Regular Article

Metabolic Extraction of Nifedipine during Absorption from the Rat Small Intestine

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Summary: Nifedipine is one of drugs that have been suggested to undergo significant first-pass metabolism by cytochrome P450 (CYP) 3A in the intestine, based mainly on pharmacokinetic analyses of in vivo observations. To further substantiate this suggestion, we examined the metabolic extraction of nifedipine from the rat small intestine, using intestine perfused in situ by a single-pass technique and microsomes in vitro. When the intestinal lumen was perfused with nifedipine solution (30 μM) at the flow rate of 0.15 mL/min and steady-state was achieved, the fraction that disappeared from the intestinal lumen ($F_a$) and the fraction absorbed into the mesenteric venous blood ($F_{a,b}$) was 0.26 and 0.13, respectively. Thus, $F_{a,b}$ was 50% smaller than $F_a$, indicating a significant extraction of nifedipine during passage through the intestinal mucosa. When ketoconazole (40 μM), a specific inhibitor of CYP3A, was added to the perfusion solution, $F_{a,b}$ was increased to a level comparable with $F_a$, while $F_a$ remained unchanged, suggesting the complete inhibition of metabolic extraction by CYP3A. A similar result was obtained for cyclosporin A (40 μM), another specific CYP3A inhibitor. In intestinal microsomes, the metabolic degradation of nifedipine (1 μM) was almost completely inhibited by ketoconazole (10 μM) and cyclosporin A (10 μM), consistent with the results in the perfused intestine. It was also found in intestinal microsomes that anti-rat CYP3A2 antibody can inhibit nifedipine metabolism completely. Thus, the present study demonstrates that nifedipine undergoes significant extraction during passage through the intestinal mucosa, and provides substantial evidence that CYP3A2 is responsible for that.

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

Introduction

Nifedipine, a dihydropyridine calcium channel blocker, is a substrate of CYP3A and its first-pass intestinal metabolism has been attracting increasing attention.1-3 It is one of the first drugs for which the bioavailability after oral administration was found to be increased by concomitant ingestion of grapefruit juice in humans.4-6 Because the elimination of nifedipine from the systemic circulation, which is mainly mediated by hepatic metabolism, was not altered by grapefruit juice ingestion8 and CYP3A was found to exist not only in the liver but also in the small intestine,9-11 the increase in its bioavailability has been believed to be caused by inhibition of the first-pass metabolism by intestinal CYP3A and, hence, the contribution of intestinal metabolism has been presumed to be significant as far as its first-pass extraction (about 50%) is concerned.6,12,13 Consistent with the postulate, it has been suggested that nifedipine can be almost completely absorbed from the gastrointestinal tract and, also in the rat, it has been suggested that intestinal metabolism could play a greater role than hepatic metabolism as far as first-pass nifedipine extraction is concerned.14,15 This is coincidently comparable with that in humans: the first-pass extraction is reduced, without an alteration in hepatic extraction, by the coingestion of grapefruit juice,16-18 and CYP3A is present in the small intestine.17,19

However, all these suggestions about the metabolic extraction of nifedipine in the small intestine are mostly based on indirect evaluation by pharmacokinetic analyses of in vivo observations. Evaluation of the metabolism in intestinal tissue in situ specifically and in microsomes in vitro in more detail, as conducted in the present study, should help further substantiate these hypotheses regarding nifedipine. It could also provide useful information for devising strategies to manage problems arising from intestinal metabolism and also
for developing a method or model for quantitative prediction of intestinal extraction.

**Materials and Methods**

**Chemicals:** Nifedipine was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), nitrendipine was from Yoshitomi Pharmaceutical Industries Ltd. (Osaka, Japan), 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). Anti-rat CYP antisera preparations (anti-rat CYP2C11 prepared from goat and anti-rat CYP3A2 prepared from rabbit) were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). All other reagents were of analytical or HPLC grade and commercially obtained.

