Mechanism of Renal Excretion of Carumonam in Rats, Rabbits, Dogs, and Monkeys

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The mechanism of the renal excretion of carumonam (CRMN) was investigated in rats, rabbits, dogs, and monkeys. Stop-flow analysis in dogs demonstrated that CRMN is exclusively excreted by glomerular filtration. There was no specific CRMN peak corresponding to the peak of p-aminohippuric acid (PAH) secretion or to the trough of Na⁺-K⁺ reabsorption in the stop-flow pattern. Although the PAH peak disappeared when probenecid was administered, the CRMN stop-flow pattern showed no change. In rabbits, however, the CRMN concentration peak corresponding with the PAH peak was detected in the stop-flow pattern; the CRMN peak disappeared when probenecid was administered. The pharmacokinetic parameters in plasma, such as the area under the concentration-time curve, the half-life, and the clearance rate, were affected by probenecid in rats, rabbits, and monkeys, but not in dogs. The results suggest that the renal excretion of CRMN in dogs takes place exclusively through glomerular filtration. In rats, rabbits, and monkeys, however, CRMN is excreted through both glomerular filtration and renal tubular secretion.

Carumonam (CRMN), a new synthetic N-sulfonated monocyclic β-lactam antibiotic, has potent antibacterial activities against gram-negative bacteria (2). In experimental animals (3) and humans (11), CRMN is eliminated predominantly by renal excretion after parenteral administration and is metabolized only to a small extent (3).

In the present study, the mechanism of renal CRMN excretion in dogs and rabbits was examined by renal clearance and stop-flow analysis. The effects of probenecid administration on the plasma pharmacokinetics in rats and monkeys as well as in rabbits and dogs were also examined.

MATERIALS AND METHODS

Antibiotic and chemicals. CRMN was prepared in our research division. Other chemicals were obtained commercially: inulin, mannitol, and creatinine (CR) from Wako Pure Chemical Industries, Ltd.; sodium p-aminohippurate (PAH) and probenecid from Sigma Chemical Co. Ltd.; and sodium pentobarbital (Nembutal) from Abbott Laboratories.

Animals. Five-week-old male Slc:ICR mice weighing 20 to 25 g, 7-week-old male Icl:SD rats weighing 210 to 250 g, 4-month-old male New Zealand White rabbits weighing 2.5 to 3.5 kg, 10-month-old male and female beagle dogs weighing 10 to 12 kg, and male and female cynomolgus monkeys weighing 2.8 to 3.7 kg were used. They were deprived of feed for 16 to 18 h before the antibiotic was administered; water was given ad libitum.

Operative procedures. Animals (dogs and rabbits) were anesthetized with 30 mg of sodium pentobarbital per kg administered intravenously. After the trachea was cannulated, the abdomen was opened with a midline incision and both ureters were cannulated with polyethylene tubing through which urine was collected. The left kidney only was used for occlusion. The left femoral vein and the right femoral artery were catheterized with polyethylene tubing through which the drug solutions were administered and the blood was sampled, respectively.

Renal clearance. (i) Dogs. Three male dogs were used. After the operative procedures, 100 mg of CR per kg was injected via the left femoral vein as a priming dose. Sustaining solution 1 (15% mannitol, 0.9% NaCl, 0.25% CR) was then infused with an infusion pump at 0.3 ml/min per kg. When the urine flow had stabilized, urine was collected during a 10-min interval. After 60 min of infusion, 10 or 20 mg of CRMN per kg was injected intravenously as a priming dose, and then sustaining solution 2 (solution 1 plus 167 µg of CRMN per ml) or 3 (solution 1 plus 333 µg of CRMN per ml) was infused, respectively. Urine samples were collected during three successive 10-min intervals, beginning 30 min after the start of each infusion. Blood samples were taken at the midpoint of the urine collections. After these procedures were completed, a single dose of 30 mg of probenecid per kg was administered intravenously, and urine and blood samples were collected again as described above.

