ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM OF CEFMETAZOLE IN CYNOMOLGUS MONKEYS

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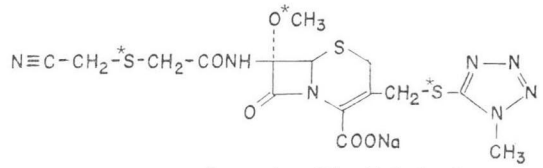
The pharmacokinetics and metabolic fate of cefmetazole (CS-1170, CMZ) were studied in cynomolgus monkeys by means of combination of radio- and bioassay techniques and that of three radioactive drugs labeled at the different moiety of the molecule. The plasma level of radioactivity after intramuscular injection of cefmetazole[methoxyl-^{14}C] (50 mg/kg) reached the maximum level (185 μg/ml) after 10 minutes and declined with a half-life of about 48 minutes. In the thin-layer chromatography of the plasma extracts, a single radioactive spot was detected corresponding to unchanged cefmetazole. Repeated intravenous administration for one and two weeks showed no effect on the plasma levels and half-lives. More than 75% of the intravenous or intramuscular dose was recovered in the urine by both radio- and bioassay, the most part being excreted during the first 3 hours period. On the TLC of urine, 93~97% was detected as unchanged cefmetazole. In the bile, the most part (>80%) was detected as unchanged cefmetazole. In the feces, about 15% of the dose was recovered, in which 30~40% was detected as intact cefmetazole. The metabolic fate of [3-^{35}S] and [7-^{35}S]cefmetazole was described and discussed. The whole-body autoradiography in the monkeys was also presented.

Cefmetazole (CMZ, CS-1170, **I**) is a new cephamycin antibiotic developed in these laboratories.[1] It has been clarified[2] that cefmetazole has a broad antimicrobial spectrum, particularly a high potential against Gram-negative organisms, and has a high stability against β-lactamases. On the other hand, its toxicity has been shown to be quite low in many experimental animals[3] and in clinical studies.[4]

It has been reported previously[5] that a large species difference was found in the blood levels and urinary excretion when cefmetazole was administered parenterally to mice, rats, rabbits, dogs and monkeys, and that monkey is the most suitable model animal for human pharmacokinetics of cefmetazole. In the present works, therefore, pharmacokinetics and metabolic fate of cefmetazole were investigated in cynomolgus monkeys in details, by means of combination of radioassay and bioassay techniques and that of three radioactive drugs labeled at the different moiety of the molecule (**I**).



I (* : Site of labeling)

Materials and Methods

Labeled Compounds

[^{14}C]Cefmetazole Na salt labeled at 7-methoxyl group was prepared in these laboratories[6] starting from [^{14}C]CH_3OH (Radiochemical Center, Amersham, England). The specific activity was 5.8 μCi/mg (2.86 mCi/mmole) and the radiochemical purity was 96.65% based on thin-layer chromatography. [7-^{35}S]Cefmetazole labeled at the side chain at 7 position was prepared in these laboratories[7] starting from [^{35}S]thiourea (New England Nuclear Corp., Boston, U.S.A.). The compound was purified and

used as dicyclohexylamine (DCHA) salt. The specific activity was 2.73 μCi/mg (1.78 mCi/mmole) and the radiochemical purity was 95.84% (TLC). [3-^{35}S] Cefmetazole labeled at the substituent at 3 position was prepared starting from [^{35}S]-5-mercaptomethyltetrazol which was prepared in Daiichi Pure Chemicals Co., Tokyo. The specific activity was 37.11 μCi/mg (18.29 mCi/mmole) and the radiochemical purity was 97.09% (TLC).

Animals and Drug Administration

Three female cynomolgus monkeys (*Macaca fascicularis*) bred in the Safety Evaluation Center of this company (Fukuroi, Shizuoka Pref.) weighing 2.9 to 3.1 kg (estimated 5~9 years old) were used. The animals were provided for the experiments fed with a constant food under the laboratory conditions: temperature of 23~24°C and humidity of 50~60%. All the experiments except for whole-body autoradiography were performed with the same animals at an interval of at least 1 month. Additional two female monkeys weighing about 2.2 kg were sacrificed for the autoradiography.

Labeled cefmetazoles were diluted with unlabeled cefmetazole to 1 μCi/mg and an aqueous solution of 200 mg/ml was administered at a dose of 50 mg/kg (50 μCi/kg), otherwise notified, to each monkey after fasting for 16 hours intramuscularly from the left femoral muscle or intravenously from the inferior saphenous vein. For the experiment of multiple administration, [^{14}C]cefmetazole was administered intravenously at the first, 7th and 14th day and unlabeled cefmetazole for the days between them at a daily dose of 50 mg/kg.

Determination of Urinary and Fecal Excretion

After administration of the labeled drug, each animal was fixed in a monkey chair and the urine and feces were collected separately for 0~3, 3~6 and 6~24 hours. After the period, each animal was placed in an individual metabolic cage and the urine and feces were collected for each 24 hours period up to 168 hours (7 days) after the administration. The urine samples, after being measured the volume, were subjected for the assay and thin-layer chromatographic analysis after an appropriate dilution. The feces were homogenized in 3 volumes of 0.1 M phosphate buffer (pH 6.0) with Polytron homogenizer, after allowing to stand for 24 hours in a cold room. The supernatant after centrifugation (3,000 rpm, 10 minutes) was assayed and analyzed. All samples were stored at −20°C until the analysis.

Determination of Plasma Concentration

The experiments were conducted separately from the excretion experiments using the same monkeys. After administration of [^{14}C]cefmetazole at a dose of 50 mg/kg, 2.5 ml of the blood samples were withdrawn from the femoral vein at 5, 10, 20, 30, 60, 120, 180 and 360 minutes. The plasma samples were obtained by an immediate centrifugation at 3,000 rpm for 10 minutes to be assayed. The thin-layer chromatographic analysis was performed after extraction of radioactive substances with ethanol. For two weeks multiple dose study, the blood samples were obtained on the first, 7th and the last days in the same way as above.

