MECHANISM OF RENAL EXCRETION OF FK027 IN DOGS AND RABBITS

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The mechanism of renal excretion of FK027, a new oral cephalosporin, was investigated in dogs and rabbits. In dogs, FK027 was mainly cleared by glomerular filtration, and approximately 50% of the filtered drug was reabsorbed through the proximal tubules. This tubular reabsorption and a high binding ratio to serum protein lead to the exceptionally long serum half-life of the drug. The facts that the clearance ratio of FK027 declined slightly from 58.0 to 49.2% by the addition of probenecid, and that the effect of probenecid was less marked in the stop-flow study, along with no significant change in serum half-life, may account for the scarcely detectable secretion from the renal tubules.

In rabbits, the addition of probenecid caused a decrease of the clearance ratio of FK027, disappearance of FK027 peak in the stop-flow study, and extended the serum half-life. These facts are evidence that FK027 is excreted by both tubular secretion and glomerular filtration in rabbits.

FK027, a new oral cephalosporin, was synthesized and evaluated in our laboratories. FK027 differs in structure from commercially available products, and its antibacterial activity against the common Gram-negative bacteria is considerably more potent than that of other oral β-lactam antibiotics such as cephalexin, cefaclor and amoxicillin[1]. Serum half-lives of FK027 in rats, dogs and human are longer than those of other oral β-lactam antibiotics[2,3].

In this study, the mechanism of renal excretion of FK027 was examined by renal clearance and stop-flow analysis in dogs and rabbits.

Materials and Methods

Reagents

Inulin (5%), mannitol (10 or 15%), creatinine (10%; Merck & Co.), probenecid (6%, solubilized with NaOH; Sigma Chemical Co.), and sodium *para*-aminohippurate (PAH; 40 or 60% solution; Nakarai Chemicals, Ltd., Kyoto, Japan) were all of special grade.

Operative Procedures

Animals were anesthetized with sodium pentobarbital (30 mg/kg) intravenously (iv). The left ureter was exposed by a flank incision and catheterized with a polyethylene tube. Urine was collected through the cannula.

In dogs, blood was collected from the right femoral vein, and the test compounds and fluid were injected through the left femoral vein. In rabbits, blood was collected from the right femoral vein, and the test compounds and fluid were injected through the ear vein.

Analysis of Urine and Plasma Samples

Urine and plasma samples were analyzed for creatinine by the method of BONSNES and TANSSKEY[4]; for inulin by the method of BROWN and NOLPH[5]; for PAH by the method of SMITH, *et al.*[6]; and for sodium and potassium by atomic absorption analysis.

FK027 in urine and plasma samples in the renal clearance and stop-flow studies in dogs was assayed by high-performance liquid chromatography, performed on a model ALC/GPC 204 equipped with a μBondapak C_{18} column (4.0 mm × 30 cm) (Waters Assoc.). The mobile phase consisted of 22% acetonitrile in 0.01 M KH_2PO_4 - Na_2HPO_4 buffer (pH 7.0) supplemented with 45 mM tetra-*n*-butylammonium hydrogen sulfate as counter ion. The detector monitored absorption at 245 nm. In the other studies, FK027 concentrations were determined by a disc diffusion bioassay using *Escherichia coli* ATCC 39188 as the test organism.

Renal Clearance

Dogs: Three 9.0 to 12.5 kg male beagle dogs were used. After the operation, 40 mg of inulin/kg (50 mg/ml, 0.8 ml/kg) and 20 mg of PAH/kg (40 mg/ml, 0.5 ml/kg) were iv injected *via* the left femoral vein as priming doses. Sustaining solution (mannitol 10%, NaCl 0.9%, inulin 0.12%, PAH 0.17%) was then infused with an infusion pump at the rate of 0.3 ml/kg/minute. After 45 minutes FK027 was given by infusion at progressively increased rates of 0.25, 0.5 and 1.0 mg/kg/hour. Before each infusion, 2.2 mg/kg (20 mg/ml, 0.11 ml/kg), 4.2 mg/kg (20 mg/ml, 0.21 ml/kg) and 8.0 mg/kg (20 mg/ml, 0.4 ml/kg) of FK027 were injected iv. Beginning 45 minutes after the infusion of FK027, urine was collected three times at intervals of 5 minutes. Blood was collected midway during urine collection. This procedure was repeated for each of the three doses. After completing these procedures with all doses of FK027, 30 mg of probenecid/kg (60 mg/ml, 0.5 ml/kg) was iv administered, and urine and blood samples were again collected as described above.

