JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 805–813

Received September 18, 2000
Accepted February 2, 2001
ISSN 0022-3573

Effect of experimental renal dysfunction on bioavailability of ajmaline in rats

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Abstract

The effect of renal dysfunction on the bioavailability of ajmaline has been investigated in rats, where experimental renal dysfunction was induced by subcutaneous injection of uranyl nitrate (10 mg kg^{-1}). Renal dysfunction did not cause any change in the blood ajmaline concentration after intravenous administration (2 mg kg^{-1}), but it increased the blood ajmaline concentration by approximately 2.8-fold after intraduodenal administration (10 mg kg^{-1}). The availability of ajmaline in control rats was 16.7%, whereas the availability was increased to 41.1% in rats with renal dysfunction. The unbound fraction in the blood and the metabolic activity in the liver, was assessed with the 10000-*g* supernatant fraction and with isolated hepatocytes, respectively. The values were found to be similar in both groups. The blood concentration following intraportal infusion was only slightly increased in rats with renal dysfunction, but the hepatic first-pass extraction was infusion rate-dependent and saturable. The initial absorption rate of ajmaline from the small intestine in rats with renal dysfunction was significantly greater compared with control rats. These results indicated that the increased availability of ajmaline in renal dysfunction was mainly a result of partially saturated extraction in the liver, which was caused by an increased absorption rate in the intestine and non-linear extraction in the liver.

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Funding: This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Sciences and Culture of Japan, and by a grant from Japan Research Foundation for Clinical Pharmacology.

Introduction

Renal failure is commonly thought to have its sole effect on the renal elimination of drugs. On the contrary, renal failure has a variety of influences on drug kinetics: it may reduce non-renal elimination, influence protein binding and alter the volume of distribution of some drugs (Gibson 1986). The absolute bioavailability of most drugs in patients with renal failure is unknown, but the first-pass metabolism of drugs such as propranolol (Lowenthal et al 1974; Bianchetti et al 1976), (+)-propoxyphene (Gibson et al 1977) and bufuralol (Balant et al 1980) has been reported to be reduced in patients with renal failure. In addition, we reported that the bioavailability of an immunosuppressive agent, tacrolimus, was increased in rats with experimental renal failure (Okabe et al 2000).

The increased availability of propranolol, which is subjected to an avid first-pass extraction in the liver, has been investigated in rats with experimental renal failure, following single oral administration (Terao & Shen 1983; Katayama et al 1984; Laganière & Shen 1987) or during repetitive dosing (Terao & Shen 1984). In spite of the decreased presystemic clearance of propranolol in rats with renal failure, there was no alteration in the oxidative metabolic activity of the rat liver (Hori et al 1985). Decreased hepatic uptake (Hori et al 1985) and reduced hepatic metabolism due to the presence of an inhibitory factor in uraemic blood

(Terao & Shen 1985) have been suggested to be responsible for the decreased presystemic clearance of propranolol.

This investigation was designed to evaluate the bioavailability of a model drug, ajmaline, in rats with nephrotoxic uranyl nitrate-induced acute renal dysfunction. Ajmaline is a *Rauwolfia* alkaloid with class I antiarrhythmic properties (Bojorges et al 1975; Okumura et al 1988; Hashimoto et al 1989; Padrini et al 1993), and undergoes presystemic clearance after oral administration (Hori et al 1984; Yamada et al 1986). We examined the mechanisms responsible for the altered presystemic clearance of ajmaline in renal dysfunction.

Materials and Methods

Materials

Ajmaline was obtained from Nippon Chemiphar (Gilurytmal, 25 mg mL^{-1}; Tokyo, Japan). Collagenase type I (from *Clostridium histriticum*, EC 3.4.24.3) was purchased from Sigma (St Louis, MO). Analytical reagents for a JEOR Automated Assay System were obtained from Iatoron (Tokyo, Japan). All other chemicals were of the finest grade available.

Animals

Male Wistar rats (310–390 g) were used in this study. The experiments were performed in accordance with institutional guidelines for animal experiments complying with governmental ethical regulations.

Induction of renal dysfunction in rats

Renal dysfunction was induced by subcutaneous injection of uranyl nitrate (10 mg kg^{-1}) (Katayama et al 1984). Control animals received vehicle (isotonic saline, 1 mL kg^{-1}) alone. The experiments were performed three days after the uranyl nitrate injection. To characterize renal dysfunction, the plasma urea nitrogen and creatinine concentrations were determined (Katayama et al 1984).