Determination of Cefmetazole and Its Metabolites

The plasma samples (1 ml) were extracted with 4 ml of ethanol by shaking for 3 minutes, followed by centrifugation at 3,000 rpm for 5 minutes. The extraction rate was over 95% by radioassay. For the fecal samples, 30 ml of the supernatants after extraction was lyophilized and the residues were shaken with 20 ml of methanol for 5 minutes followed by centrifugation at 3,000 rpm for 10 minutes. After the extraction was repeated twice, the combined extracts were concentrated to dryness *in vacuo* and the residues were redissolved in 3 ml of methanol to be subjected for TLC separation. The extraction ratio into methanol layer was more than 95%. Twenty μl of the plasma and fecal extracts and the urine sample were spotted on silica gel TLC plate (F_{254}, Merck) and developed with a solvent system of *n*-butanol - acetic acid - water - ethanol (15: 2.5: 3: 5). The radioactive spots were localized by autoradiography on exposure to X-ray film (Sakura Type N) for 1 week. After identification of cefmetazole with an authentic sample, the plate was divided into eight zones so as to separate unchanged cefmetazole and other minor radioactive spots with an appropriate width. Each zone was scraped off and transferred into counting vials quantitatively and assayed for the radioactivity. For the urine samples, two-

dimensional thin-layer chromatography was also performed using a system of acetone - ethyl acetate - acetic acid - water (16: 8: 1.5: 3) as the second solvent system.

Bioautograms were obtained from both one and two dimensional TLC plates using *Staphylococcus aureus* 209-P as a test organism.

Determination of Respiratory $^{14}CO_2$

A monkey was fixed in a monkey chair with a plastic helmet which was connected by a vinyl tube to a high-sensitive β-ray air monitor (Triton 955B, Johnston Lab., U.S.A.). After intramuscular administration of [^{14}C]cefmetazole (50 mg/kg), the respiratory air was introduced into an ionization chamber by a flow rate of 2 liters/minute and the radioactivity was detected and recorded continuously. The total amount excreted was estimated by an integral area for 24 hours period.

Whole-body Autoradiography

[^{14}C]Cefmetazole was administered intravenously to two monkeys at a dose of 50 mg (150 μCi)/kg. The animals were anesthetized with Ketalal® (5 mg/kg, i.m.) and were deep-frozen rapidly by immersing into a mixture of dry ice and hexane at 15 and 60 minutes after the administration. The frozen animals were cut off their limbs, embedded in 8% carboxymethylcellulose gel, fixed on a microtome stage and sagittal sections of 50 μ thickness were obtained in PMV 450 Cryo-microtome (Plamstieraus Mekaniska Verksted, Sweden). The sections were freeze-dried overnight in a freezing room at -14°C and brought to contact on X-ray films (Sakura ^{3}H-Type, Konishiroku Photo Ind., Tokyo) to be exposed for 14 days. The films were developed to obtain the autoradiograms.

From the remained halves of the frozen animals, two test samples each of 100 to 200 mg were obtained from the blood, liver, kidney, lung, brain and skeletal muscle in a frozen state. After weighing, they were counted for the radioactivity by combustion with a Packard Tri-Carb Sample Oxidizer (Model 306). From the animal sacrificed 60 minutes after administration, the bile sample was obtained from the gall bladder in a frozen state. Five μl sample was spotted on TLC plate and analyzed for cefmetazole and its metabolites as described above.

Microbiological Assay

Antibacterial activity of cefmetazole in biological fluid or extracts was assayed by paper-disk agar plate technique with *Bacillus subtilis* PCI 219 as a test organism. Thin-layer plates were prepared from 5 ml of Nutrient agar of pH 6.0 containing 10^7 cells/100 ml in 90×20 mm plastic dish. The specimen was diluted appropriately with 0.1 M phosphate buffer of pH 6.0 and immersed onto a paper disc (Toyo, 8 mm thick), which was incubated at 37°C for 18 hours on the agar plate. The diameter of inhibitory zone was measured and the concentration was calculated from the standard curves made from a series of diluted solution of cefmetazole ($0.8\sim50.0$ μg/ml) in 0.1 M phosphate buffer of pH 6.0. The lowest detectable concentration in a sample was 0.8 μg/ml.

Radioactivity Measurement

The radioactivity in the specimen was counted by a Packard Model 3380 liquid-scintillation spectrometer. The plasma samples (0.2 ml) were counted in 10 ml of toluene - Triton X100 scintillator (1: 1, 8 g 2,5-diphenyloxazole (PPO) and 200 mg 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1 liter). The urine samples (0.5 ml) were counted in 10 ml of toluene - ethanol scintillator (1: 1, 8 g PPO and 200 mg POPOP in 1 liter). The samples transferred from TLC plates to the vials were allowed to stand for 3 hours with 0.5 ml of methanol and counted in 10 ml of toluene - ethanol scintillator. The fecal extracts and tissue samples were counted after combustion with a Packard Model 306 Tri-Carb Sample Oxidizer. The counting efficiencies were determined by ^{137}Cs external standard method and the dpm values were converted to μg equivalent of cefmetazole based on the specific activity.

