Intestinal Absorption and Hepatic Extraction of Propranolol and Metoprolol in Rats with Bilateral Ureteral Ligation

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To investigate the mechanism responsible for the increased bioavailability of propranolol in bilateral ureter-ligated (BUL) rats, the intestinal absorption and hepatic extraction of propranolol and metoprolol were evaluated. The initial absorption rate of these drugs after intra-intestinal administration was only slightly increased in the BUL rats, whereas the blood drug concentration in these rats was higher than that in control rats. The blood propranolol and metoprolol concentrations during intra-portal infusion in the BUL rat were significantly higher than that in the control rat. In the presence of NADPH, the intrinsic metabolic activity of metoprolol in hepatic microsomes was not altered by BUL. On the other hand, the NADPH generation rate in the hepatic cytosol in the BUL group was lower than that in the control group. These results indicate that the absorption rate-dependent decrease in hepatic first-pass clearance of propranolol and metoprolol due to saturation kinetics is marginal, and that the hepatic metabolic activity and extraction of the drugs is significantly decreased in BUL rats probably due to the reduced NADPH generation rate in the liver.

Key words  bilateral ureteral ligation; β-blocker; intestinal absorption; hepatic extraction

The intestinal absorption of orally administered propranolol is essentially complete, and the metabolism of this drug does not occur in the gut. After oral administration of propranolol, the liver is the principal site of extensive pre-systemic and systemic metabolism, and less than 1% of the intact drug is found in the urine. However, Bianchetti et al. showed that the area under the concentration-time curve for orally administered propranolol in renal failure patients not on hemodialysis is 7- to 8-fold higher than that in healthy volunteers. The pharmacokinetics of propranolol has been extensively investigated using uranyl nitrate-induced renal failure model rats. These studies showed the occurrence of increased bioavailability and reduced hepatic first-pass extraction of propranolol in rats with renal failure, although the precise biochemical and/or physiological mechanisms for the decreased presystemic clearance is unclear.

The injection of uranyl nitrate is most effective and the easiest method for producing renal dysfunction in laboratory animals; however, changes in government regulations regarding the production and use of radioactive substances have made uranyl nitrate less available. We therefore investigated the mechanisms responsible for the increased bioavailability of propranolol in cisplatin-induced renal failure rats. The hepatic intrinsic clearance of propranolol was not significantly altered in rats with renal failure as compared with control rats. However, hepatic first-pass extraction of propranolol was dose-dependent and saturable in both renal failure and control rats, and the initial absorption rate of the drug from the intestine in rats with renal failure was significantly greater than that in control rats. Accordingly, the increased bioavailability of propranolol in rats with cisplatin-induced renal dysfunction is mainly a result of the increased initial absorption rate in the intestine followed by the partial saturation of hepatic first-pass metabolism.

On the other hand, Laganière and Shen investigated the pharmacokinetics of intravenously and orally administered propranolol in bilateral ureter-ligated (BUL) rats, and showed that its bioavailability is increased in 36-h BUL rats. They also reported that the gastrointestinal absorption of propranolol is not altered in BUL rats as compared with control rats. Therefore, by inference, the increased bioavailability of propranolol in 36-h BUL rats is attributed to diminished hepatic first-pass metabolism.

The starting hypothesis of the present study was that the mechanism responsible for the increased bioavailability of propranolol in the BUL rat is different from that in the cisplatin-induced renal failure rat. We first examined the initial absorption rate of propranolol and metoprolol, another β-blocker with high hepatic intrinsic clearance but low plasma protein binding, in the BUL rat intestine. We then evaluated the hepatic first-pass extraction of these drugs in BUL rats using intra-portal administration. Finally, we examined the intrinsic cytochrome P450 activity in hepatic microsomes and the generation rate of NADPH, a co-factor of P450, in the hepatic cytosol fraction.

MATERIALS AND METHODS

Materials  Propranolol hydrochloride, metoprolol tartrate, NADP*, and NADPH were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest grade available.

