Pharmacokinetics and Hepatic Extraction of Metoprolol in Rats with Glycerol-Induced Acute Renal Failure

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The aim of the present study was to evaluate the effect of glycerol-induced acute renal failure (ARF) on the pharmacokinetics and hepatic extraction of metoprolol in rats. Experimental ARF in rats was induced by injections of 50% glycerol into the leg muscle (10 ml/kg). Pharmacokinetics and hepatic extraction of metoprolol was evaluated by means of intravenous, intra-intestinal, and intra-portal administration of the drug. The blood metoprolol concentration following intravenous infusion in ARF rats was similar to that in control rats. On the other hand, the blood metoprolol concentration at 5—10 min after intra-intestinal administration in ARF rats was significantly higher than that in control rats, and the oral clearance (CL/F) of the drug was significantly decreased in ARF rats. Hepatic extraction following intra-portal infusion was not altered by glycerol-induced ARF; however, hepatic first-pass extraction of metoprolol was dose-dependent and saturable in both ARF and control rats. These results suggested that the decreased CL/F of metoprolol in rats with glycerol-induced ARF is mainly a result of the increased initial absorption rate in the intestine followed by partial saturation of hepatic first-pass metabolism.

Key words metoprolol; glycerol-induced acute renal failure; intestinal absorption rate; hepatic extraction

The intestinal absorption of orally administered propranolol is essentially complete, with no metabolism of this drug occurring in the gut.1,2) After the oral administration of propranolol, the liver is the principal site of extensive presystemic and systemic metabolism, and less than 1% of the intact drug is found in urine.1,3) However, Bianchetti et al.4) 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. We investigated the mechanisms responsible for the increased bioavailability of propranolol in rats with cisplatin-induced renal failure.5) 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 rate of absorption of the drug from the intestine was significantly greater in rats with renal failure than 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.5)

Interestingly, the mechanism responsible for the increased bioavailability of propranolol in bilateral ureter-ligated (BUL) rats is different from that in rats with cisplatin-induced renal failure.2,6) We investigated the pharmacokinetics of propranolol and metoprolol in BUL rats, and found that the rate of intestinal absorption of these drugs was only slightly greater than that in control rats.6) On the other hand, the arterial blood concentrations of propranolol and metoprolol following intra-portal infusion were significantly higher in BUL rats than control rats.6) Therefore, the increased bioavailability of propranolol and metoprolol in BUL rats was attributed to diminished hepatic first-pass metabolism. The activity of CYP2D2, which is responsible for the metabolism of propranolol and metoprolol in the rat liver, was not altered by BUL, whereas the rate at which NADPH was generated in the liver cytosolic fraction was lower in BUL than control rats.6–8) In addition, endogenous uremic substances are not involved in the reduced hepatic extraction of metoprolol in BUL rats.9) Accordingly, the decrease in the hepatic metabolic activity and extraction of propranolol and metoprolol in BUL rats is mainly due to the reduced generation of NADPH in the liver.

Since the sequence and relative importance of pathophysiological components of acute renal failure in patients are not clearly defined, it is difficult to determine which experimental method for producing renal failure in animals yields results that are most representative of the clinical condition.10) We consider that the investigation of pharmacokinetics in other renal failure models is indispensable in order to understand the main mechanism for the increased bioavailability of drugs in renal failure. Glycerol-induced acute renal failure (ARF) in rats is a model of acute trauma in which intramuscular injection of 50% glycerol causes rapid myoglobinuria, oliguria, and a rapid reduction in glomerular filtration rate.11) The effect of renal dysfunction on the pharmacokinetics and pharmacodynamics of drugs has been extensively investigated by use of glycerol-induced ARF rats.12–14) However, the effect of glycerol-induced ARF on hepatic drug metabolism is unclear. The aim of this study was to evaluate the effect of glycerol-induced ARF on the pharmacokinetics and hepatic extraction of metoprolol.

MATERIALS AND METHODS

Materials Polyethylene tubes, SP-31 and PE-10, were obtained from Natsume Seisakusho (Tokyo, Japan) and Becton Dickinson (Parsippany, NJ, U.S.A.), respectively. Pentobarbital sodium (Nembutal® injection, 50 mg/ml) was obtained from Dainippon Sumitomo Pharma (Osaka, Japan). Metoprolol tartrate was purchased from ICN Biochemicals (Aurora, OH, U.S.A.). All other chemicals were of the highest grade available.

