Transport of Procainamide and *N*-Acetylprocainamide from Blood into the Intestinal Lumen and Intestinal Dialysis by Oral Activated Charcoal in Rats with Acute Renal Failure

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The characteristics of exsorption and/or excretion of procainamide and its metabolite, *N*-acetylprocainamide (NAPA), into the small intestinal lumen in both normal rats and rats with acute renal failure (ARF rats) induced by uranyl nitrate were investigated by an *in situ* single-pass perfusion technique. The exsorption of procainamide and NAPA from blood into the intestinal lumen was increased in ARF rats compared with normal rats. The mean apparent renal, biliary and intestinal clearance values of procainamide were 186, 1.83 and 73.9 ml/h/kg in normal rats, respectively, and were 2.02, 1.37 and 55.8 ml/h/kg in ARF rats respectively. Furthermore, the mean renal, biliary and intestinal clearance values of NAPA were 35.2, 19.4 and 21.1 ml/h/kg in normal rats, respectively, and were 1.12, 21.0 and 26.0 ml/h/kg in ARF rats, respectively. There was little difference in the intestinal clearance values of procainamide and NAPA between normal and ARF rats. The ratio of nonrenal clearance/total body clearance was greater in ARF rats than in normal rats. Treatment with oral activated charcoal reduced the serum NAPA levels in both normal and ARF rats, and had little effect on the serum procainamide levels in normal rats, while it reduced the serum drug levels in ARF rats. Consequently, the increase in both drugs transported into the intestinal lumen induced by renal failure may enhance the intestinal clearance of the drug by oral administration of activated charcoal.

Keywords — procainamide; *N*-acetylprocainamide; renal failure; activated charcoal; intestinal dialysis; gastrointestinal tract; serum concentration; nonrenal clearance; exsorption rate

Introduction

Procainamide is a representative antiarrhythmic drug, which exhibits a marked intersubject variation in the daily dose of the drug necessary to suppress ventricular arrhythmias. Peritoneal dialysis,¹⁾ hemoperfusion,²⁾ hemodialysis³⁾ and combined hemodialysis-hemoperfusion⁴⁾ have been used in severe procainamide or *N*-acetylprocainamide (NAPA) intoxication as methods of hemopurification.

Recently, it has been noted that the clearance of intravenously administered drugs is accelerated by intestinal dialysis which removes the drugs by adsorption of the drugs transported into the gastrointestinal (g.i.) tract onto charcoal.^{5–7)} Procainamide is partly biotransformed in the liver and changed into an active metabolite, NAPA. NAPA has an antiarrhythmic activity comparable to that of procainamide and its biological half-life is about two times longer than that of the parent drug in humans.⁸⁾ Further-

more, it has been known that the higher serum levels of procainamide and NAPA were observed in patients with renal insufficiency since renal excretion is the major route of elimination of both drugs.⁴⁾ Accordingly, in patients with a disease such as renal failure, toxicity resulting from an overdose of procainamide may lead to life-threatening consequences because of the poor endogeneous clearance of this drug. It is quite interesting to investigate the exsorption and/or excretion of the drug into the g.i. lumen in disease states in view of the application of intestinal dialysis.

We have previously reported that intravenously administered theophylline,⁹⁾ phenobarbital¹⁰⁾ and phenytoin¹¹⁾ were transported into the small intestinal lumen (exsorption) to a significant extent and into the bile (biliary excretion) to a lesser extent in rats and that the drugs can be removed by adsorption onto orally administered activated charcoal.¹²⁾

The present study was designed to investigate

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the characteristics of exsorption and/or excretion of procainamide and NAPA from blood into the small intestinal and the biliary tracts and to evaluate the effect of oral activated charcoal on the clearance of both drugs in rats with acute renal failure which may decrease systemic clearance.

Materials and Methods

Materials — Procainamide and NAPA were products of Sigma Chem. Co., St. Louis, Mo., U.S.A. Amisalin® injection (Daiichi Seiyaku Co., Tokyo, Japan) was used for intravenous injection of procainamide hydrochloride. Activated charcoal was a product of Inuhinode Seiyaku Co., Osaka, Japan and the particle size used in this study was less than 62 μm (250 mesh). All other chemicals used were of analytical grade.

