METABOLIC FATE OF CEPHACETRILE AFTER PARENTERAL ADMINISTRATION IN RATS AND RABBITS

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(Received for publication July 1, 1975)

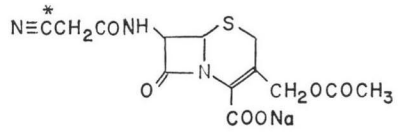
1. The metabolic fate of ^{14}C-cephacetrile was studied in rats and rabbits. The plasma level of intravenously injected cephacetrile decreased with half-lives of 17 and 22 minutes in rats and rabbits respectively, this decline being associated with a rapid appearance of the active metabolite, desacetylcephacetrile. Intramuscularly injected cephacetrile was rapidly absorbed by rats with a maximum plasma level at 20 minutes and a half-life of 16 minutes. Cephacetrile and desacetylcephacetrile did not enter erythrocytes. Cephacetrile was weakly bound to the plasma protein in the rat, rabbit and man.

2. Both in rats and rabbits, almost all of the injected radioactivity was excreted in the urine within 72 hours as the intact antibiotic and desacetylcephacetrile, only small amounts appearing in feces *via* bile. Neither the γ-lactone of desacetyl-7-cyanacetamidocephalosporanic acid nor the violet-reddish pigment (CGP-695) produced from cephacetrile were detectable in the plasma or urine of the animals.

3. In rats given the labeled antibiotic intravenously, the radioactivity was widely distributed with concentrations being high in the kidney, plasma and liver, and lowest in the brain. The radioactivity crossed the rat placenta and appeared in the fetus. Radioactivity in these tissues disappeared as the plasma level declined.

4. During daily intramuscular injection of ^{14}C-cephacetrile to rats, no significant changes were observed in the peak level of the plasma radioactivity or in the half-lives. In addition, dosing of the labeled antibiotic for 7 days caused no increase in tissue levels of radioactivity.

Cephacetrile (sodium salt of 7-cyanacetamidocephalosporanic acid) has a broad antibiotic spectrum against gram-positive as well as gram-negative bacteria and no serious toxicity, including nephrotoxicity, has been reported in the wide spread clinical use[1,2,3].



^{14}C-Cephacetrile * Labeled position

The metabolism of this antibiotic has been partly elucidated both in laboratory animals and in man (Dr. SCHMID *et al*, personal communication, CIBA-GEIGY Ltd., Basel, Switzerland).

The present study was undertaken, using ^{14}C labeled cephacetrile, to clarify in more detail the physiological disposition of the antibiotic in rats and rabbits after a single or repeated administration.

Materials and Methods

1. Materials

The following samples were supplied by CIBA-GEIGY Ltd. (Basel, Switzerland): sodium salt of 7-cyan-^{14}C-acetamidocephalosporanic acid (^{14}C-cephacetrile, sp. radioactivity; 11.2 μCi/mg),

non-labeled cephacetrile, desacetylcephacetrile, the γ-lactone of desacetyl-7-cyanacetamidocephalosporanic acid and CGP-695, a violet-reddish pigment produced from cephacetrile[4,5]. The radiochemical purity of ^{14}C-cephacetrile was 98.8 % as checked by TLC using solvent systems 1 and 2 (described below).

2. Animals

Male Sprague-Dawley rats (JCL-SD, CLEA Japan, Inc., Tokyo) weighing 200~250 g and male albino rabbits (a native kind, Saito Ikuseijyo, Tokyo) weighing 3.4~3.6 kg were used. Pregnant rats (JCL-SD, CLEA Japan, Inc., Tokyo) weighing 365~410 g were used on 18th to 19th day of gestation. They were maintained on an usual chow diet (CE-2 for rats and CR-2 for rabbits, CLEA Japan, Inc., Tokyo).

3. Collection of body fluids and excreta

The labeled antibiotic was dissolved in 0.9 % NaCl solution for intravenous (i.v.) or intramuscular (i.m.) injection at a dose of 50 mg (50 μCi for rats and 33 μCi for rabbits) per kg of body weight. Blood was obtained from the tail vein or inferior cava vein in rats and from the marginal ear vein in rabbits. Urine, feces, gastric juice and bile of rats were collected as described elsewhere[6]. Urine and feces of rabbits were collected by using the usual metabolism cage.

4. Measurement of radioactivity

Radioactivity was measured by a liquid scintillation counter, Aloka model LSC-502 (Japan Radiation and Medical Electronics Ltd., Tokyo) with an automatic quenching monitor. Aliquots of urine, bile, gastric juice, plasma and homogenates of liver, kidney, adipose tissue and feces were diluted with water to a final volume of 1.5 ml, then 3 ml of non-ionic detergent (Nissan Nonion NS-210, Nippon Yushi, Ltd., Tokyo) was added followed by a 6-ml aliquot of scintillation phosphor mixture consisting of 15 g of 2,5-diphenyloxazole, 1 g of *p*-bis(*o*-methylstyryl) benzene and 1 liter of toluene. Blood radioactivity was determined in the same way after decolorizing with H_2O_2 as described previously[7]. Radioactivity in other tissues was determined by using a sample oxidizer, model 306 (Packard Instrumental Co., Inc., U.S.A.).

