CEFTEZOLE, A NEW CEPHALOSPORIN C DERIVATIVE
I. *IN VITRO* AND *IN VIVO* ANTIMICROBIAL ACTIVITY

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Ceftezole, a new cephalosporin antibiotic similar to cefazolin, has the following chemical structure: (6*R*,7*R*)-8-oxo-7-[2-(1H-tetrazol-1-yl)acetamido]-3-[(1,3,4-thiadiazol-2-ylthio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid. Ceftezole was found to be a broad-spectrum antibiotic, active *in vitro* against many species of gram-positive and gram-negative bacteria except *Pseudomonas aeruginosa*, *Serratia marcescens* and *Proteus vulgaris*. The activity of ceftezole against clinical isolates of *Escherichia coli* and *Klebsiella* spp. appeared to be nearly equal to that of cefazolin and higher than those of cephaloridine and cephalothin. Cross-resistance was observed between ampicillin and cephaloridine, but not between ampicillin and ceftezole, in susceptibility tests on clinical isolates of *P. mirabilis*. The *in vitro* activity was little affected by the inoculum size, the presence of human serum or the test medium. Ceftezole exhibited apparent bactericidal activity at the concentrations above the minimum inhibitory concentration (MIC) against both *S. aureus* and *E. coli*. The development *in vitro* of resistance by *S. aureus* 209P and *E. coli* NIHJ to ceftezole after 16 transfers was similar to or somewhat slower than that to other drugs tested. Ceftezole was relatively stable in nutrient broth and minimally degraded in the serum or tissue homogenates of rats.

Ceftezole, in a single subcutaneous administration, exhibited somewhat less efficacy in mice against intraperitoneal infections with *Streptococcus pyogenes*, *S. pneumoniae*, *E. coli*, *K. pneumoniae* or *P. mirabilis* than either cephaloridine or cefazolin. However, ceftezole exhibited efficacy similar to that of cephaloridine or cefazolin when administered in three doses. Furthermore, ceftezole was as effective as cefazolin in the treatment of experimental abscesses in mice caused by subcutaneous inoculation with *S. aureus*.

Ceftezole, a new analog of cephalosporin C recommended for parenteral use,[1,2] has the chemical structure shown in Fig. 1. Both ceftezole and cefazolin have a tetrazolyl-acetyl group on the amino group of 7-aminocephalosporanic acid (7ACA), but ceftezole has a thiadiazolyl-thiomethyl group on the 3-position of 7ACA in place of the 5-methyl-thiadiazolyl-thiomethyl group possessed by cefazolin.

This report describes the results of *in vitro* and *in vivo* evaluations of the antimicrobial activities of ceftezole as compared with those of cefazolin and other related antibiotics.

Materials and Methods

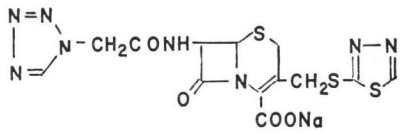
1. Antibiotics.

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

lothin sodium (CET, Eli Lilly and Co.), cephalexin (CEX, Glaxo Laboratories), ampicillin sodium (ABPC, Meiji Seika Co.) and benzylpenicillin potassium (PC G, Meiji Seika Co.).

Ceftezole sodium is a white powder, freely soluble in water.

Fig. 1. Chemical structure of ceftezole sodium



Sodium (6*R*, 7*R*)-8-oxo-7-[2(1H-tetrazol-1-yl) acetamido]-3-[(1,3,4-thiadiazol-2-ylthio) methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
$C_{13}H_{11}N_8O_4S_3Na$ (M.W. 462.47)

2. Bacterial strains.

Reference strains obtained from the Research Laboratories, Chugai Pharmaceutical Co. were used. Clinical isolates were obtained from several hospitals near Tokyo. Most of the strains were stab-cultured in nutrient agar (0.5% agar) medium, and stored at room temperature with tight stoppers. Strains of streptococci, pneumococci, *Corynebacterium diphtheriae* and *Brucella* were cultured on heart infusion agar slants containing 10% horse serum, and stored at room temperature. *Neisseria* was cultured on egg yolk medium and stored in an incubator at 36°C. *Hemophilus* strains were stored after lyophilization.