Rabbits: Three 2.7 to 3.0 kg male Japanese white rabbits were used. After the operation, 40 mg of inulin/kg (50 mg/ml, 0.8 ml/kg) was iv injected *via* the ear vein as priming dose. Sustaining solution (5% mannitol, 0.9% NaCl, 0.12% inulin) was then infused with an infusion pump at the rate of 0.3 ml/kg/minute. After 30 minutes FK027 was given by infusion at progressively increased rates of 3.25, 6.5 and 13.0 mg/kg/hour. Before each infusion, 2.9 mg/kg (20 mg/ml, 0.14 ml/kg), 5.8 mg/kg (20 mg/kg, 0.29 ml/kg) and 10.8 mg/kg (20 mg/kg, 0.54 ml/kg) of FK027 were injected iv. Urine and blood sampling, and probenecid inhibition experiment were performed by essentially the same procedure as in dogs.

Stop-flow Method

Dogs: Priming doses of 20 mg of PAH/kg (0.5 ml/kg), 60 mg of creatinine/kg (0.5 ml/kg) were iv administered *via* the left femoral vein. Sustaining solution (10% mannitol, 0.9% NaCl, 0.17% creatinine, 0.05% PAH) was then infused at the rate of 0.5 ml/kg/minute. The priming dose of FK027 was 8.8 mg/kg (0.5 ml/kg) and the sustaining dose was 1.0 mg/kg/hour. About 1 hour after starting the infusion, when the urine flow stabilized at 0.3 to 0.5 ml/kg/minute, control urine samples were collected twice at 3-minute intervals for the determination of free-flow clearance. Blood samples were collected at the same time. The catheter was occluded by clamping with a hemostat for 6 minutes and then released. At the moment of release, 0.5 ml of spurting urine samples was collected in polyacrylic resin tubes, consecutively for 30 tubes. One minute before releasing the clamp, inulin was administered iv in a dose of 50 mg/kg. One hour after completion of the control experiment, 30 mg of probenecid/kg (0.5 ml/kg) was administered iv, and the infusion was continued. After 30 minutes, the experimental maneuvers described above were repeated.

Rabbits: The experiment was performed in the same manner as for dogs. However, the priming dose (5.9 mg/kg) and sustaining dose (6.5 mg/kg/hour) of FK027 were given *via* the ear vein. Urine samples (0.5 ml each) were collected in 20 tubes.

Effect of Probenecid on Serum Concentrations of FK027

FK027 was administered iv at a dose of 20 mg/kg to three male beagle dogs weighing 11.0 to 13.0 kg and five male rabbits weighing 2.5 to 3.7 kg. Venous blood samples were taken at designated time intervals. One week later, the same experimental maneuvers were performed in the same animals, but probenecid (30 mg/kg) was administered iv 20 minutes before the administration of FK027 to dogs, and 20 minutes before and 1.5 hours after the administration of FK027 to rabbits. Probenecid was injected twice in rabbits because the compound is eliminated rapidly in this species. Serum

Table 1. Renal excretion of FK027 in dogs.

