Effect of Experimental Renal Failure on the Pharmacokinetics of Losartan in Rats

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The purpose of this investigation was to determine whether the pharmacokinetics of the angiotensin II receptor antagonist losartan is altered in renal failure. Male Wistar rats were pretreated with uranyl nitrate or subjected to bilateral ureteral ligation to produce acute renal failure (ARF). Saline-injected and sham-operated rats, respectively, served as controls. Uranyl nitrate-treated rats showed significantly higher serum concentrations of losartan after oral administration and the area under the serum concentration–time curve (AUC) of losartan increased about 3-fold compared to control rats. The systemic clearance of losartan significantly decreased from 410±254 ml/h/kg in control to 177±112 ml/h/kg in uranyl nitrate-treated rats. In order to investigate the mechanisms of reduced clearance of losartan associated with ARF, a hepatic microsome fraction was prepared from normal and ARF rats. No significant difference was found in the metabolism of losartan by hepatic microsomes prepared from ARF and control rats. In addition, the metabolic activity of microsomes was examined in the presence of uremic rat serum. The unbound clearance of losartan and the unbound clearance associated with the formation of EXP3174 in the presence of uremic serum were significantly lower than those in the presence of control serum. Furthermore, the metabolism of losartan was inhibited by indoxyl sulfate, a uremic toxin, in an uncompetitive manner. These results suggest that ARF is associated with reduced clearance of losartan due to the inhibition of hepatic metabolism by accumulated uremic toxin(s).

Key words losartan; renal failure; pharmacokinetics; uremic toxin; indoxyl sulfate; rat

Losartan is a potent, orally active, angiotensin II receptor antagonist and is used as an antihypertensive agent. Losartan binds selectively and specifically to the AT₁ subtype of angiotensin receptor. In contrast to angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists directly abolish the actions of angiotensin II in the body.

Losartan is metabolized to EXP3174, 2-n-butyl-4-chloro-1-[(2-[(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazole-5-carboxylic acid, which contributes to the overall in vivo activity of losartan in rats and humans. EXP3174 has a longer half-life than losartan. The conversion of losartan to EXP3174 is catalyzed by two cytochrome P450 subfamilies: CYP2C9 and CYP3A4. In normal volunteers, renal clearance accounts for about 12 and 55% of the systemic clearance of losartan and EXP3174, respectively.

Progressive renal insufficiency is commonly accompanied by hypertension, which frequently results in the use of antihypertensive agents to reduce blood pressure or, secondarily, to provide renoprotection; accordingly, it is necessary to investigate the effect of renal failure on the pharmacokinetics of antihypertensive drugs to establish when dosage adjustment is necessary. Very limited information is available on the pharmacokinetics of losartan in humans with renal dysfunction. Sica et al. reported that the steady-state areas under the curve (AUC) of losartan and EXP3174 were not significantly changed with renal impairment. On the other hand, Saruta et al. reported that the maximum serum concentration (Cmax) and AUC0→24 in patients with renal impairment were higher than those in patients with normal renal function.

Renal failure is commonly thought to have its sole effect on the renal elimination of drugs. However, in fact, renal failure has a variety of influences on drug kinetics: it reduces nonrenal drug elimination (which includes renal as well as hepatic drug metabolism), and influences protein binding and alters the volume of distribution of some drugs. Renal failure reduces the bioavailability of some drugs and increases that of others. Even for drugs which are not renally eliminated, renal failure can lead to the accumulation of toxic metabolites.

The purpose of this investigation was to determine the effects of experimental renal failure on the pharmacokinetics of losartan in rats.