Results

Plasma Levels and Metabolites

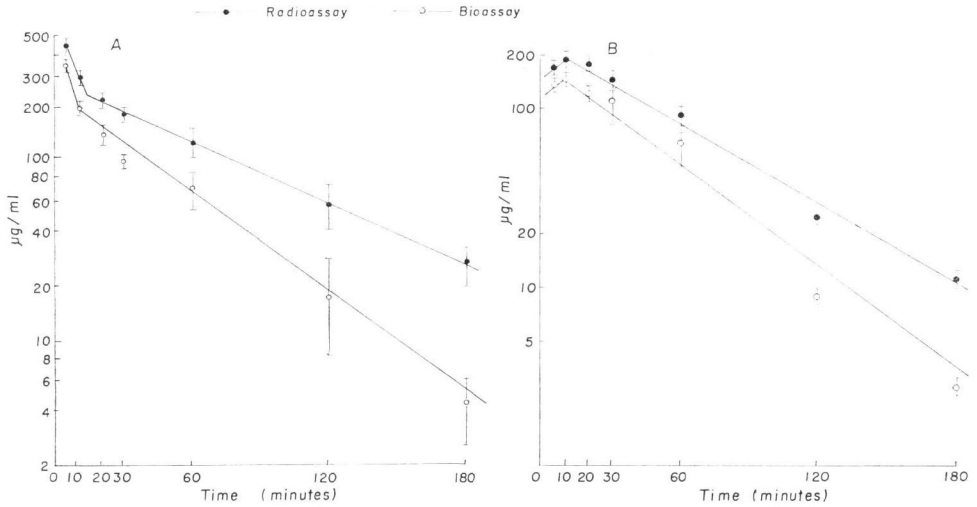
The plasma levels of cefmetazole assayed by the two methods after intravenous or intramuscular administration (50 mg/kg) of [^{14}C]cefmetazole to monkeys are shown in Table 1 and Fig. 1. The

Table 1. Plasma concentrations of cefmetazole after intravenous and intramuscular administration of [^{14}C]cefmetazole to cynomolgus monkeys.

Route of administration	Concentration, μg/ml (Mean±S.E.)*1							
	5 minutes	10 minutes	20 minutes	30 minutes	1 hour	2 hours	3 hours	6 hours
Intravenous								
Radioassay	436.2 ±37.3	292.6 ±23.9	224.0 ±19.3	186.7 ±25.1	123.7 ±25.4	55.3 ±15.9	26.3 ±6.4	13.0 ±0.8
Bioassay	352.0 ±50.8	197.3 ±17.5	142.9 ±17.2	100.8 ±16.0	68.0 ±17.9	17.3 ±10.2	4.2 ±1.8	n.d*2
Intramuscular								
Radioassay	168.5 ±4.2	184.5 ±5.6	178.2*3	139.9 ±1.3	88.8 ±13.7	24.2 ±2.6	11.2 ±1.4	4.4 ±0.4
Bioassay	132.5 ±9.0	147.5 ±10.7	122.0	113.8 ±29.3	61.2 ±9.2	8.4 ±0.6	2.7 ±0.2	n.n.

*1 Average from three experiments±standard error; dose=50 mg/kg.
*2 n.d.=Not detected.
*3 Average from two experiments.

Fig. 1. Plasma concentrations of cefmetazole after intravenous (A) and intramuscular (B) administration to cynomolgus monkeys.
Average±standard error from three experiments; dose=50 mg/kg.



half-life for the second phase after intravenous administration was about 48 and 32 minutes by radioassay and bioassay, respectively. After intramuscular administration, a peak level was achieved after 10 minutes and declined afterward. The half-life was about 37 and 32 minutes by radioassay and bioassay, respectively, being in good accordance with those after intravenous administration.

The plasma levels after multiple intravenous administrations for 1 and 2 weeks at a daily dose of 50 mg/kg are shown in Fig. 2, in which the radioactive cefmetazole was administered on the first, 7th and 14th day. By both radioassay and bioassay, no significant difference was observed in both the levels and half-lives between those after a single and multiple administrations. The half-lives observed were constant values of 43~49 minutes and 27~32 minutes by radioassay and bioassay, respectively.

Fig. 2. Plasma concentrations and half lives of [^{14}C]cefmetazole after repeated intravenous administrations to cynomolgus monkeys (50 mg/kg, n=3).

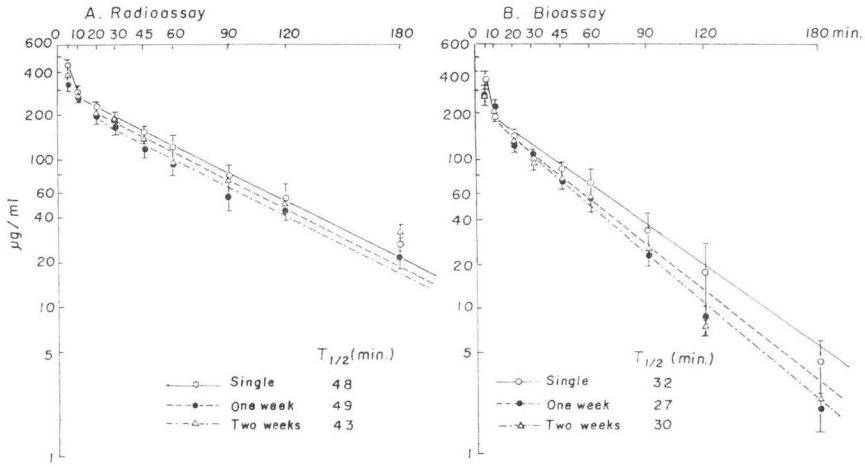
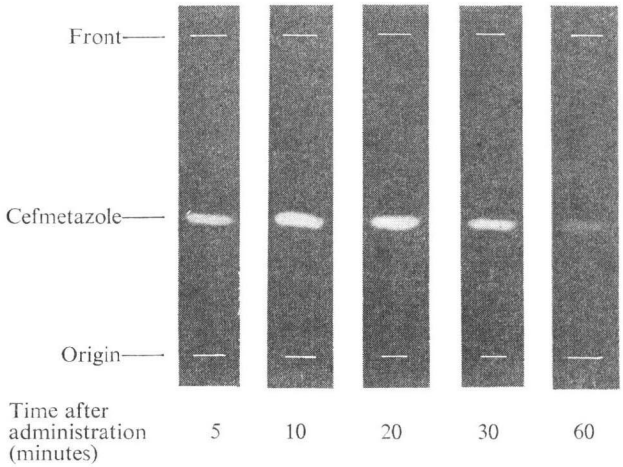


Fig. 3. TLC Autoradiograms of plasma radioactivity after intramuscular administration of [^{14}C]cefmetazole to cynomolgus monkeys (Dose: 50 mg/kg).