(ii) Rabbits. Four male rabbits were used. The experimental design and procedure were the same as those just described for dogs, except that the concentration of mannitol was 10%. Urine and blood samples were taken as described above beginning 30 min after each infusion of CRMN.

Stop-flow analysis experiments. The method used for stop-flow analysis was essentially the same as that first described by Malvin et al. (4).

(i) Dogs. Three male dogs were used. Priming doses of PAH (20 mg/kg) and CR (100 mg/kg) were administered via the left femoral vein. A sustaining solution (15% mannitol, 0.9% NaCl, 0.1% PAH, 0.25% CR) was then infused at 0.5 ml/min per kg. The priming dose of CRMN was 10 mg/kg, and the sustaining dose was 5 mg/kg per h. About 1 h after the infusion was started, when the urine flow rate had become constant (8 to 10 ml/min per dog), three urine samples were collected at 10-min intervals to determine the free-flow clearance. Blood samples were taken at the same time. The urine flow was then stopped by applying a hemostat clamp to the ureter; the clamp was removed 6 min later. After the clamp was removed, the spurring urine was collected serially in 40 polyacrylic resin tubes (about 0.4 ml per tube). One minute before the clamp was removed, inulin was...
intravenously administered at a dose of 50 mg/kg. About 1 h after the completion of the control experiment, 30 mg of probenecid per kg was administered intravenously, keeping the infusion rate of the sustaining solution at 0.5 ml/min per kg. Thirty minutes later, the experimental maneuvers described above were repeated.

(ii) Rabbits. Three male rabbits were used. Urine samples of about 0.3 ml were collected serially in 30 tubes after 6 min of occlusion; otherwise, the procedure was the same as that outlined above for dogs.

Effect of probenecid on pharmacokinetics of CRMN. CRMN was administered intramuscularly at a dose of 20 mg/kg to rabbits, dogs, and monkeys. Blood samples were taken at designated time intervals. One week later, this procedure was repeated with the same animals, except that 100 mg of probenecid per kg was administered orally 30 min before CRMN was administered. The experiment in rats was performed with different animals killed at various times.

Analysis of plasma and urine specimens. Blood samples were centrifuged at 2,000 × g for 10 min in the cold, and the plasma was separated. The serial urine sample volumes were measured by weighing. Plasma and urine samples were stored at −80°C until assayed.

CRMN in the plasma and urine samples was assayed by the agar well method with *Escherichia coli* NIHJ as the test organism (3). The sensitivity of this method was about 0.1 μg/ml. The precision in terms of variation coefficient of replicate analyses (n = 8) was ±7.0%. In addition, high-pressure liquid chromatography (3) was used to confirm the concentrations of CRMN in samples. The detection limit for CRMN was 1 μg/ml with a precision of <2%. There was good agreement between the results obtained by the bioassay and those obtained by high-pressure liquid chromatography. Plasma and urine samples were analyzed for inulin by the method of Schreiner (8); for CR by the Folin and Wu method (1), using a CR test kit (Wako); for PAH by the method of Waugh and Beall (10); and for sodium and potassium by flame photometry (model 205 photometer; Hitachi, Ltd.).

The degree of protein binding of CRMN in dog and rabbit sera was determined as 10.5% and 21.4%, respectively, in a previous study (3), and these values were adopted for the data analysis of renal clearance.

Pharmacokinetic analysis. Pharmacokinetic evaluation of the plasma concentration-time data was performed by the computer program reported previously (3, 5, 7); the optimum values of parameters for the one- or two-compartment open model were determined by the iterative least-squares method.

The pharmacokinetic parameters for the antibiotic in rats and rabbits were calculated from the mean levels in plasma by the one-compartment open model which satisfactorily fit for their calculations; parameters for the antibiotic in dogs and monkeys were calculated by the two-compartment open model, as elimination-phase curves for these animals did not fit the one-compartment open model.

