COMPARISON OF THE RENAL EXCRETORY MECHANISMS OF CEFMENOXIME AND OTHER CEPHALOSPORINS: EFFECT OF *PARA*-AMINOHIPPURATE ON RENAL CLEARANCE AND INTRARENAL DISTRIBUTION OF CEPHALOSPORINS IN RABBITS

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The renal excretory mechanism of cefmenoxime in rabbits was compared with that of 6 other cephalosporins (cefotaxime, deacetylcefotaxime, cefotiam, cefazolin, cephaloridine, and cefsulodin).

The clearance ratios ($C_{f\text{-}Drug}/C_{Inulin}=CR_f$) of cefmenoxime (337) and cefazolin (73) were considerably higher than those of the 5 other cephalosporins (0.9~20). When *p*-aminohippurate (PAH) was administered concurrently with each of the cephalosporins, the CR_f values of the cephalosporins except for cefsulodin were significantly decreased. These findings indicate that cefmenoxime and the 5 other cephalosporins except cefsulodin are actively incorporated in the proximal tubular cells and secreted into the tubular lumen.

In the case of cefotiam and cefsulodin, glomerular filtration tended to exceed urinary excretion with the highest dose of PAH (40 mg/kg/minute), suggesting the possibility of tubular reabsorption of these drugs. On the other hand, glomerular filtration of cefmenoxime and the 4 other cephalosporins did not exceed urinary excretion.

The drug concentration ratio of the cortex to medulla indicated that the tubular cell level of cefmenoxime was lower than, higher than, and similar to those of cephaloridine, cefotaxime, and the remaining cephalosporins, respectively.

These results demonstrate that the renal excretory mechanism of cefmenoxime is similar to that of cefazolin but not to that of the remaining cephalosporins.

Proximal tubular secretion by the anion transport system, in addition to glomerular filtration, plays an important role in the renal excretory mechanism of organic acids including cephalosporins[1]. *Para*-aminohippuric acid (PAH) is a typical compound of those secreted by this system: PAH anions in blood are taken up actively into the proximal tubular epithelial cells by a carrier and then secreted into the tubular lumen by passive diffusion[2~4]. The behavior of organic acids in these processes has entirely different effects on the tubular cells. When organic acids taken up into the cells are not secreted into the tubular lumen or when their rate of uptake exceeds that of secretion, they will accumulate in the cells to a toxic level, and cellular necrosis will ensue; as happens with cephaloridine[5~10]. The renal lesion caused by cephaloridine can be prevented by competitively blocking the uptake of this drug into cells with other organic acids such as PAH or probenecid[11~13]. On the other hand, when organic acids in the cells are secreted into the lumen at the same rate as they are taken up into cells from the blood, the acids do not accumulate to a toxic level in the cells[10].

Previous studies, in which we used a renal clearance technique in rabbits, suggested tubular secretion of 6 out of 7 cephalosporins examined including cefmenoxime (7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid), a novel broad-spectrum cephalosporin[14]. In the present study, the renal excretory mechanism of cef-

menoxime was quantitatively compared with that of 6 other cephalosporins by blocking competitively uptake from blood into cells with PAH[7,9,15]. Furthermore, the extent of the intracellular accumulation of these cephalosporins was estimated by comparing drug concentrations in the cortex to those in plasma; similar comparisons were made between medulla and plasma, and cortex and medulla.

Materials and Methods

Compounds

Cefmenoxime, cefotaxime (7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-acetoxymethylceph-3-em-4-carboxylic acid), deacetylcefotaxime (7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-hydroxymethylceph-3-em-4-carboxylic acid), cefotiam (7β-[2-(2-aminothiazol-4-yl)-acetamido]-3-[[[1-(2-dimethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid), and cefsulodin (3-(4-carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetamido)ceph-3-em-4-carboxylate monosodium salt) were prepared in our Central Research Division. Cefazolin (Fujisawa Pharmaceutical, Japan) and cephaloridine (Eli Lilly, U.S.A.) were commercial preparations. All cephalosporins, except cefotiam and cephaloridine, were used as the sodium salt. Cefotiam was used as the dihydrochloride.

Inulin (reagent grade; Wako Pure Chemical, Japan) and PAH (sodium salt, reagent grade; Sigma, U.S.A.) were commercial preparations.

The cephalosporins, inulin, and PAH were dissolved in physiological saline on use and the pH of the solutions was adjusted to 7.4 with sodium hydroxide solution.

Animals

Male New Zealand white rabbits (Nippon Seibutsuzairyo Center) weighing 2.7~3.3 kg were used. All animals were acclimated to the laboratory environment more than 1 week before the experiment with free access to a diet (GM-1; Funabashi Nojo, Japan) and water. Rabbits were deprived of the diet but not of water for 16 hours before the experiment.