Animals All animal experiments were performed in ac-
cordance with the Guidelines for Animal Experiments of University of Toyama. Male Wistar rats (240—300 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan), and housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. ARF was induced by injections of glycerol dissolved in saline (50% w/v, 10 ml/kg) into the leg muscle after 24 h period of water deprivation.5,7,14) Saline-treated rats served as controls. Animals were used for experiments 72 h after treatment.

Pharmacokinetics of Metoprolol Rats were anesthetized with 25—50 mg/kg pentobarbital sodium. Body temperature was maintained with appropriate heating lamps. The femoral artery was cannulated with a polyethylene tube (SP-31) for blood sampling. The jugular vein was cannulated with a polyethylene tube (PE-10) for 15-min intravenous infusion of metoprolol (5 mg/kg). Metoprolol solution (2 mg/ml) was intravenously infused with a constant rate infusion pump. Arterial blood samples for the measurement of metoprolol concentration were obtained at 7, 15, 20, 30, 60, 90, 120, and 150 min after initiation of 15-min intravenous infusion. For intra-intestinal administration study (15, 30 mg/kg), a 3-cm middle incision was made in the abdomen, and the upper end of the duodenum was ligated twice with silk sutures. The drug solution (7.5, 15 mg/ml) was injected into the duodenal lumen using a syringe with a 26 G needle. Blood samples were obtained at 5, 10, 15, 30, 60, 90, 120, and 150 min. For the intra-portal administration study (0.2, 1 mg/min/kg), a catheter with a 26 G needle was carefully inserted into the portal vein and held in place with surgical glue. Blood samples were obtained at 30 min after the start of intra-portal infusion of drug solutions (2, 10 mg/ml) with a constant rate infusion pump.

Analytical Methods The plasma concentrations of urea nitrogen and creatinine were measured using kits obtained from Wako Pure Chemical Industries (Osaka, Japan). The blood metoprolol concentration was measured with an HPLC-based method as reported previously.15) Briefly, metoprolol was extracted from blood samples (0.1 ml) with 5 ml of diethylether after alkalization with 1 ml of glycine buffer (0.1 M, saturated with NaCl, pH 10.6), and back-extracted from the organic phase with 0.5 ml 0.01 N HCl. A 50-μl aliquot of HCl solution was injected into a HPLC system.5) The column was COSMOSIL 5C18-AR-II (15 cm × 4.6 mm; i.d. 4.5 μm particle size; Nacalai Tesque). The mobile phase consisted of 10 mM KH2PO4 that contained 0.6% (w/v) triethylamine adjusted to pH 3.3 with phosphoric acid and acetonic (90.5/9.5, v/v), the flow rate was 1.6 ml/min and the column temperature was 45 °C. The peaks were monitored at an excitation wavelength of 272 nm and an emission wavelength of 303 nm.

Data Analysis The pharmacokinetic parameters of metoprolol following intravenous infusion were estimated using the software package NONMEM Version V.16) The two-compartment model was parameterized in terms of systemic clearance (CL), central volume of distribution (Vc), intercompartmental clearance (Q), and volume of distribution at steady state (Vdss). The area under the blood concentration—time curve (AUC) after intra-intestinal administration was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the last measurable metoprolol concentration to the mean terminal disposition rate constant. The oral clearance (CL/F) values following intra-intestinal administration were calculated from dose/AUC.

Statistical Analysis Values are expressed as the mean± S.E. for n animals. The statistical significance of differences between mean values was tested using an unpaired t-test provided that the variances of the groups were similar. If this was not the case, the Mann-Whitney U-test was applied. p values of less than 0.05 were considered significant.

RESULTS

Renal Function of Glycerol-Induced ARF Rats The plasma concentrations of urea nitrogen and creatinine were measured to assess the development of renal dysfunction in rats. The plasma urea nitrogen concentrations were 26.2±1.6 and 271±19 mg/dl in the control and ARF rat, respectively (p<0.05). The plasma creatinine concentrations were 0.57±0.04 and 3.93±0.28 mg/dl in the control and ARF rat, respectively (p<0.05).

