# CEFTEZOLE, A NEW CEPHALOSPORIN C DERIVATIVE

## II. DISTRIBUTION AND EXCRETION IN PARENTERAL ADMINISTRATION

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(Received for publication June 1, 1976)

The distribution of ceftezole in blood and tissues and its excretion after intramuscular or intravenous administration of single doses of 10 and 20 mg/kg were compared with those of cefazolin, cephaloridine and cephalothin. Blood levels of ceftezole in rats and rabbits were lower than those of cefazolin, and higher than those of cephaloridine and cephalothin. Retention time of ceftezole in the blood was somewhat shorter than that of cefazolin. However, blood levels of ceftezole in dogs were nearly the same as those of cefazolin and cephaloridine. The rate of urinary excretion of ceftezole in 24-hour urine after administration in rats and rabbits was found to be higher than those of the other antibiotics tested. In dogs, however, the rate of urinary excretion of ceftezole was nearly the same as that of cefazolin and higher than those of cephaloridine and cephalothin. The biliary excretion of ceftezole in rats and dogs was much higher than those of cephaloridine and cephalothin, but lower than that of cefazolin. Tissue distribution of ceftezole in rats was compared with that of the other antibiotics by intramuscular and intravenous administration. The initial level of ceftezole in the kidneys was found to be substantially higher than those of the other antibiotics. The initial level of ceftezole in the liver and lungs was also slightly higher than those of the other drugs when administered intramuscularly. Tissue levels of ceftezole were somewhat lower than those of cefazolin in rabbits after intravenous administration. Ceftezole attained a higher maximum level in rat lymph by intramuscular administration than the other antibiotics tested. The maximum concentration of ceftezole present in the exudate in the rat inflammatory pouch was higher than that of cefazolin. In rabbits with cerebrospinal meningitis induced by infection of *Streptococcus pyogenes*, the level of ceftezole in the cerebrospinal fluid was several times higher than that in normal rabbits. The serum level and urinary excretion of ceftezole was examined in 6 healthy male volunteers after intramuscular administration of a single dose of 500 mg. Ceftezole attained a mean maximum serum level of 22.9 $\mu$g/ml 30 minutes after administration and disappeared from the blood in about 6 hours. It was excreted rapidly in the urine. The concentration in 1-hour urine was the highest (mean level: 2,667 $\mu$g/ml) and the total excretion rate was 92.6%. No metabolites with antimicrobial activity were observed in the urine. No changes in the pattern of plasma level and urinary excretion and no accumulation in the tissues were observed after repeated intramuscular administration of 20 mg/kg of ceftezole in rabbits, 26 times, for 14 days.

As described in the preceding report,[1] investigations of *in vitro* and *in vivo* antimicrobial activities of ceftezole confirmed that ceftezole was highly active against a wide range of gram-positive and gram-negative bacteria, except *Serratia*, *Pseudomonas* and some other cephalosporin-resistant groups.

This report describes investigations of the blood level, urinary and biliary excretion, and tissue distribution of ceftezole compared with other related antibiotics in rats, rabbits and dogs, as well as healthy human volunteers.

## Materials and Methods

**1.** Antibiotics.

Ceftezole sodium (CTZ, Fujisawa Research Laboratories and Chugai Research Laboratories), cefazolin sodium (CEZ, Fujisawa Pharmaceutical Co.), cephaloridine (CER, Eli Lilly and Co.), and cephalothin sodium (CET, Eli Lilly and Co.).

**2.** Animals.

Rats: Sprague-Dawley (S.D.) strain, male, weighing 150~310 g or Wistar-Imamichi strain, male, weighing 200~280 g.

Rabbits: JW/CSK, male, weighing 2.0~3.2 kg.

Dogs: Beagles, female, weighing 8.6~11.2 kg.

**3.** Microbioassay method.

The concentration of antibiotics was determined by the agar-plate diffusion method using paper-discs. A nutrient agar plate containing $2\times10^6$ spores per ml of *Bacillus subtilis* ATCC 6633 was used. Paper-discs (Toyo Roshi Co., diameter 8 mm, "thick" type) were dipped in the standard or the test solution. After excess solution was removed, the discs were placed on the agar plate. The diameters of inhibitory zones were measured after incubation at 37°C for 20 hours, and the concentration of antibiotics was calculated from a standard curve.

**4.** Determination of serum, plasma and urinary levels of antibiotic in animals.

After each antibiotic was administered intramuscularly or intravenously, blood and urine samples were collected at fixed times. Serum or plasma was separated from the blood, the latter by centrifuging a mixture consisting of nine volumes of blood and one volume of 3.9% sodium citrate solution. The concentration of each antibiotic was determined by the paper-disc method described. In the preparation of the standard dilution series of each drug, serum and plasma of the test animals were used for determination of serum and plasma levels, and 1/15 M phosphate buffered saline (pH 7.0) (abbreviated as PBS) was used for determination of urinary levels.

**5.** Serum level and urinary excretion in human volunteers.

Six healthy male volunteers (body weight 55~57 kg) were given a single dose of 500 mg of ceftezole intramuscularly, and serum and urine samples were collected at fixed times. In the preparation of the standard dilution series of the drug, human serum or PBS was used.

**6.** Identification of active substances in human urine.

Ceftezole was administered intramuscularly to two healthy volunteers in a single dose of 500 mg. The urine was collected for the first 2 hours and examined by thin-layer chromatography and bioautography. In the thin-layer chromatography, the following solvent systems were used.