Infusion rate of FK027 (μg/kg/hour)	Plasma concentration of FK027 (μg/ml)		Inulin clearance C_{IN} (ml/minute)	Renal plasma flow (ml/minute)	FK027 glomerular filtration (μg/minute)	FK027 urinary excretion (μg/minute)	FK027 clearance, C_{FK027} (ml/minute)		Clearance ratio (%) C_{FK027}/C_{IN}	
	Total	Unbound					Total	Unbound	Total	Unbound
250	20.7±1.87	2.67±0.18	16.6±1.12	87.9±4.66	45.0±4.41	22.9±1.00	1.16±0.08	8.55±0.19	7.80±0.69	53.4±3.65
500	44.0±3.70	7.98±0.31	16.3±0.82	80.4±2.46	130±7.80	73.9±5.15	1.75±0.15	9.29±0.68	11.0±1.08	57.1±2.80
1,000	75.5±6.06	20.2±0.81	14.2±0.54	72.0±2.29	287±10.8	167±11.9	2.28±0.20	8.30±0.68	16.1±1.22	58.0±3.12
After probenecid										
1,000	72.5±6.29	20.2±0.81	13.1±0.36	—	266±11.2	130±5.60	1.87±0.11	6.43±0.19	14.5±1.15	49.2±1.71

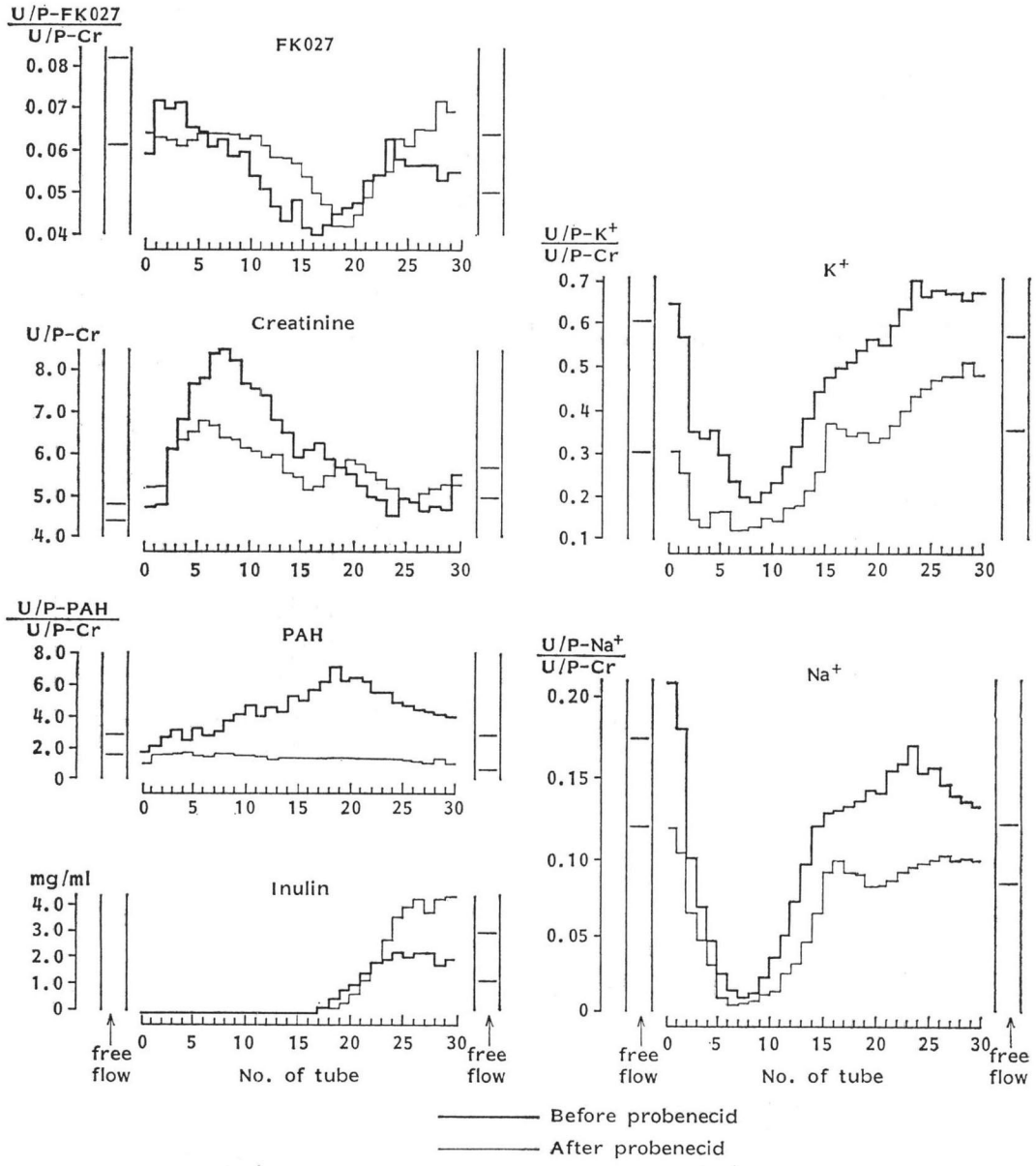
Mean±S.E. (n=3).

