CEFAZOLIN, A NEW SEMISYNTHETIC CEPHALOSPORIN ANTIBIOTIC. III

ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION IN PARENTERAL ADMINISTRATION

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Serum levels of cefazolin and related antibiotics were compared in rats, rabbits and dogs at a single parenteral dose of 10 or 20 mg per kg. The results in rats and rabbits showed that cefazolin gave higher serum levels than other related antibiotics by all the routes of administration used. In dogs, however, the serum level of cefazolin was nearly equal to those of cephaloridine and ampicillin when given intramuscularly. The urinary excretion of cefazolin and related antibiotics was also studied by the identical schedule. Amount of cefazolin recovered from the 24-hour urine of the animals accounted for 75 % or more of the dose given, and the rate of recovery was found to be higher than those of any other related antibiotics. The maximum level of cefazolin was obtained in the initial 3-hour urine, the rate of excretion being comparable to those of cephaloridine and ampicillin. The therapeutically effective level of cefazolin was maintained at least for 6 hours after administration. Biliary excretion of cefazolin was similarly studied in rats, rabbits and dogs. In rats, using a single dose of 20 mg/kg, about 20 % of the given dose was respectively recovered in the initial 8-hour bile. In dogs, when the dose was given intramuscularly, 3.3 % was recovered in the 8-hour bile. The biliary excretion of cefazolin seemed to be of similar order to that of ampicillin, and manifestly higher than that of cephaloridine or cephalothin. Tissue distribution of cefazolin was similarly estimated in rats and rabbits. The levels of cefazolin in the kidney and liver were lower than those of ampicillin and cephaloridine. On the other hand, the levels in the lung and heart were slightly higher than those of other antibiotics tested. Cefazolin and cephaloridine were administered intramuscularly to five healthy volunteers in a cross-over fashion. A mean peak serum level of 44.6 mcg/ml was obtained in the group receiving cefazolin at a single dose of 500 mg, which was about 3 times higher than that of cephaloridine (17.2 mcg/ml). In this experiment, therapeutically effective serum levels were maintained as long as 10 hours after administration. The urinary levels of both of the antibiotics were determined simultaneously. The rate of cefazolin recovered in the 24-hour urine was 95.9 % for a single intramuscular dose of 250 mg, and 82.4 % for that of 500 mg. On the other hand, with cephaloridine at a

single intramuscular dose of 500 mg, 70.2 % was recovered in the 24-hour urine. Thin-layer chromatography of the human urine sample showed no other biologically active metabolites present after intramuscular injection of cefazolin.

The *in vitro* and *in vivo* activity of cefazolin was reported in our previous paper[1], in which it was confirmed that cefazolin is a bactericidal antibiotic with a wide range of activity against Gram-positive and Gram-negative pathogens, and is also active against penicillinase-producing staphylococci in common with cephaloridine and cephalothin.

The present paper concerns a study with cefazolin and related antibiotics on serum levels, urinary and biliary excretion and tissue distribution in rats, rabbits and dogs, and in healthy volunteers.

Materials and Methods

1. Antibiotics tested

The antibiotics tested were: cefazolin (CEZ, 954 mcg/mg, Fujisawa Research Laboratories), cephalothin (CET, 971 mcg/mg, Eli Lilly and Company), cephaloridine (CER, 988 mcg/mg, Glaxo Laboratories), and ampicillin (AB-PC, 840 mcg/mg, Beecham Research Laboratories).

2. Animals used

Animals used in these experiments were as follows:

Rats; Sprague Dawley white male rats, weighing 200~270 g

Rabbits; male rabbits, weighing 2.0~2.5 kg

Dogs; female (mongrel) dogs, weighing 10~12 kg

All animals were fasted overnight before use, but supplied water *ad libitum*.

3. Method for assay of drug

Levels of the aforementioned antibiotics in the serum, urine, bile and tissues were determined by the disc method.

One hundred ml of melted nutrient agar media, inoculated with 0.1 ml of the spore suspension (10^8 spores per ml) of *B. subtilis* strain ATCC 6633, was used for the bioassay of cefazolin. Paper discs (diameter: 6 mm) were dipped in the standard solutions or the samples. After the excess solution was allowed to run off, the discs were placed on the surface of the inoculated agar plate. The diameters of inhibitory zones were measured after incubation at 37°C for 20 hours, and the amount of each drug in the test samples was calculated from the results.