Bioavailability of ajmaline

The rats were anaesthetized with 35–50 mg kg^{-1} sodium pentobarbital. The femoral artery was cannulated for blood sampling and for measuring systemic arterial blood pressure by means of a capacitance transducer (Toyo-Baldwin MPU-0.5–290–0-III, Tokyo, Japan). Arterial blood pressure and heart rate were monitored by use of a polygraph (San-ei Model 366, Tokyo, Japan). Body temperature was maintained with appropriate heating lamps. For the intravenous (i.v.) study, ajmaline (2 mg kg^{-1}) was administered into the femoral vein at 1 mL kg^{-1} as an isotonic saline solution. Blood samples (0.15 mL) were collected in heparinized glass tubes at 2, 5, 10, 30, 60, 90, and 120 min after the administration. For the intraduodenal study, a 3-cm middle incision was made in the abdomen and 10 mg kg^{-1} ajmaline (1 mL kg^{-1}) was injected into the lumen of the duodenum. Blood samples (0.15 mL) were withdrawn at 3, 8, 15, 30, 60, 90, and 120 min after the injection.

Metabolic activity of hepatic 10000 g *supernatant fraction*

Under ether anaesthesia, the livers of rats were perfused with ice-cold saline via the portal cannula for 15 min. The livers were excised, and homogenized with 4 vols 0.15 M KCl solution by a Potter glass homogenizer. The homogenate was then centrifuged (10 000 *g*) at 4°C for 15 min. The 10000-*g* supernatant fraction (0.2 mL, containing approximately 8 mg protein) was pre-incubated with Tris-HCl buffer (0.1 M, pH 7.4, 0.4 mL), isotonic saline (1.0 mL) and a NADPH-generating system (5 mM NADP, 50 mM $MgCl_2$, 50 mM D-glucose-6-phosphate; 0.2 mL) at 37°C for 5 min in the air. The 1-min reaction was initiated by the addition of ajmaline solution (0.2 mL of a 3.26 μg mL^{-1} in isotonic saline). Control tubes contained 0.2 mL distilled water instead of the NADPH-generating system. The extent of ajmaline metabolism was determined by subtracting the experimental reading from the control reading.

Metabolic activity of isolated hepatocytes

The isolated hepatocytes of rats were prepared by the collagenase perfusion method (Eaton & Klaassen 1978), and then the isolated hepatocytes were suspended in 30 mL Tris incubation buffer (in mM: NaCl 131, KCl 5.2, $MgSO_4$ 0.9, $CaCl_2$ 0.12, Na_2HPO_4 3.0, Tris 10; pH 7.4). Cell viability was approximately 85% in both groups of rats as determined by means of the trypan blue exclusion method (Eaton & Klaassen 1978). The cell suspension (0.4 mL, containing approximately 30 mg protein) was pre-incubated with 0.5 mL saline at 37°C for 5 min in the air, and the reaction was started by the addition of ajmaline solution (3.26 μg mL^{-1}, 0.1 mL). No cofactor was added to the reaction mixture, and the reaction time was 30 s. The control experiment was performed at 4°C, and the activity was determined as described above.

Plasma protein binding and erythrocyte partitioning of ajmaline

Rat arterial blood was collected in a heparinized container. A small volume of isotonic ajmaline solution was added to 5 mL blood to obtain a final ajmaline concentration of 1 μg mL^{-1}. After 15-min incubation at 37°C, the haematocrit value was measured by a Kubota Model KH-120 system (Tokyo, Japan). Plasma was separated by centrifugation, and plasma protein binding of ajmaline was determined by ultrafiltration (Yasuhara et al 1987).

Hepatic extraction of ajmaline following intraduodenal and intraportal infusion

Under pentobarbital anaesthesia, the femoral artery was cannulated for blood sampling, and the femoral vein was cannulated for intravenous infusion. A catheter with a 26-gauge needle was carefully inserted into the portal vein or the duodenum, with the use of surgical glue (Morita et al 1986). Ajmaline solution was infused via the catheter at 0.1 mL min^{-1} with a constant rate infusion pump. The administration rate of ajmaline was 0.25 mg min^{-1} kg^{-1} for the intrafemoral dose, 1.0 mg min^{-1} kg^{-1} for the intraduodenal dose, and 0.05 or 0.25 mg min^{-1} kg^{-1} for the intraportal dose. Arterial blood samples (0.25 mL) were obtained for assay of ajmaline at 2, 5, and 10 min after the start of infusion.

Intestinal metabolism of ajmaline

Ajmaline metabolism in the intestinal tissue of normal rats was examined using the everted sac technique described by Hashimoto et al (1998). A 10-cm portion of the upper region of the small intestine was excised, and the everted intestine sac was washed gently in ice-cold phosphate buffer (pH 7.4). The average wet weight of the everted sac was 1.32 ± 0.31 g (mean $\pm$ s.e. of four experiments). One millilitre incubation buffer (100 mM NaH_2PO_4, 50 mM Na_2HPO_4, 0.3% glucose, pH 7.4) was then put into the serosal side, and the everted sac was pre-incubated in 30-mL incubation buffer at 37°C for 5 min. The incubation was started by the addition of ajmaline solution to the incubation buffer. The final concentration of ajmaline was 10 μM (or 3.26 μg mL^{-1}). After a 60-min incubation period, the solution in the serosal and the mucosal sides was collected for the determination of ajmaline concentration. The intestinal tissue was homogenized with 9 vols incubation buffer for assay of ajmaline.