5. Measurement of antibacterial activity

Antibiotic concentration in urine and plasma was determined by a cylinder plate method after appropriate dilution with 0.1 M potassium phosphate buffer (pH 7.0) and with homologous plasma obtained from non-treated animals, respectively. The test organism used was *Bacillus subtilis* ATCC 6633. As described in the "Results", antibacterial activities in urine and plasma were derived from cephacetrile and its active metabolite, desacetylcephacetrile. The antibacterial activity of cephacetrile is about 4 times higher than for desacetylcephacetrile and therefore the antibacterial activity in the samples for all practical purpose indicates the amount of the intact antibiotic.

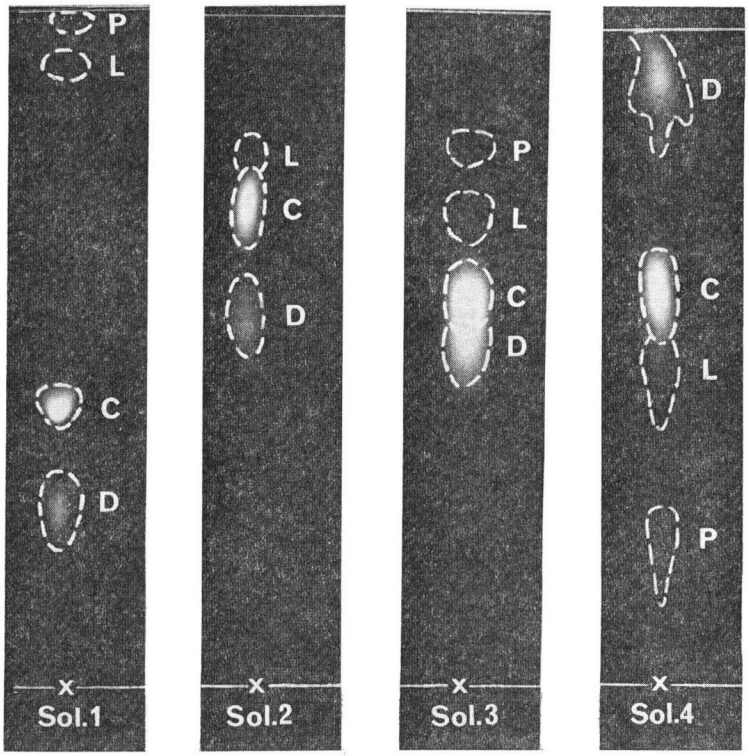
6. Determination of cephacetrile and metabolites by TLC

Radioactivity was extracted from plasma with an equal volume of acetone with a recovery of about 90 %. Aliquots of the plasma extracts, urine and bile were then subjected to TLC, followed by counting each radioactive spot in the detergent-phosphor mixture. Thin-layer plates (20×20 cm, 0.75 mm thickness) of silica gel GF_{254} (E. Merck AG, Darmstadt) were prepared by the method of STAHL[8].

The solvent systems were as follows: system 1, acetonitrile-ethyl acetate-water (3:1:1, v/v); system 2, ethyl acetate-glacial acetic acid-water (3:1:1, v/v); system 3, *n*-butanol-pyridine-glacial acetic acid-water (21:12:2:15, v/v); and system 4, 0.1 M sodium sulfate. System 4 was used for reversed phase chromatography, in which the plates were pre-coated with silicone DC-200 (350 cs) by developing using the solvent system of 5 % silicone DC-200 dissolved in diethyl ether. In these TLC analysis, cephacetrile, desacetylcephacetrile, the γ-lactone of desacetyl-7-cyanacetamidocephalosporanic acid and CGP-695 were added to the samples as internal standards. Fig. 1 shows typical thin-layer chromatograms showing separation of cephacetrile and its metabolites. Authentic compounds could be located by UV absorption or iodine vapor.

Fig. 1. Representative radioautograms of the sample by TLC showing separation of cephacetrile and metabolites.

The sample was the 4-hour urine of rats given ^{14}C-cephacetrile (50 mg/kg) intravenously (See also Fig. 5-A). Spots encircled by dotted lines denote UV absorption of the authentic samples of cephacetrile (C), desacetylcephacetrile (D), the lactone of desacetylcephacetrile (L) and CGP-695 (P).



The antimicrobially active compounds were also examined by a bioautographic technique using *Bacillus subtilis* ATCC 6633 as the test organism. In this case, pre-coated silica gel F_{254} sheets (20×20 cm, 0.25 mm thickness, E. Merck AG, Darmstadt) were used with solvent systems 1 or 2.

7. Whole-body radioautography

Whole-body sections (40 μ thickness) of pregnant rats given ^{14}C-cephacetrile (50 mg-261 μCi/kg) intravenously were prepared according to the method of ULLBERG[9] and exposed to X-ray film (Industrial type, Konishiroku Photo Chemical Industries Ltd., Tokyo) for 6 days at 4°C.