Measurement of Renal Clearance and Collection of Renal Specimens

The technique of determining renal clearance for each cephalosporin alone or with PAH was similar to that reported previously[14]. The rabbits were anesthetized with 30 mg/kg of sodium pentobarbital (Somnopentyl®; Pitman-Moor, U.S.A.) intravenously and tracheotomized. The jugular vein, carotid artery, and both urethrae were cannulated with polyethylene tubes for infusion, blood collection, and urine collection, respectively. The rabbits were primed with 5 ml/kg of inulin solution (15 mg/ml in saline) (solution A). This was followed by an intravenous infusion of solution A at a rate of 18 ml/kg/hour. Two consecutive 30-minute urine specimens were collected beginning 2 hours after the start of infusion. The infusion was stopped, and the animals were primed with 1 ml/kg of a saline solution (solution B) containing one of the cephalosporins (20 mg/ml) and PAH (0, 200, 400 and 800 mg/ml). This was followed by an intravenous infusion of another solution (0.3 mg of one of the cephalosporins, 0, 10, 20, and 40 mg of PAH in 0.3 ml of solution A) (solution C) at the rate mentioned above. Three consecutive 30-minute urine specimens were collected beginning 30 minutes after the start of the solution C infusion. Three ml blood specimens were drawn at the midpoint of each urine collection before and after the priming with solution B, and the plasma was obtained by centrifugation (3,000 rpm × 10 minutes) immediately. The urine and plasma specimens were stored as described previously[14].

Rabbits were given 1 ml (1,000 U) of heparin intravenously after the end of the infusion and totally bled through the carotid arterial cannula. Both kidneys were excised and 1 g each of cortex and medulla was removed from each kidney. The cephalosporins and inulin in each of these tissue samples were extracted by homogenization with 9 volumes of 0.1 M phosphate buffer (pH 7.0) in ice cold and by centrifugation. Methanol was used instead of the phosphate buffer when cefotaxime and deacetylcefotaxime were extracted. The recovery rate of each cephalosporin was more than 95%. The extracts were stored at −20°C.

Measurements of Cephalosporins, Inulin, and PAH

The concentrations of cephalosporins and inulin in the urine and plasma were assayed as described

previously[14]. The concentrations of these drugs in the kidney extracts were assayed by the same method as was used for plasma specimens. PAH concentrations in plasma were determined by the colorimetric technique of WAUGH and BEALL[16].

Effect of PAH on Binding of Cephalosporins to Rabbit Serum Proteins

The extent of binding of cephalosporins to the proteins of rabbit serum was measured by the equilibrium dialysis technique reported previously[14] except that PAH (0~8 mg/ml) was added to the cephalosporin solutions.

Calculation of Renal Clearance

Renal clearances of inulin and cephalosporins were calculated from the standard formula reported previously[14].

Statistical Treatment

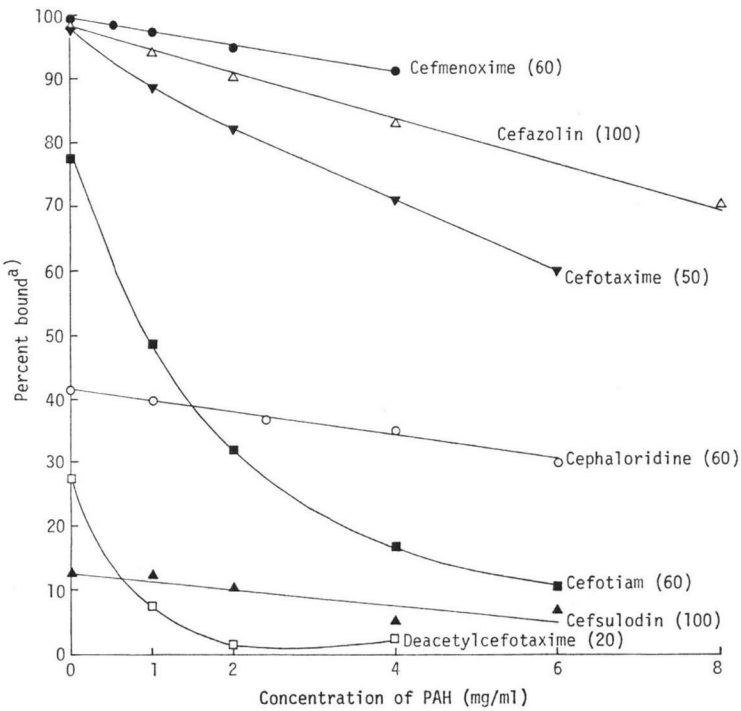
Statistical significances were evaluated by the Student's t-test.

Results

Effect of PAH on Binding of Cephalosporins to Rabbit Serum Proteins

The rate of serum protein binding of each cephalosporin examined was reduced by adding PAH concurrently (Fig. 1). The rate was markedly reduced in the case of cefotiam, cefotaxime, and deacetylcefotaxime, and slightly reduced in the case of cephaloridine and cefsulodin.