Intestinal absorption of ajmaline

Under pentobarbital anaesthesia, the absorption of ajmaline in rats was determined by the in-situ closed loop method. Ajmaline (10 mg kg^{-1}, 1 mL kg^{-1}) was injected into the closed loop (10 cm) of the upper region of the small intestine. At the end of the specified period, a blood sample (0.25 mL) was obtained and the loop was dissected. The loop was washed with isotonic saline, and the luminal content and washings were combined. The intestinal tissue was homogenized with 9 vols saline for assay of ajmaline.

Analytical method

Ajmaline concentration was determined by HPLC as described by Hashimoto et al (1986). Analysis of plasma urea nitrogen and creatinine was performed by an automated assay system (JEOR JCA-SIM 6R, Tokyo, Japan) with Hyland Control Serum II (Travenol, Bannockburn, IL). Protein concentration was determined with a dye-binding assay (Bradford 1976) by using a protein assay kit (Bio-Rad, Richmond, CA) with bovine gamma globulin as standard.

Pharmacokinetic analysis

The pharmacokinetic analysis was carried out according to the procedure previously reported (Hashimoto et al 1986). Briefly, the disposition of ajmaline after intravenous administration was analysed with the biexponential equation:

$$Cb = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \quad (1)$$

where Cb is the whole blood concentration. The parameters were estimated by the non-linear iterative least-squares method (Yamaoka et al 1981). Total blood clearance (CL), central (V_1), and steady state (Vd_{ss}) distribution volume were calculated as follows:

$$CL = Dose_i/((A/\alpha) + (B/\beta)) \quad (2)$$

$$V_1 = Dose_i/(A + B) \quad (3)$$

$$Vd_{ss} = (CL^2 \cdot ((A/\alpha^2) + (B/\beta^2)))/Dose_i \quad (4)$$

where $Dose_i$ is the intravenous dose (Gibaldi & Perrier 1982). The area under the concentration curve from time zero to infinity following intraduodenal administration (AUC_O) was calculated by the trapezoidal method for the measured values and then extrapolated to infinity using the terminal phase. The mean bioavailability (F) of ajmaline was determined by the equation:

$$F = AUC_O \cdot (CL/Dose_O) \quad (5)$$

where $Dose_O$ is the intraduodenal dose.

Statistics

All values were expressed as mean ± s.e. for n experiments. Statistical significance of difference between mean values was calculated using non-paired *t*-test provided that the variances were similar. If this was not the case, the Mann-Whitney U-test was applied. *P* values less than 0.05 (two-tailed) were considered to be significantly different.

Results

The plasma concentrations of creatinine and urea nitrogen were measured to assess the development of renal dysfunction (Table 1). Plasma creatinine and urea nitrogen concentrations increased approximately 3- to 4-fold after uranyl nitrate injection. The heart rate and mean arterial blood pressure (MABP) were determined before ajmaline administration in the experiments for the bioavailability of the drug (Table 1). The heart rate of rats with renal dysfunction was slightly slower than that of control rats, whereas there was no significant difference in MABP between the two groups.

Table 1 Comparison of biochemical values in plasma and haemodynamics between rats with renal dysfunction and control rats.

Biochemical parameter	Control	Renal dysfunction
Creatinine (mg dL^{-1})	0.68 ± 0.02	2.85 ± 0.18**
Urea nitrogen (mg dL^{-1})	20.6 ± 1.5	70.8 ± 6.7**
Heart rate (beats min^{-1})	356 ± 10	304 ± 13*
Mean arterial blood pressure (mmHg)	120 ± 3	122 ± 2

Values are expressed as mean ± s.e. of five to ten animals. *$P < 0.05$, **$P < 0.01$ compared with the control.

Figure 1 shows the mean blood concentration–time course of ajmaline following intravenous administration in rats with renal dysfunction and control rats. After ajmaline was injected intravenously, the blood concentration declined rapidly in a biexponential manner. No significant difference was observed in the time course of the mean blood concentration between the rats with renal dysfunction and the control rats (Figure 1). Table 2 shows the mean pharmacokinetic parameters derived from the biexponential fitting of whole blood concentration–time data of individual rats. The calculated systemic blood clearance (CL) was very high. The systemic blood clearance, the volume of the central compartment (V_1) and the steady-state volume of distribution (Vd_{ss}) tended to be smaller in the renal dysfunction group, but these changes were not statistically significant. Figure 1 shows the mean blood concentration–time course of ajmaline following intra-