8. Distribution of blood radioactivity between plasma and erythrocytes

Radioactivity in whole blood and plasma was determined on the same blood sample and % distribution of radioactivity in erythrocytes was calculated according to the following formula:

$$\% \text{ Distribution} = 100 - \frac{(100 - \text{Ht}) \times \text{plasma level}}{\text{blood level}},$$

where Ht is hematocrit value.

9. Plasma protein binding

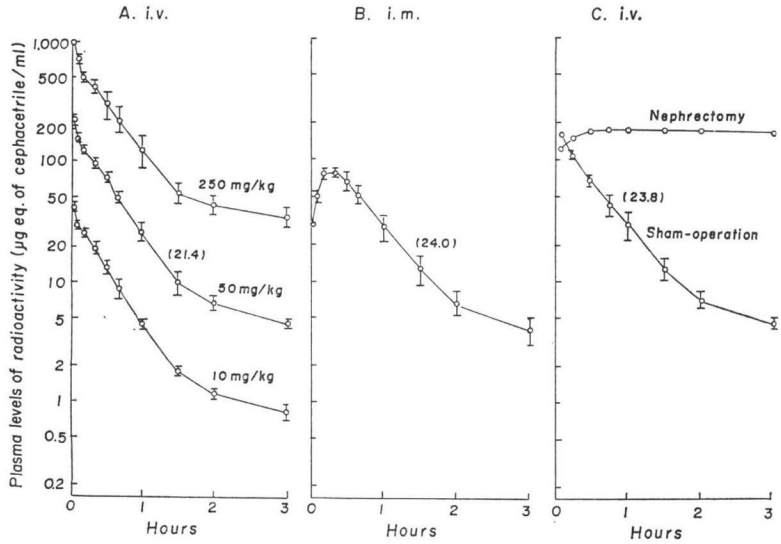
The plasma samples of rats, rabbits or man (lyophilized human plasma, Nihon Seiyaku Ltd., Tokyo) to which ^{14}C-cephacetrile was added *in vitro* were centrifuged for 6 hours at 300,000×g using an ultracentrifuge (Hitachi model 65 P and a swing rotor, Hitachi model RPS-65 T). After centrifugation, the colorless supernatant containing only about 1.3 % of the total plasma proteins, was carefully pipetted off for counting. Percentage binding was calculated by the following equation:

$$\% \text{ Binding} = \left(1 - \frac{\text{supernatant level}}{\text{plasma level}}\right) \times 100 .$$

The protein binding was also examined using the 0.5-hour plasma of rats given the labeled antibiotic intravenously.

Fig. 2. Plasma levels of radioactivity after intravenous or intramuscular injection of ^{14}C-cephacetrile in rats.

Data are expressed in mean ± S.D. of three rats in each experiment. Figures in parentheses denote the half-life in the second phase (see the text). Sham-operated rats (C) were laparotomized only.



Results

1. Plasma Kinetics

Distribution of Radioactivity

During the first 3 hours after intravenous or intramuscular injection of ^{14}C-cephacetrile to rats and rabbits, almost all of the radioactivity in the blood was present in the plasma fraction.

Protein Binding

Binding of ^{14}C-cephacetrile to rat plasma proteins was studied both *in vitro* and *in vivo* (Table 1). In the *in vitro* experiment, 21~40 % of the antibiotic were bound to the plasma protein at a concentration of less than 340 μg/ml. In the 0.5-hour plasma of rats given ^{14}C-cephacetrile intravenously, about 34 % of radioactivity was bound to protein. The protein binding in rabbits and man seemed to be somewhat less extensive than that in rats (Table 1).

Table 1. Binding of cephacetrile to rat, rabbit and human plasma protein

Experimental condition	Concentration of cephacetrile (μg/ml)	% Binding		
		Rat	Rabbit	Man
In vitro	13	39.6	24.0	15.2
	50	29.2	26.7	15.9
	130	30.6	23.2	14.4
	340	21.1	18.3	14.1
In vivo	38	33.8	n.d.	n.d.

In the *in vitro* experiment, ^{14}C-cephacetrile at a concentration of 13~340 μg/ml was added to plasma samples for a subsequent ultracentrifugation as described in the text. In the *in vivo* experiment, the 0.5-hour plasma was obtained from three rats given the labeled antibiotic (50 mg/kg) intravenously. Concentration of the radioactivity is expressed in cephacetrile equivalent. n.d., Not determined.