Fig. 1. Effect of *p*-aminohippurate (PAH) on protein binding rate of cefmenoxime and other cephalosporins in rabbit sera.



Data are expressed in mean values of three experiments.
Number in parentheses denote drug concentration applied (μg/ml).
a) Determined by the equilibrium dialysis method.

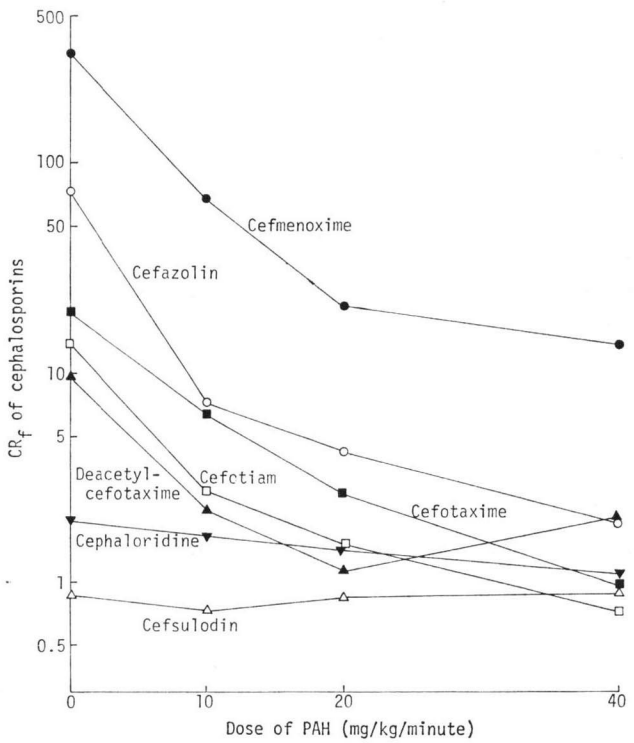
Effect of PAH on Renal Clearance in Rabbits

The effect of concurrently administered PAH on the renal clearance of cefmenoxime and the other cephalosporins is shown in Table 1. Since cefotaxime is known to be partly metabolized to deacetylcefotaxime in the body, a less active metabolite[14], parameters for both are shown separately.

The clearance ratios ($C_{f\text{-}Drug}/C_{Inulin}=CR_f$) of cefmenoxime (337) and cefazolin (73) were considerably higher than those of the 5 other cephalosporins [cefotaxime (19.7), cefotiam (13.9), deacetylcefotaxime (9.7), cephaloridine (1.95), and cefsulodin (0.86)].

In the case of cefmenoxime, when plasma PAH concentrations were increased, the free drug concentration in plasma ($P_{f\text{-}Drug}$) and the amount of glomerular filtration ($P_{f\text{-}Drug}\cdot C_{Inulin}$) were increased, while the renal clearance ($C_{f\text{-}Drug}$) and the clearance ratio were decreased. On the other hand, the inulin clearance(C_{Inulin}) was unchanged and the amount of urinary excretion($U_{Drug}\cdot V$) was little influenced. In the case of cefotaxime, deacetylcefotaxime, cefotiam, and cefazolin these findings were changed in a similar manner to those of cefmenoxime. Although the results for cephaloridine showed a similar tendency to those of cefmenoxime, the effect of PAH on these parameters was much smaller with cephaloridine than with the other cephalosporins. The findings for cefsulodin were not significantly influenced by PAH. With concurrent infusion of the highest dose of PAH (40 mg/kg/minute), the CR_f values for cefmenoxime, cefazolin, cefotaxime, cefotiam, and cephaloridine were lowered to 13.8, 1.94, 0.99, 0.73, and 1.10, respectively (Fig. 2). The CR_f for deacetylcefotaxime was reduced with PAH and almost reached a constant value ($CR_f=1.15\sim2.22$) with $10\sim40$ mg/kg/minute.

Fig. 2. Effect of *p*-aminohippurate (PAH) on renal clearance ratio of cefmenoxime and other cephalosporins in rabbits.



Data were obtained from Table 1 and expressed as mean values.

Table 1. Effect of *p*-aminohippurate (PAH) on the renal clearance of cefmenoxime and other cephalosporins in rabbits.