MATERIALS AND METHODS

Materials Losartan and EXP3174 were gifts from Merck Research Laboratories (Rahway, NJ, U.S.A.), β-Naphthoxyacetic acid (β-NAA) was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). NADPH was purchased from Oriental Yeast Co. Ltd. (Osaka, Japan). Indoxyl sulfate (IS), indole acetate (IA), β-aminoisobutyric acid (BAIBA), creatinine hydrochloride (Cr), cystathionine (Cst) and guanidinosuccinic acid (GSA) were purchased from Sigma Co. (St Louis, MO, U.S.A.). Hippuric acid sodium (HA), methylguanidine hydrochloride (MG) and L-tryptophan (Trp) were obtained from Nakalai Tesque Inc. (Kyoto, Japan). All other chemicals were of reagent grade.

Animals Male Wistar rats (8 weeks) weighting 250—302 g, were purchased from SLC Japan (Hamamatsu). Before the experiments, the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. The animal experiments were performed in accordance with The Guidelines for Animal Experiments of Tokyo Medical and Dental University.

Induction of Acute Renal Failure To produce experimental acute renal failure (ARF), rats received a subcutaneous injection of uranyl nitrate, 10 mg/kg as a 1% solution in normal saline, about 72 h before the experiment. Control animals received an injection of normal saline. ARF was produced by bilateral ligation of ureters (two tight ligatures

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around each ureter and the ureters cut between the ligatures) about 24 h before the experiment. Sham-operated animals served as controls.

**In Vivo Studies. Oral Administration** About 24 h before the experiments, under light ether anesthesia, the carotid artery was cannulated with polyethylene tubing (PE50, Becton Dickinson and Company) which passed subcutaneously to the dorsal side of the neck and then rats were fasted with free access to water. Losartan was dissolved in saline and administered orally by means of a feeding tube at a dose of 10 mg/kg to rats. Blood samples were collected via the carotid artery at 0 (to serve as a control), 1, 2, 3, 4, 6, 8, 10, 22 and 24 h after oral administration of losartan. Blood samples were centrifuged and an aliquot (0.1 ml) of the serum was stored at −20 °C until HPLC analysis of losartan and EXP3174.

**Intravenous Administration** About 24 h before the experiments, under light ether anesthesia, the carotid artery and the jugular vein were cannulated with PE50 and both cannulae were passed subcutaneously to the dorsal side of the neck and then rats were fasted with free access to water. Losartan, 2 mg/kg, was given by intravenous injection via the jugular vein. Blood samples were collected via the carotid artery at 0 (to serve as a control), 2, 5, 15 and 30 min and at 1, 2, 4, 6, 8 and 10 h after intravenous administration of losartan.

**In Vitro Studies. Preparation of Hepatic Microsomes** The rats were fasted overnight before the experiment. The hepatic microsomes were prepared by the methods of Omura and Sato\(^{1}\) and suspended in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA to yield the final protein concentration of 2.56—3.56 mg/ml in the incubation medium.

**Metabolism of Losartan in Rat Hepatic Microsomes** The metabolism of losartan in rat hepatic microsomes was evaluated by the elimination rate of losartan or the formation rate of EXP3174 under the following conditions.

1. **Effect of ARF in Rat Hepatic Microsomes**: 0.5 ml of microsome suspension was preincubated with 0.25 ml of NADPH solution (final concentration 1 mg/ml) for 5 min at 37 °C. The reaction was initiated by adding 0.25 ml of losartan solution (final concentration 1.25—50 μM) and incubated for 5 min at 37 °C. The Michaelis–Menten constant (\(K_m\)) and the maximum velocity of metabolism (\(V_{max}\)) for the formation rate of EXP3174 from losartan were calculated by using nonlinear least-squares methods.\(^{2}\)

2. **Effect of Uremic Serum on the Metabolism of Losartan**: 0.5 ml of microsome suspension was preincubated with 0.3 ml of NADPH solution (final concentration 1 mg/ml) and 0.1 ml of rat serum obtained from ARF or control animals 5 min at 37 °C before starting the metabolic reaction. The reaction was initiated by adding 0.1 ml of losartan solution (final concentration 10 or 25 μM) and incubated for 10 min at 37 °C.