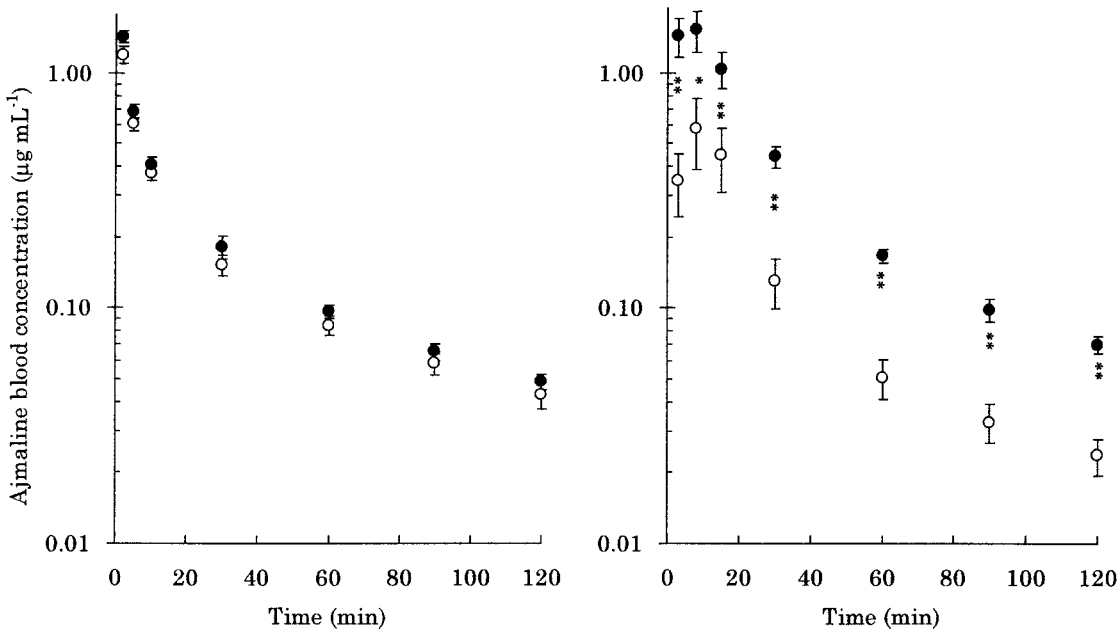

Figure 1 Mean blood concentration–time course of ajmaline after an intravenous dose (2 mg kg^{-1}, left) or an intraduodenal dose (10 mg kg^{-1}, right) to the renal dysfunction group (●) and control group (○). Bars represent ± s.e. of five animals. *$P < 0.05$; **$P < 0.01$ compared with the corresponding value of the control.

Table 2 Pharmacokinetic parameters of ajmaline in rats with renal dysfunction and control rats.

Pharmacokinetic parameters	Control	Renal dysfunction
Intravenous dose		
Systemic clearance (mL min^{-1} kg^{-1})	95.7 ± 8.0	82.5 ± 5.5
Volume of the central compartment (L kg^{-1})	1.13 ± 0.13	0.87 ± 0.05
Vd_{ss} (L kg^{-1})	4.24 ± 0.32	3.56 ± 0.31
Intraduodenal dose		
AUC_O (μg min mL^{-1})	17.5 ± 4.1	49.8 ± 5.3*

Values are expressed as mean ± s.e. of five animals. Vd_{ss}, steady-state apparent volume of distribution. AUC_O, area under the concentration-time curve for infinity. *$P < 0.01$ compared with control.

Table 3 Influence of renal dysfunction on metabolism of ajmaline.

Specimens	Control	Renal dysfunction
Liver weight (g kg^{-1})	38.6 ± 1.1	31.3 ± 1.2*
Metabolic activity (pmol min^{-1} (mg protein)$^{-1}$) 10000-*g* supernatant	103.6 ± 7.7	99.1 ± 15.5
Isolated hepatocytes	24.1 ± 1.0	24.8 ± 2.8

The activity was measured at an ajmaline concentration of 0.326 μg mL^{-1} (1 μM). Values are expressed as mean ± s.e. of four animals. *$P < 0.01$ compared with control.

duodenal administration in rats with renal dysfunction and control rats. The peak blood concentration was reached within 15 min. There was a slight difference in the time course of ajmaline absorption, and the blood concentration ratios between renal dysfunction and control animals were 4.1, 2.6, 2.3 and 3.4 at 3, 8, 15 and 30 min, respectively. The terminal elimination curves for the two groups were nearly parallel (Figure 1). The area under the concentration–time curve (AUC_O) was approximately 2.8-fold higher in rats with renal dysfunction compared with control rats (Table 2). The dose-normalized mean bioavailability (F) was 41.1% and 16.7% in rats with renal dysfunction and control rats, respectively. The in-vivo studies clearly established an increase in the availability of ajmaline after intraduodenal administration in renal dysfunction. Subsequent experiments were designed to elucidate the mechanism of this change.

The intrinsic metabolic activity of the liver was investigated by means of the 10000-*g* supernatant fraction and isolated hepatocytes at the ajmaline concentration of 0.326 μg mL^{-1} (1 μM). Both groups of rats metabolized ajmaline at the same rate, although the mean liver weight of the rats with renal dysfunction was approximately 19% lower compared with the control rats (Table 3). Table 4 shows the plasma unbound fraction (fu) and blood/plasma concentration ratio at the whole blood concentration of 1 μg mL^{-1}. The fu values tended to increase in rats with renal dysfunction, but this change was not statistically significant. The blood/plasma concentration ratio and haematocrit value were nearly identical in the two groups.