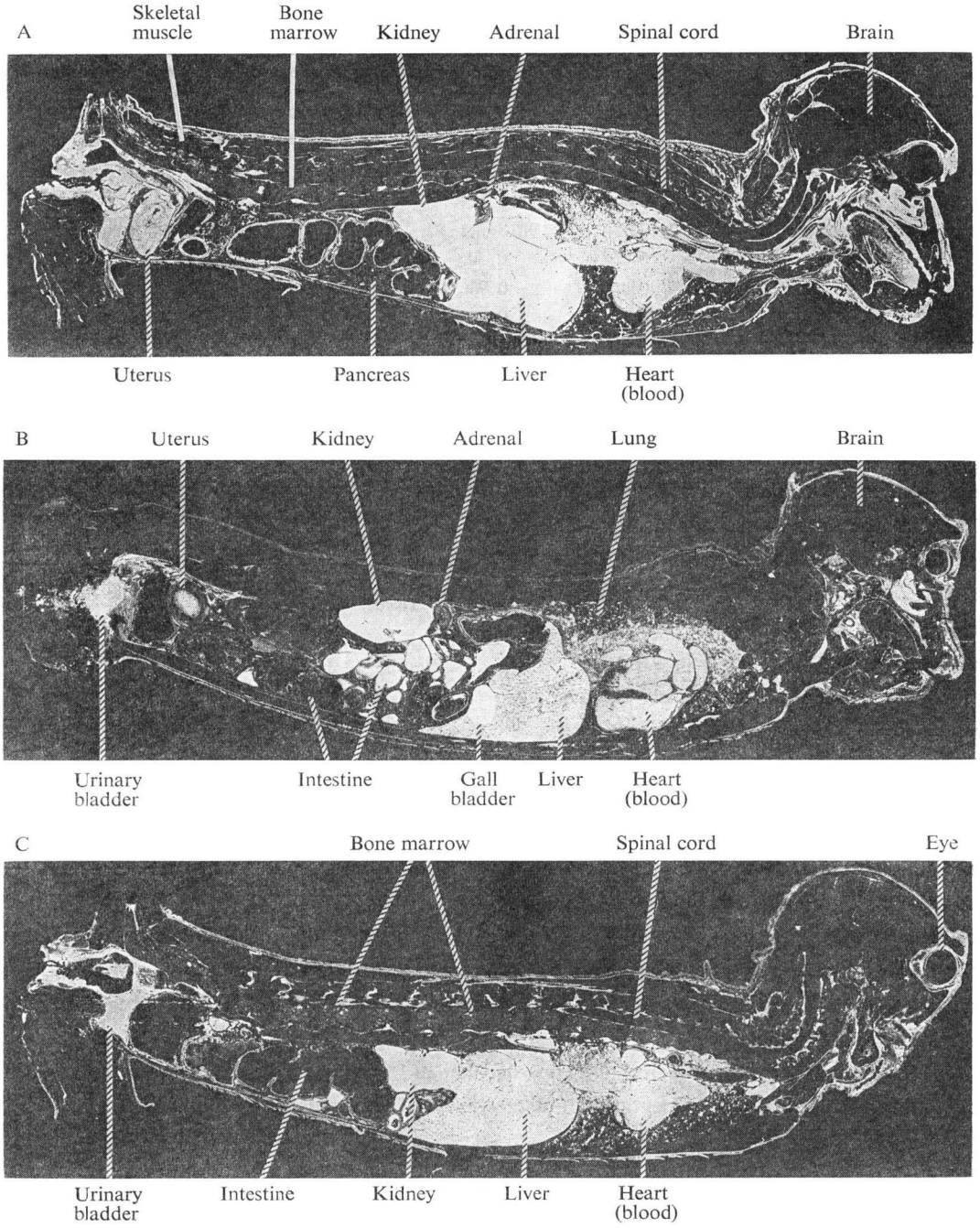
In all cases, the radioassay gave appreciably higher values as compared to bioassay. This is interpreted as being due to the effect of serum protein binding rather than the metabolic degradation. The radioassay represents the total amount in plasma, while the bioassay does mainly the "free" fraction because the calculation was made based on the standard curve from buffer solutions and cefmetazole binds to monkey plasma to an extent of about 80%.[5]

Thin-layer chromatography of the plasma extracts revealed that, as shown in Fig. 3, only a single radioactive spot was detected corresponding to unchanged cefmetazole. Counting of the radioactivity showed that unchanged cefmetazole accounted for 94 to 99% of the plasma total radioactivity in all samples during the period of 5 to 60 minutes after intramuscular administration (Table 2).

Distribution

Whole-body autoradiograms obtained from monkeys 15 and 60 minutes after intravenous administration of [^{14}C]cefmetazole are examplified in Fig. 4. Fifteen minutes after administration, the highest

Fig. 4. Whole-body autoradiograms from cynomolgus monkeys 15 minutes (A) and 60 minutes (B, C) after intravenous administration of [^{14}C]cefmetazole.



radioactivity was observed in the kidney, followed by the liver, blood, lung and intestinal mucosa. A high radioactivity distributed also in the interstitial fluid such as that in the skeletal muscle. A high concentration of radioactivity was observed in the urinary bladder, indicating a rapid excretion into the urine. A prominent radioactivity was observed in the uterus, a higher activity being localized in the

Table 2. Plasma metabolites of cefmetazole after intramuscular administration of [^{14}C]cefmetazole to cynomolgus monkeys.