Cephalosporin	PAH(n)[a]	Body Wt. (kg)	C_{Inulin} (ml/min)	P_{PAH} (mg/ml)	Corrected for binding[b]				$U_{Drug} \cdot V$[d] (μg/min)
					$P_{f\text{-}Drug}$ (μg/ml)	$C_{f\text{-}Drug}$ (ml/min)	CR_f[c]	$P_{f\text{-}Drug} \cdot C_{Inulin}$ (μg/min)	
Cefmenoxime	0 (7)	3.06±0.26	9.68±2.63	0	0.29± 0.03	3048 ±190	337 ±100	2.82± 0.97	883± 96
	10 (3)	2.85±0.04	12.8 ±2.1	0.62±0.03	1.09± 0.17	853 ± 80	67.3 ± 5.6	13.7 ± 2.0	918± 81
	20 (3)	2.82±0.06	11.6 ±3.1	1.71±0.55	3.10± 0.95	239 ± 62	20.9 ± 3.3	34.1 ± 6.5	703± 71
	40 (4)	3.10±0.10	13.1 ±3.1	3.65±2.18	5.63± 5.12	185 ± 95	13.8 ± 6.9	66.1 ± 48.0	675± 84
Cefotaxime[e]	0 (8)	3.07±0.05	9.56±1.12	0	(A) 1.49± 0.42	183 ± 56	19.7 ± 7.7	14.3 ± 4.6	265± 92
					(B) 6.65± 2.81	92.7 ± 41.0	9.70± 3.84	63.6 ± 29.6	527± 84
	10 (4)	3.00±0.07	11.5 ±1.8	1.05±0.15	(A) 6.06± 1.80	71.9 ± 14.0	6.40± 1.67	68.6 ± 19.9	426± 95
					(B) 24.8 ± 4.2	24.5 ± 4.2	2.22± 0.72	289 ± 94	596± 52
	20 (3)	3.02±0.10	12.2 ±3.3	2.99±0.55	(A) 12.2 ± 4.0	33.1 ± 11.3	2.67± 0.21	143 ± 25	381± 81
					(B) 33.1 ± 1.52	13.2 ± 2.1	1.15± 0.41	406 ±124	434± 51
	40 (3)	2.91±0.10	7.78±0.91	9.03±1.15	(A) 38.5 ± 5.9	7.80± 2.18	0.99± 0.20	300 ± 62	306±127
					(B) 16.7 ± 8.0	16.4 ± 7.7	2.07± 0.82	130 ± 70	246± 99
Cefotiam	0 (6)	3.00±0.07	7.35±2.25	0	8.25± 2.56	103 ± 35	13.9 ± 0.9	56.2 ± 5.6	780± 62
	10 (4)	2.94±0.10	12.3 ±3.8	1.39±0.30	30.6 ± 7.7	30.9 ± 9.0	2.73± 1.43	355 ± 28	941±405
	20 (3)	3.10±0.10	13.6 ±3.2	3.24±0.29	34.2 ± 4.4	20.3 ± 3.2	1.52± 0.21	456 ± 57	686± 21
	40 (3)	3.05±0.09	14.6 ±6.0	9.28±0.46	76.0 ± 1.6	9.72± 0.59	0.73± 0.24	1116 ±486	739± 48
Cefazolin	0 (8)	3.09±0.19	9.11±1.50	0	1.21± 0.47	672 ±226	73.0 ± 18.6	10.6 ± 3.1	733±131
	10 (4)	3.05±0.26	8.22±1.84	1.10±0.11	7.68± 0.99	58.0 ± 19.8	7.35± 3.08	63.7 ± 19.9	432± 97
	20 (4)	3.08±0.12	8.12±2.42	2.53±0.62	16.2 ± 4.50	36.2 ± 17.5	4.28± 0.98	124 ± 16	530±135
	40 (4)	3.19±0.08	10.6 ±2.28	6.96±1.60	33.6 ±12.0	31.4 ± 10.4	1.94± 0.55	332 ± 41	627±117
Cephaloridine	0 (5)	2.99±0.20	10.3 ±1.7	0	27.2 ± 4.2	19.3 ± 5.2	1.95± 0.77	284 ± 80	513± 91
	10 (3)	2.95±0.06	11.2 ±1.1	0.68±0.12	29.9 ± 1.3	18.4 ± 2.6	1.66± 0.27	334 ± 42	548± 65
	20 (3)	3.03±0.05	11.5 ±4.7	1.56±0.36	40.2 ±10.7	15.8 ± 3.8	1.43± 0.22	427 ± 51	606± 41
	40 (5)	3.20±0.04	11.4 ±2.0	4.25±1.60	59.4 ± 7.4	12.5 ± 3.8	1.10± 0.31	560 ±108	596±109
Cefsuldin	0 (6)	3.16±0.10	14.7 ±3.8	0	65.3 ±14.0	12.2 ± 2.4	0.86± 0.17	944 ±287	780±135
	10 (3)	3.02±0.13	10.3 ±5.9	2.35±0.99	117 ±12	6.69± 1.32	0.74± 0.27	1180 ±572	774± 79
	20 (4)	2.90±0.04	14.9 ±1.8	3.52±0.94	67.2 ± 3.3	12.5 ± 1.1	0.85± 0.11	1002 ±141	840± 80
	40 (4)	3.04±0.06	14.0 ±3.3	7.57±1.20	65.6 ±11.0	12.4 ± 2.8	0.89± 0.15	897 ± 95	790± 87

Data are expressed as the mean±S.D. a) PAH: mg/kg/min, n: Number of animals. b) Subscript f represents the value corrected for serum protein binding of drug. c) $C_{f\text{-}Drug}/C_{Inulin}$. d) $U_{Drug} \cdot V$: amount of drug excreted. e) A: cefotaxime, B: deacetylcefotaxime.