Table 4 Plasma unbound fraction, blood/plasma concentration ratio of ajmaline, and haematocrit value in rats with renal dysfunction and control rats.

Binding and partition parameters	Control	Renal dysfunction
Plasma unbound fraction (fu, %)	33.6 ± 1.8	41.6 ± 2.2
Blood/plasma concn ratio (B/P)	1.02 ± 0.05	1.06 ± 0.05
Haematocrit value (%)	42.6 ± 0.4	43.8 ± 0.6

The fu and B/P were measured at the ajmaline blood concentration of 1 μg mL^{-1}. Values are expressed as mean ± s.e. of six animals.

To evaluate the effect of renal dysfunction on the first-pass hepatic extraction of ajmaline, the blood ajmaline concentration was measured following the intravenous and the intraportal infusions. After the intraduodenal infusion the pharmacokinetics were examined in renal rats and control rats. Table 5 shows the ajmaline concentration in the femoral arterial blood determined at 2, 5 and 10 min after starting the drug infusion into the femoral vein, the duodenum, or the portal vein. When ajmaline was infused into the femoral vein, there was no significant difference in the blood concentration of ajmaline between rats with renal dysfunction and control rats. In contrast, intraduodenal infusion of ajmaline produced a significantly higher drug concentration in rats with renal dysfunction compared with control rats. When ajmaline was infused into the portal vein, the blood concentration of ajmaline was only slightly (25 to 43%) higher in rats with renal dysfunction than in control rats, although not significantly. The blood concentration of ajmaline during intraportal infusion at a rate of 0.25 mg min^{-1} kg^{-1} was less than one-half of that during intrafemoral vein infusion at the same rate, and was about 10-fold higher compared with intraportal infusion at 0.05 mg min^{-1} kg^{-1}. The results indicated that ajmaline was extracted by the liver in a non-linear fashion, and that the hepatic extraction of ajmaline at the same drug delivery rate

Table 5 Effect of renal dysfunction on the blood concentration of ajmaline following constant rate infusion.

Route of administration	Infusion rate (mg min^{-1} kg^{-1})	Time (min)	Blood concn (μg mL^{-1})	
			Control	Renal dysfunction
Femoral vein	0.25	2	1.617±0.168	1.711±0.155
		5	1.926±0.199	2.097±0.219
		10	2.231±0.262	2.449±0.244
Duodenum	1.00	2	0.028±0.006	0.112±0.029*
		5	0.133±0.033	0.406±0.083**
		10	0.354±0.049	1.163±0.170**
Portal vein	0.05	2	0.061±0.008	0.081±0.008
		5	0.063±0.010	0.086±0.008
		10	0.072±0.011	0.095±0.011
	0.25	2	0.475±0.018	0.677±0.107
		5	0.682±0.072	0.852±0.139
		10	0.817±0.099	1.076±0.139

The ajmaline concentration in the femoral arterial blood was determined after starting the drug infusion. Values are expressed as mean±s.e. of four to five animals. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 6 Experimental data for the metabolism of ajmaline in the everted sac of small intestine in normal rats.

Sampling site	Ajmaline recovery (% of dose)
Mucosal side solution	92.5±1.10
Serosal side solution	0.65±0.15
Intestinal tissue	8.71±0.85
Total	101.8±1.2[a]

Values are expressed as mean±s.e. of four experiments. [a]The 95% confidence interval for total recovery was 98.0–105.7%.

Table 7 Effect of renal dysfunction on the intestinal absorption of ajmaline.

Measurements at 5 or 20 min	Control	Renal dysfunction
At 5 min		
Absorption from lumen (%)	31.0±2.6	55.1±3.7**
Tissue distribution (%)	16.7±0.9	20.2±0.6*
Net absorption from loop (%)	14.2±2.4	34.9±4.3**
Blood concn (μg mL^{-1})	0.436±0.128	1.956±0.405*
At 20 min		
Absorption from lumen (%)	69.1±8.0	73.5±1.5
Tissue distribution (%)	9.8±1.3	9.0±2.1
Net absorption from loop (%)	59.3±7.0	64.4±2.4
Blood concentration (μg mL^{-1})	0.460±0.089	1.115±0.125**

Intestinal absorption of ajmaline (10 mg kg^{-1}) was assessed by means of an in-situ closed-loop method. Values are expressed as mean±s.e. of four to five animals. *$P < 0.05$, **$P < 0.01$ compared with control.

through the portal vein was not diminished in renal dysfunction rats by as much as was expected from the intraduodenal infusion experiment.