TLC Zone*2	% to total radioactivity*1				
	5 minutes	10 minutes	20 minutes	30 minutes	60 minutes
1 (Origin)	0.05	0.40	0.51	0.29	0.39
2	0.02	0.47	0.53	0.78	1.17
3	0.53	0.67	0.32	0.70	1.22
4	—	0.12	0.55	0.08	0.20
5 (Cefmetazole)	98.73±1.17	96.88±1.50	95.86*3	94.64±0.95	93.95±2.08
6	0.43	0.50	0.34	0.88	0.18
7	0.17	0.93	0.83	0.87	1.21
8	0.08	0.03	0.88	1.75	1.66

*1 Average from three experiments±standard error; dose=50 mg/kg.

*2 TLC condition: silica gel F_{254} plate with a solvent system of *n*-butanol - acetic acid - water - ethanol (150: 25: 30: 50).

*3 n=2

mucosa. A low radioactivity distributed in the bone marrow and in the adrenal cortex, with devoid of activity in the medulla. Almost no radioactivity was detected in the parenchymal tissues of the central nervous system and skeletal muscle.

After 60 minutes, the highest radioactivity was observed in the kidney, urinary and gall bladders and intestinal contents, indicating that biliary excretion occurs to an appreciable extent in monkey. The concentration in the liver, blood and lung decreased considerably as compared to those after 15 minutes. In the uterus, an appreciable radioactivity remained only in the mucosa. A low concentration of radioactivity continued in the adrenal cortex, while no radioactivity was detected in the pancreas, bone marrow, spleen, skeletal muscle and brain. No radioactivity was detected in the eyeball, indicating that this drug has no affinity to melanine pigment.

Table 3. Tissue concentrations of radioactivity after intravenous administration of [^{14}C]cefmetazole to cynomolgus monkeys.

Tissue	Cefmetazole equivalent (μg/g)*	
	15 minutes	60 minutes
Blood	98.67	17.67
Liver	170.20	16.40
Kidney	825.09	77.12
Lung	83.92	16.98
Brain	1.16	0.27
Skeletal muscle	6.58	1.37

* Average from two samples; Dose=50 mg/kg.

The counting of the tissue radioactivity revealed that, at 15 minutes after the administration, the concentration was in the order: kidney ≫ liver > blood ~ lung ≫ skeletal muscle ~ brain (Table 3).

Urinary and Fecal Excretion

The urinary and fecal recoveries after intravenous and/or intramuscular administration of cefmetazole labeled at different positions are summarized in Tables 4 and 5. During the first 6 hours period after intravenous administration of [^{14}C]cefmetazole (50 mg/kg), about 70 and 63% of the dose was recovered in the urine by radioassay and bioassay, respectively. The corresponding values after intramuscular administration were 73 and 76% of the dose, respectively. The total recovery in the urine by 96 hours was 76~78% of the dose by radioassay, irrespective of the route of administration. It is apparent from these results that renal excretion was the major route of elimination of cefmetazole and a

Table 4. Urinary and fecal excretion of [^{14}C]cefmetazole after intravenous and intramuscular administration to cynomolgus monkeys.

Collection (hours)	Excretion (% to dose)*1							
	Intravenous				Intramuscular			
	Radioassay		Bioassay		Radioassay		Bioassay	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0~ 3	60.44	—	55.98	—	67.93	—	72.47	—
3~ 6	8.93	—	6.94	—	4.97	—	3.69	—
6~24	2.86	0.68	0.27	0.14	2.32	0.02	0.35	n.d.*2
24~48	1.57	0.31	n.d.	0.05	0.99	2.67	n.d.	2.21
48~72	1.08	14.21	n.d.	2.64	1.20	11.62	n.d.	3.19
72~96	0.94	2.96	n.d.	0.43	0.65	1.36	n.d.	0.09
Total excretion	75.82 ±3.69	18.16 ±2.53	63.19 ±2.66	3.26 ±1.44	78.06 ±7.73	15.66 ±5.81	76.51 ±8.58	5.48 ±1.86
Total recovery	93.99±1.23		66.44±2.06		93.72±6.26		81.99±12.53	

*1 Average from three experiments±standard error; dose=50 mg/kg.
*2 n.d.=not detected.

Table 5. Urinary and fecal excretion of [^{35}S]cefmetazole labeled at the 7- and 3-substituents after intramuscular administration to cynomolgus monkeys.

Collection (hours)	Excretion (% to dose)*			
	7-^{35}S		3-^{35}S	
	Urine	Feces	Urine	Feces
0~ 3	68.78	—	67.05	—
3~ 6	9.57	1.76	11.72	1.25
6~24	7.22		7.50	
24~48	0.49	1.78	5.64	0.35
48~72	0.28	2.15	1.53	0.16
72~96	0.26	0.78	0.31	0.11
Total excretion	86.60±4.40	6.47±2.80	93.75±0.76	1.86±0.20
Total recovery	93.07±1.97		95.60±0.82	

* Average from three experiments±standard error; dose=10 mg/kg (7-^{35}S), 50 mg/kg (3-^{35}S).

good accordance between radioassay and bioassay results suggests that cefmetazole is excreted into the urine mostly as an active form.