The area under the plasma concentration-time curve (AUC) was calculated as follows: AUC = dose/(kₑ₋₁ · V) (for one-compartment open model) and AUC = dose/(β · Vₑ) (for two-compartment open model), where kₑ₋₁ is the elimination rate constant; β is the rate constant of the elimination phase, and Vₑ and V₀ are apparent volumes of distribution in the body compartment and at the β phase, respectively.

The AUCs were used to calculate the plasma clearance (CL): CL = kₑ₋₁ · V (for one-compartment open model) and CL = β · Vₐ (for two-compartment open model).

Statistical analysis. Results are expressed as the mean ± standard error. The degree of significance between means was determined by the Student t test.

RESULTS

**Dogs.** The renal clearance of CRMN in dogs is shown in Table 1. The urinary excretion of CRMN was almost equal to the estimated glomerular filtration of CRMN. The ratio of renal clearance of CRMN to that of CR was approximately 1.0 and was not dependent on the concentration in plasma. Probenecid had almost no effect on the renal clearance ratio of CRMN; the clearance ratios were approximately 1.0.

To further evaluate the contribution of the renal tubules in CRMN excretion, we conducted a stop-flow analysis. The location of excretion from the proximal renal tubules and of reabsorption through the distal renal tubules was determined by using PAH, sodium and potassium, respectively, as markers. Inulin was administered as a marker of glomerular urine. The ratio of the CR concentration in urine to that in plasma (U/P CR) was calculated as a parameter of concentrated urine. A typical stop-flow pattern is shown in Fig. 1. The ratio of the urine to plasma concentration (U/P) of each component divided by U/P CR is plotted on the ordinate.

In the stop-flow pattern of CRMN, no specific CRMN peak or trough was found corresponding to the PAH peak or to the sodium and potassium trough area. When probenecid was administered, the stop-flow pattern of CRMN showed no significant change, whereas the PAH peak disappeared.

**Rabbits.** Table 2 shows the renal clearance of CRMN in rabbits. The clearance ratio of CRMN to CR was approximately 1.7 and was not dependent on the concentration in plasma, which ranged from 21 to 55 μg/ml. Probenecid induced a marked reduction in the clearance of CRMN.

A typical stop-flow pattern is shown in Fig. 2. The CRMN peak corresponded with the PAH peak. After probenecid was administered, both the CRMN and PAH peaks disappeared. These results indicate that CRMN is excreted by glomerular filtration (about 60%) and tubular secretion (about 40%) in rabbits (Table 2).

**Effect of probenecid on concentrations of CRMN in plasma.** To investigate further whether probenecid inhibits the renal data were obtained.
excretion of CRMN in the species studied, we analyzed the concentrations in plasma pharmacokinetically. The mean concentrations of CRMN in plasma in the four species both with and without probenecid administration are shown in Fig. 3; the pharmacokinetic parameters (AUC, half-life, and CL) are listed in Table 3. When probenecid was administered, there were no significant changes in the concentration of CRMN in plasma in dogs, whereas that in rats, rabbits, and monkeys increased significantly, as compared with those observed when probenecid was not administered. The biological half-life in rats, rabbits, and monkeys was 0.28, 1.61, and 0.96 h with CRMN alone; probenecid extended the half-life to 0.36, 1.97, and 1.31 h, respectively.

**DISCUSSION**

The results of the present investigations in dogs suggest that the renal excretion of CRMN takes place exclusively through glomerular filtration. The renal clearance of CRMN in dogs was almost equal to the CR clearance (Table 1).