Table 6 shows the experimental data for the intestinal metabolism of ajmaline in the everted sac of normal rats. After a 60-min incubation period, the ajmaline concentration in the incubation buffer (mucosal side) decreased to approximately 92.5% of the initial concentration. The absorbed ajmaline from the mucosal side was completely recovered from the serosal side and intestinal tissue, indicating that ajmaline was not metabolized in the intestine.

Table 7 shows the results of the intestinal absorption experiments. Five minutes after ajmaline injection into the intestinal lumen, the absorption rate from the lumen of rats with renal dysfunction was higher compared with control rats. Approximately 40 to 50% of the ajmaline that had been absorbed from the lumen was found in the intestinal tissue at this time point in both groups of rats. The net absorption of ajmaline from the intestinal loop in rats with renal dysfunction was approximately 2.5-fold higher compared with control rats. In the renal dysfunction group ajmaline blood concentration was approximately 4.5-fold higher compared with the control group. The difference in absorption rate between renal dysfunction and control rats disappeared at 20 min after drug administration. However, the blood con-

centration in renal dysfunction rats was still approximately 2.4-fold higher compared with control rats at 20 min after dosing.

These results indicated that the increased absorption rate followed by the partially saturated hepatic extraction was mainly responsible for the increased availability of ajmaline in the rats with renal dysfunction.

Discussion

This study showed no alteration in either the systemic clearance or the apparent volume of distribution of ajmaline in rats with uranyl nitrate-induced acute renal dysfunction, whereas the bioavailability of ajmaline after intraduodenal administration was markedly increased (Table 2).

After intravenous administration of ajmaline, urinary recovery of unchanged drug was only 5% of the dose in mouse (Iven 1977) and less than 4% in man (Kleinsorge & Gaida 1961; Padrini et al 1993). In our previous study using isolated liver recirculation, we found a rapid disappearance of ajmaline from the perfusate with an elimination half-life of approximately 2 min at the dose of 0.4 mg per liver (Yamada et al 1986). In this study, the in-vivo hepatic extraction of ajmaline was investigated by infusing the drug into the femoral vein and the portal vein (Table 5). The blood concentration of ajmaline during intraportal infusion was less than one-half of that during intrafemoral vein infusion. The apparent hepatic extraction ratio (E_{app}) of ajmaline was calculated by the following equation:

$$E_{app} = 1 - ((Cb_{pv} \cdot Dose_{fv})/(Cb_{fv} \cdot Dose_{pv})) \quad (6)$$

where the subscripts pv and fv represent the drug infusion into the portal vein and femoral vein, respectively. The average E_{app} of ajmaline at the infusion rate of 0.05 mg min^{-1} kg^{-1} was 78.8% and 82.9% in rats with renal dysfunction and control rats, respectively. At the higher infusion rate (0.25 mg min^{-1} kg^{-1}), it decreased to 58.6% and 66.2% in rats with renal dysfunction and control rats, respectively (Table 5). These results indicated that the hepatic extraction of ajmaline was a non-linear process, and that the hepatic extraction was only slightly different between the rats with renal dysfunction and control rats. In addition, ajmaline showed a rapid elimination from the blood after intravenous administration and its systemic clearance exceeded 90 mL min^{-1} kg^{-1} in control rats (Table 2). These findings suggested that the systemic clearance of ajmaline was not restricted by the unbound fraction in the blood or by the metabolic activity in the liver, but was largely dependent on the hepatic blood flow rate (Wilkinson & Shand 1976). Thus, it seemed reasonable to expect little effect of renal dysfunction on the blood concentration of ajmaline after intravenous administration, provided that the splanchnic blood flow was not altered by renal dysfunction (Katayama et al 1984).

The bioavailability of ajmaline after intraduodenal administration in rats with renal dysfunction was increased approximately 2.5-fold compared with control rats (Table 2). The increase could be attributed to either a decrease in hepatic extraction and/or a decrease in the intestinal metabolism (an increase in the extent of intestinal absorption).

The 10000-*g* supernatant fraction of rat liver and the isolated hepatocytes did not show any signs of altered metabolic activity for ajmaline (Table 3). It was found also that there were no significant differences either in the plasma protein binding or in the blood/plasma concentration ratio of ajmaline between the two groups (Table 4). In addition, the hepatic extraction ratio of ajmaline showed no significant difference between rats with renal dysfunction and control rats, as described above (Table 5).

The experiment to investigate intestinal metabolism used ajmaline at a concentration of 10 μM, much less than the expected concentration of 10 mg kg^{-1} used when ajmaline is administered intraduodenally. However, it was shown that ajmaline was not metabolized in the intestinal tissue (Table 6). The extent of intestinal absorption seemed not to be altered by renal dysfunction, since approximately 70% of the dose had disappeared from the intestinal lumen by 20 min. No significant difference was found between the rats with renal dysfunction and control rats in the amount absorbed from the intestinal lumen or in the intestinal tissue content of ajmaline at 20 min (Table 7).