Animals  The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Toyama Medical and Pharmaceutical University. Male Wistar rats (240—260 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). Before the experiments, the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. The abdominal cavity was opened under pentobarbital anesthesia, and BUL was performed by isolating the ureters, placing two ligatures tightly around each ureter, and cutting between the ligatures. The experiments were performed 24, 28, or 32 h after the operation. Sham-operated animals served as controls.

Effect of BUL on the Intestinal Absorption of Propra-
Propranolol and Metoprolol Rats were anesthetized with 50 mg/kg of sodium pentobarbital, and body temperature was maintained with appropriate heating lamps. The femoral artery in anesthetized rats was cannulated with a polyethylene tube (SP-31, Natsume Seisakusyo, Tokyo, Japan) for blood sampling. The abdominal cavity was opened, and the upper site of the duodenum and 15 cm from the upper end were ligated with silk sutures. Propranolol hydrochloride and metoprolol tartrate were dissolved in saline (10 mg/ml as a free base), and the drug solution was injected (20 mg/kg) into the closed loop of the duodenum. A blood sample was obtained for the measurement of drug concentration, and the loop was removed at a specified time after the drug injection. The luminal contents were collected with 7.5 ml of 0.01 N HCl, and intestinal tissue was homogenized with 9 volumes of saline for quantitation of the drugs. The net amount absorbed was calculated by subtracting the amounts remaining in the luminal contents and in the tissue from the applied dose.

Hepatic Extraction of Propranolol and Metoprolol in BUL Rats The femoral artery in anesthetized rats was cannulated with a polyethylene tube. For the intra-portal infusion of propranolol and metoprolol, a catheter with a 24 G needle was carefully fixed with cyanoacrylate glue into the portal vein, and the drug solution (10 ml/kg) was infused over 30 min by means of an automatic infusion pump at a dose of 10 mg/kg. Blood samples for the measurement of drug concentrations were obtained 8, 15, and 30 min after the initiation of drug administration. For the intra-intestinal administration of metoprolol, the drug solution (20 mg/kg) was injected into the duodenum. Blood samples for the measurement of drug concentrations were obtained 8, 15, and 30 min after administration. In addition, in order to evaluate the renal excretion of metoprolol in the rats, sham-operated control rats with bilateral renal artery occlusion (RAO) induced after administration. In RAO rats, the liver was removed from the rat and homogenized with 4 volumes of ice-cold 1.15% potassium chloride dissolved in isotonic phosphate buffer (pH 7.4) using a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 9000 g for 20 min, and the supernatant was transferred and centrifuged again at 105000 g for 60 min. The supernatant after 105000 g centrifugation (SUP) was collected as the hepatic microsomal fraction, and the precipitate (the microsomal fraction) was re-suspended in 3 volumes of the original liver weight of ice-cold 1.15% potassium chloride dissolved in isotonic phosphate buffer (pH 7.4). The microsomal suspensions and SUP were stored at −85 °C until use.

Metabolism of Metoprolol in Hepatic Microsomes The reaction mixture consisted of 200 µl of microsomal suspension (0.2 mg of protein), 50 µl of NADPH solution (final concentration of 1000 µM), and 200 µl of Krebs–Henseleit bicarbonate buffer (KHBB), which was preincubated for 5 min at 37 °C. The reaction was started by adding of 50 µl of metoprolol solution (final concentration of 30 µM), and was allowed to run for 30 min at 37 °C. The amount of metabolized metoprolol was calculated by subtracting the amount remaining in the incubation buffer from the applied amount. In addition, to evaluate the effect of endogenous substance(s) on the metabolic activity, KHBB was replaced by the SUP ultrafiltrate, which was obtained by centrifugal ultrafiltration (ULTRAFA®-MC 10,000 NMWL Filter Unit, Millipore Co., MA, U.S.A.). To evaluate the effect of NADPH concentration on the metabolic activity of metoprolol, 50 µl of standard NADPH solution (final concentration of 1000 µM) was replaced by a solution with a lower NADPH concentration (final concentrations of 30 and 300 µM).