3. **Effects of Uremic Toxins on the Metabolism of Losartan**: To determine the effects of uremic toxins on the metabolism of losartan, 0.5 ml of microsome suspension was preincubated with 0.3 ml of NADPH solution (final concentration 1 mg/ml) and 0.1 ml of uremic toxin solution (final concentration 250 or 1000 μM) for 5 min at 37 °C. The reaction was initiated by adding 0.1 ml of losartan solution (final concentration 10 μM) and terminated by adding 125 μl of ice-cold 1.0 M phosphoric acid and 7 ml of tert-butylmethylether (TBME).

The unbound fraction (\(f_u\)) of losartan in the incubation medium was measured by the ultrafiltration method with a Visking cellulose tube. In brief, 0.8 ml of 100 mM phosphate buffer (pH 7.4), 0.1 ml of rat serum (from ARF or controls) and 0.1 ml of losartan solution (final concentration 10 or 25 μM) were incubated at 37 °C. After 10 min, the incubation mixture was transferred into a Visking cellulose tube and centrifuged for 40 min at 3500 rpm to obtain the ultrafiltrate. The unbound clearance (\(CL_{un}\)) of losartan was calculated according to the following equation:

\[
CL_{un} = \frac{v(f_u \cdot S)}{S}
\]

where \(v\) is the elimination rate of losartan, \(f_u\) is the unbound fraction of losartan and \(S\) is the substrate concentration, respectively.

The unbound clearance associated with the formation of EXP3174 (\(CL_{un, m(EXP)}\)) was calculated by the following equation:

\[
CL_{un, m(EXP)} = \frac{V_{m(EXP)}}{f_u \cdot S}
\]

where \(V_{m(EXP)}\) is the formation rate of EXP3174.

**Assay Methods** The concentrations of losartan and EXP3174 were determined by a modification of the reported HPLC method.\(^{16}\) To 0.1 ml of serum or 1.0 ml of reaction mixture with microsomes, 125 μl of 1.0 M phosphoric acid were added. β-NAA (as internal standard), 25 μl (2 μg/ml) were added to each and the analytes were extracted with 7 ml of TBME by shaking for 5 min. The samples were centrifuged for 5 min at 3000 rpm. The organic layer, containing the analytes, was decanted to clean tubes containing 200 μl of 0.05 N NaOH and shaken for 5 min. The samples were centrifuged for 5 min at 3000 rpm. The organic layer was discarded. The aqueous layer was acidified with 75 μl of pH 4.3, 0.5 M citrate buffer and washed by adding 6 ml of hexane and mixing for 5 min. After centrifuging the samples, the hexane was discarded. To improve the solubility, isopropanol was added to the aqueous layer. A 50 μl aliquot out of the total volume was then injected onto the HPLC for analysis. The chromatographic system consisted of a system controller SCL-10A, an autosampler SIL-10A, a column oven CTO-10A, a pump LC-10AD, a spectrofluorometric detector RF-550A and a UV spectrophotometric detector SPD-10A (Shimadzu, Kyoto, Japan). The column was a 5 μm Hiber LiChroCART CN 250×4 mm i.d. (Cica-MERCK, Tokyo, Japan) and was maintained at 35 °C. The mobile phase was phosphoric acid (pH 2.3; 0.015 M)–acetonitrile (80 : 20, v/v). The flow rate was set to 1.0 ml/min and the column effluent was monitored by fluorescence and ultraviolet at excitation, emission and UV wavelength of 250, 370 and 254 nm, respectively.

The serum concentration of creatinine was measured using the Jaffé method with kits obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Microsomal protein concentrations were determined by the methods of Lowry et al.\(^{17}\) using bovine serum albumin as standard protein.