Urinary excretion ($U_{Drug} \cdot V$) was significantly ($p < 0.001$) larger than glomerular filtration for all cephalosporins except cefsulodine (Table 1). When PAH was infused concurrently, the glomerular filtration of all the cephalosporins except cefsulodin increased but the value for cefmenoxime and cefazolin was about one-tenth and one-half of the urinary excretion, respectively, even with the highest dose of PAH. Glomerular filtration tended to be lower than urinary excretion for cefotaxime, deacetylcefotaxime, and cephaloridine, and higher for cefotiam and cefsulodin when the highest dose of PAH was administered, but the differences were not statistically significant ($p > 0.05$).

Either PAH alone or concurrently with each of the cephalosporins increased urine volume dose-dependently. Without PAH, the urine volume before cephalosporin priming was 10~22 ml/hour and increased twice to three times after priming. The urine volume after the cephalosporin priming increased dose-dependently and reached 20~50 times (180~230 ml/hour) with the highest dose of PAH (40 mg/kg/minute). The renal weight, however, was unchanged with PAH infusion.

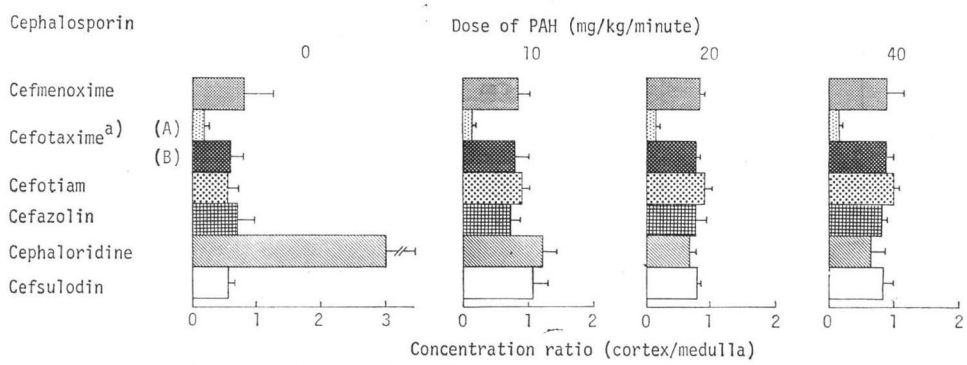
Effect of PAH on Cephalosporin Concentrations in the Renal Cortex and Medulla

The ratio of cephalosporin concentration in the renal cortex (C/P) and medulla (M/P) to that in plasma, and in cortex to that in medulla (C/M) are shown in Table 2 and Fig. 3.

The C/P for cefmenoxime (5.49) was lower ($p < 0.05$) than those for deacetylcefotaxime (13.2) and cephaloridine (12.5), similar to those for cefotiam (2.46) and cefazolin (2.32), and higher ($p < 0.05$) than those for cefsulodin (1.62) and cefotaxime (0.31). The M/P ratios were in the same order as the C/P. The C/M for cefmenoxime (0.82) was lower than that for cephaloridine (3.03), higher than that for cefotaxime (0.19), and similar to those for the remaining cephalosporins (0.54~0.72). The C/P and C/M for inulin were similar among the cephalosporins (Table 2).

The C/M values for all the cephalosporins and for inulin were not affected by concurrent infusion with PAH, except for cephaloridine (Table 2 and Fig. 3). The C/M for cephaloridine was reduced with PAH, and reached the same level as that for the other cephalosporins, except for cefotaxime, with 20 mg/kg/minute of PAH. The C/M value for all the cephalosporins, except cefotaxime (0.16), approximated 1.0 with the highest dose of PAH. The C/M for inulin showed a tendency to increase slightly with PAH in all the cephalosporin treated groups.

Fig. 3. Effect of *p*-aminohippurate (PAH) on concentration ratio of cortex to medulla for various cephalosporins in rabbits.



Data were obtained from Table 2 and expressed as the mean ±S.D.

a) A : cefotaxime, B : deacetylcefotaxime.

Table 2. Effect of *p*-aminohippurate (PAH) on the intrarenal distribution of cephalosporins and inulin in rabbits.