Pharmacokinetics of Metoprolol Following Intravenous Infusion The blood metoprolol concentration following 15-min intravenous infusion at a dose of 5 mg/kg is shown in Fig. 1. The blood metoprolol concentration in ARF rats was similar to that in control rats. The pharmacokinetic parameters of metoprolol in rats after intravenous infusion are listed in Table 1. The CL, Vc, Q, and Vdss values in ARF were not significantly different from those in control rats.

Pharmacokinetics of Metoprolol Following Intra-intestinal Administration Figure 2 shows the time course of blood concentration of metoprolol after intra-intestinal administration at a dose of 15 mg/kg in ARF and control rats. The blood metoprolol concentration at 5—10 min after dose was significantly higher in ARF rats than in control rats, indicating that the initial absorption rate of metoprolol in the intestine was significantly increased in ARF rats. In addition, we investigate the pharmacokinetics of the drug following intra-intestinal administration at a dose of 30 mg/kg. As shown in Fig. 3, the blood metoprolol concentration in ARF rats at a dose of 30 mg/kg was also significantly higher than that in control rats. The pharmacokinetic parameters of metoprolol in rats after intra-intestinal administration are listed in Table 1. The CL/F values in ARF rats were significantly dif-

![Fig. 1. Time Course of Blood Metoprolol Concentration Following 15-min Intravenous Infusion at a Dose of 5 mg/kg to ARF (Closed Circle) and Control (Open Circle) Rats](image-url)

Lines are simulation curves obtained by pharmacokinetic analysis in ARF and control rats. Each symbol and bar represents the mean±S.E. for 5 rats. *p<0.05 compared with control rats.
different from those in control rats at doses of 15 and 30 mg/kg. The mean bioavailability (F) values, which were calculated from CL and CL/F values, were dose-dependent in both ARF and control rats (Table 1). In addition, the mean F value at a dose of 15 mg/kg in ARF rats (50%) was higher than that in control rats (37%). However, the mean F value at a dose of 30 mg/kg in control rats was considerably high (63%), and was only slightly lower than that in ARF rats (69%).

### Pharmacokinetics of Metoprolol Following Intra-portal Infusion

To evaluate the effect of ARF on the hepatic extraction of metoprolol, we measured the arterial blood metoprolol concentration following 30-min intra-portal infusion at a rate of 0.2 and 1 mg/min/kg. Figure 4 shows the ratio of (infusion rate)/(blood metoprolol concentration) in ARF and control rats. The ratio in ARF rats was only slightly less than that in control rats; however, the difference was not statistically significant. This finding indicated that ARF had little effect on the hepatic extraction of metoprolol. In addition, the ratio at the higher dose (1 mg/min/kg) was significantly smaller than that at the lower dose (0.2 mg/min/kg) in both ARF and control rats ($p<0.05$), indicating that hepatic first-pass extraction of metoprolol was dose-dependent and saturable in both ARF and control rats.

## DISCUSSION

The aim of the present study was to evaluate the effect of glycerol-induced ARF on the pharmacokinetics of metoprolol. The blood metoprolol concentration following intravenous infusion in ARF rats was similar to that in control rats (Fig. 1). On the other hand, the blood metoprolol concentration at 5—10 min after intra-intestinal administration in ARF rats was significantly higher than that in control rats (Figs. 2, 3). Hepatic extraction following intra-portal infusion was not significantly altered by glycerol-induced ARF, whereas hepatic first-pass extraction of metoprolol was dose-dependent and saturable in both ARF and control rats (Fig. 4). These results indicated that the increased intestinal absorption rate significantly contributed to the decreased CL/F of metoprolol in rats with glycerol-induced ARF, and that the mechanism responsible for the increased bioavailability of drugs in rats with glycerol-induced ARF was different from that in BUL rats and similar to that in cisplatin-induced ARF rats.5,6)