**Determination of Kinetic Parameters** The following pharmacokinetic parameters were determined for both losartan and EXP3174 from serum concentrations after administration of losartan. The elimination rate constant was esti-
mated by regression of the terminal log-linear serum concentration time points, as $t_{1/2} = \ln 2/\beta$. The area under the serum concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was carried out in two steps ($AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty}$). The area under the serum concentration–time curve from zero to the last time point ($AUC_{0-t}$) was calculated by means of the linear trapezoidal rule and the last time point to infinity ($AUC_{t-\infty}$) was estimated as follows: $AUC_{t-\infty} = C_p/\beta$, where $C_p$ is the last measured serum concentration. Both the time to maximum serum concentration ($T_{max}$) and the maximum serum concentration ($C_{max}$) were determined directly from the concentration–time curves.

**Statistical Analysis** The statistical analysis was performed by using the unpaired $t$-test. Data are reported as the mean±S.D.

**RESULTS**

**In Vivo Studies** The serum concentration–time profiles of losartan and EXP3174 after oral administration of losartan at a dose of 10 mg/kg to rats are shown in Fig. 1 and the pharmacokinetic parameters are listed in Table 1. The induction of ARF by the s.c. injection of uranyl nitrate was confirmed by the elevated concentration of serum creatinine. The $C_{max}$ and $AUC$ of losartan were significantly higher in uranyl nitrate-treated rats than in control rats. On the other hand, the $C_{max}$ and $AUC$ of EXP3174 were significantly lower in uranyl nitrate-treated rats than in control rats.

The serum concentration–time profiles of losartan and EXP3174 after intravenous administration of losartan at a dose of 2 mg/kg to rats are shown in Fig. 2 and the pharmacokinetic parameters are listed in Table 2. Uranyl nitrate-treated rats showed slower elimination of losartan from serum compared to controls and the total body clearance ($CL_{un}$) of losartan was significantly lower in uranyl nitrate-treated rats than in control rats. No significant difference was found in the $AUC$ of EXP3174 between two groups of rats.

To avoid misleading results referable to one particular type of experimental model of renal failure, the surgically produced model of ARF was employed. About 24 h after bilateral ureteral ligation, the rats showed elevated concentration of serum creatinine (Table 3). The serum concentration–time profiles of losartan and EXP3174 after intravenous administration of losartan at a dose of 2 mg/kg to rats are shown in Fig. 3 and the pharmacokinetic parameters are listed in Table 3. The rats with ligated ureters had slower elimination of losartan from serum compared to corresponding control animals and the $CL_{tot}$ of losartan was significantly lower in ureteral-ligated rats than in control rats.

**In Vitro Studies** In order to investigate the mechanisms of reduced clearance of losartan associated with renal failure, a microsome fraction was prepared from rat liver with normal and impaired renal function.

No significant difference was found in $K_m$ ($26.7 \pm 2.1$ vs. $28.3 \pm 5.7 \mu M$) and $V_{max}$ ($87.4 \pm 3.8$ vs. $68.5 \pm 7.5$ pmol/min/mg protein) between uranyl nitrate-treated rats and control rats. Furthermore, ureter-ligated rats and corresponding control rats showed similar values of $K_m$ ($23.4 \pm 4.6$ vs. $28.3 \pm 2.8 \mu M$) and $V_{max}$ ($46.5 \pm 4.8$ vs. $48.8 \pm 2.7$ pmol/min/mg protein). Thus, the affinities and activities of hepatic microsomes were not affected by experimental renal failure.

The effect of uremic serum on the metabolic activity of hepatic microsomes was examined by adding the rat serum to the incubation medium and evaluated in terms of $CL_{un}$ and $CL_{un, m(EXP)}$ of losartan. As shown in Table 4, $CL_{un}$ and...
of losartan in the presence of uremic serum obtained from uranyl nitrate-treated rats or ureteral ligated rats were significantly lower than those in the presence of corresponding control serum, respectively. These results suggest that uremic serum contains some compounds which inhibit the oxidative metabolism of losartan by rat hepatic microsomes.

The effects of some known uremic toxins on the metabolic activity of rat hepatic microsomes were investigated at the concentration of 1 mM. As shown in Fig. 4, IS showed the
The concentration of losartan was 10 μM. Results are reported as the mean ± S.D. (n = 3).

