administration. This is consistent with its well recognized absorption characteristics with a bioavailability close to 100%. The mechanism for the increased $CL_{m}$ of theophylline at higher concentrations needs to be investigated in the future, although an increase of about 50% would not be meaningful as long as it was highly absorbed. The concentration-dependent decrease in the $CL_{m}$ of diltiazem, which suggests involvement of carrier-mediated transport, will also require investigation in the future.

Diltiazem is reportedly a substrate of P-glycoprotein (P-gp), a secretory transporter acting at the brush border membrane to prevent various xenobiotics, including drugs, from entering the intestinal mucosa. The saturable nature of P-gp-mediated secretory transport could lead to an increase in $F_i$, which represents the mucosal uptake, with an increase in the substrate concentration. However, we did not see any sign of that for diltiazem. Furthermore, we did not see any significant inhibition of mucosal diltiazem uptake by ketoconazole and cyclosporin A, which are potent inhibitors of P-gp as well as CYP3A. Thus, the results of the present study suggest that, apparently, P-gp is not involved in mucosal diltiazem uptake. A possible explanation for this is that the P-gp-mediated transport of diltiazem was totally saturated at the concentrations examined and negligible compared with absorptive

### Table 3. Membrane permeability clearances of diltiazem and theophylline in the rat small intestine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mM)</th>
<th>$CL_{m,app}$ ($\mu$L/min/cm)</th>
<th>$CL_{m}$ ($\mu$L/min/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem</td>
<td>0.05</td>
<td>2.24 ± 0.15</td>
<td>4.11 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.90 ± 0.22</td>
<td>1.04 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.12 ± 0.17</td>
<td>4.26 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.10 ± 0.07**</td>
<td>6.18 ± 0.17**</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.1</td>
<td>3.12 ± 0.17</td>
<td>4.26 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.10 ± 0.07**</td>
<td>6.18 ± 0.17**</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. (n = 3). **Significantly different from the value for 0.05 mM (diltiazem) or 0.1 mM (theophylline) at p < 0.01. $CL_{m,app}$ and $CL_{m}$ are the apparent membrane permeability clearance and the membrane permeability clearance corrected for the resistance of the unstirred water layer, respectively.
transport by diffusion and, if involved, the carrier. A similar case apparently lacking any sign of significant involvement of P-gp-mediated secretory transport has recently been reported for another P-gp substrate, tacrolimus, in a perfusion study using the rat small intestine.\cite{Takara, Ohnishi, Horibe, Yokoyama: 2002} It is also possible that the inhibition of P-gp by the metabolites of diltiazem may be in part involved in the apparent lack of secretory transport of diltiazem.

In conclusion, the present study demonstrates the extensive metabolic extraction of diltiazem in the perfused rat small intestine, supporting earlier suggestions based on pharmacokinetic analyses of in vivo observations. CYP3A is suggested to be involved in the intestinal metabolism of diltiazem, although its contribution appears to be only minor. Some esterases, other than CES2 and BNPP-sensitive carboxylesterases, which were suggested not to be involved, may actually make a significant contribution. It should be also noted that the perfused rat intestine model is useful for evaluating intestinal metabolism and examining the various factors involved. It can also provide useful information for devising strategies to manage problems arising from intestinal metabolism and also for developing methods or models for the quantitative prediction of intestinal extraction.

**References**