**Animals:** Male Wistar rats, weighing about 300 g, were purchased from Nihon SLC (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 and 0.01% HCO-60 (pH 6.4), and contained nifedipine (30 μM) and a trace amount of [³H]PEG 4000 (1.85 kBq/0.033 g/mL) as a nonabsorbable marker. A set of experiments was also conducted using nitrendipine (30 μM) instead of nifedipine. In inhibition experiments, ketoconazole or cyclosporin A was added at a concentration of 40 μM to the perfusion solution. The outflow solution and the total mesenteric venous blood draining the perfused segment were collected at 5-min intervals. Blood samples (100 μL aliquots) were subjected to hemolysis by addition of 50 μL distilled water.

Nifedipine in the samples of perfusate and the hemolysate of mesenteric venous blood was analyzed by HPLC, according to the method of Horvath et al. Briefly, nitrendipine as the internal standard (3 μM in 100 μL acetonitrile), 1 M NaOH (200 μL) and 6 mL of an extraction solvent (hexane:dichloromethane = 7:3) were added to each sample (100 μL perfusate or 150 μL hemolysate). After shaking for 10 min at a rate of 300 strokes/min (SR-2s recipro shaker, Taitec Co., Ltd., Nagoya, Japan) and subsequently centrifuging (1,600 g, 10 min, 4°C), 5 mL extraction solvent (the upper, organic layer) was transferred to a fresh sample tube and evaporated at 40°C under vacuum (CC-105 centrifugal concentrator, Tomy Seiko Co., Ltd., Tokyo, Japan). The residue was reconstituted in 200 μL mobile phase (65% methanol in 20 mM acetate buffer, pH 4.0) before being injected (100 μL) into the HPLC system: column, Wakosil-II 5C18 RS 4.6 mm × 150 mm (Wako Pure Chemical Industries, Ltd., Osaka, Japan); flow rate, 0.8 mL/min; detector, UV 350 nm (SPD-10A, Shimadzu Co., Kyoto, Japan). Nitrendipine was analyzed by the same procedure, using nifedipine as the internal standard.

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.

Throughout the experiments, care was taken to prevent photodegradation of nifedipine and nitrendipine.

**Analysis of intestinal perfusion data:** The fraction absorbed (Fᵢ), 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:

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

where \( C_{\text{in}} \) and \( C_{\text{out}} \) are the concentrations of nifedipine in inflow and outflow solutions respectively; \( C_{\text{in}}' \) and \( C_{\text{out}}' \) are those of PEG 4000.

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

\[ F_{a,b} = \frac{C_{\text{b}}/Q_{\text{b}}}{C_{\text{in}}'/Q} \]

where \( C_{\text{b}} \) is the concentration of nifedipine in mesenteric venous blood; \( Q_{\text{b}} \) is the flow rate of mesenteric venous blood draining the perfused segment (0.39 mL/min on average). The \( Q_{\text{b}} \) 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. The values of \( Fᵢ \) and \( F_{a,b} \) were, after examining their changes with time, determined as the average of three 5-min sampling periods at steady-state (15 to 30 min after the start of perfusion) in each rat and then averaged for 3 animals.

The multiplication of \( Fᵢ \) by the intestinal availability (\( Fᵢ \)) during passage through the mucosa gives \( F_{a,b} \). Therefore, \( Fᵢ \) was estimated by dividing \( F_{a,b} \) by \( Fᵢ \).

\[ Fᵢ = \frac{F_{a,b}}{Fᵢ} \]

The apparent membrane permeability clearance for
the unit length of intestinal segment \( (CL_{m,\text{app}}) \) was estimated by the following equation, using a tube model:\(^{22-24}\)

\[
CL_{m,\text{app}} = \frac{-Q}{L} \ln (1 - F_a) \quad (4)
\]

where \( Q \) is the perfusion rate (0.15 mL/min) and \( L \) 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 \( CL_{m,\text{app}} \). This resistance can be taken into account by assuming an unstirred water layer (UWL) adjacent to the intestinal surface. In the UWL model, \( CL_{m,\text{app}} \) is related to the membrane permeability clearance \( (CL_m) \) and the permeability clearance of UWL \( (CL_{\text{aq}}) \) as follows:\(^{23}\)

\[
\frac{1}{CL_{m,\text{app}}} = \frac{1}{CL_m} + \frac{1}{CL_{\text{aq}}} \quad (5)
\]