Cephalosporin	PAH (n)[a]		Drug				Inulin		
			Plasma level (μg/ml)	Cortex/Plasma Ratio	Medulla/Plasma Ratio	Cortex/Medulla Ratio	Cortex/Plasma Ratio	Medulla/Plasma Ratio	Cortex/Medulla Ratio
Cefmenoxime	0 (7)		44± 5	5.49±4.16	7.10± 3.55	0.82±0.44	2.83±0.91	4.28±3.26	0.81±0.35
	10 (3)		56± 7	3.40±0.64	4.17± 0.66	0.82±0.17	2.29±0.65	3.57±1.08	0.65±0.10
	20 (3)		76± 9	1.62±0.44	1.98± 0.65	0.83±0.08	1.11±0.22	1.64±0.73	0.73±0.23
	40 (4)		64±21	0.92±0.34	1.02± 0.44	0.93±0.28	1.16±0.28	0.89±0.32	1.36±0.26
Cefotaxime[b]	0 (8)	(A)	45±13	0.31±0.14	1.66± 0.65	0.19±0.06	2.89±0.58	3.68±1.39	0.88±0.18
		(B)	9± 4	13.2 ±6.6	23.2 ±14.2	0.60±0.20			
	10 (4)	(A)	59±12	0.06±0.04	0.49± 0.34	0.12±0.03	2.37±0.36	2.41±0.48	1.00±0.15
		(B)	27± 4	2.75±0.91	3.48± 0.44	0.79±0.19			
	20 (3)	(A)	52± 9	0.07±0.04	0.55± 0.15	0.14±0.05	1.79±0.39	1.41±0.24	1.27±0.15
		(B)	33± 2	1.59±0.35	2.09± 0.43	0.76±0.06			
	40 (3)	(A)	60±13	0.03±0.01	0.07± 0.02	0.16±0.07	1.45±0.01	0.97±0.14	1.52±0.21
		(B)	17± 8	1.04±0.85	1.37± 0.75	0.89±0.12			
Cefotiam	0 (6)		37±11	2.46±0.82	5.08± 2.39	0.54±0.17	1.98±0.43	2.44±0.63	0.82±0.10
	10 (4)		53±12	1.82±0.25	2.07± 0.46	0.90±0.11	2.38±0.41	2.25±0.40	1.06±0.10
	20 (3)		43± 5	0.43±0.52	0.47± 0.54	0.90±0.13	1.57±0.24	1.47±0.17	1.09±0.28
	40 (3)		78± 1	0.75±0.16	0.73± 0.12	1.03±0.07	1.96±0.46	1.21±0.38	1.65±0.16
Cefazolin	0 (8)		65±25	2.32±1.18	3.87± 2.57	0.72±0.25	2.32±0.32	2.82±1.30	0.95±0.35
	10 (4)		120± 9	0.60±0.14	0.83± 0.13	0.73±0.15	1.09±0.11	1.45±0.15	0.75±0.05
	20 (4)		137±18	0.60±0.17	0.79± 0.24	0.77±0.15	1.25±0.28	1.11±0.26	1.14±0.27
	40 (4)		126±23	0.38±0.05	0.45± 0.03	0.84±0.08	0.87±0.19	0.67±0.07	1.28±0.21
Cephaloridine	0 (5)		46± 7	12.5 ±5.6	7.58± 5.70	3.03±2.74	2.31±0.34	4.08±1.56	0.63±0.22
	10 (3)		50± 2	2.56±0.46	2.18± 0.61	1.21±0.25	2.26±0.48	3.14±1.01	0.74±0.10
	20 (3)		65±17	0.66±0.08	1.03± 0.29	0.66±0.12	1.44±0.30	1.38±0.39	1.07±0.15
	40 (5)		74±12	0.46±0.16	0.74± 0.13	0.64±0.21	0.97±0.10	0.74±0.29	1.40±0.37
Cefsulodin	0 (6)		75±16	1.62±0.92	3.02± 2.02	0.57±0.18	2.67±0.64	2.65±0.57	1.04±0.30
	10 (3)		129±12	1.02±0.16	0.98± 0.27	1.07±0.23	3.67±2.56	1.21±0.24	1.47±0.87
	20 (4)		73± 3	0.81±0.07	1.01± 0.04	0.80±0.05	2.21±0.36	1.78±0.28	1.25±0.06
	40 (4)		67±11	0.86±0.05	0.74± 0.13	0.86±0.13	1.82±0.63	1.11±0.37	1.65±0.10

Data are expressed as the mean±S.D. a) PAH: mg/kg/min, n: Number of animals. b) A: cefotaxime, B: deacetylcefotaxime.

Discussion

When PAH was not administered concurrently with cephalosporins, the clearance ratio (CR_f) of cefmenoxime was similar to that of cefazolin and considerably higher than that of the other cephalosporins. The renal excretion of cefmenoxime and cefazolin is, therefore, largely the result of tubular secretion and some glomerular filtration.