Animals — Wistar strain male rats, weighing 290–440 g, were used in the present studies. Acute renal failure (ARF) was induced by subcutaneous injection of uranyl nitrate (10 mg/kg) in physiologic saline solution. The pharmacokinetic experiment using rats with ARF was started 48 h after the treatment. The development of renal dysfunction was monitored by measurement of total protein, albumin, blood urea nitrogen and serum creatinine concentration after the termination of the study by RABA Super Kit® (Chugai Pharmaceutical Co., Tokyo). The biochemical data obtained in normal and ARF rats are shown in Table I.

Exsorption Study — Intestinal exsorption experiments were performed by *in situ* single-pass perfusion technique reported previously.⁹⁾ The rats were anesthetized by an intraperitoneal injection of ethyl carbamate (1.2 g/kg). The

small intestine was exposed by a midline abdominal incision. The upper duodenum and the ileocecal junction were cannulated with polyethylene tubing and the entire small intestine was washed with saline maintained at 37 °C. Isotonic 0.1 M phosphate buffered solution, pH 6.0, maintained at 37 °C, was used for perfusion at a rate of approximately 1.3 ml/min from the duodenum through the small intestine to the ileocecal junction using a perfusion pump (GM-24, Tokyo Rikakikai, Tokyo). Procainamide solution (10 mg/ml) was injected at a dose of 10 mg/kg over about 1 min into the right femoral vein. After injection, perfusates were collected every 15 min from the ileal outflow into a 25 ml volumetric flask and diluted with the buffered solution to 25 ml before assay. Exsorption rate was calculated as the amount exsorbed into the intestinal lumen per min. The bile was collected during the experimental period from the cannula introduced into the common bile duct. Blood samples for the determination of serum drug concentrations were taken from the cannula introduced into the left femoral artery at the mid-time of the perfusate collection period (7.5, 22.5, 37.5, 52.5, 67.5, 82.5, 97.5, and 112.5 min). At the end of all experiments, 2 h after administration of drug solution, urine was collected from the urinary bladder with a syringe.

In Vivo Study — Rats were fasted overnight with free access to water. Procainamide solution (10 mg/ml) was administered intravenously at a dose of 50 mg/kg *via* the caudal vein. In the case of treatment with activated charcoal, the activated charcoal suspended in water (150 mg/ml) was administered orally with a catheter at an initial dose of 300 mg (2 ml) at time zero and additional doses of 150 mg (1 ml)

TABLE I. Biochemical Data in Normal and ARF Rats

		Normal rats	ARF rats
Total protein	(g/dl)	6.46 $\pm$ 0.16	6.20 $\pm$ 0.14
Albumin	(g/dl)	3.61 $\pm$ 0.23	3.43 $\pm$ 0.13
Serum Cr.	(mg/dl)	1.08 $\pm$ 0.026	4.14 $\pm$ 0.46 ^{b)}
BUN	(mg/dl)	24.8 $\pm$ 1.62	61.9 $\pm$ 3.98 ^{b)}

Each value represents the mean $\pm$ S.E.M. of 4 rats. ^{b)} $p < 0.01$.

at 1, 2, 3, and 4 h after the intravenous (i. v.) administration of procainamide. In the case of the controls, water corresponding to the volume used for the activated charcoal suspension was administered orally at each dosing time.

Analytical Method — Procainamide and NAPA in the serum and the bile juice were determined by a homogeneous immunoassay technique (Ames TDA, Miles Sankyo Co., Tokyo) after adding drug-free human serum. This assay showed a coefficient of variation less than 5% within the range of 1–16 $\mu\text{g/ml}$ and 1–20 $\mu\text{g/ml}$ for procainamide and NAPA, respectively. Concentrations above or less than the range were also assayed within the range of calibration by changing the dilution ratio with the buffered solution of the kit. The drugs in the perfusate and the urine were determined by high-pressure liquid chromatography (HPLC) with metoclopramide hydrochloride as an internal standard. A 2 ml portion of the perfusate or 0.1 ml portion of the urine was extracted with 5 ml ethyl ether containing the internal standard after addition of 1 ml of 1 M NaOH. The mixture was shaken for 15 min and then centrifuged for 10 min. The organic layer was transferred to a clean tube and evaporated under reduced pressure at room temperature. After evaporation, 100 μl of acetonitrile were added to the residue, mixed on a vortex mixer and 20 μl were injected into an HPLC. Separation was carried out using 5 μm in a 250 mm $\times$ 4.6 mm octadecylsilane column (TSK-GEL ODS-80TM, Tosoh, Japan). The mobile phase consisted of acetonitrile and 0.03 M sodium acetate buffer, pH 7.4 (75:25). At a flow rate of 1.0 ml/min, the eluate was monitored with an ultraviolet (UV)-detector for absorbance at 290 nm.