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

\[
CL_{\text{aq}} = CL_{\text{aq,glc}} \frac{M_{\text{glc}}}{M} \quad (6)
\]

where \( CL_{\text{aq,glc}} \) is the \( CL_{\text{aq}} \) for D-glucose, a highly permeable marker. The \( M \) 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 weight\(^{25}\) and so is \( CL_{\text{aq}} \), 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 \( CL_{m,\text{app}} \) 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 could be varied depending on anesthetic regimens, was previously determined to be 11.9 \( \mu \)L/min/cm for pentobarbital anesthesia.\(^{23}\) Therefore, the \( CL_{\text{aq}} \) for nifedipine \((M, 346)\) and nitrendipine \((M, 360)\) is estimated to be 8.6 and 8.4 \( \mu \)L/min/cm, respectively. From the \( CL_{\text{aq}} \) and \( CL_{m,\text{app}} \) from experimentally determined \( F_a \) (Eq. 4), the \( CL_m \) was estimated using Eq. 5.

**Preparation of intestinal and hepatic microsomes:**

Intestinal and hepatic microsomes were prepared by the method of Mohri et al.\(^{17}\)

For the preparation of intestinal microsomes, a 40-cm jejuno-midgut segment (about 6 g) was isolated and internally rinsed with solution A (96 mM NaCl, 1.5 mM KCl, 8 mM KH\(_2\)PO\(_4\), 5.6 mM Na\(_2\)HPO\(_4\)-12H\(_2\)O, 27 mM trisodium citrate dihydrate, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.4). The intestinal segment was filled with solution A (8 mL), closed at both ends by clamping and incubated for 10 min at 37°C and shaken at a rate of 100 cycles/min in 100 mL saline (0.9% NaCl solution). The solution in the intestinal lumen was replaced with 10 mL ice-cold solution B (20.1 mM Na\(_2\)HPO\(_4\)-12H\(_2\)O, 47.0 mM KH\(_2\)PO\(_4\), 101.0 mM NaCl, 1.5 mM disodium ethylenediaminetetraacetate (EDTA), 0.5 mM dithiothreitol (DTT), 0.1 mM PMSF, 5 U/mL heparin, pH 7.4), and the intestinal segment was placed on a petri dish on ice and tapped gently several times to liberate epithelial cells. The suspension of the liberated cells was drained into a 50-mL centrifuge tube, centrifuged at 180 g and 4°C for 10 min and the cells from the resultant pellet were resuspended in 20 mL ice-cold solution C (5 mM L-histidine, 250 mM sucrose, 0.5 mM EDTA, 0.1 mM PMSF, 20 mM Tris-HCl, pH 7.4). This procedure of centrifugation and resuspension was repeated once more to purify the cells. The cells were homogenized in ice-cold solution C (10 mL) with a glass/Teflon Potter homogenizer and centrifuged at 10,000 g and 4°C for 20 min. After adding calcium chloride solution (100 mM) to give a final concentration of 10 mM, the supernatant was centrifuged at 1,600 g and 4°C for 15 min to obtain intestinal microsomes as a pellet.

For preparation of hepatic microsomes, the whole liver (about 10 g) was isolated, minced on a petri dish on ice and homogenized in 20 mL ice-cold solution C with a glass/Teflon Potter homogenizer. The homogenate was centrifuged at 700 g and 4°C for 10 min and then the supernatant was centrifuged at 10,000 g and 4°C for 20 min. After adding calcium chloride solution (100 mM) to give a final concentration of 10 mM, the supernatant was centrifuged at 1,600 g and 4°C for 15 min to obtain hepatic microsomes as a pellet.

Microsomal pellets from intestine and liver were resuspended, respectively, in 2.5 and 4 mL solution D (100 mM Tris-HCl, 10 mM EDTA, 20% glycerol, pH 7.4) and, after measurement of protein concentrations by Bio-Rad Protein Assay kit II* (Bio-Rad Laboratories, Hercules, CA, USA) with BSA as the standard, stored at −80°C until use. Protein concentrations were roughly 2.5 and 6 mg/mL, respectively, for intestinal and hepatic microsomes.