Kimura et al. investigated the intestinal absorption rate of various drugs in glycerol-induced ARF.17) They reported that the intestinal absorption rate of drugs (sulfanilic acid, procainamide ethobromide, cefazoline, sulfafurazole, quinine, salicylic acid, imipramine, cefadroxil, cicaclillin etc.) was significantly increased in rats with glycerol-induced ARF.17)
The increased intestinal absorption rate of drugs has been observed in other renal failure models.\textsuperscript{5,18–20} That is, we found that the intestinal absorption rate of propranolol and tacrolimus is significantly increased in rats with cisplatin-induced renal dysfunction.\textsuperscript{5,18} We also reported that the intestinal absorption rate of ajmaline is significantly increased in rats with uranyl nitrate-induced renal failure.\textsuperscript{19} Moreover, Kimura et al.\textsuperscript{20} reported that the intestinal absorption rate of sulfanilic acid is increased in rats with HgCl\textsubscript{2}-induced renal failure.\textsuperscript{22} However, there may be decreases the protein expression of CYP3A1, 3A2, 2C6, and 2C11 in normal rat hepatocytes.\textsuperscript{23} In addition, hepatic drug metabolism may be decreased in patients with renal failure.\textsuperscript{5} In fact, Lowenthal et al. reported that the mean serum concentration of propranolol at 30 min (the initial period of absorption) after single oral administration in patients with chronic renal failure was approximately 10-fold higher than that in healthy volunteers.\textsuperscript{21}

The activity of CYP2D2, which is responsible for the metabolism of cationic drugs in the rat liver, may not be altered in renal failure.\textsuperscript{5,7,19} That is, we previously reported that debrisoquine 4-hydroxylation activities in rat liver microsomes were not altered in rats with glycerol-, cisplatin-, nephrectomy-, and BUL-induced renal failure.\textsuperscript{7} We have investigated the effect of renal failure on the hepatic extraction of cationic drugs following intra-portal infusion.\textsuperscript{5,19} The hepatic extraction of ajmaline in rats with uranyl nitrate-induced renal dysfunction was not significantly different from that in control rats.\textsuperscript{5,19} Similarly, the hepatic extraction of propranolol was not altered significantly in rats with cisplatin-induced ARF.\textsuperscript{5}

In the present study, we found that the hepatic extraction of metoprolol in glycerol-induced ARF rats was similar to that in control rats (Fig. 4). On the other hand, hepatic CYP2C and 3A activities are altered in some renal failure rats.\textsuperscript{7} That is, we reported that hepatic CYP2C activity in rat liver microsomes is decreased in rats with cisplatin-induced renal failure, and that hepatic CYP3A activities are decreased in rats with nephrectomy- and glycerol-induced renal failure.\textsuperscript{7}

Although the precise mechanism responsible for the decrease in hepatic metabolic activity during renal failure was unclear in our previous study,\textsuperscript{7} Guévin et al. reported that the serum of rats with chronic renal failure contains mediator(s) that decreases the protein expression of CYP3A1, 3A2, 2C6, and 2C11 in normal rat hepatocytes.\textsuperscript{22} However, there may be other possible explanations for the diminished hepatic extraction of drugs.\textsuperscript{8,23} We reported that the decrease in the generation of NADPH in BUL rats was mainly caused by the decreased concentration of endogenous substrate(s) and/or the increased concentration of endogenous inhibitor(s) for the pentose phosphate pathway.\textsuperscript{5} Sun et al. reported that uracil toxin directly inhibited the uptake of erythromycin by rat hepatocytes, mainly by inhibiting the liver uptake transporter.\textsuperscript{23}

In addition, hepatic drug metabolism may be decreased in patients with renal dysfunction. That is, Fukatsu et al. reported that the CL of tacrolimus in adult recipients with renal dysfunction, defined as a serum concentration of creatinine over 1 mg/dl, was 80.9% of that in recipients with serum concentration of creatinine below this level.\textsuperscript{24} It was also reported that the hepatic metabolism of erythromycin and verapamil was decreased in patients with renal dysfunction.\textsuperscript{25,26} Further studies may be necessary to determine the mechanism responsible for the alteration of hepatic drug metabolism in patients with renal failure.\textsuperscript{7}

In conclusion, the mechanism responsible for the decreased CL/F of metoprolol in rats with glycerol-induced ARF is mainly a result of the increased initial absorption rate in the intestine followed by partial saturation of hepatic first-pass metabolism. This may provide a new insight into the altered bioavailability of drugs during renal failure.

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**REFERENCES**