In the feces, 16~18 and 3~6% of the dose was recovered by radioassay and bioassay, respectively, indicating that an appreciable biliary excretion occurs and that at least a part is excreted in the feces as a biologically active form. Thus, the total recovery of radioactivity in the urine and feces exceeded 93% of the dose by 96 hours after administration. Determination of respiratory $^{14}CO_2$ revealed that cumulative excretion of the radioactivity for 24 hours period was only 1.3% of the dose, indicating that 7-methoxyl group in cefmetazole is quite stable in animal body.

After intramuscular administration of [7-^{35}S]cefmetazole (10 mg/kg), about 78 and 86% of the dose was recovered in the urine during the first 6 and 24 hours periods, while about 5% of the dose was re-

Table 6. Urinary metabolites of cefmetazole after intravenous and intramuscular administration of [^{14}C]-cefmetazole to cynomolgus monkeys.

TLC zone	% to total radioactivity*							
	Intravenous				Intramuscular			
	0~3 hours		3~6 hours		0~3 hours		3~6 hours	
	% on TLC	% to dose	% on TLC	% to dose	% on TLC	% to dose	% on TLC	% to dose
1 (Origin)	0.05	0.03	0.97	0.06	0.93	0.59	4.20	0.17
2	0.37	0.22	2.18	0.20	0.66	0.41	2.94	0.15
3	0.34	0.20	2.26	0.16	1.94	1.16	8.58	0.43
4	0.50	0.31	2.65	0.20	1.52	0.94	7.01	0.27
5 (Cefmetazole)	97.29 ±0.36	58.82 ±2.83	87.18 ±2.59	7.98 ±3.64	92.97 ±3.90	63.59 ±7.94	71.50 ±3.93	3.67 ±1.56
6	1.12	0.67	2.78	0.24	1.42	0.89	3.53	0.18
7	0.20	0.12	1.15	0.08	0.33	0.21	1.44	0.06
8 (Front)	0.12	0.07	0.30	0.02	0.23	0.16	0.82	0.04

* Average from three experiments±standard error; dose=50 mg/kg.

covered in the feces during 96 hours period. After administration of [3-^{35}S]cefmetazole, on the contrary, about 80% of the dose was recovered in the urine during the first 6 hours period and the recovery increased with time, about 86 and 94% of the dose being recovered by 24 and 96 hours, respectively. In consistence, only about 2% of the dose was recovered in the feces. As will be discussed later, this delayed and higher excretion of radioactivity at 3-position is due to the excretion of [^{35}S]methyltetrazolthiol.

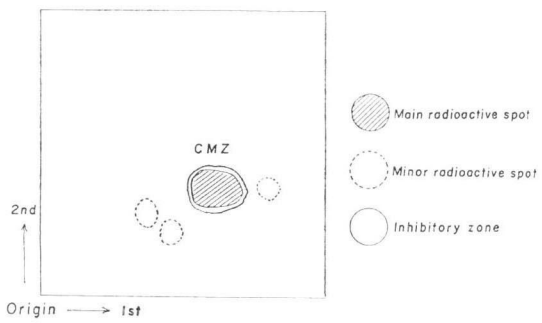
Cefmetazole and Metabolites in Urine

TLC bioautograms of the first 3 hours and 3~6 hours urine after intravenous and intramuscular

Fig. 5. Two-dimensional TLC bio- and radioautogram of monkey urine after intramuscular administration of [^{14}C]cefmetazole.

Solvent system: 1st: acetone - ethyl acetate - acetic acid - water (160: 80: 15: 30). 2nd: *n*-butanol - acetic acid - water - ethanol (150: 25: 30: 50).

Dose=50 mg/kg, 0~3 hours urine.



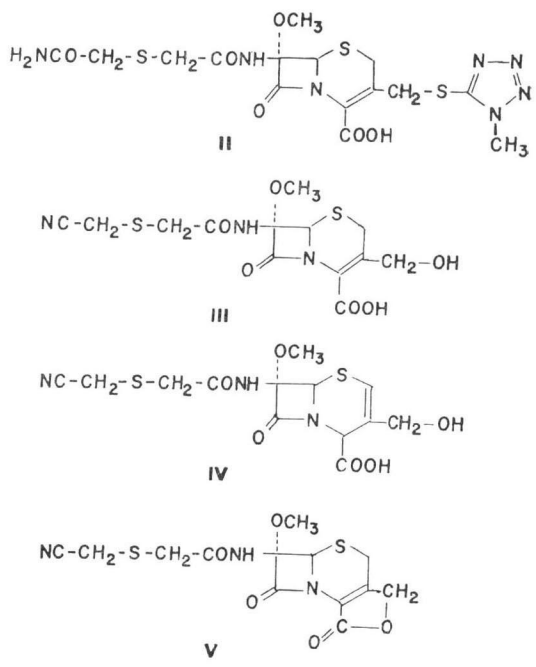


Table 7. Urinary cefmetazole and its metabolites after intramuscular administration of [^{35}S]cefmetazole labeled at the 7- and 3-substituents to cynomolgus monkeys.

TLC zone	% to total radioactivity*1				
	7-^{35}S		3-^{35}S		
	0~3 hours	3~6 hours	0~3 hours	3~6 hours	6~24 hours
1 (Origin)	0.13	1.04	0.18	2.43	8.62
2	0.54	1.66	0.34	0.34	4.28
3	0.35	2.03	0.48	2.43	4.50
4 (Cefmetazole)	90.36±0.64	93.22±2.77	95.23±0.68	71.92±1.20	36.45±2.33
5	0.45	1.58	0.74	1.77	2.74
6	0.14	0.39	0.30	0.97	2.53
7 (Me-TZT)*2	—	—	2.63±0.51	19.95±0.32	40.68±4.02
8	0.03	0.02	0.10	0.19	0.18

*1 Average from three experiments±standard error; dose=10 mg/kg (7-^{35}S), 50 mg/kg (3-^{35}S).
*2 Methyltetrazolthiol.

administration of [^{14}C]cefmetazole showed a single inhibitory zone corresponding to unchanged cefmetazole. The autoradiograms showed the main radioactive spot corresponding to unchanged cefmetazole, but two or three faint radioactive spots were also detected. The counting of the TLC plates revealed, however, that the most part, 97 and 93% of the total radioactivity in 3 hours urine after intravenous and intramuscular administration, respectively, was detected as unchanged cefmetazole (Table 6). In two-dimensional TLC autoradiograms, as shown in Fig. 5, three minor radioactive spots were detected, but none of them corresponded to any of four authentic samples of the postulated metabolites (**II**~**V**).