**In vitro metabolism in microsomes:**

To 0.2 mL suspension of intestinal microsomes, was added 0.2 mL substrate solution consisting of a substrate (nifedipine or nitrendipine) at a required concentration, 7.5 mM MgCl\(_2\) and 200 mM Tris-HCl (pH 7.4) to give a mixture 0.4 mL in volume. To 0.04 mL suspension of hepatic microsomes, was added 0.36 mL substrate solution consisting of a substrate, 4.2 mM MgCl\(_2\) and 200 mM Tris-HCl (pH 7.4). In inhibition experiments, one of the CYP inhibitors (ketoconazole, cyclosporin A, sulfaphenazole or quinidine) was included in the substrate solution. After preincubation of the mixture for 5 min at 37°C and shaking at a rate of 100 cycles/min, the metabolic reaction was initiated by addition of 6.7 mM
Absorption and extraction from the perfused intestine: As shown in Fig. 1, the absorption of nifedipine (30 μM) had reached steady-state 15 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, accordingly, its intestinal availability (F_i=F_{a,b}/F_a). The F_a, F_{a,b} and F_i for steady-state are summarized in Table 1. The F_i value was 0.51, indicating that about 50% of nifedipine was extracted during its passage through the intestinal mucosa.

The F_{a,b} of nifedipine was found to be increased by specific CYP3A inhibitors of ketoconazole (40 μM) and cyclosporin A (40 μM), while the F_a was unaffected, and consequently the F_i reached about unity (Fig. 1 and Table 1). These results suggest that metabolism by CYP3A is responsible for the intestinal extraction of nifedipine and it can be almost completely inhibited by these inhibitors. Although HCO-60 was used to solubilize these highly lipophilic inhibitors, it was confirmed that HCO-60 does not affect the absorption and extraction of nifedipine (Table 2). It was also found that the values of F_a, F_{a,b} and F_i for 30 μM nifedipine were com-
Table 1. Absorption and extraction of nifedipine and nitrendipine at steady-state during single-pass perfusion of the rat small intestine: effects of inhibitors.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inhibitor</th>
<th>( F_a )</th>
<th>( F_{a,b} )</th>
<th>( F_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine (30 ( \mu )M)</td>
<td>None (control)</td>
<td>0.26 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (40 ( \mu )M)</td>
<td>0.24 ± 0.04</td>
<td>0.21 ± 0.01*</td>
<td>0.91 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td>Cyclosporin A (40 ( \mu )M)</td>
<td>0.31 ± 0.03</td>
<td>0.31 ± 0.03**</td>
<td>1.00 ± 0.03*</td>
</tr>
<tr>
<td>Nitrendipine (30 ( \mu )M)</td>
<td>None (control)</td>
<td>0.24 ± 0.02</td>
<td>0.08 ± 0.003</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (40 ( \mu )M)</td>
<td>0.24 ± 0.03</td>
<td>0.13 ± 0.02*</td>
<td>0.56 ± 0.02**</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. (n = 3). Levels of statistical significance compared with the control: *p < 0.05, **p < 0.01. \( F_a, F_{a,b}, F_i \) are noted in the legend of Fig. 1.

Table 2. Absorption and extraction of nitrendipine at steady-state during single-pass perfusion of the rat small intestine: effects of HCO-60 and nifedipine concentration.

<table>
<thead>
<tr>
<th>Nitrendipine concentration (( \mu )M)</th>
<th>HCO-60 concentration (%)</th>
<th>( F_a )</th>
<th>( F_{a,b} )</th>
<th>( F_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.01</td>
<td>0.26 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0.32 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.32 ± 0.03</td>
<td>0.19 ± 0.01</td>
<td>0.60 ± 0.05</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. (n = 3). \( F_a, F_{a,b}, F_i \) are noted in the legend of Fig. 1.

bioavailability in the systemic circulation after oral administration.\(^{14,15}\) Although CYP3A activity could be lower in the ileum than in the jejunomidgut region used in this study, as suggested in humans,\(^5\) it is likely that nifedipine is mainly absorbed in the jejunomidgut region and/or the reduction in CYP3A activity in the ileum is moderate, leading to a similar level of \( F_i \) for oral administration. Thus, this finding for \( F_i \) would support the earlier suggestion\(^{15} \) that the contribution of the liver is minimal during first-pass extraction unless \( F_i \) after oral administration is elevated by a significant contribution of the ileum with markedly low CYP3A activity.