Pharmacokinetic Analysis — The disappearance of procainamide from serum followed a one compartment model. The elimination rate constant (k_{el}) was determined by a linear regression analysis of the elimination curve of serum levels. The serum half-life ($t_{1/2}$) was calculated from the relationship $t_{1/2} = 0.693/k_{\text{el}}$. An intercept of ordinate at time zero was used to estimate the initial serum concentration (C_0) of procainamide. The apparent volume of distribution ($V_d = \text{dose}/C_0$) and the total body clearance (Cl_{tot}

$= k_{\text{el}} \cdot V_d$) were also calculated. The area under the serum concentration–time curve (AUC) of procainamide and NAPA from time 0 to 4 and 0 to 6 h, respectively, were calculated by the trapezoidal rule. The unpaired t -test was used to assess the effect of charcoal treatment on pharmacokinetic parameters. The apparent renal, intestinal and biliary clearance values of procainamide and NAPA were calculated by dividing the overall amount of the drugs excreted into the urine, the perfusate or the bile in 2 h by the appropriate AUC value of both drugs obtained over the same period of time.

Results

Transport of Procainamide and NAPA into the Intestinal, Biliary and Urinary Tract

The concentrations of procainamide in the serum and the bile and the exsorption rate of the drug from blood into the perfusate across the small intestinal mucosa following i. v. administration of procainamide to normal and ARF rats were examined by the *in situ* single pass perfusion technique at a dose of 10 mg/kg. Figure 1 shows the results following i. v. administration of procainamide to normal and ARF rats. The serum procainamide levels decreased in a manner obeying first-order kinetics after i. v. administration. The concentrations of procainamide in the serum and bile were significantly higher in ARF rats than those in normal rats. The exsorption rate was decreased as the concentration of the drug in the serum was decreased. Moreover, the exsorption rate of the drug into the perfusate tended to be larger in ARF rats than that in normal rats.

Figure 2 shows the concentrations of NAPA in the serum and the bile, and the exsorption rate of the drug into the perfusate following i.v. administration of procainamide to rats at the dose of 10 mg/kg by the *in situ* single-pass perfusion technique. The concentration of NAPA in the serum became significantly higher in ARF rats with lapses of time. Moreover, the concentration of NAPA in the bile juice was approximately ten-fold higher than that in the serum and the concentration–time profiles of NAPA in serum and bile were very similar. Moreover, the

Fig. 1. The Concentrations of Procainamide in Serum and Bile Juice and the Exsorption Rate of the Drug into the Perfusate at pH 6.0 after i.v. Administration of the Drug at the Dose of 10 mg/kg to Normal (Open Symbols) and ARF (Closed Symbols) Rats during *in Situ* Single-Pass Perfusion

Each value represents the mean $\pm$ S.E.M. of 4 rats. a) $p < 0.05$. b) $p < 0.01$.

Fig. 2. The Concentrations of NAPA in Serum and Bile and the Exsorption Rate of the Drug into the Perfusate at pH 6.0 after i. v. Administration of Procainamide at the Dose of 10 mg/kg to Normal (Open Symbols) and ARF (Closed Symbols) Rats during *in Situ* Single-Pass Perfusion

Each value represents the mean $\pm$ S.E.M. of 4 rats. a) $p < 0.05$. b) $p < 0.01$.

exsorption rate of NAPA into the perfusate increased with and increase in serum level of NAPA. Compared with normal rats, the exsorption rates of NAPA in ARF rats were markedly increased with lapses of time. Thus, it was suggested that NAPA was transported to a notable extent into the intestinal lumen *via* the mucosal membrane or the bile duct.