Table 2. Renal excretion of FK027 in rabbits.

Infusion rate of FK027 (μg/kg/hour)	Plasma concentration of FK027 (μg/ml)		Inulin clearance C_{IN} (ml/minute)	FK027 glomerular filtration (μg/minute)	FK027 urinary excretion (μg/minute)	FK027 clearance, C_{FK027} (ml/minute)		Clearance ratio (%) C_{FK027}/C_{IN}	
	Total	Unbound				Total	Unbound	Total	Unbound
3,250	15.9±1.49	7.94±0.75	8.81±1.48	64.8±6.87	102±14.4	6.93±1.48	13.9±2.95	79.8±9.11	160±18.2
6,500	33.1±2.63	16.6±1.31	10.6±1.63	166±14.0	248±18.4	7.91±1.14	15.8±2.28	75.3±2.62	151±5.23
13,000	58.5±1.05	29.3±0.53	4.95±0.68	144±19.1	248±24.1	4.24±0.43	8.49±0.85	88.9±5.92	178±11.8
After probenecid									
6,500	68.4±2.41	34.2±1.20	6.28±0.60	214±21.4	214±16.7	3.13±0.23	6.27±0.45	50.6±1.63	101±3.26

Mean±S.E. (n=3).

Fig. 1. Stop-flow pattern in dogs.
FK027 was given in a sustaining dose of 1.0 mg/kg/hour.