Generation Rate of NADPH in the Hepatic Cytosol Fraction The reaction mixture, which consisted of 100 µl of SUP (1.5 mg of protein) and 300 µl of phosphate buffer (pH 7.4), was preincubated for 10 min at 30 °C. After 50 µl of NADP+ solution (final concentration of 3 mM) was added to the mixture, the reaction was started by adding 50 µl of glucose-6-phosphate solution (final concentration of 3 mM) or glucose solution (final concentration of 5 mM). The reaction was allowed to run for a specified time at 30 °C. At the end of the reaction, an equal amount of acetonitrile was added to precipitate the protein and allow for measurement of the NADPH concentration. In some experiments, the SUP ultrafiltrate (200 µl) described above was used instead of an equal volume of phosphate buffer (pH 7.4) to check for endogenous uremic inhibitors.

Analytical Methods The plasma concentrations of urea nitrogen, creatinine, and glutamic oxaloacetic transaminase (GOT) were measured using kits obtained from Wako Pure Chemical Industries (Osaka, Japan). Propranolol and metoprolol were assayed by an HPLC method as previously reported.11,14] NADPH was assayed spectrophotometrically based on the absorbance at 340 nm.15 The protein contents of the hepatic microsomal suspension and SUP were determined using Bio-Rad protein assay dye reagent (Bio-rad, Munich, Germany).

Data Analysis Statistical analysis was performed using a non-paired t-test provided that the variances were similar. If this was not the case, the Mann–Whitney U-test was applied. Multiple comparisons were performed using a Tukey-type test following Kruskal–Wallis analysis. p values less than 0.05 (two-tailed) were considered to be statistically significant.

RESULTS

Biochemical Parameters of BUL Rats The plasma concentrations of urea nitrogen and creatinine were measured to assess the development of renal dysfunction in the rats. Table 1 shows these biochemical parameters as well as the plasma GOT concentration in the 24-, 28-, and 32-h BUL rats. The plasma urea nitrogen and creatinine concentrations increased approximately 6.9- to 9.3-fold in BUL rats as compared with control rats. In contrast, the plasma GOT concentration in BUL rats was not significantly different from that in control rats (Table 1).

Intestinal Absorption of Propranolol and Metoprolol in BUL Rats Figure 1 shows the blood propranolol concentration versus net intestinal absorption in individual BUL and control rats. Eight minutes after propranolol injection into
The blood metoprolol concentration was between 3.8—5.4 mcg/ml in BUL rats as compared with that in control rats (Fig. 2B). In contrast, the blood metoprolol concentration was significantly higher in 32-h BUL rats as compared with control rats, suggesting the diminished hepatic first-pass extraction in BUL rats.

To further evaluate the hepatic extraction of metoprolol in BUL rats, we compared the time courses of blood metoprolol concentration following intra-portal infusion during intra-intestinal administration in BUL rats can not be solely explained by slight alterations in the initial drug absorption rate in the intestine.

**Hepatic Extraction of Propranolol and Metoprolol in BUL Rats**

The mean blood concentration of propranolol at 8, 15, and 30 min after the start of intra-portal infusion was 2.06±0.17, 2.96±0.17, and 3.93±0.19 mcg/ml, respectively (Fig. 3). On the other hand, the mean blood concentration of propranolol at 8, 15, and 30 min after the start of intra-portal infusion was 1.21±0.23, 1.62±0.24, and 2.29±0.17 mcg/ml, respectively (Fig. 3). The blood concentration of propranolol in BUL rats was significantly higher than that in control rats, suggesting the diminished hepatic first-pass extraction in BUL rats.

