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Effects of Itraconazole, Dexamethasone and Naringin on the Pharmacokinetics of Nadolol in Rats

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Summary: The aim of the present study was to clarify the involvement of P-glycoprotein (P-gp) or organic anion transporting polypeptide (Oatp) 1a5 in the pharmacokinetics of nadolol (NDL), a non-metabolized hydrophilic β-adrenoceptor blocker, in rats. Pretreatment with itraconazole (ICZ, P-gp inhibitor, 50 mg/kg) for 30 min before oral administration of NDL (10 mg/kg) significantly increased the area under the plasma concentration-time curve (AUC0–∞) of NDL by 1.7-fold compared with control. Intragastric administration of dexamethasone (DEX, 8 mg/kg) for 4 consecutive days increased P-gp level in the intestine and the liver. In line with this, DEX pre-treatment decreased maximum plasma concentration (Cmax) of NDL by 28% of control. To inhibit the intestinal Oatp1a5, naringin (NRG, 0.145 mg/kg) was preadministered orally for 30 min before the oral administrations of NDL or celiprolol (CEL, 10 mg/kg, Oatp1a5 substrate). Although NRG markedly reduced Cmax and AUC0–∞ of CEL by 60% and 65% of control, respectively, little difference was observed in the plasma concentration of NDL between NRG and control. These results suggest that P-gp is greatly involved in the pharmacokinetics of NDL, while the involvement of Oatp1a5 in the pharmacokinetics of NDL may be less than that of celiprolol in rats.

Keywords: celiprolol; dexamethasone; itraconazole; nadolol; naringin; Oatp1a5; P-gp

Introduction

Drug transporters in the cell membrane are responsible for cellular uptake or export of vital materials and/or xenobiotics.1) While being involved in the drug disposition, activities of drug transporters can be modulated by a large number of drugs, resulting in clinically important drug interactions.2)3) So far, such drug transporter-mediated drug interactions have been reported in humans with some herbal medicines and fruit juice such as St. John’s wort and grapefruit juice (GFJ).4,5) For instance, both single and repeated ingestion of GFJ markedly reduced the oral bioavailability of talinolol, a hydrophilic β-blocker, in a human study.6) A recent report has shown that naringin, a flavonoid compound derived from GFJ, has a significant inhibitory effect on not only P-glycoprotein (P-gp)-mediated drug efflux, but also organic anion transporting polypeptide (Oatp)-mediated uptake of drugs in the rat intestine.7) Drug interactions in animals and humans have been investigated using a probe drug to evaluate the activity of a drug transporter. However, the in vivo probe drugs currently in use have serious limitations for the specificity for individual drug transporters.7,8) For example, talinolol, an in vivo probe drug for P-gp, has been reported also to be a substrate for drug transporters other than P-gp.9) In addition, talinolol is not approved for clinical use in countries including Japan and the USA.9,10) Therefore, it is of great importance to develop a novel in vivo probe drug with a high specificity and good feasibility for a better understanding of drug transporter-mediated drug interaction.

Nadolol, a nonselective β-blocker, is used for the treatment of hypertension and angina pectoris.11) Since it is hydrophilic, nadolol is not metabolized by drug metabolizing enzymes such as cytochrome P450 (Cyp), and is excreted primarily into the bile with up to 70% of its dose in unchanged form. The bioavailability of nadolol given orally is approximately 20% in rats.12) According to the biopharmaceutical classification system (BCS), nadolol is categorized as a class 3 drug, which is characterized by high solubility and low permeability in the gastrointestinal tract.13) Owing to the poor permeability in the intestinal epithelial cells, an active transport is thought to play a pivotal role in the membrane permeation of class 3 drugs.14)15) Previously, it has been shown that nadolol is a substrate for human P-gp and OATP1A2 in vitro.14,15)
Those findings suggest that nadolol could be a candidate for the evaluation of drug transporter activities in vivo. Moreover, if the pharmacokinetics of nadolol is regulated by one particular drug transporter, nadolol may be a more specific probe drug for the drug transporter than currently used probe drugs. Therefore, it is necessary to clarify whether P-gp and/or Oatp are involved in the absorption, distribution or excretion of nadolol by assessing whether the inhibition or induction of these drug transporters affects the pharmacokinetics of nadolol.