*n*-Butanol - pyridine - water (3:2:1)

Methanol - *n*-propanol - water (6:1:2)

*n*-Butanol - ethanol - water (4:1:2)

Cellulose thin-layer plate (Avicel SF, 5×20 cm, Funakoshi Yakuhin Co.) was used as adsorbent. In the bioautography, thin-layer plates developed with the above solvent systems were dried, and the plates were placed on the nutrient agar plates containing $10^6$ spores of *Bacillus subtilis* ATCC 6633 per ml for 20 minutes. After removing the thin-layer plates, the agar plates were incubated at 37°C for 18 hours to detect the active substances.

**7.** Determination of biliary excretion in animals.

Rats and dogs were operated under anesthetization with ether and pentobarbital, respectively, and the biliary ducts were cannulated with polyethylene tubes for collecting the bile samples. Rats were administered 20 mg/kg of ceftezole and related antibiotics intramuscularly or intravenously after awakening from anesthesia. Dogs were administered 20 mg/kg of the antibiotics intramuscularly under anesthetization. Bile samples were collected at fixed intervals for 24 hours after administration. PBS was used for preparation of the standard dilution series of the drugs.

**8.** Preparation of tissues.

Three to 12 rats per group were used. After each antibiotic was administered intramuscularly or intravenously in the rats, the animals were sacrificed by cervical dislocation at fixed intervals. The

liver, kidneys, lungs, heart and spleen were excised, washed with PBS and homogenized with four-fold volumes of the buffer, in a Waring Blender. The homogenates were then centrifuged at 5,000$\times g$ for 10 minutes to obtain the supernatant. Blood samples were simultaneously collected and separated into sera.

A similar procedure was applied in examining the tissue distribution after intravenous administration in rabbits (3 rabbits per group).

Rat serum or rabbit plasma and the supernatant of rat and rabbit tissues were used in preparation of the standard dilution series.

**9.** Determination of lymph levels in rats.

According to the method of BOLLMAN *et al.*,[2] rats were operated under anesthetization with ether, and the thoracic lymph ducts under the inferior aorta were cannulated with polyethylene tubes. Six hours after the operation, 20 mg/kg of each antibiotic was administered intramuscularly in the rats, and lymph samples were collected at fixed intervals. Simultaneously, blood samples were soaked into paper-discs which had been dipped in 10% sodium citrate solution and dried, to determine the antibiotic concentration in the plasma. In preparation of standard dilution series of drugs, rat lymph and plasma were used.

**10.** Determination of the exudate levels in the aseptic inflammatory pouch of rats.

According to the method of ROBERT *et al.*,[3] an inflammatory pouch was induced in rats by injecting 25 ml of air and then 1 ml of olive oil containing 1% croton oil subcutaneously on their backs. On the 5th~8th days after induction of inflammation, 20~80 mg/kg of each antibiotic was administered intramuscularly in the rats. At fixed intervals, 0.25~0.4 ml of each exudate was extracted. The exudate of the pouch was used to prepare the standard dilution series of the drugs.

**11.** Determination of the level in the cerebrospinal fluid of rabbits.

The level of ceftezole in the cerebrospinal fluid after intravenous administration of 50 mg/kg was determined in rabbits infected with *S. pyogenes* and in normal ones.

An overnight culture of *S. pyogenes* strain JU-13 in heart infusion broth containing 1% of yeast extract, diluted ten-fold with physiological saline, was used as the bacterial suspension for infection. Experiments were carried out on rabbits anesthesized with urethane.

In the experiments with infected rabbits, ceftezole was administered 24 hours after inoculation with 0.1 ml of the bacterial suspension ($1\times10^6$ viable units/ml) into the cerebrospinal fluid and the cerebrospinal fluid was extracted at fixed intervals. Similarly, ceftezole was administered intravenously to normal rabbits and the cerebrospinal fluid was also extracted. PBS was used for preparation of the standard dilution series of the drugs.

Rabbit blood agar plates were used to count the viable cells of *S. pyogenes* in the fluid. Body temperature was measured in the rectum.

**12.** Plasma levels and urinary excretion during repeated long-term administration in rabbits.

Twenty mg/kg of ceftezole was administered intramuscularly in rabbits, twice a day as a rule, for 14 days (26 times in total). Plasma levels were determined on the 1st, 7th, 17th and the last day of administration, and urinary excretion on the 1st and the last day of administration.

The rabbits were sacrificed 24 hours after the final administration and the blood, urine, liver, lungs, spleen, heart and kidneys were collected and residual antibacterial activities were determined.

## Results

### 1. Plasma Levels in Rabbits and Dogs

Table 1 shows the results of the determination of plasma levels after ceftezole, cefazolin, cephaloridine and cephalothin were administered intramuscularly and intravenously in rabbits and dogs.

(1) Intramuscular administration

When ceftezole was administered at 20 mg/kg to rabbits, the maximum plasma level of ceftezole was observed 15 minutes after administration; ceftezole was approached the minimum at 3 hours. The maximum plasma level was the highest for cefazolin, followed by ceftezole, cephaloridine and

Table 1. Mean concentrations of ceftezole and related antibiotics in the plasma of rabbits and dogs