In the urine after administration of [^{35}S]cefmetazole labeled at 7-side chain, three extremely faint radioactive spots were detected at the same positions as those from [^{14}C]cefmetazole in addition to the main spot of unchanged drug, while no radioactive spot was detected corresponding to any of five reference compounds (**VI**~**X**) of the postulated metabolites derived from the side chain degradation. The counting of the plates revealed that unchanged cefmetazole accounted for 98.4 and 93.2% of the total radioactivity in the first 3 hours and 3~6 hours urine, respectively, while the minor spots accounted for less than 2% (Table 7).

In considering the fact that a few % of radioactive impurity is contained in the labeled compounds, these results indicate that cefmetazole is not suffered from any appreciable metabolic change.

VI $NC-CH_2-S-CH_2-COOH$

VII $NC-CH_2-S-CH_2-CONH_2$

VIII $NC-CH_2-S-CH_2-CONHCH_2-COOH$

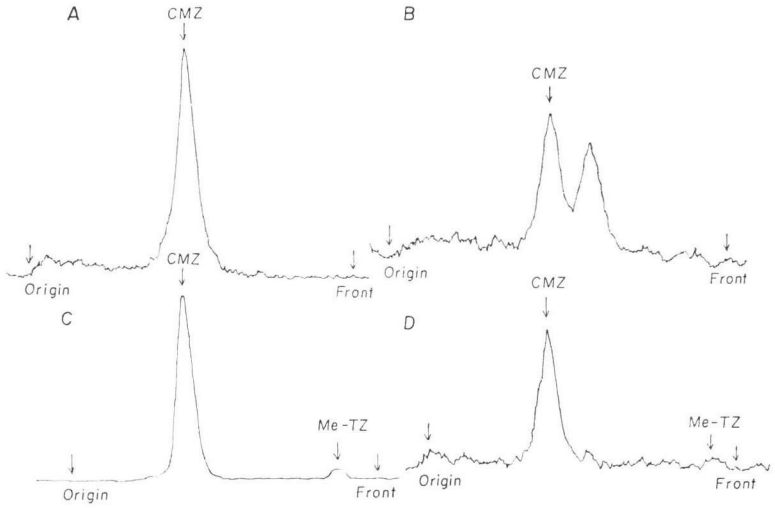
IX $NC-CH_2-S-CH_2-CONHCH_2-COOCH_3$

X
$$NC-CH_2-S-CH_2-CONH-\underset{\displaystyle OCH_3}{\underset{|}{CH}}-CHO$$

In the urine after administration of [^{35}S]cefmetazole labeled at 3-substituent, on the other hand, a new radioactive spot appeared at a high Rf region of the TLC autoradiograms corresponding to *N*-methyltetrazolthiol (Fig. 6-C). In the first 3 hours urine, 95.2% of the total radioactivity was detected as unchanged cefmetazole, while 2.6% as *N*-methyltetrazolthiol. In the urine collected during later periods, the percentage of the latter increased to 20 and 40.7% in 3~6 and 6~24 hours urine, respecti-

Fig. 6. TLC radiochromatograms of urinary (A, C) and fecal (B, D) radioactivity after intravenous and intramuscular administration of [^{14}C] (A, B) and [3-^{35}S]cefmetazole (C, D), respectively, to cynomolgus monkeys.

Urine: 0~3 hours, feces: 48~72 hours, dose=50 mg/kg, silica gel thin-layer plate F_{254}, solvent system: *n*-butanol - acetic acid - water - ethanol (150: 25: 30: 50).



vely (Table 7). The total excretion of [^{35}S]-*N*-methyltetrazolthiol during 24 hours period was estimated to be about 8.3% of the total radioactivity and 7.2% of the dose, while those of unchanged [^{35}S]cefmetazole to be about 87.0 and 75.0%, respectively.

Cefmetazole and Metabolites in Feces and Bile

In the TLC autoradiograms of the fecal extracts (48~72 hours) after administration of [^{14}C]cefmetazole, two main radioactive spots were detected and one of them corresponded to unchanged cefmetazole (Fig. 6-B). The counting revealed that about 37 and 33% of the total radioactivity was unchanged cefmetazole and the main metabolite of Rf 0.6, respectively (Table 8). The bioautogram revealed only a single inhibitory zone corresponding to unchanged cefmetazole, indicating that the main metabolite or decomposed product is biologically inactive.

In the fecal extracts from [^{35}S]cefmetazole labeled at the 3-position, on the other hand, only a single peak was detected as the main radioactive spot corresponding to unchanged cefmetazole (Fig. 6-D). Several minor spots including that of *N*-methyltetrazolthiol were present, but almost no radioactivity at the position corres-

Table 8. Fecal metabolites of cefmetazole after intravenous administration of [^{14}C]cefmetazole and intramuscular administration of [3-^{35}S]cefmetazole to cynomolgus monkeys.