4. Determination of serum levels and urinary excretion in animals

(1) Serum level: Serum levels of cefazolin and related antibiotics were determined in three administration routes.

a) Intravenous administration: Cefazolin was given to 4 rabbits at a single dose of 20 mg/kg, and blood samples were withdrawn at 5, 15, 30, and 60 minutes from the auricle vein and allowed to clot. The separated sera were used for the assay by the disc method. The same procedure was done with cephaloridine, cephalothin and ampicillin.

b) Intramuscular administration: Each antibiotic was given to 15 rats and 4 rabbits at a single dose of 20 mg/kg, and to 5 dogs at 10 mg/kg. In dogs, the blood sample was collected from the cephalic vein.

c) Subcutaneous administration: Each antibiotic was given to 15 rats and 4 rabbits at the same dose level as in the intravenous administration. At 0.5, 1, 2, 3 and 5 hours after dosage, the blood samples were obtained from 3 rats by heart-puncture and from 4 rabbits by venepuncture.

(2) Urinary excretion: The urinary excretion of the antibiotics were investigated as follows: The urine samples were collected using metabolic cages, at certain intervals over a 24-hour period, from all the animals which were used for the determination of serum levels of the antibiotics. The concentrations of antibiotics in each urine sample were determined by the disc method.

5. Determination of serum levels and urinary excretion in healthy volunteers

The serum and urinary levels were determined in 5 healthy volunteers according to a cross over procedure. Five adult male volunteers, ranging from 60.0 to 70.5 kg in weight, were each administered intramuscularly cefazolin at a single dose of 250 mg and 500 mg, and cephaloridine at a single dose of 500 mg. Blood specimens were obtained at 0.5, 1, 3, 6, 8 and 10 hours after administration. Urine samples were collected over the periods 0~1, 1~3, 3~6, 6~8, 8~10 and 10~24 hours following the administration. The concentrations of cefazolin and cephaloridine in the serum and urine were assayed by the disc method.

6. Determination of biliary excretion in animals

Biliary levels were determined in white male rats (Sprague Dawley strain, each weighing 200~270 g), male rabbits (2.0~2.5 kg) and male mongrel dogs (10~12 kg). The animals were anesthetized with ether and cannulated with a polyethylene tube by a standard laboratory procedure. Cefazolin and the related antibiotics were administered by various routes to test animals at a single dose of 20 mg/kg. Bile samples were collected at certain intervals over a period of 24 hours after administration.

7. Determination of tissue distribution in animals

Sprague Dawley white male rats each weighing 200~270 g were used. The rats were fasted 24 hours prior to use, water always being available. Cefazolin and related antibiotics were given subcutaneously or intramuscularly to 12 rats. At 0.5, 1, 2 and 3 hours after dosage, 3 rats in each group were sacrificed by cervical dislocation. The liver, kidneys, lungs, heart and spleen were each removed, washed with normal saline and homogenized with 3-fold volumes of 99 % ethanol in a Waring Blendor. The homogenates were then centrifuged at 8,000 rpm for 10 minutes to obtain the supernatants for bioassay. Blood samples were simultaneously collected to determine the antibiotic activity in the sera. A similar procedure was applied to rabbits to examine the tissue distribution after intravenous, subcutaneous and intramuscular administration.

8. Identification of active substances excreted into human urine

Cefazolin was administered intramuscularly to two healthy volunteers at a single dose of 250 mg. The urine was collected over a period of 24 hours after administration, and examined by thin-layer chromatography and bioautography. The standard cefazolin solution (1 mg/ml), and urine samples (0~3, 3~6 and 6~24 hour) were run for chromatographic examination by the use of the Chromagram Sheet (Eastman). The sheet was then dried and developed to a height 12~15 cm at room temperature by ascending technique.

a) Thin-layer chromatography

Solvent system;

n-BuOH – AcOH – water (4 : 1 : 5, top layer)
n-BuOH – EtOH – water (4 : 1 : 5)
MeOH – *n*-PrOH – water (6 : 1 : 2)

Adsorbent;

Eastman Chromagram Sheet No. 6061 (Silica gel)
" " " No. 6064 (Cellulose)

b) Bioautography: The dried sheet was then plated on the agar plate which had been seeded with 0.2 % of the spore suspension (2×10^8 spores per ml) of *B. subtilis* strain ATCC 6633.

Results

1. Serum Levels in Rats, Rabbits and Dogs

Serum levels were determined in rats, rabbits and dogs by intravenous, intramuscular and subcutaneous routes at a single dose of 10 or 20 mg/kg (Table 1).

(1) Intravenous administration

When cefazolin was administered intravenously at 20 mg/kg to 4 rabbits, the serum levels reached a maximum (166 mcg/ml, mean value) 5 minutes after administration, and the peak level was twice as high as with cephaloridine (73.5 mcg/ml), 6 times that with cephalothin (24.3 mcg/ml) and 4 times that with ampicillin (37.3 mcg/ml). However, serum levels of cefazolin declined rapidly, with little antibiotic activity remaining in the 2-hour serum, as was the case with cephalothin and ampicillin. On the other hand, a measurable level of activity was found in the 3-hour serum sample after administration of cephaloridine.