Plasma Level

The plasma levels of cephacetrile and metabolites were studied in rats and rabbits after intravenous or intramuscular injection (Figs. 2 and 3, Table 2). Following intravenous injection of the labeled antibiotic to rats, the plasma level of radioactivity decreased in three phases (Fig. 2-A). In rats given 50 mg/kg of the antibiotic, for example, the initial rapid decrease in the plasma level with a half-life of 6 minutes (first phase, 0~5 minutes) was followed by declines with half-lives of 21 minutes (second phase, 5~90 minutes) and 90 minutes (third phase, 90~180 minutes), respectively. Fig. 2-A also indicates that the plasma levels of radioactivity were proportional to the dose. Intramuscular injection of ^{14}C-cephacetrile to rats resulted in the immediate presence of radioactivity in the plasma, reaching the maximum concentration (78 μg eq. of cephacetrile/ml) at 20 minutes followed by decline with a half-life of 24 minutes (Fig. 2-B). These findings clearly indicate a rapid absorption of cephacetrile after the intramuscular injection.

In bilaterally nephrectomized rats given the labeled antibiotic intravenously, the plasma level of radioactivity did not decline during the first 3 hours after the injection (Fig. 2-C). It must be mentioned that, at any time, the plasma levels in the nephrectomized rats were approximately the same as the initial concentration of radioactivity in the second phase in the intact rats (Fig. 2-A). These results suggest that the distribution of radioactivity from plasma to tissues was complete in the first phase and the decline in the second phase depended solely upon the renal excretion.

The composition of the plasma radioactivity was then examined by TLC or bioassy (Fig. 3,

Fig. 3. Plasma levels of cephacetrile and desacetylcephacetrile after intravenous or intramuscular injection of ^{14}C-cephacetrile in rats and rabbits.

Cephacetrile, desacetylcephacetrile and antibacterial activity were estimated on the pooled samples from three animals given the labeled antibiotic. Plasma levels of radioactivity are mean values, except for rabbits in which data are expressed in mean ±S.D. Note that the concentrations of total antibacterial activity are quite similar to those of the intact antibiotic in rabbits (C).

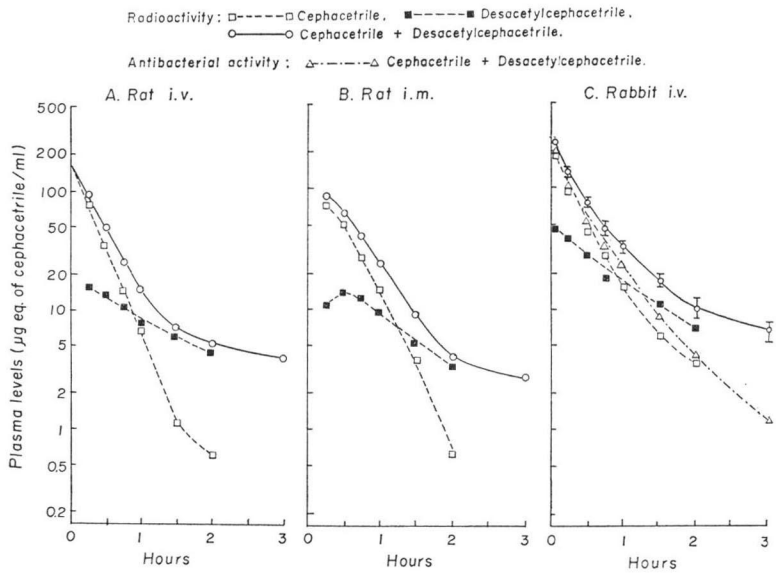


Table 2. Plasma levels of cephacetrile, desacetylcephacetrile and antibacterial activity after intravenous injection of ^{14}C-cephacetrile in rats

Time after dosage (min.)	μg eq. of cephacetrile/ml			
	Total radioactivity	Cephacetrile	Desacetyl-cephacetrile	Antibacterial activity
5	150	126	20	130
30	46.6	27	19	31
60	15.7	6	10	8

The plasma levels were determined on the pooled samples from three rats given ^{14}C-cephacetrile (50 mg/kg) intravenously.

Table 2). In rats given ^{14}C-cephacetrile intravenously (Fig. 3-A) or intramuscularly (Fig. 3-B), all of the plasma radioactivity was accounted for by the intact antibiotic and its active metabolite, desacetylcephacetrile; the γ-lactone of desacetyl-7-cyanacetamidocephalosporanic acid and CGP-695 were not detectable. After the intravenous injection, the plasma level of cephacetrile declined with a half-life of 17 minutes (Fig. 3-A). The plasma level of the antibiotic injected intramuscularly reached a peak at 15 minutes and declined thereafter with a half-life of 16 minutes (Fig. 3-B). The level of desacetylcephacetrile after the intravenous and intramuscular injections decreased with half-lives of 72 and 42 minutes, respectively, in this species. The plasma levels of cephacetrile and metabolite composition in rabbits were similar to those in rats, although the levels of desacetylcephacetrile were somewhat higher in rabbits (Fig. 3-C). Both

Table 3. Tissue levels of radioactivity after intravenous injection of ^{14}C-cephacetrile in rats