When PAH was infused concurrently with each of the cephalosporins, the CR_f values were significantly decreased except that for cefsulodin (Fig. 2). This finding indicates that cefmenoxime and the other cephalosporins, except cefsulodin, are actively secreted at the proximal tubules by the same carrier system as PAH. However, with the highest dose of PAH the CR_f of cefmenoxime was 13.8 indicating that the tubular secretion of this drug was not blocked completely even by a large amount of PAH, with which the concentration ratio of PAH to cefmenoxime in plasma rose to as high as 650. There are two possible explanations for this failure: a very high affinity of the drug to PAH carrier or the drug may be secreted by a transport system other than the anion. The latter explanation is unlikely for the following reason. According to ITAKURA (personal communication) the values of pKa_1 (for carboxyl group at position 4) and of pKa_2 (for ammonium group of position 7 side chain) for cefmenoxime are 3.0 and 3.5, respectively. These *pKa* values indicate that in plasma (pH 7.4) a greater part of the carboxyl group at position 4 exists as the anionic form and the ammonium group at the side chain of position 7 exists as the nonionic form. Therefore, it is unlikely that cefmenoxime is secreted by nonionic diffusion or cation transport system.

To calculate the extent of reabsorption of drugs from the renal clearance, the CR_f must be constant at less than 1.0, or the tubular secretion must be completely blocked, and glomerular filtration must be larger than urinary excretion. In the present study, the values of CR_f for cefotiam and cefsulodin with the highest dose of PAH were less than 1.0, and their glomerular filtrations were somewhat larger than their urinary excretions, suggesting the possibility of reabsorption. However, since the differences between glomerular filtration and urinary excretion were not significant ($p > 0.05$), the reabsorption of these drugs, if any, is not large. The findings for cefmenoxime, cefotaxime, deacetylcefotaxime, cefazolin, and cephaloridine did not satisfy the criteria for calculation of the reabsorption. Furthermore, the values of C/M for cefotaxime (0.54) and cefsulodin (0.57) without PAH were less than the corresponding values of C/M for inulin, 0.82 and 1.04, respectively. This observation supports the possibility of reabsorption of these drugs by distal tubules in the renal cortex. On the other hand, the values of C/M for cefmenoxime (0.82) and cefazolin (0.72) were about the same as the corresponding values for inulin, 0.81 and 0.95, respectively, which indicates that reabsorption of these drugs is less likely.

The C/P and C/M values for cephaloridine without PAH were 12.5 and 3.3, respectively. These were far higher than those of the other cephalosporins, but were reduced to the same level as those of the others with the concurrent administration of PAH (Table 2 and Fig. 3). On the other hand, the corresponding values of C/P and M/P for inulin in the cephaloridine clearance experiment were about the same as those for inulin in the experiments with the other cephalosporins. Furthermore, the reduction in the CR_f of cephaloridine with concurrent administration of PAH was minute (Table 1 and Fig. 2). These results indicate that a large amount of cephaloridine is actively taken up into the proximal cells but only a small amount is secreted into the tubular lumen; as a consequence, the drug accumulates in the cells. This observation is in accord with many of the earlier reports on the renal excretion of cephaloridine[7,9,13].

Small differences in the protein binding rate of highly protein-bound drugs have more influence on the CR_f value than those of poorly protein-bound drugs. Therefore, only semi-quantitative analysis is allowed to assess the renal excretory mechanism of extremely highly protein-bound drugs for the following two reasons. First, there is a difficulty in determining glomerular filtration and tubular secretion of protein-bound drugs. Although only unbound drugs can be filtered and secreted, the apparent clearance of a drug by this secretory process finally approaches the clearance of the total drug in the plasma, bound and unbound. This results from the recycling process in which, when the unbound drug has been secreted, a re-equilibration between the remaining bound and unbound drugs is effected. The net degree of tubular secretion then depends upon the number of secretory sites, the flow rate of plasma, and the time of passage of the plasma flow in contact with these sites[17]. On the other hand, since the

Table 3. Comparisons of postulated renal excretory mechanisms of cefmenoxime and other cephalosporins.

	Cefmenoxime	Cefotaxime	Deacetylcefotaxime	Cefotiam	Cefazolin	Cephaloridine	Cefsulodin
Glomerular filtration	+	+	+++	++	+	+++	+++
Tubular secretion	+++	++	++	++	+++	+	±
Reabsorption	−	*	*	±	−	*	±
Intracellular accumulation	+	±	+	+	+	+++	+

*: Could not be postulated, −: No, ±: Little and no, +: Low, ++: Moderate, +++: High

plasma flow at the glomerular filtration process is rapid, only unbound drugs are thought to be filtered. However, the shift of bound to unbound drugs could also occur to a small extent at the filtration process. The shift, even to a small extent, would affect the CR_f for an extremely highly protein-bound drug, and create a large discrepancy between the actual CR_f and the calculated CR_f. Second, there is a problem in determining the protein binding of the drugs. Since the extent of plasma protein binding of a drug cannot be determined practically under the same condition as in the glomerular capillaries[18], the value determined by an *in vitro* technique such as equilibrium dialysis is used as an approximation. However, the discrepancy between the value in the glomerular capillaries and that determined by an *in vitro* technique would greatly affect the CR_f for extremely highly protein-bound drugs.