The rate of intestinal absorption is another variable that may be involved in the change in presystemic clearance. As described above, hepatic extraction of ajmaline was non-linear (saturable), being dependent on the drug delivery rate to the liver (Table 5). It follows that the extent of presystemic hepatic clearance could be modulated by variation in the rate of intestinal absorption of ajmaline without an actual change in the intrinsic metabolic activity of the liver. Several researches have investigated the alterations in intestinal function and drug absorption associated with the renal disease. McDermott et al (1971) reported that epithelial cell division was suppressed in the mouse with acute renal failure. Chau et al (1977) suggested that the initial absorption fraction of pindolol increased with decreasing kidney function in patients with chronic renal failure.

Kimura et al (1984) found the absorption rate of sulfanilic acid and the morphological abnormalities of the intestinal mucosa to be increased in rats with renal failure, and suggested that the increased absorption rate of sulfanilic acid was due to the reduced barrier function of the intestinal mucosa. In fact, we found that the initial absorption rate of ajmaline from the small intestine was significantly faster in rats with renal dysfunction than in control rats (Table 7). It is reasonable to expect that the enhanced absorption rate of ajmaline will result in a substantial increase in its bioavailability due to the non-linearity of the hepatic extraction.

Previously we found that the presystemic clearance of orally administered propranolol was decreased in rats with uranyl nitrate-induced renal dysfunction and the AUC_O of propranolol increased approximately 2.5-fold (Katayama 1984). In this study we used the same method to induce renal dysfunction and the biochemical perturbations were similar to those obtained in the previous study (Katayama 1984). As shown in Table 2, AUC_O of ajmaline increased 2.8-fold in renal dysfunction rats. Thus, uranyl nitrate-induced acute renal dysfunction had a pronounced inhibitory effect on the presystemic clearance of ajmaline as well as on propranolol. In addition, we have recently reported that the bioavailability of tacrolimus was significantly increased in rats with cisplatin-induced renal dysfunction (Okabe et al 2000). Combined with the clinical observations in uraemic patients (Lowenthal et al 1974; Gibson et al 1977; Balant et al 1980), these results indicated that renal dysfunction was associated with an increased bioavailability of drugs with high and non-linear hepatic extraction. Additional studies are necessary to delineate the relationship between intestinal absorption rate and hepatic extraction of these drugs in renal dysfunction.

In summary, the bioavailability of ajmaline in rats with renal dysfunction was increased approximately 2.5-times compared with control rats. The most likely cause of this was an increase of the intestinal absorption rate followed by partially saturated hepatic extraction. Our finding indicated that when a drug with a narrow therapeutic index (e.g. tacrolimus) is administered to patients with renal dysfunction, the concentration of drug in the blood should be monitored carefully, even though the drug is metabolized mainly by the liver.

References

Balant, L., Francis, R. J., Tozer, T. N., Marmy, A., Tschopp, J. M., Fabre, J. (1980) Influence of renal failure on the hepatic clearance of bufuralol in man. *J. Pharmacokinet. Biopharm.* **8**: 421–438

Bianchetti, G., Granziani, G., Brancaccio, D., Morganti, A., Leonetti, G., Manfrin, M., Sega, R., Gomeni, R.,. Ponticalli, C., Morselli, P. L. (1976) Pharmacokinetics and effects of propranolol in terminal uraemic patients and in patients undergoing regular dialysis treatment. *Clin. Pharmacokinet.* **1**: 373–384

Bojorges, R., Pastelin, G., Sanchez-Perez, S., Mendez, R., Kabela, E. (1975) The effects of ajmaline in experimental and clinical arrhythmias and their relation to some electrophysiological parameters of the heart. *J. Pharmacol. Exp. Ther.* **193**: 182–193

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254

Chau, N. P., Weiss, Y. A., Safar, M. E., Lavene, D. E., Georgess, D. R., Milliez, P. L. (1977) Pindolol availability in hypertensive patients with normal and impaired renal function. *Clin. Pharmacol. Ther.* **22**: 505–510

Eaton, D. L., Klaassen, C. D. (1978) Carrier-mediated transport of ouabain in isolated hepatocytes. *J. Pharmacol. Exp. Ther.* **205**: 480–488

Gibaldi, M., Perrier, D. (1982) In: Gibaldi, M., Perrier, D. (eds) *Pharmacokinetics*. 2nd edn, Marcel Dekker, New York, pp 45–111

Gibson, T. P. (1986) Influence of renal disease on pharmacokinetics. In: Evans, E. W. E., Schentag, J. J., Jusko, W. J. (eds) *Applied Therapeutics*. 2nd edn. Applied Pharmacokinetics, Washington, pp 83–115

Gibson, T. P., Giacomini, K. M., Briggs, W. A., Whitman, W., Levy, G. (1977) Pharmacokinetics of *d*-propoxyphene in anephric patients. *Clin. Pharmacol. Ther.* **21**: 103

Hashimoto, Y., Hori, R., Okumura, K., Yasuhara, M. (1986) Pharmacokinetics and antiarrhythmic activity of ajmaline in rats subjected to coronary artery occlusion. *Br. J. Pharmacol.* **88**: 71–77