Fig. 4. Effects of Uremic Toxins on the Formation of EXP3174 in Rat Hepatic Microsomes
The concentration of losartan was 10 μM. Results are reported as the mean ± S.D. (n = 3).

Fig. 5. Inhibitory Effect of Indoxyl Sulfate on the Formation of EXP3174 in Rat Hepatic Microsomes Based on a Dixon Plot
The concentrations of losartan were 10 (open symbols) and 25 μM (closed symbols).

most remarkable inhibition of losartan metabolism by rat hepatic microsomes among uremic toxins tested. A Dixon plot, as shown in Fig. 5, indicated that IS inhibits the losartan metabolism in an uncompetitive manner.

DISCUSSION

In healthy male subjects, the average plasma clearance of losartan was 610 ml/min and the renal clearance (70 ml/min) accounted for 12% of plasma clearance. In general, the extent to which decreased renal function influences drug elimination is a function of the percentage of circulating drug cleared through the kidney unchanged and the pharmacologic activity of its metabolites. Therefore, the effect of renal failure on the pharmacokinetics of losartan might be marginal. In fact, Sica et al. investigated the pharmacokinetics of losartan in 18 patients with various degrees of renal function and found that the steady-state AUC of losartan and EXP3174 were not significantly changed with renal impairment in spite of decreased renal clearance of losartan and EXP3174. On the other hand, Tagawa et al. reported higher Cmax and AUC of losartan and slower elimination half-life in patients with hemodialysis than in normal subjects after a single administration of losartan. In addition, Saruta et al. reported higher values of Cmax and AUC in patients with renal impairment than in hypertensive patients with normal renal function after repeated administration of losartan for 7 d. These limited findings in clinical studies warrant the basic animal studies to explore the effect of renal diseases on the pharmacokinetics of losartan.

The pharmacokinetic characteristics of losartan in the rat resembles those reported in man. The urinary excretion of losartan within 24 h after oral administration was 0.3% of dose in male rats, which indicates the small contribution of renal clearance to the total body clearance of losartan in rats. The oral bioavailability of losartan ranged from 31.5 to 38.2% in male rats and 33% in humans. In both man and rats, the active metabolite, EXP3174 was found in serum. These data indicate that the rat is an appropriate animal model for studies relating to losartan pharmacokinetics.

Urinary excretion of EXP3174 after oral administration of losartan was not detected in male rats and was 0.1% of dose in female rats. Biliary excretion of EXP3174 was 17.7% of dose in male rats and 20.9% of dose in female rats, respectively. In incubations with liver slices, three primary routes of losartan metabolism have been identified: 1) oxidation of the alcohol to the carboxylic acid, 2) monohydroxylation of the butyl side chain, and 3) glucuronidation of the tetrazole moiety. Of these, only the first pathway generates a metabolite, the carboxylic acid EXP3174, which accounted for more than 25% of all metabolites in incubations with rat liver slices.

The results of the present investigation show, in two different experimental models of renal failure, that the systemic clearance of losartan is decreased in rats with renal failure. These data support the hypothesis that renal failure can disturb the pharmacokinetics of drugs not only by reducing the renal excretion but also by affecting the hepatic elimination.

Control values of Clun in ureteral ligation study tended to be lower than those in uranyl nitrate study, which
may reflect the effect of laparotomy on the hepatic function.

The effect of uranyl nitrate-induced ARF on the pharmacokinetics of propranolol was investigated in rats and shown to be dependent on the route of drug administration. 

Because the systemic clearance of propranolol is rate-limited by hepatic blood flow, no significant change was found in the serum clearance of the drug after i.v. administration. In contrast, serum propranolol concentration after oral administration was much higher in ARF rats than in controls, indicating the increased availability of propranolol associated with ARF. The observed values of CLun in control animals were much slower than the hepatic blood flow (ca. 50 to 70 ml/min/kg), suggesting that the systemic clearance of losartan is not rate-limited by blood flow to the liver. In fact, ARF-induced elevated concentration of losartan was found after i.v. as well as oral administration of the drug.