| Route of administration | Animal species | Dose (mg/kg) | No. of test animals | Antibiotic* | Mean plasma level ($\mu$g/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 5 min | 15 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| Intramuscular | Rabbits (2.4~2.7 kg) | 20 | 6 | CTZ | 51.4 | 54.5 | 41.6 | 20.7 | 5.1 | 1.6 | ND** |
| | | | | CEZ | 37.1 | 59.2 | 52.1 | 30.3 | 9.2 | 2.2 | ND** |
| | | | 4 | CER | 18.9 | 26.0 | 35.6 | 22.6 | 9.0 | 2.7 | ND** |
| | | | | CET | 21.1 | 26.5 | 22.2 | 7.5 | 1.2 | 0.1 | ND** |
| | Dogs (8.6~11.2 kg) | 10 | 4 | CTZ | 8.9 | 18.5 | 20.5 | 15.0 | | 2.5 | 0.2 |
| | | | | CEZ | 12.8 | 21.4 | 21.4 | 15.5 | | 3.0 | 0.2 |
| | | | | CER | 8.7 | 18.8 | 21.8 | 13.7 | | 1.9 | 0.2 |
| | | | | CET | 14.0 | 18.5 | 13.6 | 5.7 | | 0.3 | <0.1 |
| | Dogs (9.3~10.9 kg) | 20 | 4 | CTZ | 17.9 | 33.7 | 32.5 | 26.6 | | 6.2 | 0.7 |
| | | | | CEZ | 27.9 | 46.1 | 47.9 | 32.4 | | 8.0 | 0.9 |
| | | | | CER | 19.1 | 32.9 | 37.0 | 29.5 | | 4.7 | 0.6 |
| | | | | CET | 24.6 | 30.9 | 28.0 | 16.5 | | 1.1 | <0.1 |
| | | | | | 15 min | 30 min | 45 min | 60 min | 120 min | 180 min | 360 min |
| Intravenous | Rabbits (2.6~3.3 kg) | 10 | 3 | CTZ | 22.1 | 11.3 | | 4.9 | 0.5 | ND** | |
| | | | | CEZ | 33.6 | 16.2 | | 5.9 | 1.5 | ND** | |
| | | | | CER | 17.8 | 12.3 | | 4.8 | 1.1 | ND** | |
| | | | | CET | 11.0 | 4.1 | | 0.6 | <0.1 | ND** | |
| | Rabbits (2.6~3.3 kg) | 20 | 3 | CTZ | 56.5 | 31.2 | 19.0 | 12.2 | | 0.5 | ND** |
| | | | | CEZ | 66.4 | 40.2 | 22.9 | 13.4 | | 0.4 | ND** |
| | | | | CER | 34.9 | 28.7 | 18.0 | 13.0 | | 2.6 | ND** |
| | | | | CET | 25.3 | 8.0 | 2.7 | 1.4 | | <0.1 | ND** |

* CTZ (ceftezole), CEZ (cefazolin), CER (cephaloridine), CET (cephalothin). **ND: Not done.

Table 2. Urinary excretion of ceftezole and related antibiotics by rats, rabbits and dogs

| Route of administration | Animal species | Dose (mg/kg) | No. of test animals | Antibiotic | Mean urinary level and recovery | | | | | | Total recovery in 24 hours (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0~3 hrs | | 3~6 hrs | | 6~24 hrs | | |
| | | | | | μg/ml | % | μg/ml | % | μg/ml | % | |
| Intramuscular | Rats (S.D. strain, 180~200 g) | 10 | 6 | CTZ | 279 | 80.1 | 25.1 | 1.4 | 5.6 | 0.7 | 82.2 |
| | | | | CEZ | 249 | 67.1 | 48.2 | 2.5 | 8.6 | 2.1 | 71.7 |
| | | | 3 | CER | 698 | 60.8 | 49.3 | 2.7 | 5.0 | 0.6 | 64.1 |
| | | | | CET | 205 | 27.3 | 11.5 | 0.5 | 0.2 | 0.04 | 27.8 |
| | Rats (S.D. strain, 180~200 g) | 20 | 6 | CTZ | 626 | 76.5 | 76.6 | 1.1 | 16.8 | 1.3 | 78.9 |
| | | | | CEZ | 464 | 67.0 | 87.2 | 2.2 | 9.0 | 0.8 | 70.0 |
| | | | 3 | CER | 807 | 63.5 | 16.0 | 0.5 | 0.1 | 0.02 | 64.0 |
| | | | | CET | 275 | 17.8 | 23.7 | 0.7 | 2.5 | 0.1 | 18.6 |
| | Rabbits (2.4~2.7 kg) | 20 | 5 | CTZ | 395 | 84.1 | 48.2 | 8.1 | 0.9 | 0.4 | 92.6 |
| | | | | CEZ | 301 | 82.1 | 22.1 | 6.6 | 1.6 | 0.4 | 89.1 |
| | | | 4 | CER | 167 | 49.9 | 13.3 | 2.8 | 0.9 | 0.3 | 53.0 |
| | | | | CET | 191 | 36.7 | 0.7 | 0.1 | 0.1 | 0.03 | 36.7 |
| | Dogs (9.3~10.9 kg) | 10 | 4 | CTZ | 234 | 76.5 | 541 | 7.3 | 14.0 | 0.6 | 84.4 |
| | | | | CEZ | 234 | 77.3 | 527 | 5.0 | 20.0 | 0.8 | 83.1 |
| | | | | CER | 203 | 69.3 | 357 | 5.5 | 14.0 | 0.6 | 75.4 |
| | | | | CET | 131 | 46.6 | 54 | 0.6 | 3.0 | 0.1 | 47.3 |
| | Dogs (9.3~10.9 kg) | 20 | 4 | CTZ | 410 | 69.7 | 928 | 8.2 | 51.0 | 1.3 | 79.2 |
| | | | | CEZ | 412 | 72.8 | 736 | 7.7 | 56.0 | 1.6 | 82.1 |
| | | | | CER | 338 | 60.4 | 688 | 6.8 | 36.0 | 1.0 | 68.2 |
| | | | | CET | 272 | 49.8 | 165 | 1.5 | 6.0 | 0.1 | 51.4 |
| Intravenous | Rats (S.D. strain, 220~230 g) | 10 | 3 | CTZ | 390 | 66.4 | 32.5 | 3.4 | 3.4 | 0.6 | 70.4 |
| | | | | CEZ | 310 | 47.8 | 190 | 6.3 | <0.2 | — | 54.1 |
| | | | | CER | 250 | 35.2 | 76.0 | 1.7 | 2.4 | 0.7 | 37.6 |
| | | | | CET | 160 | 10.0 | 36.0 | 2.4 | 0.4 | 0.2 | 12.6 |
| | Rats (S.D. strain, 220~230 g) | 20 | 3 | CTZ | 1,060 | 73.4 | 45.0 | 0.9 | <0.2 | — | 74.3 |
| | | | | CEZ | 630 | 49.5 | 320 | 9.5 | 31.5 | 6.8 | 65.8 |
| | | | | CER | 560 | 44.0 | 225 | 5.8 | 14.0 | 1.4 | 51.2 |
| | | | | CET | 220 | 9.1 | 76.0 | 2.8 | 5.8 | 0.3 | 12.2 |
| | Rabbits (2.6~3.3 kg) | 20 | 3 | CTZ | 219 | 84.4 | 13.1 | 3.2 | 2.1 | 0.4 | 91.0 |
| | | | | CEZ | 333 | 83.0 | 16.1 | 4.4 | 3.0 | 1.0 | 88.4 |
| | | | | CER | 191 | 47.4 | 18.9 | 8.2 | 3.3 | 0.8 | 56.4 |
| | | | | CET | 136 | 34.5 | 2.2 | 0.5 | 0.1 | 0.02 | 35.0 |