Time	5 min.	30 min.	1 hr.	6 hrs.	24 hrs.	72 hrs.
Brain	4.9± 1.0	2.9±0.2	1.6± 0.3	0.5±0.1	0.1±0.0	0.0±0.0
Spinal cord	15.3± 2.3	5.2±1.3	1.8± 0.2	0.5±0.1	0.1±0.0	n.d.
HARDER's gland	22.5± 0.8	9.2±0.3	3.1± 0.3	0.8±0.2	1.1±0.2	0.3±0.1
Eye	20.0± 1.6	8.1±0.4	3.8± 0.6	1.1±0.3	0.1±0.0	n.d.
Salivary gland	31.4± 0.8	11.0±0.8	3.7± 0.3	0.8±0.2	0.4±0.2	n.d.
Thymus	18.7± 1.5	6.2±1.2	2.3± 0.3	0.6±0.2	0.2±0.0	0.1±0.1
Lung	55.9± 5.8	17.8±4.8	8.2± 4.0	1.1±0.2	0.7±0.1	0.4±0.1
Heart	25.5± 1.5	7.3±0.9	3.4± 0.5	0.8±0.2	0.2±0.0	0.2±0.0
Liver	59.7± 7.9	20.9±5.3	6.6± 0.6	1.1±0.4	0.5±0.1	0.2±0.1
Stomach	20.0± 0.6	16.0±8.7	14.6±11.8	0.9±0.2	0.8±0.1	0.5±0.1
Intestine	5.3± 0.5	8.6±2.7	4.9± 1.5	0.7±0.2	0.4±0.1	0.4±0.2
Spleen	14.3± 1.4	6.8±0.5	3.6± 1.5	0.7±0.2	0.5±0.1	0.2±0.0
Pancreas	35.5± 2.0	10.0±3.1	4.6± 0.7	0.7±0.2	0.3±0.0	0.1±0.0
Adrenal gland	18.9± 1.4	8.5±1.2	2.3± 0.8	0.6±0.1	0.3±0.1	0.2±0.1
Kidney	360.7±30.6	113.3±0.8	41.6±10.4	3.9±0.3	2.3±0.1	1.1±0.1
Testis	15.4± 3.8	6.9±0.9	3.3± 0.4	0.6±0.1	0.2±0.0	0.1±0.0
Adipose	16.6± 3.6	6.6±0.1	3.1± 0.5	0.5±0.0	0.2±0.0	0.2±0.0
Skeletal muscle	15.9± 0.3	5.0±0.1	2.4± 0.4	0.5±0.2	0.1±0.0	0.1±0.0
Skin	38.8± 2.7	13.8±1.2	5.1± 0.3	1.0±0.1	0.6±0.0	0.5±0.1
Blood	97.7± 8.8	30.2±1.8	10.2± 1.4	1.9±0.4	0.4±0.0	0.3±0.0
Plasma	146.4±10.0	45.8±3.9	15.6± 2.3	2.3±0.4	0.6±0.1	0.3±0.1

The tissue concentrations (μg eq. of cephacetrile/g or ml) are expressed in mean ± S.D. of three rats given the labeled antibiotic (50 mg/kg) intravenously. n.d., Not determined.

in rats and rabbits, the antibacterial activity in plasma was largely derived from cephacetrile, since the activity of desacetylcephacetrile was only one-quarter of that of the intact antibiotic (Table 2, Fig. 3-C).