**TABLE 2. Renal excretion of CRMN in rabbits**

<table>
<thead>
<tr>
<th>Infusion rate of CRMN (mg/kg/h)</th>
<th>Conc of CRMN in plasma (µg/ml)</th>
<th>CRMN urinary excretion (µg/min)</th>
<th>CRMN glomerular filtration (µg/min)</th>
<th>CRMN tubular secretion (%)ᵃ</th>
<th>CLCRMN (ml/min)</th>
<th>CLCR (ml/min)</th>
<th>CL ratio (CLCRMN/CLCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>Total: 20.8 ± 2.8</td>
<td>Unbound: 16.4 ± 2.2</td>
<td>211 ± 7.7</td>
<td>33.7 ± 2.7</td>
<td>13.7 ± 2.4</td>
<td>9.0 ± 1.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>6.0</td>
<td>Total: 54.7 ± 9.0</td>
<td>Unbound: 43.0 ± 7.0</td>
<td>599 ± 49.6</td>
<td>315 ± 24.9</td>
<td>47.1 ± 3.0</td>
<td>14.9 ± 2.0</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>6.0 (after probenecid)</td>
<td>Total: 43.1</td>
<td>Unbound: 33.9</td>
<td>250</td>
<td>225</td>
<td>7.4</td>
<td>6.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

ᵃ Values represent means ± standard errors (n = 4).
ᵇ Protein binding of CRMN was 21.4% (2).
ᶜ [(Urineal excretion – glomerular filtration) × 100]/urinary excretion.
ᵈ P < 0.001 versus CL ratio after probenecid.
ᵉ P < 0.05 versus the corresponding CLCR.
ᶠ Mean (n = 2).
FIG. 3. Mean concentrations of CRMN in plasma after a single intramuscular (i.m.) administration of 20 mg/kg in rats, rabbits, and monkeys with (*) or without (○) probenecid. Probenecid (100 mg/kg) was administered orally at 30 min before the administration of CRMN. 

- $b$, $P < 0.1$
- $c$, $P < 0.05$
- $d$, $P < 0.001$

versus the corresponding control.

The stop-flow pattern of CRMN in dogs (Fig. 1), no definite peak of CRMN was found corresponding to the peak of PAH. When probenecid was administered the PAH peak disappeared, but the stop-flow pattern of CRMN showed no change. In addition, there were no significant changes in the pharmacokinetic parameters of CRMN in dogs given probenecid (Table 3; Fig. 3).

These results indicate that the renal excretion of CRMN in
dogs takes place mostly through glomerular filtration and that there is little or no contribution from renal tubular secretion.

In rabbits, however, both glomerular filtration and renal tubular secretion are involved in the excretion of CRMN. The renal clearance ratio of CRMN to CR was about 1.7, indicating that renal tubular secretion plays a part in the excretion process of CRMN (Table 2). In the stop-flow analysis in rabbits, CRMN showed a peak corresponding with the PAH stop-flow pattern, and both the CRMN and PAH peaks disappeared when probenecid was administered. Renal tubular secretion was found to be about 40% of the renal excretion of CRMN. The pharmacokinetic parameters of CRMN in rats, rabbits, and monkeys were significantly changed by probenecid, indicating that renal tubular secretion, in addition to glomerular filtration, contributes to renal excretion of CRMN in these animals (Table 3; Fig. 3).

Recently, Yoshida et al. (12) reported that [14C]CRMN was eliminated by both glomerular filtration (67%) and tubular secretion (33%) in rats. In mice, probenecid caused slight changes in the pharmacokinetic parameters, suggesting that the tubular secretion is also involved, as in rats (unpublished data). In humans, probenecid administered concomitantly to healthy volunteers does not affect the renal clearance or urinary excretion of CRMN (11), indicating that the renal elimination of this antibiotic occurs exclusively by glomerular filtration, as in dogs.

Thus, the mechanisms of renal excretion of CRMN differ with animal species. In humans and dogs, CRMN is excreted mainly through glomerular filtration, and in rats, rabbits, and monkeys, it is excreted through both renal tubular secretion and glomerular filtration.

The renal excretion mechanisms of CRMN in dogs and rabbits are similar to those for ceftriaxone (6) and moxalactam (9). The results of similar studies with aztreonam closely resemble those obtained with CRMN (unpublished data).

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LITERATURE CITED