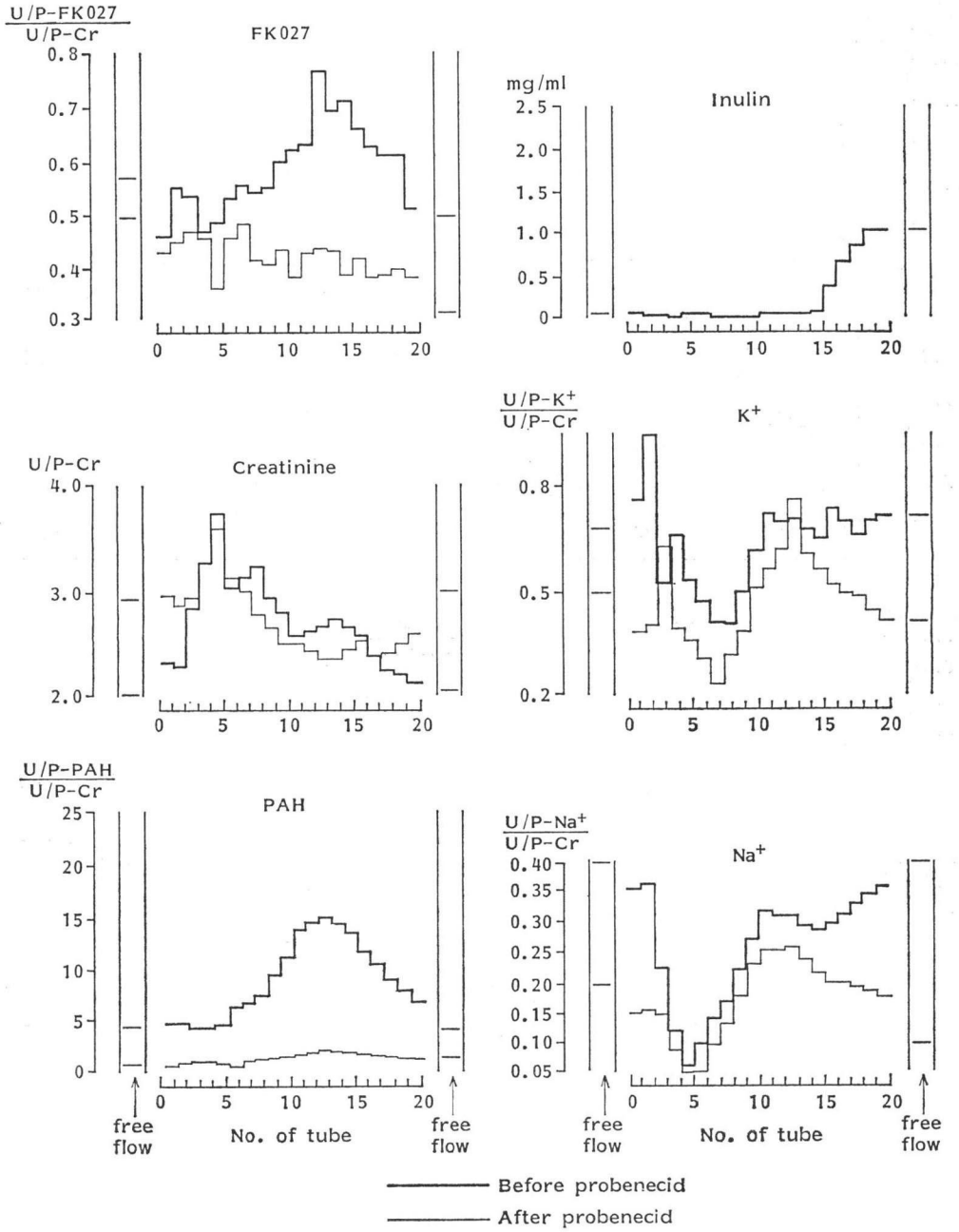


levels of FK027 were bioassayed using standard solution prepared with control serum. The mean serum concentration-time data were analyzed by two-compartment model. The serum level curves of FK027 with and without probenecid were treated statistically by analysis of covariance with ANOVA procedure in SAS (SAS Institute Inc., North Carolina, U.S.A.).

Determination of Free Concentrations of FK027

Protein-bound and unbound FK027 were separated by ultrafiltration. A 15-cm section of Visking tubing (seamless cellulose tubing, 6.4-mm diameter) was filled with 1 to 2 ml of the plasma collected in the clearance and stop-flow studies, put into a polyethylene tube, and centrifuged at 4°C and 1,000 $\times g$ for about 20 minutes. The concentration of FK027 in the ultrafiltrate was determined by bioassay.

Fig. 2. Stop-flow pattern in rabbits.
FK027 was given in a sustaining dose of 6.5 mg/kg/hour.