(2) Intramuscular administration

In this experiment, cefazolin and the other antibiotics were administered intramuscularly to groups each consisting of 3 rats and 4 rabbits at a single dose of 20

Table 1. Mean serum levels after a single dose of cefazolin and related antibiotics (20 or 10 mg/kg) in rats, rabbits and dogs

Route and dose	Animal species	No. of test animals	Antibiotic	Mean serum level (mcg/ml)					
				5 min.	15 min.	30 min.	1 hr.	2 hrs.	3 hrs.
Intravenous administration (20 mg/kg)	Rabbit 2.0~2.5 kg	4	CEZ	166.0	83.3	31.3	8.8	<2.0	<1.0
			CER	73.5	44.1	25.3	17.9	5.4	3.5
			CET	24.3	12.6	5.5	2.0	<0.4	<0.4
			AB-PC	37.3	14.5	5.5	1.7	<0.5	<0.5
Intramuscular administration (Rat & Rabbit; 20 mg/kg, Dog; 10 mg/kg)	Rat 200~270 g	3	CEZ	70.0	65.0	19.0	4.5	<2.0	—
			CER	24.5	10.0	1.3	0.5	<0.25	—
			CET	7.2	1.6	<0.1	<0.1	<0.1	—
			AB-PC	14.3	2.5	0.2	0.1	<0.1	—
	Rabbit 2.0~2.5 kg	4	CEZ	72.3	33.7	8.4	2.7	<1.1	<1.1
			CER	30.0	15.8	3.6	1.3	0.2	<0.1
			CET	8.1	3.6	<0.4	<0.4	<0.4	<0.4
			AB-PC	16.3	8.7	2.7	<0.5	<0.5	<0.5
	Dog 10~12 kg	5	CEZ	23.4	16.6	7.5	2.9	<0.5	<0.5
			CER	20.4	16.0	6.1	2.2	0.6	<0.3
			CET	7.4	4.2	1.1	0.6	<0.1	<0.1
			AB-PC	16.2	10.0	3.5	0.8	0.2	<0.02
				30 min.	1 hr.	2 hrs.	3 hrs.	5 hrs.	7 hrs.
Subcutaneous administration (20 mg/kg)	Rat 200~270 g	3	CEZ	64.0	40.0	16.0	4.5	<2.0	—
			CER	16.5	9.9	1.9	0.3	<0.25	—
			CET	6.9	1.4	<0.1	<0.1	<0.1	—
			AB-PC	9.6	3.8	0.6	0.2	<0.2	—
	Rabbit 2.0~2.5 kg	4	CEZ	33.1	25.6	15.7	11.1	<1.3	<1.3
			CER	19.5	15.5	5.6	1.8	0.5	0.1
			CET	10.5	5.6	1.3	0.4	<0.4	<0.4
			AB-PC	21.7	10.1	<3.2	<0.5	<0.5	<0.5

mg/kg, and to a group of 5 dogs at 10 mg/kg.

In rats and rabbits, the serum levels depended to a great extent on the kind of antibiotic tested, and were comparable to the results after subcutaneous administration. For instance, in the rats, the maximum concentrations were 70.0 mcg/ml (mean value) for cefazolin, 24.5 mcg/ml for cephaloridine, 14.3 mcg/ml for ampicillin and 7.2 mcg/ml for cephalothin. However, in dogs, no significant difference in serum levels was found between cefazolin and cephaloridine.

(3) Subcutaneous administration

Cefazolin and related antibiotics, each at a single dose of 20 mg/kg, were administered subcutaneously to groups each consisting of 3 rats and 4 rabbits. The maximum serum levels were found 30 minutes after administration with all the antibiotics tested. Furthermore, the maximum serum levels of cefazolin were higher than those of other antibiotics tested. The mean maximum serum concentration for cefazolin was 64.0 mcg/ml in the rats and 33.1 mcg/ml in the rabbits, and these values were 4 and 1.5 times higher than those obtained with cephaloridine in the same species of animals. Therapeutically effective levels of cefazolin were also attained in the 3-hour sera of both species of animals, while little or no activity remained at this time when the other antibiotics were administered. Thus, subcutaneous administration provided

Table 2. Urinary excretion after a single dose of cefazolin and related antibiotics (20 or 10 mg/kg) in rats, rabbits and dogs