cephalothin.

When administered to dogs at 10 and 20 mg/kg, maximum plasma levels of ceftezole were observed 15~30 minutes after administration; ceftezole was essentially absent from the blood at 6 hours. At a dose of 10 mg/kg, the maximum levels of each antibiotics were almost the same except for cephalothin; the level for cefazolin was slightly higher than those of the others.

(2) Intravenous administration

When 10 and 20 mg/kg of ceftezole and related antibiotics were administered to rabbits, determination of the plasma levels of each antibiotic 15 minutes after administration revealed that cefazolin had the highest, followed by ceftezole, cephaloridine and cephalothin. Thereafter, rapid decreases in plasma levels of each drug were observed with each drug disappearing from the blood within 3 hours after administration.

### 2. Urinary Excretion in Rats, Rabbits and Dogs

Urinary excretion of ceftezole and related antibiotics after intramuscular and intravenous administration in rats, rabbits and dogs was examined. The results are shown in Table 2.

(1) Intramuscular administration

When 10 and 20 mg/kg of each antibiotic were administered to rats, at each dosage level of each drug, most of the antibiotic was excreted in the urine within 3 hours after administration. The percentage of ceftezole excreted in 24-hour urine was the highest, followed by cefazolin, cephaloridine and cephalothin.

Similar results were observed when 20 mg/kg of each antibiotic was administered to rabbits.

When 10 and 20 mg/kg of each drug were administered to dogs, the amounts of ceftezole and cefazolin excreted were nearly the same, but higher than those of cephaloridine and cephalothin.

(2) Intravenous administration

When 10 and 20 mg/kg of each antibiotic were administered to rats, most of the drug was excreted in the urine within 3 hours, with the excretion of ceftezole was faster than those of the other antibiotics. The total excretion of ceftezole in 24-hour urine was also higher than those of the other antibiotics.

### 3. Serum Level and Urinary Excretion after Intramuscular Administration in Human Volunteers

A single dose of 500 mg of ceftezole was administered intramuscularly to six healthy adult male volunteers and the serum and urine levels were examined.

As shown in Table 3, the serum level of ceftezole reached its maximum (mean: 22.9 $\mu$g/ml) 15~30 minutes after administration, and decreased almost below the assay range after 6 hours. When the standard dilution was carried out with PBS, lower values than with human serum were obtained.

The urine levels of antibiotic for the six subjects are shown in Table 4. The percentage of ceftezole excreted in 24-hour urine was high (mean: 92.6%), and particularly high concentrations of antibiotic were observed in the initial period after administration, reaching 2,667 $\mu$g/ml (mean) in 0~1-hour urine. Of additional interest, a mean antibiotic level of 5.8 $\mu$g/ml was still observed in 6~24-hour urine.

### 4. Identification of Microbiologically Active Substances in Human Urine

By thin-layer chromatography of human urine using three different developing solvent systems, spots of the same Rf value as ceftezole were observed on the bioautogram as shown in Fig. 1. These results suggested that no metabolite with antimicrobial activity was formed from ceftezole in the human body.

Table 3. Serum levels after intramuscular administration of 500 mg of ceftezole to human volunteers

| Volunteer | Body weight (kg) | Serum level ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 hr | 2 hrs | 4 hrs | 6 hrs |
| A (male) | 75 | 18.0*(8.4)** | 22.0 (10.0) | 20.8 (9.5) | 16.0 (7.3) | 5.5 (2.6) | 1.9 ( 0.9) |
| B (male) | 69 | 20.9 ( 9.8) | 20.5 ( 9.6) | 16.4 (7.5) | 8.2 (3.9) | 1.3 (0.6) | <0.4 (<0.2) |
| C (male) | 60 | 28.3 (13.3) | 22.3 (10.4) | 16.8 (7.7) | 8.8 (4.3) | 2.0 (0.9) | <0.4 (<0.2) |
| D (male) | 60 | 25.3 (11.9) | 26.3 (12.1) | 18.0 (8.2) | 10.3 (4.8) | 1.8 (0.9) | <0.4 (<0.2) |
| E (male) | 56 | 22.0 (10.4) | 22.5 (10.5) | 17.5 (8.0) | 10.6 (5.0) | 2.2 (1.0) | <0.4 (<0.2) |
| F (male) | 55 | 22.0 (10.4) | 23.9 (11.2) | 19.0 (8.7) | 10.2 (4.8) | 1.7 (0.8) | <0.4 (<0.2) |
| Mean | 62.5 | 22.8 (10.7) | 22.9 (10.6) | 18.1 (8.3) | 10.7 (5.0) | 2.4 (1.1) | <0.7 (<0.4) |
| ±S.D. | 7.9 | 3.6 ( 1.7) | 2.0 ( 0.9) | 1.6 (0.7) | 2.8 (1.2) | 1.5 (0.7) | |

* Standard dilution with human serum. ** Standard dilution with PBS (pH 7.0).