In this study, we aimed to clarify the involvement of P-gp or Oatp1a5 in the pharmacokinetics of nadolol in rats. To modulate P-gp activity, we used itraconazole and dexamethasone as an inhibitor and inducer of P-gp, respectively. We measured the P-gp protein level in the small intestine and liver following the pretreatment with dexamethasone over 4 days to examine the induction of P-gp by dexamethasone. As an in vivo Oatp1a5 inhibitor, naringin was used to investigate the pharmacokinetic profile of nadolol as well as celiprolol, a previously reported Oatp1a5 substrate.

Methods

Reagents and chemicals: Nadolol was purchased from Sigma Aldrich (St. Louis, MO). Itraconazole, dexamethasone and metoprolol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Celiprolol was provided from Nihon Shinyaku (Kyoto, Japan). Naringin was purchased from Tokyo Chemical Industry (Tokyo, Japan). All the other reagents and solvents used were commercially available and of analytical or high-performance liquid chromatography (HPLC) grade.

Animals: Male Sprague-Dawley (SD) rats (250–350 g) were purchased from SLC (Shizuoka, Japan). The rats were housed under identical conditions in a pathogen-free environment with a 12-h light/dark cycle and free access to standard chow and water. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Fukushima Medical University.

Pharmacokinetic studies of nadolol and celiprolol: In the nadolol study, rats were divided into four groups: control, itraconazole, dexamethasone and naringin. After an overnight fast with free access to water, itraconazole (50 mg/kg) dissolved in saline and adjusted to pH 4.0 by phosphoric acid, naringin (0.145 mg/kg) dissolved in phosphate-buffered saline or saline (control) were orally administered 30 min before the oral administration of nadolol (10 mg/kg). In the dexamethasone group, rats were pretreated orally for 4 days with dexamethasone suspended in 0.1% carboxymethyl cellulose at a daily dose of 8 mg/kg before the administration of nadolol (10 mg/kg). The liver sample was collected from the same lobe for each rat. In the celiprolol study, rats were divided into two groups: control, itraconazole, dexamethasone and naringin. After an overnight fast with free access to water, naringin (0.145 mg/kg) or saline (control) were orally administered 30 min before the oral administration of celiprolol (10 mg/kg).

Blood was periodically collected through the tail vein up to 6 h after nadolol or celiprolol administration. The blood samples were immediately centrifuged to obtain plasma. The urine was collected cumulatively from 0 to 24 h in fractions from 0–3 h, 3–6 h, 6–9 h, 9–12 h and 12–24 h after nadolol or celiprolol administration using a metabolic cage. The plasma and urine samples were stored at −80°C until analysis.