Route and dose	Animal species	No. of test animals	Antibiotic	Mean urine level (mcg/ml)			Total % in 24 hrs.
				0~3 hrs.	3~6 hrs.	6~24 hrs.	
Intravenous administration (20 mg/kg)	Rabbit 2.0~2.5 kg	4	CEZ	1149.2	20.7	1.3	88.4
			CER	1546.0	62.5	0.1	55.2
			CET	1516.0	74.5	—	33.6
			AB-PC	1435.9	65.5	0.1	70.5
Intramuscular administration (Rat & Rabbit; 20 mg/kg, Dog; 10 mg/kg)	Rat 200~270 g	3	CEZ	3400.0	677.0	3.4	81.8
			CER	3125.0	487.0	5.7	47.9
			CET	1885.0	45.3	1.2	30.1
			AB-PC	2850.0	446.0	2.3	60.8
	Rabbit 2.0~2.5 kg	4	CEZ	2780.0	166.5	0.5	96.7
			CER	2451.3	109.4	0.3	45.3
			CET	1503.1	56.9	—	32.8
			AB-PC	1074.0	116.5	0.2	65.0
	Dog 10~12 kg	5	CEZ	1512.0	91.0	13.0	80.4
			CER	1180.0	120.0	2.0	71.9
			CET	2438.0	207.0	0.3	49.6
			AB-PC	1026.0	42.3	5.6	29.1
Subcutaneous administration (20 mg/kg)	Rat 200~270 g	3	CEZ	3150.0	380.0	7.4	74.7
			CER	3088.0	162.0	4.9	54.3
			CET	845.0	85.0	≦ 2.8	25.1
			AB-PC	2125.0	712.0	2.8	53.6
	Rabbit 2.0~2.5 kg	4	CEZ	1356.8	334.6	8.4	76.3
			CER	1126.1	128.4	0.2	37.4
			CET	880.6	471.9	0.1	36.8
			AB-PC	995.0	87.0	0.3	43.2

different results in serum levels from those of intravenous administration.

2. Urinary Excretion in Rats, Rabbits and Dogs

Urinary levels of cefazolin and related antibiotics were determined in rats, rabbits, and dogs, by intravenous, subcutaneous and intramuscular administration at a single dose of 20 mg/kg. The results are shown in Table 2.

(1) Intravenous administration

Groups each consisting of 4 rabbits were used in this experiment. The average rate of recovery in the 24-hour urine samples was 88.4 % for cefazolin, 55.2 % for cephaloridine, 33.6 % for cephalothin and 70.5 % for ampicillin. Irrespective of the antibiotics tested, the maximum urinary concentrations exceeding 1,000 mcg/ml were established in the initial 3-hour urine samples. The levels declined rapidly thereafter, only traces being recovered in the 6 to 24-hour urine.

(2) Intramuscular administration

Cefazolin and related antibiotics were each administered intramuscularly to groups of 3 rats and 4 rabbits at a single dose of 20 mg/kg, and to 5 dogs at 10 mg/kg. Higher amounts of cefazolin were recovered in the 24-hour urines of each species in comparison with the other antibiotics. In dogs, the total recovery was 80.4 % for the administered dose of cefazolin, 71.9 % for cephaloridine, 49.6 % for cephalothin and 29.1 % for ampicillin.

(3) Subcutaneous administration

Cefazolin and related antibiotics were given to groups of 3 rats and 4 rabbits at 20 mg/kg. As shown in Table 2, higher recoveries of cefazolin was noted in the 24-hour urine of rats as compared with other antibiotics. For instance, the total recovery in the rat urine was 74.7 % for cefazolin, 54.3 % for cephaloridine, 25.1 % for cephalothin and 53.6 % for ampicillin. On the other hand, the maximum urinary concentrations of cefazolin and cephaloridine, above 3,000 mcg/ml, were established in the initial 3-hour urine and therapeutically effective levels were maintained for at least 6 hours. Rather similar results were also obtained in rabbits.

Table 3. Serum levels after intramuscular administration of cefazolin and cephaloridine in five healthy volunteers

Antibiotic	Volunteer	Serum level (mcg/ml) 1/2 hr.	1 hr.	3 hrs.	6 hrs.	8 hrs.	10 hrs.
CEZ 250 mg	T. ♂ 70.5 kg	22.0	29.0	14.0	3.8	2.0	<1.0
	F. ♂ 60.0	26.0	29.0	15.0	3.9	1.0	<1.0
	K. ♂ 60.0	30.0	31.0	20.0	5.1	2.9	1.0
	A. ♂ 62.0	24.0	29.0	14.0	5.4	1.4	<1.0
	N. ♂ 61.0	25.0	31.0	15.0	6.6	3.3	1.0
	Mean	25.4* ±1.3	29.8 ±0.5	15.6 ±1.1	5.0 ±0.5	2.1 ±0.4	
CEZ 500 mg	T. ♂ 70.5 kg	34.0	45.0	27.0	8.3	3.9	2.3
	F. ♂ 60.0	40.5	42.0	25.5	8.4	4.9	2.3
	K. ♂ 60.0	41.8	48.0	34.5	11.2	7.7	4.6
	A. ♂ 62.0	54.0	52.0	29.5	7.5	3.9	2.1
	N. ♂ 61.0	34.0	36.2	31.0	9.8	6.2	4.4
	Mean	40.1 ±4.1	44.6 ±2.7	29.5 ±1.6	9.0 ±0.7	5.3 ±0.7	3.1 ±0.6
CER 500 mg	T. ♂ 70.5 kg	16.0	15.0	5.3	1.2	0.6	0.2
	F. ♂ 60.0	15.8	14.2	7.0	1.8	0.7	0.3
	K. ♂ 60.0	13.0	9.8	7.4	2.6	1.3	0.5
	A. ♂ 62.0	19.0	18.0	9.0	1.5	0.9	0.5
	N. ♂ 61.0	22.0	17.0	9.5	4.0	1.9	0.9
	Mean	17.2 ±1.5	14.8 ±1.4	7.6 ±0.8	2.2 ±0.5	1.1 ±0.2	0.5 ±0.1