The percentages of dose of procainamide and

NAPA transported into the urine, intestinal perfusate and bile juice from time 0 to 2 h were then computed. In ARF rats, only a small amount of procainamide was excreted in the urine. The amount of procainamide exsorbed into the intestinal lumen was approximately one-third of that excreted into the urine in 120 min in normal rats (7.63% vs. 20.9% of dose), while in ARF rats the amount of procainamide ex-

sorbed into the intestinal lumen was approximately 28-fold larger than that excreted into the urine (11.0% vs. 0.39% of dose). The amount of procainamide excreted into the bile was less than 0.3% of dose in each treated rat. In addition, the amount of NAPA exsorbed into the intestinal lumen and excreted into the bile was approximately one half of that excreted into the urine in 120 min in normal rats (4.47% and 4.11%, respectively, vs. 7.64% of dose), while in ARF rats the amounts of NAPA exsorbed into the intestinal lumen and excreted into the bile were 24 and 20 fold larger, respectively, than that excreted into the urine (6.85% and 5.70%, respectively, vs. 0.29% of dose).

To express the ability of a system to excrete the drug, the concept of clearance was used. Renal, biliary and intestinal clearance values are defined as volumes of serum from which the drug is removed by excretion into the urine or bile and by exsorption into the intestine in 1 h. The renal clearance values of procainamide and NAPA were markedly reduced in ARF rats, however, there were little differences in the intestinal and biliary clearance values of both

Fig. 3. Renal (□), Intestinal (▨), and Biliary (■) Clearance of Procainamide and NAPA after i. v. Administration of Procainamide at the Dose of 10 mg/kg to Normal and ARF Rats during *in Situ* Single-Pass Perfusion. Each bar represents the mean $\pm$ S.E.M. of 4 rats.

Fig. 4. Serum Procainamide Levels after i. v. Administration of Procainamide at the Dose of 50 mg/kg to Normal and ARF Rats with or without Treatment with Activated Charcoal

Each point represents the mean $\pm$ S.E.M. of 5 rats. a) $p < 0.05$. Key; ○, no charcoal treatment; ●, treatment with multiple doses of charcoal.

drugs between the two groups of rats (Fig. 3). Additionally, the intestinal clearance values of procainamide were about 30 to 40-fold higher than biliary clearance values in both groups of rats. Moreover, in ARF rats, the intestinal clearance value were considerably higher than the renal or biliary clearance values. These results suggested that the intestinal lumen may be considered to be one of the organs of drug secretion; in other words, a route of drug removal unless the drug is reabsorbed.

Effect of Activated Charcoal on the Pharmacokinetic Behavior of Procainamide and NAPA

Figures 4 and 5 show the time course of procainamide and NAPA levels after i. v. administration of procainamide (50 mg/kg) to normal and ARF rats with or without oral activated charcoal. As shown in Fig. 4, treatment with oral activated charcoal had little effect on the serum procainamide levels in normal rats, but tended to reduce the serum drug levels in ARF rats. Serum NAPA concentration in ARF rats following i.v. administration was also higher and remained higher than that in normal rats (Fig. 5). Additionally, treatment with oral activated charcoal significantly reduced the serum NAPA

Fig. 5. Serum NAPA Levels after i.v. Administration of Procainamide at the Dose of 50 mg/kg to Normal and ARF Rats with or without Treatment with Activated Charcoal

Each point represents the mean $\pm$ S.E.M. of 5 rats. a) $p < 0.05$. Key: Δ , no charcoal treatment; $\blacktriangle$, treatment with multiple doses of charcoal.

TABLE II. Pharmacokinetic Parameters of Procainamide and NAPA after i.v. Administration of Procainamide at the Dose of 50 mg/kg to Normal and ARF Rats with or without Treatment with Activated Charcoal

	Procainamide				NAPA
	$t_{1/2}$ (h)	V_d (l/kg)	Cl (ml/h/kg)	AUC_{0-4h} (h $\cdot$ μ g/ml)	AUC_{0-6h} (h $\cdot$ μ g/ml)
Normal rats					
Without charcoal	0.597 ± 0.067	4.66 ± 0.27	5520 ± 650	9.28 ± 0.99	23.9 ± 1.38
With charcoal	0.519 ± 0.033	3.87 ± 0.91	5220 ± 1300	11.6 ± 2.10	21.6 ± 1.29
ARF rats					
Without charcoal	1.07 ± 0.21	2.01 ± 0.14	1410 ± 150	37.6 ± 5.18	92.8 ± 4.01
With charcoal	0.793 ± 0.05	2.25 ± 0.20	$1960^a \pm 180$	25.7 ± 1.06	$76.9^a \pm 2.73$

Each value represents the mean $\pm$ S.E.M. of 5 rats. a) $p < 0.05$, significantly different from the treatment without charcoal in ARF rats.

levels in both normal and ARF rats.