2. Tissue Distribution

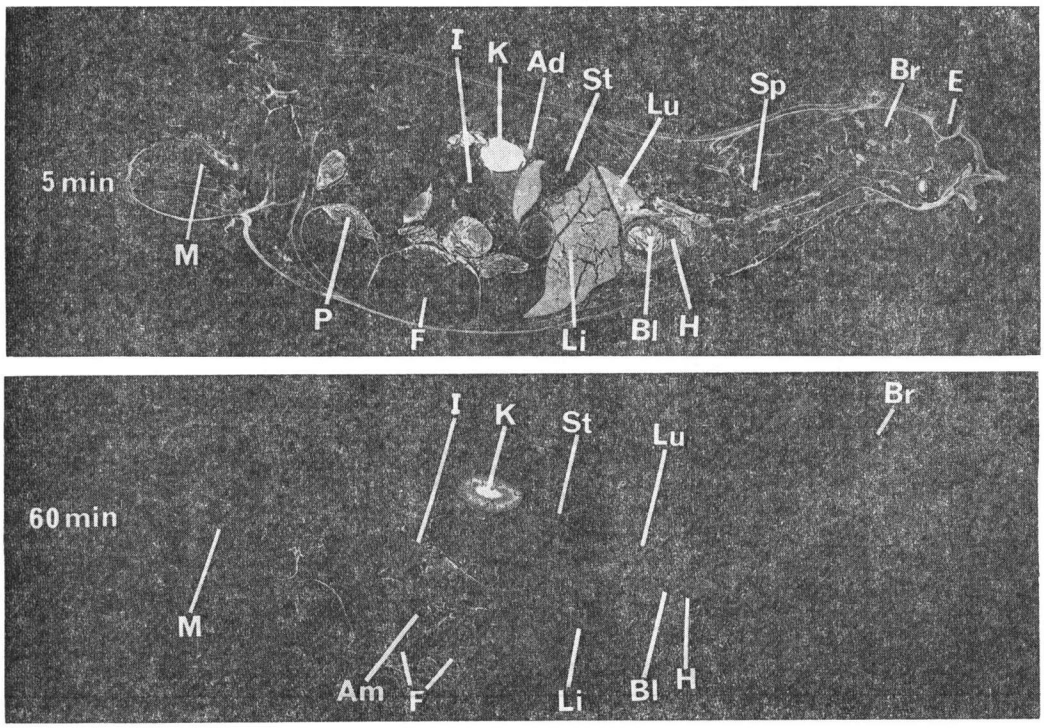
Intact Rats

After intravenous injection of ^{14}C-cephacetrile in rats, radioactivity was found to be widely distributed in tissues (Table 3). Invariably, the highest concentration was noted in the kidney, followed by plasma, liver and lung, while the lowest was found in the brain. The radioactivity in most tissues disappeared as the plasma level declined, showing that there was no trend towards an accumulation of the radioactivity in tissues. This was further comfirmed by the experiments described below (Tables 5 and 6).

Pregnant Rats

Placental transfer of radioactivity was studied in pregnant rats using a whole-body autoradiographic technique (Fig. 4). At 5 minutes after intravenous injection of the labeled antibiotic, the concentration of radioactivity in the fetus was much lower than that in placenta and other maternal tissues, indicating that a placental barrier to cephacetrile and desacetylcephacetrile existed. Radioactivity was detectable at 1 hour in the fetus and was evenly distributed in tissues including the brain, liver, blood and kidney. The distribution pattern of radioactivity in maternal tissues was similar to that in the intact male rats both at 5 minutes and 1 hour

Fig. 4. Radioautography in pregnant rats after intravenous injection of ^{14}C-cephacetrile. Female rats on the 18th to 19th day of gestation were injected with the labeled antibiotic (50 mg/kg) intravenously. Key to tissues: Ad; adrenal gland, Am; amniotic fluid, Bl; blood, Br; brain, E; eye, F; fetus, H; heart, I; intestine, K; kidney, Li; liver, Lu; lung, M; skeletal muscle, P; placenta, Sp; spinal cord, St; stomach.



(Table 3). No appreciable amounts of radioactivity remained in maternal or fetal tissues at 6 and 24 hours.

3. Excretion

Excretory patterns of radioactivity were studied in rats and rabbits given ^{14}C-cephacetrile intravenously or intramuscularly (Fig. 5). In these two animals, excretion of radioactivity in urine and feces was complete within 72 hours after the administration, more than 80 % of the dose being excreted in the first 4 hours into urine. After intravenous injection of the labeled antibiotic to rats, excretion of the radioactivity in gastric juice and bile was only 0.1 and 1.6 %, respectively, of the dose in 24 hours. Fecal excretion of radioactivity was much less in the biliary-cannulated rats than in the intact ones, indicating that the fecal radioactivity was derived from the bile in this species.

Almost all of the urinary radioactivity both in rats and rabbits was accounted for by cephacetrile and desacetylcephacetrile as evidenced by radioassay as well as bioassay, the γ-lactone of desacetyl-7-cyanacetamidocephalosporanic acid and CGP-695 not being detected (Fig. 1, Table 4). Only small amounts of cephacetrile and desacetylcephacetrile were excreted in the rat bile together with the unknown polar metabolite(s).

Fig. 5. Cumulative excretion of radioactivity in urine and feces after intravenous or intramuscular injection of ^{14}C-cephacetrile in rats and rabbits.

Data are expressed as the means ± S.D. or with range for the number of animals shown in parentheses. Dose of ^{14}C-cephacetrile was 50 mg/kg.

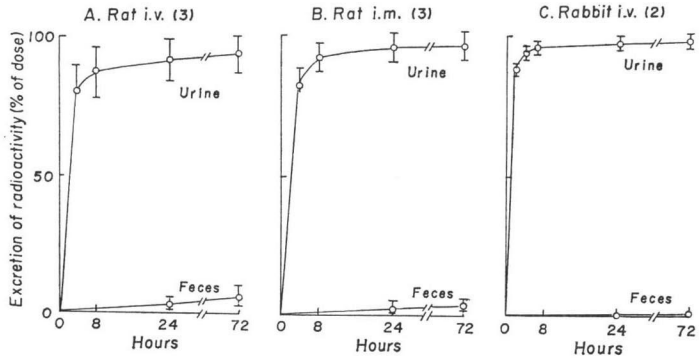


Table 4. Urinary concentrations of cephacetrile, desacetylcephacetrile and antibacterial activity after intravenous or intramuscular injection of ^{14}C-cephacetrile in rats and rabbits

Species	Route of administration	Time after dosage (hrs.)	Excretion of radioactivity (% of dose)	Concentration (μg eq. of cephacetrile/ml)			
				Total radioactivity	Cephacetrile	Desacetylcephacetrile	Antibacterial activity
Rat (n=3)	i.v.	0~4	80.3	3,060	1,830	1,100	2,100
		4~8	6.7	194	93	89	110
	i.m.	0~4	82.1	3,190	1,930	1,080	2,400
		4~8	10.0	294	147	106	170
Rabbit (n=2)	i.m.	0~2	88.5	9,820	4,010	4,930	4,600
		2~4	5.8	823	240	480	300
		4~6	1.9	276	62	166	100

Data are expressed in mean values of the animals corresponding to Fig. 5.