* Standard error

Five male adult volunteers, 60.0~70.5 kg in weight, were each administered intramuscularly cefazolin at a single dose of 250 mg and 500 mg, and cephaloridine at a single dose of 500 mg.

Table 4. Urinary excretion after intramuscular administration of cefazolin and cephaloridine in five healthy volunteers

Antibiotic	Volunteer	0~1 hr.		1~3 hrs.		3~6 hrs.		6~8 hrs.		8~10 hrs.		10~24 hrs.		Total in 24 hrs.	
		mcg/ml	mg	mcg/ml	mg	mcg/ml	mg	mcg/ml	mg	mcg/ml	mg	mcg/ml	mg	mg	%
CEZ 250 mg	T.	520	54.6	860	94.6	770	65.5	260	19.8	130	6.1	20	9.2	249.6	99.8
	F.	810	55.1	950	86.5	330	56.1	145	22.3	62	7.7	12	9.4	237.1	94.8
	K.	1410	46.5	1720	72.2	1120	67.2	485	24.7	260	7.8	46	12.0	230.4	92.2
	A.	580	56.4	860	103.2	390	58.5	—	—	100	18.0	8.2	5.1	241.2	96.5
	N.	1030	43.3	940	102.5	520	55.1	260	26.5	95	4.8	29	8.7	240.9	96.4
	Mean	870	51.2 ±2.6*	1066	91.8 ±5.7	626	60.5 ±2.4	288	14.7 ±1.4	126	8.9 ±2.3	13.9	8.9 ±3.1	239.8 ±3.1	95.9 ±1.2
CEZ 500 mg	T.	420	31.5	1200	224.4	1200	114.0	510	24.5	180	10.4	18	14.2	419.0	83.8
	F.	1150	98.9	1250	133.8	780	115.4	300	25.2	90	10.3	9	7.7	391.3	78.3
	K.	960	61.4	1050	199.5	1150	126.5	500	24.5	140	17.9	23	18.4	448.2	89.6
	A.	750	85.5	1250	190.0	690	110.4	260	20.8	150	12.3	16	10.1	429.1	85.8
	N.	1400	53.2	1890	117.2	1400	64.4	720	76.3	340	43.5	40	18.0	372.6	74.5
	Mean	936	66.1 ±11.9	1328	173.0 ±20.4	1044	106.1 ±10.8	458	34.3 ±10.5	180	18.9 ±6.3	21.2	13.7 ±2.1	412.0	82.4 ±2.7
CER 500 mg	T.	1100	123.2	940	127.8	470	65.8	195	15.8	70	5.6	9	6.7	345.0	69.0
	F.	1250	76.3	1300	118.3	540	75.1	130	14.3	74	6.7	8.4	6.8	297.5	59.5
	K.	1420	61.1	1650	133.7	920	75.4	640	28.0	240	11.3	26	11.2	320.7	64.1
	A.	1400	141.4	1400	201.6	520	91.0	140	10.1	79	5.5	10	5.6	455.2	91.0
	N.	1500	72.0	1100	133.1	540	93.4	138	19.7	58	11.2	18	6.7	336.1	67.2
	Mean	1334	94.8 ±15.7	1278	142.9 ±14.9	598	80.1 ±5.2	249	17.6 ±3.0	104	8.1 ±1.3	14.3	7.4 ±0.9	350.9 ±27.2	70.2 ±5.4

* Standard error

Five male adult volunteers, 60.0~70.5 kg in weight, were each administered intramuscularly cefazolin at a single dose of 250 mg and 500 mg, and cephaloridine at a single dose of 500 mg.