Results

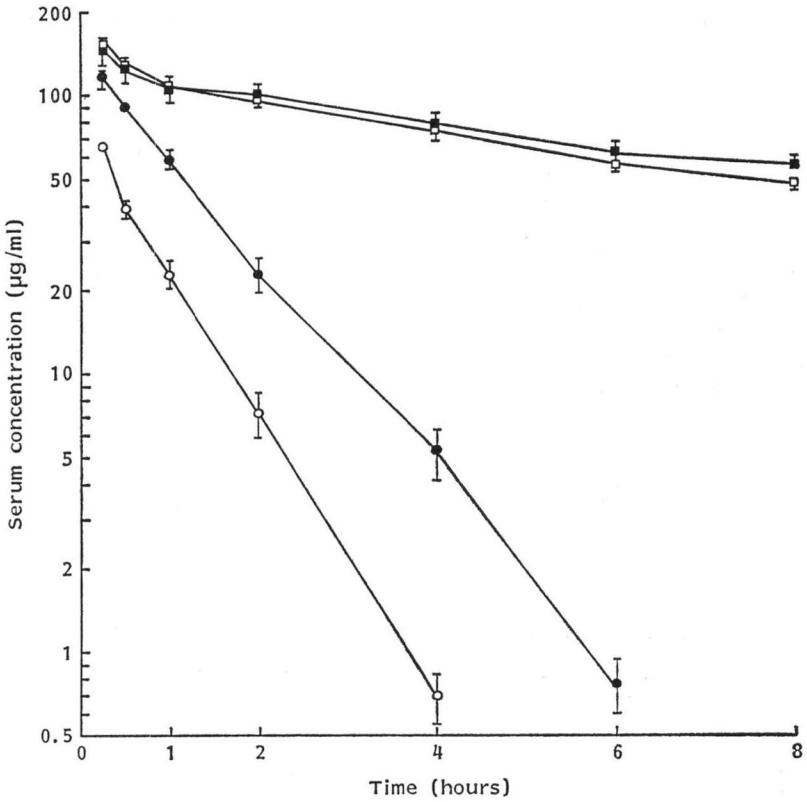
Dogs

The rate of urinary excretion of FK027 (μg/minute) and the estimated amount of FK027 filtered by the glomeruli (μg/minute) calculated from the plasma concentration of unbound FK027 and the

Fig. 3. Mean serum levels of FK027 in dogs and rabbits with or without probenecid.

FK027; 20 mg/kg, iv, probenecid; 30 mg/kg, iv, 20 minutes before FK027 injection to dogs, 20 minutes before and 1.5 hours after FK027 injection to rabbits.

□; Dog (n=3) without probenecid, ■; dog (n=3) with probenecid, ○; rabbit (n=5) without probenecid, ●; rabbit (n=5) with probenecid.



glomerular filtration rate, *i.e.* inulin clearance (C_{IN}) are shown in Table 1. The amount of FK027 excreted in the urine was almost half of the amount estimated by glomerular filtration. The clearance of FK027 unbound ($C_{FK027,unbound}$) was obtained by dividing the urinary excretion of FK027 by the plasma concentration of the unbound FK027 (using the values shown in Table 1). The clearance ratios (%) of $C_{FK027,unbound}$ to C_{IN} (C_{FK027}/C_{IN}) were 53.4, 57.1 and 58.0% for the FK027 infusion rate of 250, 500 and 1,000 μg/kg/hour, respectively.

When FK027 was infused at the rate of 1,000 μg/kg/hour with probenecid (30 mg/kg), the clearance ratio was 49.2%, slightly lower than the value without probenecid *i.e.* 58.0%. To assess the localization of nephron transport of FK027, stop-flow analysis was conducted. The urine to plasma concentration ratio (U/P) for sodium, potassium, PAH and FK027 were corrected for water absorption effects by dividing their observed U/P ratio by the U/P ratio for creatinine (U/P-Cr). The proximal tubular secretion of PAH was determined by a marked increase in U/P-PAH/U/P-Cr ratio, and the distal tubular reabsorption of sodium by a marked reduction in the U/P-Na^+/U/P-Cr ratio. Inulin was administered as a marker of glomerular urine. Fig. 1 shows a typical stop-flow pattern.

In the stop-flow pattern of FK027, a specific trough was observed corresponding to the PAH peak. This trough indicated that reabsorption was occurring in the proximal tubules. Probenecid,

Table 3. Pharmacokinetic parameters of FK027 in dogs and rabbits after a single iv injection of 20 mg/kg with or without probenecid.

Parameter	Dog		Rabbit	
	Without probenecid	With probenecid	Without probenecid	With probenecid
α (hour^{-1})	2.83	5.65	1.22	1.03
β (hour^{-1})	0.113	0.11	1.16	0.86
$t_{1/2}$-β (hour)	6.13	6.30	0.60	0.81
Ke (hour^{-1})	0.179	0.204	1.17	0.87
C_0 (μg/ml)	191	228	73.6	143
AUC (μg·hour/ml)	1,060	1,120	63.2	165
Vc (ml/kg)	105	87.7	272	140

The mean serum concentration-time data were analyzed by a two compartment open model; $C=Ae^{-\alpha t}+Be^{-\beta t}$, where C is the serum concentration: α and β are the first-order rate constants in the distribution and elimination phase, respectively. A and B are the coefficients of exponential term α and β, respectively ($t_{1/2}$-β; biological half-life).