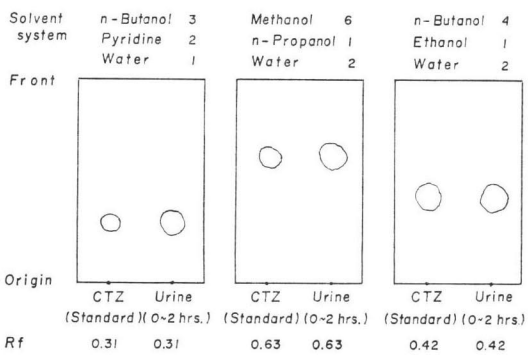
### 5. Biliary Excretion in Rats and Dogs

Biliary excretion was compared after intramuscular and intravenous administration of 20 mg/kg of ceftezole and related antibiotics in rats and dogs. As shown in Table 5, the biliary excretion of ceftezole was about 1/2 to 1/3 of that of cefazolin when administered intramuscularly and intravenously in rats, although it was much higher than those of cephaloridine and cephalothin.

When a 20 mg/kg dose of ceftezole was administered intramuscularly in dogs, biliary excretion was somewhat lower than that of cefazolin but much higher than those of cephaloridine and cephalothin. The bile level of ceftezole was 436 $\mu$g/ml in 0~2-hour bile and remained at 67 $\mu$g/ml in 4~8-hour bile.

Fig. 1. Bioautograms of human urine after intramuscular administration of ceftezole.

Urine: Healthy volunteer, 500 mg
(Urine at 0~2 hours after administration)
TLC: Cellulose thin-layer plate
(Avicel SF, Funakoshi Yakuhin)




Test organism: *Bacillus subtilis* ATCC 6633

### 6. Tissue Distribution in Rats and Rabbits

Serum and tissue distribution of ceftezole and related antibiotics was compared after intramuscular and intravenous administration of a 20 mg/kg dose in rats. The results are shown in Table 6.

Serum levels of antibiotics observed in rats were similar to those in rabbits. When administered intramuscularly in rats, ceftezole was found in the various tissues tested, particularly in the kidneys, and to a lesser extent in the liver, lungs, heart and spleen in that order. The initial level of ceftezole in the kidney was much higher than the levels of the other antibiotics tested. The initial levels of ceftezole in the liver and lung were also slightly higher than those of the other drugs. On the other hand, the disappearance of ceftezole was somewhat faster than that of the other drugs. When a dose of 20 mg/kg of either ceftezole or cefazolin was administered intravenously in rabbits, the concentration of ceftezole in the tissues was less than that of cefazolin.

Table 4. Urinary excretion of ceftezole after intramuscular administration of 500 mg to human volunteers

| Volunteer | 0~1 hr | | 1~2 hrs | | 2~3 hrs | | 3~6 hrs | | 6~24 hrs | | Total recovery in 24 hrs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | μg/ml | mg | μg/ml | mg | μg/ml | mg | μg/ml | mg | μg/ml | mg | mg | % |
| A | 855 | 115.4 | 935 | 138.4 | 730 | 88.3 | 385 | 97.4 | 12.9 | 21.3 | 460.8 | 92.2 |
| B | 1,135 | 196.4 | 845 | 148.7 | 435 | 55.7 | 200 | 51.0 | 3.9 | 4.9 | 456.7 | 91.3 |
| C | 1,340 | 193.0 | 805 | 120.8 | 685 | 61.0 | 159 | 21.4 | 3.6 | 7.0 | 403.2 | 80.6 |
| D | 8,800 | 211.2 | 4,400 | 158.4 | 1,875 | 54.4 | 515 | 44.8 | 6.4 | 6.1 | 474.9 | 95.0 |
| E | 1,665 | 176.5 | 1,265 | 161.9 | 900 | 71.1 | 355 | 85.2 | 5.1 | 7.1 | 501.7 | 100.3 |
| F | 2,205 | 196.2 | 1,230 | 174.7 | 285 | 61.0 | 89 | 42.5 | 3.1 | 5.6 | 480.0 | 96.0 |
| Mean | 2,667 | 181.5 | 1,580 | 150.5 | 818 | 65.3 | 283 | 57.1 | 5.8 | 8.7 | 462.9 | 92.6 |
| ±S.D. | 3,040 | 34.2 | 1,395 | 19.0 | 562 | 12.7 | 161 | 28.6 | 3.7 | 6.3 | 33.3 | 6.6 |

Table 5. Mean biliary excretion after administration of ceftezole and related antibiotics (20 mg/kg) to animals.