3. Serum Levels and Urinary Excretion in Healthy Volunteers after Intramuscular Administration

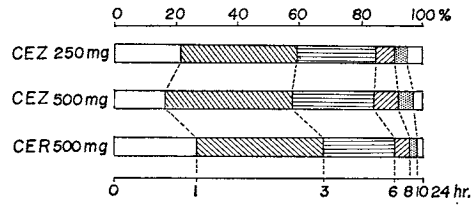
(1) Serum levels

The serum levels were determined after a single intramuscular administration to five healthy volunteers. The results are shown in Table 3. When single dose of cefazolin (250 or 500 mg) were given, mean peak serum levels of 29.8 and 44.6 mcg/ml respectively were attained 1 hour after administration and measureable concentrations persisted for 8~10 hours after administration.

On the other hand, when cephaloridine at a single dose of 500 mg was administered to the same subjects, the peak concentration of 17.2 mcg/ml was reached at 30 minutes, and the measurable activity still remained 8 hours after admisnistration.

The peak serum levels obtained after a single administration of 250 or 500 mg of cefazolin were twice or three times as high as that obtained by a single administration of 500 mg of cephaloridine. The mean serum levels of cefazolin were higher than those of cephaloridine whenever the levels were examined.

Fig. 1. Hourly percentage excretion from the total antibiotic recovery in the 24-hour urine after intramuscular administration of cefazolin and cephaloridine to five healthy volunteers.



(2) Urinary excretion

Urinary excretion was simultaneously studied in these volunteers. The results are shown in Table 4. The amounts of recovered antibiotic in the 24-hour urine samples were 95.9 % for 250 mg of cefazolin, 82.4 % for 500 mg of cefazolin and 70.2 % for 500 mg of cephaloridine.

On the other hand, the maximum urinary concentrations of cefazolin in the 3-hour urine samples were 1,066 mcg/ml for a dose of 250 mg and 1,328 mcg/ml for 500 mg. Furthermore, measurable levels of cefazolin were still excreted in the 10~24 hours' urine. Fig. 1 shows comparative results for the hourly excretion of the total amounts of antibiotics. These results indicate that the

Fig. 2. Bioautograms of urine after intramuscular administration of cefazolin.
Urine: Healthy volunteer (adult), single dose 250 mg
TLC: Adsorbent, Eastman Chromagram Sheet 6061; Solvent system, BuOH - AcOH - H_2O (4 : 1 : 5, top layer)

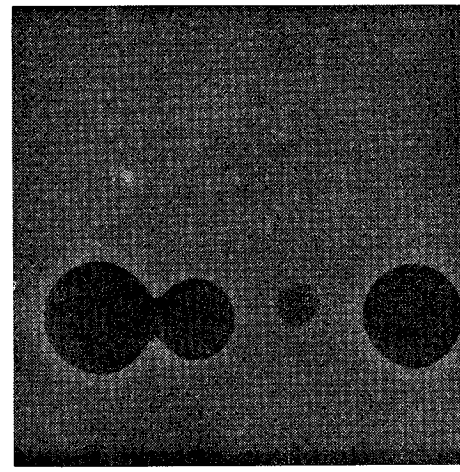


Table 5. Mean biliary excretion after a single dose (20 mg/kg) of cefazolin and related antibiotics in rats, rabbits and dogs

Route	Animal species	No. of test animals	Antibiotic	Mean bile level (mcg/ml)				Total % in 24 hrs.
				0~2 hrs.	2~4 hrs.	4~8 hrs.	8~24 hrs.	
Intravenous administration	Rabbit 2.0~2.5 kg	4	CEZ	19.1	—	—	—	1.3
			CER	8.5	1.3	0.5	0.02	0.4
			CET	6.1	0.3	—	—	0.3
			AB-PC	16.0	4.1	0.6	—	0.8
Intramuscular administration	Rat 200~270 g	3	CEZ	379.0	171.0	34.3	—	17.0
			CER	13.1	9.0	4.0	—	0.6
			CET	32.3	1.4	—	—	1.0
			AB-PC	314.3	75.5	12.1	0.9	12.0
	Rabbit 2.0~2.5 kg	4	CEZ	9.4	4.8	1.4	—	0.6
			CER	5.3	4.4	1.8	—	0.5
			CET	1.1	1.2	0.4	—	0.1
			AB-PC	15.9	17.8	6.9	—	1.2
	Dog 10~12 kg	5	CEZ	120.9	699.0	923.0	113.0	3.3
			CER	6.1	14.3	9.2	1.7	0.1
			CET	72.2	21.8	10.0	1.4	0.2
			AB-PC	71.4	252.6	212.0	47.0	1.4
Subcutaneous administration	Rat 200~270 g	3	CEZ	500.0	297.5	31.0	—	23.5
			CER	14.4	12.4	8.3	—	0.7
			CET	23.0	4.3	0.8	—	0.8
			AB-PC	240.0	190.0	28.0	—	9.6
	Rabbit 2.0~2.5 kg	4	CEZ	7.7	6.2	1.0	—	0.5
			CER	3.7	5.9	1.0	—	0.4
			CET	4.1	1.2	0.2	—	0.2
			AB-PC	12.4	15.3	3.1	—	1.4

excretion of cefazolin is somewhat slower than that of cephaloridine after intramuscular administration.