Hashimoto, Y., Yasuhara, M., Kamiya, A., Okumura, K., Hori, R. (1989) Pharmacokinetics and dromotropic activity of ajmaline in rats with hyperthyroidism. *Br. J. Pharmacol.* **96**: 163–169

Hashimoto, Y., Sasa, H., Shimomura, M., Inui K. (1998) Effects of intestinal and hepatic metabolism on the bioavailability of tacrolimus in rats. *Pharm. Res.* **15**: 1609–1613

Hori, R., Okumura, K., Inui, K., Yasuhara, M., Yamada, K., Sakurai, T., Kawai, C. (1984) Quinidine-induced rise in ajmaline plasma concentration. *J. Pharm. Pharmacol.* **36**: 202–204

Hori, R., Okumura, K., Yasuhara, M., Katayama, H. (1985) Reduced hepatic uptake of propranolol in rats with acute renal failure. *Biochem. Pharmacol.* **34**: 2679–2683

Iven, H. (1977) The pharmacokinetics and organ distribution of ajmaline and quinidine in the mouse. *Naunyn Schmiedebergs Arch. Pharmacol.* **298**: 43–50

Katayama, H., Fujiwara, J., Yasuhara, M., Okumura, K., Hori, R. (1984) Increased availability of propranolol in rats with uranyl nitrate-induced acute renal failure. *J. Pharmacobiodyn.* **7**: 536–544

Kimura, T., Ikeda, K., Kobayashi, A., Nakayama, T. (1984) Effect of experimental acute renal failure on intestinal barriers to drug absorption. *Chem. Pharm. Bull.* **32**: 2471–2473

Kleinsorge, H., Gaida, P. (1961) Ausscheidungsmengen und geschminding-keiten des Rauwolfia-Alkaloids Ajmaline nach Verschiedenen Applikationsformen. *Arzneimittelforschung* **11**: 1100–1102

Laganière, S., Shen, D. D. (1987) Altered S-(−)-propranolol disposition in bilateral ureter-ligated rats. *Nephron* **46**: 305–311

Lowenthal, D. T., Briggs, W. A., Gibson, T. P., Nelson, H., Cirksena, W. J. (1974) Pharmacokinetics of oral propranolol in chronic renal disease. *Clin. Pharmacol. Ther.* **16**: 761–769

McDermott, F. T., Nayman, J., De Boer, G. R. R. M., Path, M. C.

(1971) Effect of acute renal failure upon cell division in the jejunum: radioautographic and ultrastructural studies in the mouse. *Ann. Surg*. **174**: 274–282

Morita, K., Sugiyama, Y., Hanano, M. (1986) Pharmacokinetic study of 4-methylumbelliferone in rats: influence of dose on its first-pass hepatic elimination. *J. Pharmacobiodyn*. **9**: 117–124

Okabe, T., Hashimoto, Y., Inui, K. (2000) Pharmacokinetics and bioavailability of tacrolimus in rats with experimental renal dysfunction. *J. Pharm. Pharmacol*. **52**: 1467–1472

Okumura, K., Hashimoto, Y., Yasuhara, M., Hori, R. (1988) Regional myocardial ajmaline concentration and antiarrhythmic activity for ischaemia- and reperfusion-induced arrhythmias in rats. *Br. J. Pharmacol*. **93**: 827–832

Padrini, R., Iovan, D., Javarnaro, A., Cucchini, F., Ferrari, M. (1993) Pharmacokinetics and electrophysiological effects of intravenous ajmaline. *Clin. Pharmacokinet*. **25**: 408–414

Terao, N., Shen, D. D. (1983) Effect of experimental renal failure on the disposition kinetics of *l*-propranolol in rats. *J. Pharmacol. Exp. Ther*. **227**: 295–301

Terao, N., Shen, D. D. (1984) Pharmacokinetics of *l*-propranolol during repetitive dosing in normal and uranyl nitrate-induced renal failure rats. *J. Pharmacokinet. Biopharm*. **12**: 479–493

Terao, N., Shen, D. D. (1985) Reduced extraction of *l*-propranolol by perfused rat liver in the presence of uremic blood. *J. Pharmacol. Exp. Ther*. **233**: 277–284

Wilkinson, G. R., Shand, D. G. (1976) A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther*. **18**: 377–390

Yamada, K., Yatsuzuka, A., Yasuhara, M., Okumura, K., Hori, R., Sakurai, T., Kawai, C. (1986) Mechanisms of pharmacokinetic interaction between ajmaline and quinidine in rats. *J. Pharmacobiodyn*. **9**: 347–351

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobiodyn*. **4**: 879–885

Yasuhara, M., Hashimoto, Y., Okumura, K., Hori, R., Sakurai, T., Kawai, C. (1987) Kinetics of ajmaline disposition and pharmacologic response in beagle dogs. *J. Pharmacokinet. Biopharm*. **16**: 39–55