Determination of nadolol and celiprolol concentration: Urine samples were diluted 20 times with distilled water. The plasma and diluted urine samples (100 µL) were mixed with 1 M NaOH (1 mL), metoprolol (10 µL) and diethylether: dichloromethane = 70:30 (3 mL). The mixed solutions were shaken for 10 min and centrifuged at 3,000 × g for 3 min. The supernatants were dried up with N2 gas, and the residues were dissolved in 100 µL of 50 mM ammonium acetate with pH values being adjusted to 4.5 for nadolol detection or 9.5 for celiprolol detection. After the filtration through a 0.2-µm membrane filter (Millex-LG, Millipore, Bedford, MA), 50 µL of the solution was subjected to HPLC analysis. The HPLC system consisted of an LC-2000Plus system (JASCO, Tokyo, Japan) with a fluorescence detector. Chromatographic separation was performed using an Inertsil ODS-4C18 column (2.1 × 100 mm, particle size 3 µm; GL Sciences, Tokyo, Japan) as an analytical column and a guard column (Inertsil ODS-4C18, 2.1 × 40 mm; GL Sciences) at room temperature. For the detection of nadolol, the samples were separated using a gradient mobile phase consisting of 50 mM ammonium acetate (pH 4.5) (A) and acetoni trile (B) at a flow rate of 0.30 mL/min. The linear gradient condition of the mobile phase was 0–4.0 min, 12–30% B; 4.0–8.0 min, 30–75% B; 8.0–10.0 min, 75% B; and 10.0–20.0 min, 12% B. For the detection of celiprolol, the samples were separated using a gradient mobile phase consisting of 50 mM ammonium acetate (pH 9.5) (A) and acetoni trile (B) at a flow rate of 0.35 mL/min. The linear gradient condition of the mobile phase was 0–10.0 min, 18–40% B; 10.0–12.0 min, 40–75% B; 12.0–14.0 min, 75% B; and 14.0–24.0 min, 18% B. The fluorometric detection wavelengths were ex. 230/30 nm for nadolol, and ex. 350/480 nm for celiprolol. The limit of quantification was 1 ng/mL for both nadolol and celiprolol. The intra-assay coefficients of variance were <8.5% for nadolol detection, and <5.6% for celiprolol detection.

Analysis of P-gp protein expression: Protein levels of P-gp in the liver and intestine were analyzed by Western blotting. Rats were pretreated with dexamethasone (8 mg/kg/day) or water for 4 days. On Day 5, rats were euthanized by intraperitoneal injection of pentobarbital (40 mg/kg body weight) after overnight fasting. The liver sample was collected from the same lobe for each rat. The intestinal sample was harvested as follows: the first 20-cm segment proceeding from the pyloric sphincter was denoted as the duodenal sample, the middle 50-cm segment as the jejunum sample and the posterior 20-cm segment just preceding the colon as the ileal sample. The central portion of each of these samples was further sectioned into 2-cm segments for the Western blot. Samples were homogenized using a BioMasher II (Nippi, Tokyo, Japan). About 100 mg of the tissue was cut into small pieces and homogenized in 250 µL of homogenizing buffer (250 mM sucrose/5 mM HEPES with 2% protease inhibitor, pH 7.4). The crude homogenates were centrifuged with 500 µL of homogenizing buffer at 3,000 × g, 4°C for 10 min. The supernatant was removed and centrifuged again at 15,000 × g, 4°C for 30 min. The pellet was then reconstituted in suspending buffer (50 mM mannitol/20 mM HEPES with 2% protease inhibitor, pH 7.4). An aliquot of protein (15 µg) was loaded in each lane to detect the expression of P-gp or actin, electrophoresed on 7.5% SDS-polycrylamide gel, and transferred to a PVDF membrane (Immobilon transfer membrane, Millipore). For immunoblotting, the membranes were blocked with 1% nonfat powdered milk in TBS-T20 (20 mM Tris-HCl/137 mM NaCl/0.1% Tween 20, pH 7.6) at ambient temperature for 1 h. The blots were incubated with anti-P-gp (H-241; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or anti-actin (H-
Data analysis: All values are expressed as the mean±standard error of the mean (SEM). A non-compartmental pharmacokinetic analysis was applied to the plasma concentration vs. time data using WinNonlin software (Pharsight, Mountain View, CA). The maximum plasma concentration (C_{max}) and the time to maximum concentration (t_{max}) were estimated directly from observed plasma concentration-time data. The area under the area under the plasma concentration curve after oral administration of nadolol (AUC) was calculated using the linear trapezoidal rule up to 3 h (AUC_{0–3}), or up to the last measured plasma concentration and extrapolated to infinity (AUC_{0–∞}). The terminal elimination half-life (t_{1/2}) was determined by dividing ln 2 by the elimination rate constant at the terminal phase, which was computed by a linear least squared method using at least 3 measurement points. Amounts of excretion into urine (A_{u}) were calculated as the urinary concentration of nadolol multiplied by the urine volume. Statistical differences of the means were assumed to be significant when p < 0.05 by one-way ANOVA, followed by Dunnett’s multiple comparison test or unpaired t-test.