Ke; The first-order rate constant of elimination, C_0; the fictive serum concentration at t=0, AUC; area under serum concentration-time curve, Vc; distribution volume.

injected after a control period, had no effect on the FK027 or sodium stop-flow patterns, whereas tubular secretion of PAH was inhibited.

Rabbits

Renal clearance of FK027 in rabbits is shown in Table 2. The clearance rates of the total FK027 ($C_{FK027,total}$) ranged from 4.24 to 7.91 ml/minute, and were about 80% of C_{IN}. The clearance rates of the unbound FK027 ($C_{FK027,unbound}$) ranged from 8.49 to 15.8 ml/minute and were larger than the inulin clearance at all the infusion rates tested. When FK027 was infused at the rate of 6.5 mg/kg/hour, the clearance ratio (%) was 151%. This ratio was significantly reduced to 101% when probenecid was injected. Thus, probenecid had a marked effect on the clearance of FK027. A typical stop-flow pattern is shown in Fig. 2. A proximal secretory peak was observed in the FK027 stop-flow pattern, similar to that in PAH. After probenecid was injected, the FK027 peak disappeared, which indicated that probenecid inhibited the tubular secretion of FK027. These findings indicated that FK027 was excreted by glomerular filtration and tubular secretion in rabbits.

Effect of Probenecid on Serum Levels of FK027

To additionally investigate whether probenecid inhibits the renal excretion of FK027, the serum concentrations of FK027 were determined in dogs and rabbits given FK027 (20 mg/kg) iv with and without probenecid (30 mg/kg, iv). Fig. 3 shows the mean serum levels of FK027 in dogs and rabbits, and the pharmacokinetic parameters are listed in Table 3.

Addition of probenecid caused no significant changes in the various parameters in dogs, however in rabbits, serum levels increased significantly by the addition of probenecid. The biological half-life was 0.6 hour with FK027 alone, and the addition of probenecid extended the half-life to 0.81 hour.

Discussion

In dogs, the renal clearance of FK027 was less than the glomerular filtration rate; the clearance ratio of unbound FK027 to inulin clearance was 0.5 to 0.58. These data indicate that the renal excretion of FK027 takes place primary through glomerular filtration and about 50% of the filtered drug

is reabsorbed through the tubules.

The reabsorption is evidenced by the trough at the proximal site in the stop-flow pattern of FK027. Probenecid, an inhibitor of the proximal tubular organic acid secretory mechanism, caused no change in excretion rate, stop-flow pattern or serum levels of FK027. Therefore, the tubular secretion makes little contribution to the excretion of FK027 in dogs. Cephaloridine[7], ceftizoxime[8] and latamoxef[9] have been reported to be excreted mostly through glomerular filtration in dogs, and in our study of FK027 in dogs, glomerular filtration and reabsorption were also involved in the excretion mechanism of FK027. In rabbits, the clearance ratio was 1.51 to 1.78, greater than the glomerular filtration ratio, indicating renal tubular secretion in the excretion process of FK027. The tubular secretion of the drug is evidenced by marked changes in excretion rate, stop-flow pattern and pharmacokinetic parameters by administration of probenecid. Tubular secretion was found to be 33 to 42% in the excretion of FK027.

It is concluded that FK027 is excreted by both glomerular filtration and tubular secretion in rabbits. The renal excretion mechanism of FK027 in rabbits is similar to that of ceftizoxime[8] and latamoxef[9]. The difference in renal excretion mechanism between dog and rabbit reflect a species difference. The longer serum half-life (6.6 hours) compared with other β-lactam antibiotics (41~64 minutes)[10] in dogs is explained by both the high degree of serum protein binding (74~89%) and the tubular reabsorption.

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