4. Identification of Microbiologically Active Substances in Human Urine

Despite the use of different solvent systems and absorbents, only one inhibition zone was obtained on the bioautogram. The position of the inhibition zone was identical with that of authentic cefazolin. One of the bioautograms are shown in Fig. 2.

The results indicated that the active substance in the urine was unchanged cefazolin. There were no metabolite having antimicrobial activity in the human urine.

5. Biliary Excretion

Biliary excretion of cefazolin was compared with those of other antibiotics in rats, rabbits and dogs. As shown in Table 5, since cefazolin and other antibiotics were mostly excreted by the urinary route, their excretion into the bile was of low order. However, this study revealed that cefazolin was excreted into the bile to a greater extent than that of cephaloridine or cephalothin. For instance, when cefazolin was administered to rats subcutaneously or intramuscularly at a single dose of 20 mg/kg, as much as 20 % of the dose was recovered in the 8-hour bile.

Another notable finding was that, in dogs receiving an intramuscular dose of 20 mg/kg of cefazolin, therapeutically effective bile levels (120~923 mcg/ml) were

Table 6. Tissue distribution after intramuscular or subcutaneous administration of a single dose of cefazolin and related antibiotics (20 mg/kg) in rats

Route	Antibiotic	Time (hrs.)	Tissue levels (mcg/g or mcg/ml)					
			Liver	Kidney	Lung	Heart	Spleen	Serum
Intramuscular administration	CEZ	1/2	14.0	45.2	12.5	5.2	2.8	70.0
		1	8.8	34.8	7.4	4.8	2.2	65.0
		2	1.0	18.0	3.2	1.3	<1.0	19.0
		3	<0.2	3.0	0.6	1.1	<1.0	4.5
	CER	1/2	9.8	70.0	7.8	3.1	2.5	24.5
		1	6.8	34.4	3.5	1.5	1.2	10.0
		2	4.6	8.8	0.8	0.3	1.0	1.3
		3	1.0	1.5	0.4	0.1	0.2	0.5
	CET	1/2	3.4	8.4	0.6	0.4	<0.9	7.2
		1	<0.2	2.3	<0.3	<0.2	<0.9	1.6
		2	<0.2	<0.3	<0.3	<0.2	<0.9	<0.1
		3	<0.2	<0.3	<0.3	<0.2	<0.9	<0.1
	AB-PC	1/2	50.8	62.0	4.5	1.7	1.8	14.3
		1	23.2	19.2	1.2	0.2	0.4	2.5
		2	4.6	2.3	0.2	<0.2	<0.1	0.2
		3	1.4	1.6	0.2	<0.2	<0.1	0.1
Subcutaneous administration	CEZ	1/2	22.4	45.6	19.6	7.6	3.7	64.0
		1	8.6	38.4	9.2	3.6	2.0	40.0
		2	3.2	11.0	3.2	<2.0	<2.0	16.0
		3	<2.0	5.1	<2.0	<2.0	<2.0	4.5
	CER	1/2	7.6	84.0	7.2	3.2	2.0	16.5
		1	6.0	36.8	2.9	0.8	1.4	9.9
		2	3.2	9.6	1.0	0.4	0.9	1.9
		3	1.9	2.5	<0.5	<0.3	0.6	0.3
	CET	1/2	1.2	8.2	1.4	1.0	<0.5	6.9
		1	<0.3	1.8	<0.3	<0.3	<0.5	1.4
		2	<0.3	<0.3	<0.3	<0.3	<0.5	<0.1
		3	<0.3	<0.3	<0.3	<0.3	<0.5	<0.1
	AB-PC	1/2	30.4	30.4	3.0	1.4	1.4	9.6
		1	18.4	14.0	1.6	0.5	1.0	3.8
		2	4.8	2.2	<0.2	<0.2	<0.2	0.6
		3	1.4	0.8	<0.2	<0.2	<0.2	0.2

Sprague Dawley white male rats each weighing 200~270 g were used. Cefazolin and related antibiotics were each given subcutaneously or intramuscularly to 12 rats. At 0.5, 1, 2 and 3 hours after dosage, 3 rats in each group were sacrificed by cervical dislocation. The liver, kidneys, lungs, heart and spleen were each removed. The supernatants obtained from the homogenates were used for bioassay.

maintained as long as 8 hours. The total recovery in the 24-hour bile was 3.3 % for cefazolin, 0.1 % for cephaloridine, 0.2 % for cephalothin and 1.4 % for ampicillin. The results indicated that the biliary excretion of cefazolin was of a similar order to that of ampicillin, and higher than those of cephaloridine and cephalothin.