Results

Effect of itraconazole on the pharmacokinetics of nadolol: Plasma nadolol concentration-time profiles were obtained after oral administration of 10 mg/kg nadolol to rats in the control and pretreatment groups (Fig. 1). The corresponding pharmacokinetic parameters of nadolol are summarized in Table 1. Itraconazole pretreatment at 30 min before nadolol administration significantly increased C_{max}, AUC_{0–3} and AUC_{0–∞} of nadolol by 1.7-, 1.5- and 1.7-folds, respectively, compared with control. Median t_{max} and the average t_{1/2} for nadolol were not altered by itraconazole. The urine volume and the urinary excretion of nadolol during 24 h were not different between control and itraconazole treated rats (Fig. 2).

Effect of dexamethasone on P-gp protein level and nadolol pharmacokinetics: After repeated oral administration of dexamethasone for 4 days, there was no significant difference in the mean body weight between vehicle- and dexamethasone-treated rats (data not shown). The protein expression levels of P-gp in the intestine and the liver were analyzed by Western blotting. Dexamethasone treatment increased P-gp protein levels by 1.4- and 1.8-fold in the liver and the ileum, respectively (Fig. 3). C_{max} and AUC_{0–3} of nadolol decreased by 28% and 37%, respectively, compared to control with dexamethasone (Fig. 1 and Table 1). On the other hand, AUC_{0–∞} was significantly increased by 1.8-fold of control. Median t_{max} was not altered, while t_{1/2} was significantly prolonged by 2.1-fold by the pretreatment with dexamethasone. The urine volume was increased by 1.5-fold in the dexamethasone group, although the urinary concentration of nadolol was not significantly different between control and dexamethasone (data not shown).

![Fig. 1. Plasma concentration-time profile of nadolol in rats pretreated with saline (control, closed circles), itraconazole (50 mg/kg, open squares), dexamethasone (8 mg/kg/day, 4 days, open triangles) or naringin (0.145 mg/kg, open circles)](image1)

An oral dose of nadolol (10 mg/kg) was given at time 0. Plasma concentrations were measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h. Each value represents the mean ± SEM (n = 5–8).

![Fig. 2. Cumulative amount-time profile of nadolol in urine in rats pretreated with saline (control, closed circles), itraconazole (50 mg/kg, open squares), dexamethasone (8 mg/kg/day, 4 days, open triangles) and naringin (0.145 mg/kg, open circles)](image2)

An oral dose of nadolol (10 mg/kg) was given at time 0. Cumulative amount of nadolol during 24 h. The urine was collected cumulatively up to 24 h in fractions from 0–3 h, 3–6 h, 6–9 h, 9–12 h and 12–24 h using a metabolic cage. Each value represents the mean ± SEM (n = 5–8). * p < 0.05 and ** p < 0.01 vs. control.