| Route of administration | Animal species | Dose (mg/kg) | No. of test animals | Antibiotic | Mean biliary level and recovery | | | | | | Total recovery in 24 hrs (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0~3 hrs | | 3~6 hrs | | 6~24 hrs | | |
| | | | | | μg/ml | % | μg/ml | % | μg/ml | % | |
| Intramuscular | Rats (S.D. strain, 200~300 g) | 20 | 12 | CTZ | 104.6 | 4.6 | 2.0 | 0.08 | 0.2 | 0.02 | 4.7 |
| | | | | CEZ | 281.5 | 12.7 | 31.9 | 1.2 | 3.2 | 0.3 | 14.2 |
| | | | 6 | CER | 11.7 | 0.6 | 3.3 | 0.1 | 0.6 | 0.04 | 0.7 |
| | | | | CET | 15.8 | 0.7 | 0.1 | 0.01 | <0.1 | — | 0.7 |
| Intravenous | Rats (S.D. strain, 290~310 g) | 20 | 4 | CTZ | 110.6 | 5.4 | 0.8 | 0.03 | 0.2 | 0.03 | 5.5 |
| | | | | CEZ | 262 | 11.6 | 4.7 | 0.2 | 0.4 | 0.03 | 11.8 |
| | | | | CER | 7.5 | 0.4 | 0.8 | 0.03 | <0.1 | — | 0.4 |
| | | | | CET | 12.2 | 0.7 | <0.1 | — | <0.1 | — | 0.7 |
| | | | | | 0~2 hrs | | 2~4 hrs | | 4~8 hrs | | 8~24 hrs | | Total recovery in 24 hrs (%) |
| | | | | | μg/ml | % | μg/ml | % | μg/ml | % | μg/ml | % | |
| Intramuscular | Dogs (6.4~9.6 kg) | 20 | 3 | CTZ | 304 | 0.8 | 202 | 0.4 | 67.0 | 0.2 | 3.0 | 0.02 | 1.4 |
| | | | | CEZ | 436 | 1.0 | 345 | 0.6 | 101 | 0.3 | 21.0 | 0.1 | 2.0 |
| | | | | CER | 18.0 | 0.03 | 36.0 | 0.04 | 11.0 | 0.03 | 1.0 | <0.01 | 0.1 |
| | | | | CET | 83.0 | 0.3 | 17.0 | 0.03 | 1.0 | <0.01 | 0.1 | <0.01 | 0.3 |

Table 6. Serum and tissue distribution after administration of ceftezole and related antibiotics (20 mg/kg) to animals

| Route of administration | Animal species | No. of test animals | Antibiotic | Time (min) | Mean serum and tissue concentrations ($\mu$g/ml or $\mu$g/g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Serum | Kidney | Liver | Lung | Heart | Spleen |
| Intramuscular | Rats (S.D. strain, 200~250 g) | 12 | CTZ | 15 | 53.8 | 86.9 | 23.9 | 16.7 | 9.4 | 4.3 |
| | | | | 30 | 38.2 | 56.0 | 10.9 | 11.3 | 6.7 | 2.5 |
| | | | | 60 | 12.1 | 19.8 | 1.5 | 3.8 | 1.9 | <0.5 |
| | | | | 90 | 4.5 | 9.5 | <0.5 | 2.1 | <0.5 | <0.5 |
| | | | | 120 | 1.1 | 2.3 | <0.5 | <0.5 | <0.5 | <0.5 |
| | | | CEZ | 15 | 84.8 | 54.8 | 17.6 | 13.6 | 8.9 | 3.6 |
| | | | | 30 | 69.5 | 43.8 | 15.4 | 11.9 | 7.0 | 2.7 |
| | | | | 60 | 33.6 | 18.3 | 4.7 | 6.3 | 3.5 | 0.7 |
| | | | | 90 | 16.9 | 11.1 | 1.0 | 4.2 | <0.5 | <0.5 |
| | | | | 120 | 8.1 | 5.1 | <0.5 | 2.5 | <0.5 | <0.5 |
| | | 6 | CER | 15 | 23.5 | 50.5 | 5.3 | 11.3 | 8.0 | 3.2 |
| | | | | 30 | 24.9 | 54.5 | 7.0 | 9.3 | 6.8 | 3.4 |
| | | | | 60 | 11.2 | 25.6 | 6.9 | 4.7 | 2.8 | 2.1 |
| | | | | 90 | 5.5 | 17.5 | 5.4 | 2.3 | 1.0 | 0.8 |
| | | | | 120 | 1.9 | 5.0 | 2.2 | 0.8 | 0.3 | 0.3 |
| | | | CET | 15 | 22.5 | 20.6 | <0.1 | 5.9 | 2.4 | 2.1 |
| | | | | 30 | 7.3 | 5.2 | <0.1 | 3.2 | 0.5 | <0.1 |
| | | | | 60 | 1.0 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| | | | | 90 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| | | | | 120 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Intravenous | Rats (S.D. strain, 220~240 g) | 3 | CTZ | 5 | 95.0 | 197 | 42.0 | 18.3 | 10.7 | 5.8 |
| | | | | 15 | 54.0 | 85.0 | 25.0 | 8.8 | 5.7 | 3.0 |
| | | | | 30 | 23.0 | 28.3 | 5.0 | 4.4 | 2.8 | 0.8 |
| | | | | 60 | 7.2 | 8.9 | 0.7 | 1.3 | <0.5 | <0.5 |
| | | | CEZ | 5 | 126 | 104 | 43.0 | 11.6 | 9.4 | 4.4 |
| | | | | 15 | 96.7 | 67.5 | 27.0 | 9.5 | 6.9 | 3.3 |
| | | | | 30 | 67.7 | 32.5 | 5.4 | 6.1 | 4.2 | 0.9 |
| | | | | 60 | 32.7 | 14.4 | 0.9 | 1.2 | 1.0 | <0.5 |
| | | | CER | 5 | 52.3 | 148 | 10.4 | 20.2 | 8.7 | 4.8 |
| | | | | 15 | 34.7 | 74.2 | 9.3 | 11.0 | 5.3 | 3.9 |
| | | | | 30 | 22.3 | 51.2 | 9.2 | 10.7 | 2.9 | 2.1 |
| | | | | 60 | 4.6 | 12.6 | 4.9 | 2.4 | 0.8 | 0.7 |
| | | | CET | 5 | 29.9 | 29.0 | 2.6 | 8.4 | 2.2 | 3.0 |
| | | | | 15 | 12.4 | 8.5 | 0.5 | 5.0 | 0.8 | 1.8 |
| | | | | 30 | 1.8 | 3.2 | <0.1 | 2.3 | <0.1 | <0.1 |
| | | | | 60 | 0.2 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| | Rabbits (2.6~3.3 kg) | 3 | CTZ | 15 | 55.0 | 435 | 7.3 | 12.8 | 12.9 | 5.2 |
| | | | | 30 | 28.3 | 177 | 4.3 | 7.1 | 6.3 | 3.1 |
| | | | | 60 | 11.2 | 50.5 | 2.8 | 3.2 | 2.6 | 2.2 |
| | | | CEZ | 15 | 90.0 | 440 | 11.2 | 19.6 | 17.0 | 7.0 |
| | | | | 30 | 50.5 | 260 | 6.0 | 9.8 | 10.3 | 4.2 |
| | | | | 60 | 15.3 | 70.5 | 1.4 | 3.7 | 3.5 | 2.7 |