4. Accumulation in the Body

Rats were injected intramuscularly with the labeled antibiotic for 7 days, and plasma and tissue levels of radioactivity were observed (Tables 5 and 6). During repeated injection of ^{14}C-cephacetrile, no significant changes were noted in the plasma levels at 15 minutes and 24 hours as well as in the plasma half-life times (Table 5). In addition, tissue levels of radioactivity at 24 hours after injections 1, 3 and 7 times were only slightly increased (Table 6). These results indicate that no significant amounts of cephacetrile or its metabolite(s) were accumulated in the body during repeated medication.

Table 5. Plasma levels of radioactivity during repeated intramuscular injections of ^{14}C-cephacetrile in rats

Days on drug	No. of animals	Plasma levels (μg eq. of cephacetrile/ml)		Half-life* (min.)
		15 min.	24 hrs.	
1	9	90.4±11.8	0.6±0.1	24.3±1.9
2	6	92.5±12.7	0.8±0.1	21.4±2.1
3	6	94.2±12.5	1.2±0.1	22.0±1.3
4	3	105.6±13.1	1.3±0.1	22.4±3.1
5	3	98.0± 2.2	1.4±0.1	21.9±1.0
6	3	96.5±13.0	1.6±0.1	23.8±1.5
7	3	91.8±12.8	1.8±0.0	24.3±1.2

Rats were injected with ^{14}C-cephacetrile intramuscularly for 7 days at a daily dose of 50 mg/kg. Data are expressed in mean ± S.D.

* Calculated from the decay curves of the plasma level during the first 1.5 hours after each injection.

Table 6. Tissue levels of radioactivity during repeated intramuscular injections of ^{14}C-cephacetrile in rats

Tissue	Tissue levels (μg eq. of cephacetrile/g or ml)		
	1 day	3 days	7 days
Brain	0.1±0.0	0.1±0.0	0.2±0.0
Thymus	0.2±0.1	0.4±0.0	0.7±0.0
Lung	0.6±0.1	1.4±0.3	3.1±0.2
Heart	0.2±0.1	0.5±0.1	0.8±0.0
Liver	0.4±0.1	0.7±0.1	1.1±0.1
Stomach	0.4±0.0	1.2±0.3	2.0±0.1
Pancreas	0.2±0.0	0.7±0.1	0.9±0.1
Spleen	0.3±0.0	0.7±0.0	1.4±0.2
Intestine	0.3±0.1	0.5±0.1	1.1±0.2
Adrenal gland	0.3±0.0	0.9±0.1	1.3±0.1
Kidney	5.0±0.4	12.2±1.5	9.5±1.5
Adipose	0.3±0.1	0.8±0.1	1.1±0.1
Testis	0.1±0.0	0.3±0.0	0.6±0.0
Skeletal muscle	0.2±0.0	0.3±0.0	0.5±0.1
Skin	0.5±0.0	1.3±0.1	2.9±0.1
Blood	0.4±0.0	0.8±0.1	1.2±0.0
Plasma	0.5±0.0	1.1±0.1	1.5±0.1

Data are expressed in mean ± S.D. of the three rats corresponding to Table 5.

Discussion

The present studies have clarified the metabolic fate of cephacetrile in rats and rabbits. In rats, intravenously injected cephacetrile was widely distributed in tissues, including fetus (Table 3, Fig. 4). The level was highest in the kidney, plasma and liver, while lowest in the brain and fetus, these distribution patterns being similar to those of other cephalosporin and penicillin derivatives[10,11,12]. A rapid absorption of intramuscularly injected cephacetrile in rats was evidenced by the facts that the plasma levels and excretory patterns were closely similar to those after the intravenous injection (Figs. 2, 3 and 5). Cephacetrile was in part deacylated in the body to yield an active metabolite, desacetylcephacetrile, and this metabolite, together with the intact antibiotic, was excreted exclusively in the urine, only small amounts appearing in feces *via* bile (Fig. 5, Table 4). In rats, no significant amounts of cephacetrile or its metabolite(s) were accumulated in the body during repeated administrations (Tables 5 and 6).

Although no significant species variations were demonstrated in the plasma level, metabolic route and excretory pattern between rats and rabbits, a larger amount of the intact antibiotic was excreted in the urine in rats than in rabbits (Figs. 3 and 5, Table 4). As evidenced by one of

the present authors (FUGONO, unpublished data), cephacetrile was excreted as such exclusively in human urine, only a small amount of desacetylcephacetrile being detectable. Dr. SCHMID and his colleagues (personal communication CIBA-GEIGY Ltd., Basel, Switzerland) also observed that no significant species differences exist in the metabolism of cephacetrile in rats, mice and dogs, although in man the rates of absorption and elimination of intramuscularly injected cephacetrile were somewhat slower than those in laboratory animals.