Table 1. Pharmacokinetic parameters of nadolol in rats after a single dose of 10 mg/kg nadolol, which was administered at 30 min after saline (control), itraconazole (50 mg/kg) or naringin (0.145 mg/kg), or with dexamethasone (8 mg/kg) for 4 days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Itraconazole</th>
<th>Dexamethasone</th>
<th>Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/mL)</td>
<td>122 ± 14</td>
<td>98 ± 10</td>
<td>88 ± 10</td>
<td>123 ± 16</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>1.5 (1.0–2.0)</td>
<td>2.0</td>
<td>2.5 (0.5–4.0)</td>
<td>2.0 (1.0–2.0)</td>
</tr>
<tr>
<td>AUC_{0–3} (h·ng/mL)</td>
<td>212 ± 17</td>
<td>315 ± 28**</td>
<td>133 ± 16*</td>
<td>184 ± 23</td>
</tr>
<tr>
<td>AUC_{0–∞} (h·ng/mL)</td>
<td>297 ± 28</td>
<td>509 ± 33**</td>
<td>529 ± 69**</td>
<td>259 ± 38</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>2.0 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>4.1 ± 0.6**</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>A_{u} (µg)</td>
<td>245 ± 37</td>
<td>251 ± 47</td>
<td>411 ± 34*</td>
<td>187 ± 24</td>
</tr>
</tbody>
</table>

C_{max}, maximum plasma concentration of nadolol; t_{max}, time to maximum concentration; AUC_{0–3}, area under the plasma concentration-time curve from time 0 to 3 h; AUC_{0–∞}, area under the plasma concentration-time curve from time 0 to infinity; t_{1/2}, elimination half-life; A_{u}, the amount of nadolol excreted into urine within 24 h. Each value represents the mean ± SEM; except for t_{max} data, which are given as median and range (n = 5–8). * p < 0.05 and ** p < 0.01 vs. control.
Pharmacokinetic Interaction Study of Nadolol in Rats

Table 2. Pharmacokinetic parameters of celiprolol in rats after a single dose of 10 mg/kg celiprolol, administered at 30 min after saline (control) or naringin (0.145 mg/kg)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>364 ± 29</td>
<td>149 ± 7&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>2.0 (1.5–2.0)</td>
<td>1.3 (1.0–1.5)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0&lt;/sub&gt;–&lt;sub&gt;∞&lt;/sub&gt; (h·ng/mL)</td>
<td>418 ± 64</td>
<td>177 ± 39&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>1.1 ± 0.1</td>
<td>0.6 ± 0.1&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

C<sub>max</sub>, maximum plasma concentration of celiprolol; t<sub>max</sub>, time to maximum concentration; AUC<sub>0</sub>–<sub>∞</sub>, area under the plasma concentration-time curve from time 0 to infinity; t<sub>1/2</sub>, elimination half-life. Each value represents the mean ± SEM; except for t<sub>max</sub> data, which are given as median and range (n = 3–4). * p < 0.05 and ** p < 0.001 vs. control.

**Discussion**

It has been reported previously that nadolol was not metabolized by CYP enzymes in vivo, but could be transported by P-gp and OATP in vitro.\(^{12,14,15}\) Since the permeability of nadolol in the intestinal epithelium is poor, the active influx and/or efflux transport may contribute to the membrane permeation of nadolol at the absorption site. Nadolol could be a candidate for an in vivo probe drug for the evaluation of drug transporter activity. To date, however, there has been no evidence for the involvement of drug transporters in the pharmacokinetics of nadolol in rodents or humans. Therefore, in the present study, we aimed to clarify whether the inhibition or induction of P-gp or Oatp1a15 influences the plasma concentration profile and urinary excretion of nadolol in rats using itraconazole, dexamethasone and naringin as P-gp inhibitor, P-gp inducer and Oatp1a15 inhibitor, respectively.