The results of the present study on renal clearances and distributions in the renal cortex and medulla allow for the characterization of the renal excretory mechanisms of cefmenoxime and the other cephalosporins semi-quantitatively, as shown in Table 3. Glomerular filtration is high in the low protein-bound compounds (deacetylcefotaxime, cephaloridine, and cefsulodin) and low in the highly protein-bound drugs (cefmenoxime, cefotaxime, and cefazolin). On the other hand, the tubular secretion is high with highly protein-bound drugs such as cefmenoxime and cefazolin, low with cephaloridine and cefsulodin. The possibility of minor reabsorption of cefotiam and cefsulodin is suggested. From the results of the clearance experiments there is no evidence for the reabsorption of cefmenoxime and cefazolin. In the cells of the proximal tubule, accumulation of cephaloridine is extremely high, and low for cefmenoxime, deacetylcefotaxime, cefotiam, cefazolin, cefsulodin, and cefotaxime. These results demonstrate that the renal excretory mechanism of cefmenoxime is similar to that of cefazolin, but not that of the remaining cephalosporins.

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References

1) WEINER, I. M. & G. H. MUDGE: Renal tubular mechanisms for excretion of organic acids and bases. Am. J. Med. 36: 743~762, 1964
2) FOULKES, E. C.: Kinetics of *p*-aminohippurate secretion in the rabbit. Am. J. Physiol. 205: 1019~1024, 1963
3) BURG, M. B. & J. ORLOFF: *p*-Aminohippurate uptake and exchange by separated renal tubules. Am. J. Physiol. 217: 1064~1068, 1969
4) TUNE, B. M.; M. B. BURG & C. S. PATLAK: Characteristics of *p*-aminohippurate transport in proximal renal tubules. Am. J. Physiol. 217: 1057~1063, 1969

5) ATKINSON, R. M.; J. P. CURRIE, B. DAVIS, D. A. H. PRATT, H. M. SHARPE & E. G. TOMICH: Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. Toxicol. Appl. Pharmacol. 8: 398~406, 1966
6) SILVERBLATT, F.; W. O. HARRISON & M. TURCK: Nephrotoxicity of cephalosporin antibiotics in experimental animals. J. Infect. Dis. 128: S367~S372, 1973
7) TUNE, B. M.; M. FERNHOLT & A. SCHWARTZ: Mechanism of cephaloridine transport in the kidney. J. Pharmacol. Exp. Ther. 191: 311~317, 1974
8) SILVERBLATT, F.; M. TURCK & R. BULGER: Nephrotoxicity due to cephaloridine: a light- and electron-microscopic study in rabbits. J. Infect. Dis. 122: 33~44, 1970
9) TUNE, B. M. & M. FERNHOLT: Relationship between cephaloridine and *p*-aminohippurate transport in the kidney. Am. J. Physiol. 225: 1114~1117, 1973
10) TUNE, B. M.: Relationship between the transport and toxicity of cephalosporins in the kidney. J. Infect. Dis. 132: 189~194, 1975
11) CHILD, K. J. & M. G. DODDS: Nephron transport and renal tubular effects of cephaloridine in animals. Brit. J. Pharmacol. Chemoth. 30: 354~370, 1967
12) TUNE, B. M.; K. Y. WU & R. L. KEMPSON: Inhibition of transport and prevention of toxicity of cephaloridine in the kidney. Dose-responsiveness of the rabbit and the guinea pig to probenecid. J. Pharmacol. Exp. Ther. 202: 466~471, 1977
13) TUNE, B. M.: Effect of organic acid transport inhibitor on renal cortical uptake and proximal tubular toxicity of cephaloridine. J. Pharmacol. Exp. Ther. 181: 250~256, 1972
14) YAMAZAKI, I.; Y. SHIRAKAWA & T. FUGONO: Comparison of the real excretory mechanisms of cefmenoxime and other cephalosporins: renal clearance in rats and rabbits. J. Antibiotics 34: 1055~1063, 1981
15) ARITA, T.; R. HORI, E. OWADA & K. TAKAHASHI: Transformation and excretion of drugs in biological systems. I. Renal excretion mechanisms of sulfonamides. (1). Chem. Pharm. Bull. 17: 2526~2532, 1969
16) WAUGH, W. H. & P. T. BEALL: Simplified measurement of *p*-aminohippurate and other arylamines in plasma and urine. Kidney Int. 5: 429~436, 1974
17) GARRETT, E. R.: Pharmacokinetics and clearances related to renal processes. Int. J. Clin. Pharmacol. 16: 155~172, 1978
18) CAFRUNY, E. J.: Renal excretion of drugs. *in* "Fundamentals of Drug Metabolism and Drug Disposition" (*ed.* by LA DU, B. N., H. G. MANDEL & E. L. WAY), pp. 119~130, Williams and Willkins, Baltimore, 1971