The absorption of nitrendipine (30 \( \mu \)M), another dihydropyridine calcium channel blocker, was also examined and compared with that of nifedipine (Table 1). Nitrendipine absorption had also reached steady-state 15 min after the initiation of perfusion (data not shown). The \( F_a \) of 0.24 for nitrendipine was almost equivalent to that for nifedipine (0.26), suggesting a similar rate of mucosal uptake. However, the \( F_{a,b} \) of 0.08 for nitrendipine was smaller than that for nifedipine (0.13) and accordingly so too was the \( F_i \), suggesting more extensive metabolism in the intestinal mucosa. This metabolism was also suggested to be mediated at least in part by CYP3A, because it was significantly inhibited by ketoconazole (40 \( \mu \)M), as indicated by the increased \( F_{a,b} \) and \( F_i \) in its presence. On the other hand, \( F_a \) was unaffected by ketoconazole. The \( C_{L_{m,app}} \) and \( C_{L_m} \) corrected for the UWL resistance were 4.17 ± 0.38 and 8.79 ± 1.54 \( \mu \)L/min/cm, respectively, from the \( F_i \) in the absence of inhibitors.

A number of CYP3A substrates are known to undergo secretory transport by P-glycoprotein (P-gp) at the brush border membrane of the intestinal epithelia.\(^{28}\) However, it has been suggested that nifedipine and nitrendipine are not substrates of P-gp, although they work as P-gp inhibitors.\(^{28,29}\) Therefore, it was not surprising to find that their \( F_a \) values, which depend on transport across the brush border membrane, were unaffected by cyclosporin A and ketoconazole, which work as inhibitors of P-gp as well as CYP3A.

Metabolism in intestinal and hepatic microsomes:
Table 3. Concentration-dependence of nifedipine metabolism in rat intestinal and hepatic microsomes.

<table>
<thead>
<tr>
<th>Nifedipine concentration (mM)</th>
<th>Metabolic clearance (μL/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small intestine</td>
</tr>
<tr>
<td>1</td>
<td>6.4 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>100</td>
<td>2.8 ± 0.6*</td>
</tr>
</tbody>
</table>

The values of metabolic clearance (metabolic degradation rate/concentration) are represented as the mean ± S.E. from 4 (small intestine) or 3 (liver) preparations. *p<0.05 compared with the value for 1 mM.

To further confirm the involvement of CYP3A in the intestinal extraction of nifedipine, the metabolism of nifedipine was examined in microsomes prepared from isolated jejuno-midgut enterocytes and also compared that in liver microsomes. In preliminary experiments, the degradation of nifedipine was negligible in the absence of NADPH in both intestinal and hepatic microsomes. In the absence of inhibitors, the amount of nifedipine that disappeared due to metabolism was proportional to the time up to 30 and 1 min, respectively, in intestinal and hepatic microsomes, and was less than 20% of the initial amount in these time ranges (data not shown). We, therefore, assumed that the nifedipine concentration can be approximated by the initial concentration throughout the experimental period, and estimated the metabolic degradation rate by dividing the amount that disappeared by time.

In both intestinal and hepatic microsomes (Table 3), the metabolic clearance (metabolic degradation rate/concentration) was unchanged when the nifedipine concentration was increased to 5 mM from 1 mM, suggesting linearity in the metabolic kinetics in this concentration range, while it was reduced at a higher concentration of 100 mM, demonstrating saturability. Although the concentration of nifedipine in epithelial cells during absorption is unknown, nifedipine metabolism was examined at 1 mM in the following experiments to evaluate metabolism in the linear phase, as was the case with the evaluation of absorption in the perfused intestine.

As shown in Fig. 2, ketoconazole (10 μM) and cyclosporin A (10 μM) inhibited nifedipine metabolism in intestinal microsomes almost completely, consistent with the results in the perfused intestine and suggesting the involvement of CYP3A. In contrast, quinidine,30 a CYP2D inhibitor, did not inhibit nifedipine metabolism, and sulfaphenazole,30 a CYP2C inhibitor, did so but only slightly. It has been suggested in male rats that CYP2C11 could be involved in the metabolism of some CYP3A substrates,31–33 including nifedipine.34 Therefore, to further clarify the CYP isozymes responsible for nifedipine metabolism, we examined the effects of antibodies to CYP2C11 as well as CYP3A2, the major CYP3A isoform in the rat (Table 4). It was clearly shown in intestinal microsomes that anti-CYP3A2 antibody inhibits nifedipine metabolism completely, but anti-CYP2C11 antibody has absolutely no effect. These results strongly suggest that nifedipine is almost exclusively metabolized by CYP3A2 in the small intestine. It should be noted, however, that very recent research suggests that the major CYP3A in the rat small intestine may be a novel CYP3A2-like isozyme.35,36