### 7. Lymph Level in Rats

Concentrations of ceftezole in the lymph after administration of a single dose of 20 mg/kg to rats were compared with those of cefazolin and cephaloridine. As shown in Table 7, ceftezole reached a higher concentration 1 hour after administration than did cefazolin and cephaloridine. Thereafter, the decrease in the concentration of ceftezole was slightly faster than that of cefazolin. A considerable amount of ceftezole was found in the lymph 5 hours after administration despite its disappearance from

Table 7. Concentration of ceftezole and related antibiotics in the lymph of rats*

| Antibiotic | Mean lymph concentration ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0~1/2 hr | 1/2~1 hr | 1~2 hrs | 2~3 hrs | 3~5 hrs | 5~8 hrs |
| CTZ | 20.0 | 20.6 | 16.6 | 11.8 | 4.0 | 0.8 |
| CEZ | 15.4 | 16.6 | 15.0 | 11.0 | 6.2 | 2.5 |
| CER | 7.5 | 11.0 | 9.5 | 5.3 | 0.4 | <0.3 |

* Wistar-Imamichi strain, weighing 260~280 g, 3 rats/group.
Antibiotics were administered intramuscularly (20 mg/kg).

Table 8. Concentrations of ceftezole and related antibiotics in the exudates in rat inflammatory pouches*

| Route of administration | Antibiotic | Mean exudate concentration ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1/4 hr | 1/2 hr | 1 hr | 2 hrs | 3 hrs | 5 hrs |
| Intramuscular | CTZ | 0.8 | 2.6 | 3.4 | 3.5 | 2.5 | 1.4 |
| | CEZ | 0.6 | 1.4 | 2.2 | 2.4 | 2.6 | 1.6 |
| | CER | 3.0 | 4.0 | 5.8 | 4.3 | 2.7 | 1.2 |
| | CET | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | <0.3 |
| Intravenous | CTZ | 4.3 | 5.6 | 6.0 | 4.6 | 3.0 | 1.4 |
| | CEZ | 2.0 | 3.9 | 5.0 | 4.8 | 3.9 | 2.0 |
| | CER | 2.8 | 4.8 | 6.8 | 5.6 | 3.0 | 0.9 |
| | CET | 1.1 | 1.4 | 1.4 | 0.8 | 0.5 | <0.3 |

* Wistar-Imamichi strain rats, weighing 200~240 g, 7 rats/group
Antibiotics were injected at 20 mg/kg on the 5th day (intravenous injection) or 8th day (intramuscular injection) after induction of inflammation.

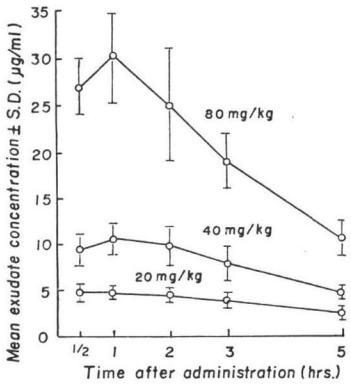
the blood.

### 8. Exudate Level in Rat Aseptic Inflammatory Pouches

On the 8th day after the induction of subcutaneous aseptic inflammatory pouches in rats, 20 mg/kg of ceftezole and related antibiotics were administered intramuscularly and the exudate levels of the antibiotics were determined. Similarly, exudate levels of the drugs were examined after intravenous administration of a 20 mg/kg dose to rats with inflammatory pouches on the 5th day after induction. As shown in Table 8, ceftezole attained a maximum 1 to 2 hours after administration, a concentration that was somewhat lower than that of cephaloridine but slightly higher than that of cefazolin. When the antibiotics were administered intravenously, the maximum concentrations in the exudates were higher than when the antibiotics were given by intramuscular administration.

Fig. 2. Concentration of ceftezole in rat exudates*

* S.D. strain rats, weighing 140~160 g, 4 rats/group, 7 day-pouch after inflammatory induction.
Antibiotics administered intravenously.