TLC zone	% to total radioactivity*1		
	[^{14}C]Cefmetazole	[^{35}S]Cefmetazole	
	48~72 hours	0~24 hours	24~48 hours
1 (Origin)	2.32	0.35	1.04
2	4.97	1.58	3.93
3	7.68	0.91	1.65
4	9.77	—*2	—
5 (Cefmetazole)	36.76 ±4.50	80.52 ±3.82	57.54*3
6 (Rf 0.6)	32.48 ±4.16	—	—
7	4.97	—	—
7′ (Me-TZT)	—	9.84	11.93
8	1.05	1.05	0.39

*1 Average from three experiments±standard error; dose=50 mg/kg.
*2 No corresponding radioactivity.
*3 n=2

ponding to the main metabolite derived from ^{14}C labeled drug, indicating that this substance did not contain 3-thiotetrazole substituent. Unchanged cefmetazole accounted for about 81 and 58% of the total radioactivity in 0~24 and 24~48 hours feces, respectively, while *N*-methyltetrazolthiol for about 10 and 12%, respectively.

On the TLC autoradiogram of the bladder bile obtained 60 minutes after intravenous administration of [^{14}C]cefmetazole, a single peak corresponding to unchanged cefmetazole was observed as the main spot, with a few faint spots in the region near the origin. The counting of the plates revealed that unchanged cefmetazole accounted for about 81% of the total radioactivity, indicating that the drug is excreted into the bile mostly as the unchanged active form.

Discussion

It has been found in the previous paper[5] by bioassay that there are significant species differences in the blood levels and urinary recoveries of cefmetazole. After subcutaneous or intramuscular administration (50 mg/kg), the urinary recovery was the lowest in rats (22%), followed by mice and dogs (60%) and rabbits (64%), while the highest in monkeys (77%). The peak plasma concentration was also the lowest in rats (~27 μg/ml) and the highest in monkeys (~150 μg/ml). The species difference was found to be mainly due to that in the participation of biliary excretion and the excretion in the cannulated animals was in the order: rabbits (1.3%) < dogs (15%) ≪ rats (60%). In phase I study with healthy volunteers[8], a high plasma level and the urinary recovery of as high as 85 to 95% of the dose have been observed and the monkey can be regarded as the most suitable animal model for human pharmacokinetics of cefmetazole. With respect to the serum protein binding, monkey was also similar to human: human (85%) > monkey (76%) > rabbit (59%) > rat ~ mouse (40~45%) > dog (25%).

In the present investigations, therefore, the pharmacokinetics and metabolic fate of cefmetazole were studied in details in monkeys. As the results, it was clarified that cefmetazole is absorbed rapidly from the site of injection, circulates in the peripheral blood as an active form and eliminated mainly *via* the urinary route mostly as an unchanged active form. The only metabolite of cefmetazole in the urine was *N*-methyltetrazolthiol which was detected when 3-^{35}S-labeled drug was administered. The total amount excreted during 24 hours period was estimated to be about 7.2% of the dose.

From the present results, it is presumed that cefmetazole excreted into the intestinal lumen *via* the bile was degraded in an alkaline media of the lumen to liberate *N*-methyltetrazolthiol gradually, which was re-absorbed from intestine and excreted into the urine as its free form. This might, in turn, give an explanation for a higher and delayed urinary recovery of radioactivity for the label at 3-position as compared to those for the label at 7-position. Thus, the amount of *N*-methyltetrazolthiol excreted in the urine may depend on the participation of the biliary excretion. In fact, in our unpublished results, when [3-^{35}S]cefmetazole was administered subcutaneously to rats, 11.0% of the radioactive dose was found to be excreted as [^{35}S]-*N*-methyltetrazolthiol in the urine during 24 hours period. It was also found, in our unpublished results by high performance liquid chromatography, that the excretion of *N*-methyltetrazolthiol in human urine was no more than 2.7% of the dose in 24 hours period, indicating that the participation of biliary excretion in human subjects must be lower than that in monkeys.

The total urinary excretion of labeled cefmetazole, except that at 3-position, was 76 to 86% of the dose by both radioassay and bioassay, irrespective of the route of administration. The total recovery of radioactivity from the urine and feces reached as high as 91 to 96% of the dose during 96 hours period in all three differently labeled compounds, indicating that there is no retention of cefmetazole and/or its metabolites in the body. This was further confirmed by the results that both the plasma levels and their half-lives were not affected to any appreciable extent after daily repeated administrations for one and two weeks. If there occurred some accumulation of cefmetazole in the blood by the repeated administration, it must be detected as an increase of the level by bioassay, while if there occurred any change in the metabolic fate of cefmetazole due to an enzyme induction or a renal toxicity, it must be detected as a change in the levels and/or the half-lives of the spiked [^{14}C]cefmetazole by radioassay. Though the half-

life of cefmetazole in monkey plasma was rather short (about 32 minutes), it has been shown that the half-life in human plasma is long enough, being about 60 minutes.[8]

In the present results, it was shown that cefmetazole was excreted in the intestinal lumen *via* the bile mostly as intact form and passed through the lumen down into the feces at least partly (about 40%) as an unchanged active form. Generally, other cephalosporin compounds are suffered from lactam cleavage by the intestinal flora and no active form can be detected in the feces.[9] In contrast, cefmetazole was shown to be quite resistant to the intestinal flora because of its high stability against β-lactamases. In fact, when [^{14}C]cefmetazole was incubated in the fecal homogenates of monkey (50%, pH 7.0) at 37°C for 1 hour, no degradation product was detected on the TLC autoradiogram. Cefmetazole undergoes easily, on the other hand, chemical decomposition in an alkaline media and the metabolite observed in the feces after administration of [^{14}C]cefmetazole is considered to be an inactive product chemically degraded in the intestinal lumen, which does not contain 3-substituent.

As judged from the present results, it might be deduced that cefmetazole is quite stable in animal body and after rapid absorption from the site of injection it is eliminated from the body as an active form without being suffered from any metabolic change, providing an evidence for the safety and efficacy in exerting its high antibacterial action clinically.

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