We also examined the metabolism of nitrendipine (1 μM) in intestinal microsomes. It was more rapid (16.3 ± 2.8 pmol/min/mg protein) than nifedipine metabolism and inhibited by ketoconazole (10 μM) to a lesser extent (4.0 ± 0.2 pmol/min/mg protein, 75% inhibition), consistent with the results in the perfused intestine showing that the extraction was larger and less inhibited for nitrendipine than for nifedipine.

The results in hepatic microsomes were in general similar to those in intestinal microsomes (Fig. 2 and

![Fig. 2. Nifedipine metabolism in rat intestinal (A) and hepatic (B) microsomes: effects of inhibitors. Data are represented as the mean ± S.E. from 3 preparations. The degradation of nifedipine (1 μM) was evaluated in the absence or presence of one of the inhibitors (10 μM). Levels of statistical significance compared with the control: *p<0.05, **p<0.01.](image-url)
Table 4. Nifedipine metabolism in rat intestinal and hepatic microsomes: effects of anti-rat CYP antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Serum volume (µL)</th>
<th>Metabolic degradation rate (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blank serum</td>
</tr>
<tr>
<td>Anti-CYP3A2</td>
<td>10</td>
<td>4.61±0.34</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.91±0.53</td>
</tr>
<tr>
<td>Anti-CYP2C11</td>
<td>10</td>
<td>6.14±0.35</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.00±0.40</td>
</tr>
</tbody>
</table>

Data are represented as the mean±S.E. from 3 preparations. Values in parentheses represent the percentage of the control (blank serum). The degradation of nifedipine (1 µM) was evaluated after pretreatment of microsomes with blank serum or antiserum. Levels of statistical significance compared with the control: *p<0.05. N.D., not determined.

Table 4). However, nifedipine metabolism was less inhibited by cyclosporin A and was reduced by about 40%, although this was not statistically significant, by anti-CYP2C11 antibody. Thus, CYP3A2 seems also to play the major role in nifedipine metabolism in the rat liver, but we cannot exclude the possibility that CYP2C11 might be involved as suggested by an earlier study.34

The activity of nifedipine metabolism (metabolic clearance) was much higher, about 20-fold, in hepatic microsomes than in intestinal microsomes, presumably reflecting a difference in the CYP3A content between the two organs.17,19) However, the high activity in hepatic microsomes could be largely compromised in vivo by the high protein binding in plasma. Assuming nifedipine is available from plasma for metabolism and the unbound fraction is as low as 0.04, as reported for humans,13) the apparent metabolic clearance on the basis of the plasma concentration would be reduced to a level comparable with that for intestine, in which there is no protein binding effect on the availability of nifedipine from the intestinal lumen. Although more detailed quantitative analysis will be required in the future, our results in microsomes do not seem to be inconsistent with the in situ and in vivo suggestion that the small intestine plays a significant role in the first-pass metabolic extraction of nifedipine.

In conclusion, the present study successfully demonstrates the significant metabolic extraction of nifedipine in the perfused rat intestine, supporting earlier suggestions based on pharmacokinetic analyses of in vivo observations. In addition, together with the findings in microsomes, these results strongly suggest that nifedipine is almost exclusively metabolized by CYP3A2 in the small intestine. The perfused rat intestine model should be useful for further elucidating the mechanism and factors involved in the metabolism of nifedipine and some other CYP3A substrate drugs. It could also provide information useful for devising strategies to manage problems arising from intestinal metabolism and also for developing a method or model for quantitative prediction of intestinal extraction.

Acknowledgment: This work was supported in part by a Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (#12672155).

References

10) Gibbs, M. A., Thummel, K. E., Shen, D. D. and Kunze, K. L.: Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of Ki...