Of pharmacological interest is the finding that cephacetrile and its active metabolite, desacetylcephacetrile, are excreted in the urine of rats and rabbits (Table 4), since these results indicate that the antibiotic could be effectively used especially in urinary-tract infections.

Cephacetrile was weakly bound to the rat, rabbit and human plasma protein (Table 1), the binding being rather less extensive than that of other cephalosporin antibiotics[13,14]. Although it is widely accepted that plasma protein binding affects the metabolism or biological activities of a drug, GILLETTE[15] has calculated that the protein binding effectively lowers the free concentration of a drug in the body, only when more than 80 % of the drug was bound to the plasma protein. It is, therefore, unlikely that the plasma protein binding significantly affects the metabolism and efficacy of cephacetrile.

Acknowledgement

We are deeply indebted to Messrs. Y. SHIRAKAWA and K. MAEDA for excellent technical assistance.

References

1) KNÜSEL, F.; E. A. KONOPKA, J. GELZER & A. ROSSELET: Antimicrobial studies *in vitro* with CIBA 36278-Ba, a new cephalosporin derivative. Antimicr. Agents & Chemoth. -1970: 140~149, 1971
2) KRADOLFER, F.; W. SACKMANN, O. ZAK, H. BRUNNER, R. HESS, E. A. KONOPKA & J. GELZER: CIBA 36278-Ba: Chemotherapy and toxicology in laboratory animals. Antimicr. Agents & Chemoth. -1970: 150~155, 1971
3) NEU, H. C. & E. B. WINSHELL: *In vitro* evaluation of cephacetrile, a new cephalosporin antibiotic. J. Antibiotics 25: 400~404, 1972
4) RUSSELL, A. D.: Interaction of a new cephalosporin, 7-cyanacetamidocephalosporanic acid, with some gram-negative and gram-positive β-lactamase producing bacteria. Antimicr. Agents & Chemoth. 2: 255~260, 1972
5) MEYER-BRUNOT, H. G.; J. KRAETZ, C. SCHENK, P. THOMANN, O. ZÁK & W. ZIMMERMANN: Studies of the formation of a violet-reddish pigment from the cephalosporin derivative celospor (C 36,278-Ba). Chemotherapy (Basel) 20: 6~17, 1974
6) TANAYAMA, S.; T. FUJITA, Y. SHIRAKAWA & Z. SUZUOKI: Metabolic fate of 5-ethoxycarbonyl-3-morpholinosydnonimine (SIN-10), 1. Absorption, excretion and tissue distribution in rats and mice. Jap. J. Pharmacol. 20: 413~423, 1970
7) TANAYAMA, S.; Y. SHIRAKAWA, Y. KANAI & Z. SUZUOKI: Metabolism of 8-chloro-6-phenyl-4*H*-*s*-triazolo[4, 3-*a*] [1, 4]benzodiazepine (D-40 TA), a new central depressant, I. Absorption, distribution and excretion in rats. Xenobiotica: 4: 33~47, 1974
8) STAHL, E.: Dünnshicht-Chromatographie. Pharmazie 11: 633~637, 1956
9) ULLBERG, S.: Studies on the distribution and fate of ^{35}S-labelled benzylpenicillin in the body. Acta Radiol., Suppl. 118: 1~110, 1954
10) KOZATANI, J.; M. OKUI, K. NODA, T. OGINO & H. NOGUCHI: Cefazolin, a new semisynthetic cephalosporin antibiotic, V. Distribution of cefazolin-^{14}C in mice and rats after parenteral administration. Chem. Pharm. Bull. 20: 1105~1113, 1972
11) SULLIVAN, H. R.; R. E. BILLINGS & R. E. MCMAHON: Metabolism of cephalexin-^{14}C in mice and in rats. J. Antibiotics 22: 195~200, 1969
12) NAKAI, Y.; Y. SHIRAKAWA, T. FUJITA, Z. SUZUOKI & T. FUGONO: The metabolic fate of α-sulphobenzylpenicillin in rats. Xenobiotica 2: 147~157, 1972.
13) OKUI, M.; K. HATTORI & M. NISHIDA: Studies on the metabolism of cephalosporins, II. Biliary excretion and metabolism of ^{14}C-cephachlomezine and its parent compound. J. Antibiotics 24: 667~674, 1971
14) WELIKY, I.; H. H. GADEBUSCH, K. KRIPALANI, P. ARNOW & E. C. SCHREIBER: Cepharadine: Absorption, excretion, and tissue distribution in animals of a new cephalosporin antibiotic. Antimicr. Agents & Chemoth. 5: 49~54, 1974
15) GILLETTE, J. R.: Factors affecting drug metabolism. Ann. N.Y. Acad. Sci. 179: 43~66, 1971