The concentrations of ceftezole in the exudates after intravenous administration of 20, 40 and 80

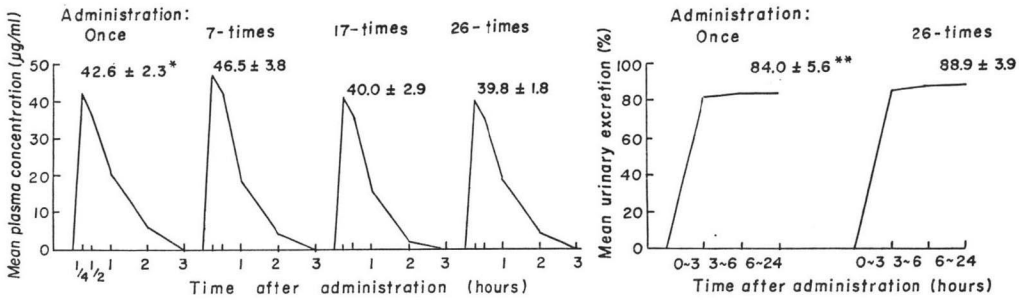
Table 9. Cerebrospinal fluid concentration after intravenous administration of ceftezole (50 mg/kg) in rabbits*

| Exp. group | Mean cerebrospinal fluid concentration±S.D. (μg/ml) | | | | | Body temp. (°C) (immediately before administration) |
|---|---|---|---|---|---|---|
| | 1/2 hr | 1 hr | 2 hrs | 3 hrs | 4 hrs | |
| *S. pyogenes* JU-13 infected rabbits** | 4.1±0.5 | 3.2±0.4 | 2.2±0.1 | 1.7±0.2 | 1.5±0.3 | 40.9~41.5 |
| Normal rabbits | 0.6±0.3 | 0.9±0.4 | 1.1±0.7 | 0.8±0.5 | 0.3±0.2 | 38.9~39.1 |

* Weighing 2.6~3.2 kg, 3 rabbits/group.

** Rabbits were injected with $10^6$ viable units (v.u.) of *S. pyogenes* JU-13 into the cerebrospinal fluid 24 hours before administration.
Viable cell count in cerebrospinal fluid immediately before administration: $5.0\times10^2$~$9.5\times10^3$ v.u./ml.

Fig. 3. Effect of repeated intramuscular administration of ceftezole to rabbits* (20 mg×2/day) on plasma concentrations and excretion in the urine.
Rabbits: Weighing 2.6~3.2 kg, 3 rabbits/group
* Mean peak level ± S.D. (μg/ml)
** Mean urinary excretion rate in 0~24 hour-urine±S.D. (%)




mg/kg doses were examined using rats with inflammatory pouches on the 7th day after induction. As shown in Fig. 2, a dose-response relationship was observed between the maximum concentration and the dose.

### 9. Level in the Cerebrospinal Fluid of Rabbits

The cerebrospinal fluid level of ceftezole was determined using normal rabbits and those with cerebrospinal meningitis induced by infecting with *S. pyogenes*.

Twenty-four hours after inoculation of *S. pyogenes*, the viable cell counts in the fluid were about $1\times10^3$ viable units/ml, and the mean body temperature was 41.2°C, about 2°C higher than that of normal rabbits, which clearly indicates the establishment of meningitis. After administration of 50 mg/kg of ceftezole intravenously to rabbits, the concentration of antibiotic in the cerebrospinal fluid was determined. The results are shown in Table 9. The concentrations present in rabbits infected with *S. pyogenes* were found to be several times higher than that in normal rabbits.

### 10. Plasma Level and Urinary Excretion in Repeated Long-term Administration in Rabbits

The changes in plasma concentrations and rate of urinary excretion were examined when 20 mg/kg of ceftezole was administered 26 times intramuscularly in rabbits for 14 days. As shown in Fig. 3, there was no change in the patterns of plasma level and urinary excretion throughout the period of

the experiment. Moreover, the levels of ceftezole in the blood, urine, kidneys, liver, lungs, spleen and heart 24 hours after the last administration were all below the assay range, indicating there were no residues in the various tissues.

## Discussion

As reported in the preceding paper,[1] ceftezole has a chemical structure similar to that of cefazolin. Antimicrobial tests conducted *in vitro* showed that ceftezole, in its activities against clinical isolates of gram-negative bacteria, especially *Klebsiella* spp. and *E. coli*, was essentially equivalent to cefazolin and more potent than cephaloridine and cephalothin. The extent of binding of ceftezole to human serum protein was somewhat lower than that of cefazolin.

In this report, a comparative study of blood, lymph and various tissue levels, as well as urinary and biliary excretion of ceftezole, cefazolin[4,5] and related antibiotics was conducted in rats, rabbits and dogs when the antibiotics were administered intramuscularly and intravenously.

In the animal studies ceftezole concentrations in the blood decreased more rapidly than did concentrations of cefazolin. Furthermore, the excretion of ceftezole was faster than that of cefazolin. When ceftezole was administered intramuscularly to human volunteers in a single dose of 500 mg, it was found that ceftezole disappeared from the blood 6 hours after administration. Ceftezole was excreted rapidly by humans, 79.5% in the first three hours. The initial concentration of antibiotics in the urine was high. It was also confirmed by bioautography that ceftezole was excreted in human urine in the active form undergoing minimal metabolism. It is concluded that ceftezole could be especially effective in the treatment of clinical urinary infections.

Biliary excretion of ceftezole in rats and dogs was found to be somewhat lower than that of cefazolin but much higher than those of cephaloridine and cephalothin.

The pattern of tissue distribution of ceftezole was found to be similar to that of cefazolin but the former was distributed more rapidly and the rate of decrease in the tissue was also faster.

Peak levels of ceftezole in lymph and the exudate of inflammatory pouches were slightly higher than those obtained with cefazolin. These results also imply good distribution of ceftezole in various tissues.

It was found that repeated administration of ceftezole to rabbits did not affect the pattern of the blood level and urinary excretion. Therefore, it is probable that repeated administration of ceftezole to humans is unlikely to cause toxic manifestation related to accumulation of the antibiotic in various tissues.

Acknowledgement

We wish to express our appreciation to the following individuals: H. ENDO, Y. SHIRAKI, S. SUGANO, T. YAMAZAKI, M. AKIMA, Y. NODA, R. NIKI, M. KOJIMA, S. SHINGAI and T. TATSUMI, for their co-operation throughout the experiments.

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