The decreased systemic clearance of losartan may reflect the changes in hepatic drug metabolism. In spite of elevated concentration of losartan, serum concentration of the active metabolite, EXP3174, after i.v. administration of losartan in ARF rats was not significantly different from that in controls. Hence, the metabolic ratio (AUC (EXP3174)/AUC (losartan)) in ARF rats was lower than in controls, suggesting the decreased formation rate of metabolites in ARF rats. A similar decrease of the metabolic ratio was also found after oral administration of losartan in ARF rats compared to controls. The T1/2 of losartan after oral administration was significantly longer in ARF rats than in controls. The gastrointestinal disturbances of renal failure such as nausea, vomiting and edematous changes of the gastrointestinal tract may affect the absorption rate of losartan. In Fig. 1, the elimination half-life of EXP3174 was estimated to be 24.4±6.0 h in controls and 28.3±11.5 h in ARF rats, respectively. Though ARF might be associated with the delayed elimination of EXP3174, it was impossible to estimate its elimination half-life precisely under the present experimental conditions.

Two plausible mechanisms for the apparent inhibitory effect of ARF on the hepatic metabolism of losartan could be considered: 1) an intrinsic alteration in the hepatic metabolic processes during renal failure, or 2) the presence of endogenous inhibitors in the uremic blood. In order to investigate the mechanisms of decreased clearance of losartan associated with ARF, a hepatic microsome fraction was prepared from ARF and control rats respectively, and losartan metabolic activity was examined. The apparent Km and Vmax of hepatic microsomes from saline-injected control rats for losartan were 28.3 μM and 68.5 pmol/min/mg protein, respectively. These kinetic parameters are similar to those reported for losartan by human liver microsomes (Km: 20 μM, Vmax: 0.06 nmol/min/mg protein).  

No significant difference was found in the metabolism of losartan by hepatic microsomes prepared from ARF and control rats. These results suggest no apparent alterations in the intrinsic activities of hepatic microsomes associated with ARF.

The effect of endogenous substance(s) in the uremic rat serum was investigated by comparing the microsomal metabolic rate of losartan in the presence of rat serum obtained from ARF and control rats. To correct the differences in serum protein binding of losartan between ARF and control serum, the metabolic activity was expressed as CLun, m(EXP) of losartan. As shown in Table 4, the CLun and the CLun, m(EXP) of losartan by hepatic microsomes were significantly reduced in the presence of uremic serum compared to those in the presence of control serum. By using a rat liver perfusion method, Terao and Shen showed that the hepatic extraction of propranolol was significantly depressed by perfusing with uremic blood, suggesting the presence of a circulating inhibitor(s) in the uremic blood.

A variety of endogenous substances are found to accumulate in uremic blood. These include among others creatinine, urea, indolic derivatives, guanidino derivatives, and amino acids. To investigate the endogenous substances responsible for inhibiting the losartan metabolism, 9 kinds of uremic toxins were selected: indolic derivatives; IS, IA and Trp, guanidino derivatives; MG and GSA, amino acids; Cst and BAIBA, others; Cr and HA. Sakai et al. reported the serum concentration of IS (4.1 vs. 104.3 μM), IA (1.5 vs. 26.3 μM) and HA (13.3 vs. 270.9 μM) in healthy volunteers and predialysis patients with renal insufficiency, respectively.  

The metabolism of losartan by hepatic microsomes was examined in the presence of these uremic toxins at a concentration of 1 mM, which was considered to be high enough to examine the inhibitory effect. Of the 9 kinds of compounds, IS showed the uncompetitive inhibition of losartan metabolism by rat hepatic microsomes.

The results of our present study suggest that the accumulation of circulating uremic toxins such as IS is one plausible explanation of reduced systemic clearance of losartan in ARF rats.

REFERENCES