Itraconazole, a triazole antifungal drug, is a potent inhibitor of P-gp and of Cyp3a.\(^{17}\) In this study, pretreatment with itraconazole (50 mg/kg) at 30 min before nadolol administration significantly increased C<sub>max</sub> and AUC<sub>0</sub>–<sub>∞</sub> of nadolol without alterations in t<sub>max</sub> or t<sub>1/2</sub> (Fig. 1). The AUC<sub>0</sub>–<sub>3</sub> value, which is more sensitive than C<sub>max</sub>, to detect differences in the absorption rate, was also significantly increased in the itraconazole group. These results could be attributed to an inhibition of P-gp by itraconazole, presumably in the apical membrane of the intestinal epithelium, because nadolol is not metabolized by Cyp3a.\(^{17}\) Concerning the P-gp–mediated itraconazole-drug interaction, a previous clinical study showed that itraconazole markedly increased the AUC value of celiprolol, possibly due to the inhibition of intestinal P-gp by itraconazole.\(^{15}\) Zolnerciks et al. recently demonstrated that the inhibitory potencies of itraconazole for prazosin and verapamil, which are typical P-gp substrates, were similar between human and rat P-gp, suggesting that the inhibition of P-gp by itraconazole is species-independent.\(^{19}\) Based on these findings, our results suggest that the bioavailability of orally administered nadolol is increased by the inhibition of intestinal P-gp by itraconazole. P-gp is also expressed in the apical membrane of renal proximal tubule cells as well as in hepatocytes.\(^{11}\) In this study, the cumulative amount of nadolol excreted into urine over 24 h was not altered between itraconazole and control rats (Fig. 2). Currently, we do not have a mechanistic explanation for the unchanged urinary excretion of nadolol by itraconazole. However, because the urinary excretion process is relatively minor in the elimination of nadolol in rats, the influence of itraconazole on renal P-gp–mediated excretion of nadolol may be less significant under our experimental conditions. On the other hand, since nadolol is mainly excreted into the bile in rats,\(^{12}\) hepatic P-gp may be involved in the biliary excretion of nadolol.
To assess the role of hepatic P-gp in the excretion of nadolol, the measurement of biliary excretion and the pharmacokinetic analysis of nadolol after parenteral administration are required in the future. Dexamethasone is known to induce P-gp via the activation of pregnane X receptor. To verify the effect of dexamethasone at a daily oral dose of 8 mg/kg for 4 days, we examined the protein level of P-gp in the liver and small intestine by Western blotting. We found that the P-gp expression in the liver, jejunum and ileum were elevated to 1.4-, 1.2- and 1.8-fold of vehicle, respectively (Fig. 3). This result was in accordance with the previous study on the effects of dexamethasone and St. John’s wort on P-gp induction in rats.

When we investigated the effect of dexamethasone on the pharmacokinetics of nadolol, we found that repeated dexamethasone significantly decreased AUC0-3 metrics of nadolol along with a decrease in Cmax (Fig. 1 and Table 1). Lin et al. reported that the plasma concentration of indinavir, a P-gp substrate, was reduced by dexamethasone treatment in rats (40 mg/kg/day, 3 days). This suggests that the induction of P-gp by dexamethasone treatment significantly increased the efflux of its substrate drug to the intestinal lumen. Therefore, the decrease in plasma concentration of nadolol during the absorption phase may be partly explained by the effect of dexamethasone on P-gp induction, which mediated efflux transport of nadolol from intestinal epithelial cells to the lumen. In contrast to the results of AUC0-3 and Cmax, the values of AUC0-∞ and t1/2 of nadolol were significantly increased in dexamethasone-pretreated rats as compared to control. These results are inconsistent with previous reports that the AUC and t1/2 of indinavir and fexofenadine were decreased by the pretreatment with a P-gp inducer such as dexamethasone or St. John’s wort.

In the present study, biochemical blood tests revealed remarkable increases in aspartate aminotransferase and alanine aminotransferase after dexamethasone treatment, indicating the impairment of rat liver function (data not shown). The elevated AUC0-∞ and prolonged t1/2 of nadolol may be ascribed to the decline of biliary excretion of nadolol from the hepatocytes. In addition, the cumulative amount of urinary-excreted nadolol was significantly increased in the dexamethasone group with little change in the urinary concentration of nadolol. Since the urine volume was also increased by dexamethasone treatment, we speculated that the increase in the urine excretion of nadolol is attributable to the increased urine volume. A similar result has been reported in which dexamethasone treatment induced a near doubling of the urine volume with a significant increase in urinary sodium excretion in rats. Because dexamethasone has multiple effects which could impair the influence of P-gp induction on the pharmacokinetics of nadolol, additional in vivo studies using other P-gp inducers will be needed to confirm the role of intestinal P-gp in the absorption of nadolol.