The pharmacokinetic parameters are shown in Table II. There were no significant differences in the parameters of procainamide and NAPA treatments with or without charcoal. On the other hand, the oral administration of activated charcoal tended to decrease the $t_{1/2}$ and the AUC of procainamide to 74% and 68%, respectively and significantly decreased the AUC of NAPA in ARF rats. The total body clearance of procainamide in ARF rats treated with charcoal was increased to 139% as compared with ARF rats not treated with charcoal.

Discussion

Our results suggest that the intestinal lumen exhibits a noticeable exsorption/excretion function for both procainamide and NAPA in the case of renal failure and that the oral administration of activated charcoal should enhance the clearance of procainamide and NAPA in severe procainamide intoxication. It is known that most of drugs are exsorbed into the g. i. lumen according to concentration gradients between blood (the side of higher drug concentration) and g. i. lumen (the side of lower drug concentration) by passive diffusion. Accordingly, the delayed elimination of serum procainamide and NAPA observed in ARF rats (Figs. 1 and 2), which resulted in the higher serum level, may promote exsorption into the g. i. lumen to compensate for their poor renal excretion. Since the total body clearance of the drug is a sum of the clearance through the renal and nonrenal routes, the decreased clearance through the renal route resulted in the decreased total body clearance in ARF rats. As a result, the ratio of nonrenal clearance/total body clearance was greater in ARF rats compared with that in normal rats (Fig. 3). Thus, it is suggested that an appreciable fraction of procainamide and NAPA may be vicariously transported by nonrenal routes, including exsorption and/or excretion, into the g. i. lumen.

Procainamide is partially metabolized to NAPA by the acetylation enzyme, N-acetyltransferase, in the liver and is normally eliminated through renal excretion. In fact, NAPA

tended to accumulate in ARF rats. Therefore, patients with renal failure may tend to develop NAPA intoxication because of a long half-life of NAPA compared with that of procainamide, even if the serum levels of procainamide are within the therapeutic range. The amount of NAPA excreted into the bile in this study was markedly greater than that of procainamide. Accordingly, the excretion of NAPA through the biliary tract may play an important role in the transport of the drug into the g.i. tract as well as in the exsorption of NAPA from blood into the g. i. tract.

Procainamide is a weak base with a pK_a of 9.4. The unionized form of the drug would be expected to permeate freely through capillary walls to the g. i. tract. It has been shown that the saliva to plasma concentration (S/P) ratio for most weakly acidic or basic compounds can be predicted from the pH-partition hypothesis. Watanabe *et al.*¹³⁾ have reported that salivary procainamide and NAPA levels were correlated with the plasma level and the S/P ratio for both drugs were fairly consistent with the calculated value according to the pH-partition hypothesis. Koup *et al.*¹⁴⁾ have also reported that marked intersubject variability was found in the S/P ratio for procainamide (0.27–8.93) and the ratio was increased with decrease of saliva pH. They have discussed the differences among patients with respect to saliva pH as one possible reason for this variation. Accordingly, they suggested that an appreciable amount of the drug may be expected to permeate into the overall digestive organs such as the oral cavity, the esophagus and the stomach according to the extent of ionization by pH-partition hypothesis. Kamath *et al.*¹⁵⁾ have reported that a small portion (3–5%) of the i.v. dose of procainamide and NAPA was excreted in the feces of rats, suggesting a possibility of enterohepatic cycling. Thus, the increase of procainamide and NAPA transported into the intestinal lumen induced by renal failure may enhance the intestinal clearance of the drug by oral administration of activated charcoal.

Consequently, if oral activated charcoal is administered in acute drug poisoning with a high procainamide or NAPA level, it may be expect-

ed that the charcoal will prevent reabsorption of both drugs exsorbed into the g.i. tract by adsorption onto charcoal and promote exsorption due to a greater concentration gradient between blood and the fluid in the g.i. lumen. This so-called g.i. dialysis¹⁶⁾ may play an important role for removal of procainamide and the active metabolite NAPA, from blood.

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