The intestinal loop, the mean net absorption in 24-h BUL rats was only slightly increased as compared with that in control rats, whereas the blood concentration in 24-h BUL rats was significantly higher than that in control rats (Fig. 1A). In addition, blood propranolol concentration was also significantly higher in 32-h BUL rats as compared with control rats, whereas there was little difference between the net absorption in 32-h BUL rats and that in control rats (Fig. 1B). The net absorption of propranolol in 32-h BUL rats was slightly greater than that in 24-h BUL rats; however, this was not statistically significant. The significant increases in blood propranolol concentration were observed in both 24-h and 32-h BUL rats, as compared with the control rat. Therefore, the subsequent experiments were performed by use of 28-h BUL rats.

Figure 2 shows the blood metoprolol concentration versus net intestinal absorption in 28-h BUL and control rats. Fifteen minutes after administration, the mean initial absorption rate in BUL rats was 1.2-fold higher than that in control rats, whereas the mean blood metoprolol concentration was 4.3-fold higher in BUL rats as compared with control rats (Fig. 2A). Thirty minutes after administration, the mean net absorption of metoprolol was only slightly increased in BUL rats as compared with that in control rats (Fig. 2B). In contrast, the blood metoprolol concentration was between 3.8—8.1 mcg/ml and 1.2—5.4 mcg/ml in BUL and control rats, respectively (Fig. 2B). These results indicate that the increased blood concentrations of propranolol and metoprolol following intra-intestinal administration in BUL rats can not be solely explained by slight alterations in the initial drug absorption rate in the intestine.

**Table 1. Biochemical Parameters of Plasma Obtained from BUL Rats**

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>BUL</th>
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<tr>
<td><strong>24-h BUL</strong></td>
<td></td>
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</tr>
<tr>
<td>Urea nitrogen</td>
<td>17.2±1.6</td>
<td>142±5**</td>
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<tr>
<td>Creatinine</td>
<td>0.567±0.035</td>
<td>3.90±0.23*</td>
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<tr>
<td>GOT</td>
<td>45.3±3.2</td>
<td>55.7±6.6</td>
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<tr>
<td><strong>28-h BUL</strong></td>
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<tr>
<td>Urea nitrogen</td>
<td>17.2±0.7</td>
<td>139±8**</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.561±0.043</td>
<td>4.53±0.18**</td>
</tr>
<tr>
<td>GOT</td>
<td>48.0±3.2</td>
<td>45.0±3.3</td>
</tr>
<tr>
<td><strong>32-h BUL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>19.2±1.3</td>
<td>178±9**</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.726±0.086</td>
<td>5.13±0.26**</td>
</tr>
<tr>
<td>GOT</td>
<td>46.6±8.8</td>
<td>68.3±14.2</td>
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Values are expressed as the mean±S.E. for 4—8 rats. *p<0.05, **p<0.01 compared with sham-operated control rats.

Fig. 1. Blood Propranolol Concentration versus Net Absorption 8 min after Intra-intestinal Injection (20 mg/kg) to 24-h (A) and 32-h (B) BUL (Closed Circle) and Control Rats (Open Circle)

Bars represent the mean±S.E. for 6—7 rats. **p<0.01 compared with blood propranolol concentration in control rats. †p<0.05 compared with net absorption in control rats.

Fig. 2. Blood Metoprolol Concentration versus Net Absorption 15 min (A) or 30 min (B) after Intra-intestinal Injection (20 mg/kg) to 28-h BUL (Closed Circle) and Control Rats (Open Circle)

Bars represent the mean±S.E. for 7 rats. **p<0.01 compared with blood propranolol concentration in control rats. †p<0.05 compared with net absorption in control rats.

Fig. 3. Blood Propranolol Concentration after Intra-portal Infusion (0.333 mg/min/kg for 30 min) to 28-h BUL Rats (Closed Circle) and Control Rats (Open Circle)

Each symbol and bar represents the mean±S.E. for 5—6 rats. *p<0.05, **p<0.01 compared with control rats.
metoprolol concentration in BUL rats was higher than that in control rats. In addition, the blood concentration in control rats with RAO was only slightly higher than that in control rats. In addition, the blood concentration in control rats. In addition, the generation rate of NADPH after the addition of glucose-6-phosphate in BUL rats was not different from that in control rats regardless of the presence of SUP ultrafiltrate (Table 2). These results indicate that the glucose-6-phosphate dehydrogenase activity is not altered in the BUL rats.