Naringin, a flavonoid component found in GFJ, inhibits either Oatp1a5 or P-gp activity depending on its concentration. Since a low dose of naringin predominantly inhibits Oatp1a5, we used a low dose of naringin as an Oatp1a5 inhibitor. When celiprolol was administered with naringin, the AUC0-∞ and Cmax of celiprolol were decreased by 50% and 50%, respectively, and tmax and t1/2 shortened by 66% and 60%, respectively (Fig. 4 and Table 2). This result is consistent with a previous report that the blood levels of talinolol were reduced when talinolol was administered in combination with naringin. Therefore, the dose of naringin applied in this study inhibited Oatp1a5 and affected the pharmacokinetics of Oatp1a5 substrates. In contrast, as shown in Figures 1 and 2, when coadministered with naringin at the same dose, the plasma concentration and urinary excretion of nadolol did not differ between the control and naringin groups. These results suggest that the contribution of Oatp1a5 to the absorption of nadolol is relatively small compared with celiprolol. Recent study has revealed that Oatp2b1 is also expressed in the small intestine in addition to Oatp1a5 in rats.

Another study showed recently that naringin at a high concentration (1,000 µM) inhibited Oatp2b1-mediated uptake of pravastatin but not pitavastatin in vitro, suggesting that naringin has a weak inhibitory effect on Oatp2b1 depending on its substrate. We do not know whether naringin at the dose of our study (0.154 mg/kg, approximately 67 µM) affects Oatp2b1 activity. It is necessary to investigate whether Oatp2b1 mediates the uptake of nadolol in the small intestine. Taken together, P-gp may play a critical role in the pharmacokinetic profile of nadolol rather than Oatp1a5 in rats. This suggests that nadolol could be a promising candidate for the evaluation of P-gp activity in vivo. However, we cannot exclude the possibility that other transporters are also involved in the pharmacokinetics of nadolol. Further study is required to identify the major determinant of the intestinal absorption of nadolol in addition to the biliary or urinary excretion.

To our knowledge, this is the first study to show the pharmacokinetic drug-drug interactions of nadolol with commonly prescribed drugs in animal models. Our data raises concern about the clinical use of nadolol, especially with respect to its efficacy and adverse effects, in combination therapy. Indeed, we have performed a clinical trial to elucidate whether the modulations of P-gp or OATP1A2 activities alter the pharmacokinetics of nadolol in healthy volunteers. The coadministration of itraconazole significantly increased the plasma concentration of nadolol after an oral administration of nadolol, while a single ingestion of grapefruit juice had a minor impact on nadolol pharmacokinetics. These results are in agreement with the present rat study, and suggest that care should be taken in the concomitant use of nadolol with P-gp inhibitors and/or inducers. At the same time, our clinical and preclinical results indicate that the pharmacokinetics of nadolol is predominantly regulated by P-gp, but not by OATP1A2/Oatp1a5, in both humans and rats, suggesting the potential usefulness of nadolol in preclinical studies for predicting P-gp-mediated drug interactions.

In conclusion, the present study was performed to examine whether P-gp or Oatp1a5 was involved in the pharmacokinetics of nadolol, and to address the possibility that nadolol can be an in vivo probe drug for P-gp or Oatp1a5. Our results show that nadolol is greatly affected by P-gp while negligibly affected by Oatp1a5 in its intestinal absorption as revealed by the pharmacokinetic experiments using itraconazole, dexamethasone and naringin. Although the role of other drug transporters on the pharmacokinetics of nadolol should be studied, we propose that nadolol could be a probe drug candidate for the evaluation or prediction of P-gp-mediated drug interactions.

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References

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