6. Tissue Distribution in Rats and Rabbits

Tissue distribution of cefazolin was compared with that of related antibiotics in rats and rabbits, after intravenous, intramuscular and subcutaneous administration at a single dose of 20 mg/kg. The results are shown in Tables 6 and 7.

Table 7. Tissue distribution at 30 minutes after a single dose of cefazolin and related antibiotics (20 mg/kg) in rabbits

Route	Antibiotic	Tissue levels (mcg/g or mcg/ml)						
		Liver	Kidney	Lung	Heart	Spleen	Muscle	Serum
I. V.	CEZ	16.3	348.0	22.8	18.4	9.3	4.1	135.0
	CER	33.2	499.0	22.0	8.6	17.6	6.9	89.0
	CET	7.6	171.0	4.0	2.7	4.0	2.0	53.0
	AB-PC	5.6	317.0	14.4	9.0	7.8	3.3	93.0
I. M.	CEZ	4.4	102.8	9.6	7.4	<1.0	<2.3	96.7
	CER	7.7	206.0	8.6	5.0	4.1	1.9	33.5
	CET	0.9	14.9	1.4	0.9	<0.5	<0.7	7.4
	AB-PC	7.1	83.0	9.4	4.6	2.2	0.2	26.5
S. C.	CEZ	3.3	100.5	7.6	7.2	<2.3	<1.6	61.8
	CER	10.4	118.0	6.1	4.6	2.8	1.8	17.2
	CET	1.6	46.3	1.9	2.0	<0.3	<0.5	15.5
	AB-PC	2.3	120.9	5.6	4.4	1.9	1.7	18.7

Each antibiotic was given to 4 rabbits at a single dose of 20 mg/kg by various routes. The rabbits were sacrificed at 30 minutes after administration to determine the tissue levels.

When administered intramuscularly to rats, cefazolin was well distributed in various tissues tested. The maximum concentrations in the kidney (45.2 mcg/g) and liver (14.0 mcg/g) were somewhat lower than those of ampicillin or cephaloridine, whereas the concentrations of cefazolin in the lung (12.5 mcg/g) and heart (5.2 mcg/g) were slightly higher than those of other antibiotics. Measurable concentrations of cefazolin were maintained in the all tissues except spleen for 2 hours after administration. A similar tendency was observed after subcutaneous administration in rats and by various routes in rabbits.

It was noteworthy that cefazolin, in spite of its high serum level, gave a lower level than that of cephaloridine in the kidney when given to rats or rabbits intramuscularly or subcutaneously.

Discussion

In our previous paper[1] concerning the laboratory evaluation of cefazolin, it was shown that cefazolin is a broad-spectrum antibiotic, active *in vitro* and *in vivo* against both Gram-positive and Gram-negative bacteria except for *Ps. aeruginosa*. It was also found that cefazolin is more active than related antibiotics against clinical isolates of Gram-negative rods, especially *Kl. pneumoniae* and *E. coli*. These results suggested the need for further study of its absorption, excretion and tissue distribution.

In the present paper, cefazolin was compared with cephaloridine, cephalothin and ampicillin with regard to serum levels, urinary[2] and biliary excretion and tissue distribution. The results in rats and rabbits revealed that the serum levels and urinary recovery of cefazolin were higher than those of the related antibiotics.[3,4,5,6] This merit of cefazolin was equally justified in experiment with healthy human volunteers. Cephalothin has been reported to be inactivated in the body, being rapidly converted into desacetyl compound.[7,8] In contrast, an extremely high recovery of unchanged cefazolin was

obtained in the urine and bile, indicating a high degree of stability in the body. Furthermore, the urinary levels of cefazolin in healthy volunteers were quite satisfactory for the treatment of urinary tract infections.

In biliary excretion, cefazolin displayed useful biliary levels as high as that with ampicillin which was far higher than those of cephaloridine or cephalothin. Accordingly, cefazolin was considered to be as effective as ampicillin for the treatment of biliary infections[9].

It may be supposed that the higher serum levels of cefazolin are inevitably produced by the inversely lower tissue levels. This assumption, however, was readily negated by comparing the tissue distribution of cefazolin and related antibiotics.

A series of our experimentations concerning cefazolin suggested the promising clinical usefulness of this new antibiotic.

Furthermore, experiments so far made, though unpublished, indicate cefazolin to have extremely low toxicity. Consequently cefazolin should be considered useful for clinical trials.

In addition to the above studies, its further investigation as an oral drug will be of a great concern, since cefazolin is absorbed gastro-intestinally to a certain extent.

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