On the other hand, the generation rate of NADPH after the addition of glucose in BUL rat liver was significantly lower than that in control rat SUP (Table 2), which suggests that glucokinase activity was markedly depressed in BUL rats as compared with control rats.

**Hepatic P450 Activity in BUL Rats** We examined the hepatic metabolism of propranolol in rat liver microsomes obtained from 28-h BUL and control rats. Figure 5 shows the microsomal metabolism of propranolol in the presence or absence of the SUP ultrafiltrate. The intrinsic metabolic activity of hepatic microsomes of BUL rats was similar to that of control rats. In addition, the metabolic activity in the presence of the SUP ultrafiltrate in the BUL rat was also similar to that in the control rat (Fig. 5). We also evaluated the metabolic activity of metoprolol at various NADPH concentrations. As shown in Fig. 6, the metabolic activity of metoprolol was well correlated with the NADPH concentration. In addition, the metabolic activity of metoprolol at NADPH concentrations of 30 and 300 μM in BUL rats was not different from that in control rats.

**Glucose-6-phosphate Dehydrogenase and Glucokinase Activity in BUL Rats** We tried to measure the endogenous NADPH concentration in the prepared SUP fraction of the 28-h BUL and control rat liver. However, no significant NADPH was detected in the prepared liver SUP, probably because endogenous NADPH was degraded during the preparation process of the liver SUP.

We then examined the generation of NADPH in the 28-h BUL rat liver. Table 2 shows the generation rate of NADPH in the liver SUP obtained from the BUL and control rats. The generation rate of NADPH after the addition of glucose-6-phosphate in BUL rat SUP was not different from that in control rat SUP regardless of the presence of SUP ultrafiltrate (Table 2). These results indicate that the glucose-6-phosphate dehydrogenase activity is not altered in the BUL rats.

**DISCUSSION**

The present findings indicate that hepatic metabolic activity and extraction of propranolol and metoprolol are significantly decreased in BUL rats, but that the absorption rate-dependent decrease in hepatic first-pass clearance due to saturation kinetics is marginal. The effect of BUL on the intestinal absorption of propranolol and metoprolol was small, whereas the blood drug concentration after intra-intestinal administra-
The rate of sulfanilic acid is increased in rats with HgCl₂-induced renal failure. We also reported that the intestinal absorption rate of imipramine, cefadroxil, ciclacilline, sulfaguanidine, sulfafurazole, quinine, salicylic acid, and other drugs (sulfanilic acid, procainamide ethobromide, cefazo-line, sulfaguanidine, sulfafurazole, quinine, salicylic acid, imipramine, cefadroxil, ciclacilline etc.) in glycerol-induced renal failure rats. We also reported on the increased intestinal absorption rate of tacrolimus is increased in rats with cisplatin-induced renal failure rats, and also 36-h BUL rats. Moreover, the intestinal absorption rate of propranolol is significantly increased in rats with cisplatin-induced renal failure.18) The present findings that the initial absorption rate of propranolol and metoprolol in the intestine was only slightly increased in BUL rats (Figs. 1, 2) may be important in further investigations on the mechanism(s) involved in the alteration of the intestinal barrier function in renal dysfunction.

Several studies have examined the increased intestinal absorption rate of various drugs in rats with experimental renal failure. Kimura et al. reported that the intestinal absorption rate of sulfanilic acid is increased in rats with HgCl₂-induced renal dysfunction and in 5/6 nephrectomized rats.16) They also reported on the increased intestinal absorption rate of drugs (sulfanilic acid, procainamide ethobromide, cefazo-line, sulfaguanidine, sulfafurazole, quinine, salicylic acid, imipramine, cefadroxil, ciclacilline etc.) in glycerol-induced renal failure rats.17) We also reported that the intestinal absorption rate of tacrolimus is increased in rats with cisplatin-induced renal failure.18) Moreover, the intestinal absorption rate of propranolol is significantly increased in rats with cisplatin- and also glycerol-induced renal dysfunction.19) The present findings that the initial absorption rate of propranolol and metoprolol in the intestine was only slightly increased in BUL rats (Figs. 1, 2) may be important in further investigations concerning the effect of BUL on the activity of enzymes involved in the glycolysis and pentose-phosphate pathway, including glucokinase. We plan to perform further studies to reveal the mechanisms involved in the alteration of glucokinase activity in BUL rats.

Several reports have suggested possible effects of endogenous uremic substance(s) on hepatic drug metabolizing activity. Yoshitani et al. reported that the unbound clearance of losartan in rat hepatic microsomes in the presence of 10% uremic serum obtained from uranyl nitrate-treated and BUL rats is significantly lower than that in the presence of control serum.20) Terao and Shen also reported that endogenous substance(s) in the blood of the uranyl nitrate-induced uremic rat is capable of inhibiting the hepatic extraction of propranolol in perfused liver, although it is not clear whether endogenous uremic substances inhibit P450 activity directly and/or the NADPH generation.8) In the present study, the activity of P450 (CYP2D2) and glucose-6-phosphate dehydrogenase was not significantly inhibited by the SUP ultrafiltrate in the BUL and control rats (Fig. 5, Table 2). Glucokinase activity was enhanced by the addition of the SUP ultrafiltrate in both BUL and control rats, probably because the ultrafiltrate supplied ATP and/or Mg²⁺ to glucokinase (Table 2). In addition, the metabolism of metoprolol in hepatic microsomes in the presence of SUP, plasma, or the filtrate of plasma in the BUL rats was not significantly different from that in control rats (data not shown). Further systematic studies may be necessary to determine the inhibitory effect of endogenous uremic substances on drug metabolizing activity.

Human studies that examined the effect of renal failure on the pharmacokinetics of propranolol and metoprolol have produced conflicting results.25) Lowenthal et al. reported that the mean maximal concentration of propranolol (oral dose of 80 mg under the fasting state) in patients with chronic renal insufficiency is 155 ng/ml, whereas that in healthy volunteers is 52 ng/ml.24) Bianchetti et al. also reported that the mean blood peak level of propranolol (single oral dose of 40 mg) in the uremic patients not on regular dialysis is 161 ng/ml, that in the dialysis patients is 47 ng/ml, and that in healthy volunteers is 26 ng/ml.20) In contrast, Wood et al. found no significant difference in oral clearance, total clearance or bioavailability of propranolol in patients with stable chronic renal failure on hemodialysis or in those not receiving regular hemodialysis as compared with age-matched controls.25) Furthermore, the mean plasma peak level of metoprolol in renal failure patients after an oral dose of 50 mg metoprolol tartrate is 231 nmol/l, while that in controls is 237 nmol/l.26) Our previous and present results that the bioavailability of propranolol and metoprolol is increased in rats with cisplatin- and/or BUL-induced renal failure, are in line with those reported by Lowenthal et al. and Bianchetti et al., but do not agree with those reported by Wood et al. and Jordö et al.4,11,23,24,26) In addition, Laganière and Shen showed that no notable difference in the pharmacokinetics of propranolol was observed between control and 24-h BUL rats, but that its bioavailability was increased in 36-h BUL rats.21) Accordingly, the degree of alteration in hepatic drug metabolizing activity and/or intestinal barrier function in renal dysfunction may be dependent on the etiology and the development of...
renal failure.

In conclusion, the mechanism responsible for the increased bioavailability of propranolol and metoprolol in BUL rats is different from that of propranolol in rats with cisplatin-induced renal failure. This may provide new insight into understanding the bioavailability and hepatic first-pass metabolism of drugs in renal dysfunction.

Acknowledgements  This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) and by a Grant-in-Aid for JSPS Fellows.

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