The strains used in the experimental infections were as follows: 6 strains of *S. aureus*, 2 strains of *S. pyogenes*, 1 strain of *S. pneumoniae*, 3 strains of *E. coli*, 2 strains of *K. pneumoniae* and 3 strains of *P. mirabilis*. The strains were passed through animals several times before use for the selection of virulent cells and adaptation. The strains were then suspended in broth containing 10% glycerol and stored in a deep freezer at −70°C to maintain virulence. Each strain was streaked and cultured on nutrient agar plates or those containing 5% horse blood immediately before use and normal colonies formed were used for challenge.

3. Determination of *in vitro* antibacterial activity.

In vitro antibacterial activities of ceftezole and related antibiotics were determined by the agar-plate dilution method. Unless otherwise specified, an overnight culture ($10^8 \sim 10^9$ viable cells/ml) of each test strain in Trypticase soy broth (BBL, abbreviated as TSB) was diluted 100-fold ($10^6 \sim 10^7$ viable cells/ml). One loopful of this culture was streaked on each of several heart infusion (Eiken, abbreviated as HI) agar plates containing twofold serially graded concentrations of each antibiotic. The agar plates were cultured at 37°C for 18 hours, and the MIC value (μg/ml) was determined. For *S. pneumoniae*, *S. pyogenes*, *C. diphtheriae* and *B. suis*, TSB containing 10% horse serum was used as the preculture medium and HI agar containing 10% defibrinated horse blood as the test medium for the determination of the MIC value. *N. gonorrhoeae* and *N. meningitidis* were cultured in GC agar containing 2% hemoglobin using a 5%-CO_2 incubator. For *H. influenzae*, chocolate agar containing 10% horse blood was used.

4. Influence of various factors on antibacterial activity.

The changes in MIC values of ceftezole and of cefazolin in response to various inoculum sizes, addition of human serum, different kinds of test media and pH values of a test medium were compared. As test strains, *S. aureus* 209P and *E. coli* NIHJ were used. The MIC value was determined by the twofold serial dilution liquid method in HI broth as the basal medium. Influence of serum on the MIC value was determined in HI broth supplemented with human serum, inactivated by heating at 60°C for 20 minutes at concentrations of 20, 30 or 40%. To determine the influence of inoculum size, an overnight culture of each test strain in TSB was diluted 10-fold serially ($10^3 \sim 10^8$ viable cells/ml) and a one-tenth volume of each dilution was inoculated and cultured in each of a series of HI broth containing graded concentrations of each antibiotic. To examine the influence of test media, nutrient broth (Eiken), HI broth (Eiken), brain-heart infusion broth (Eiken) and TSB (Eiken) were chosen. In examining the influence of the pH of the test medium, HI broth at pH values of 5, 6, 7, 8 and 9 was used.

5. Estimation of bactericidal activity.

S. aureus 209P and *E. coli* NIHJ were used as the test strains. After subculturing twice in HI broth, one volume of each was inoculated into nine volumes of the same medium and then incubated at 37°C for 2 hours with shaking. The culture was used as the inoculum at the logarithmic phase of growth.

Tubes of HI broth containing 1/2, 1, 2 or 4 times the MIC value of ceftezole or cefazolin were inoculated with the culture described above to give 10^6 viable cells/ml. These were incubated at 37°C with shaking, and sampled for determination of the numbers of viable cells by the agar plate method after 1, 3, 5, 8, 12 and 24 hours.

6. Morphological changes of bacterial cells.

A logarithmic phase culture of *E. coli* NIHJ was prepared in a similar manner as described above. HI broth containing graded concentrations of ceftezole or the related antibiotic was inoculated with the culture to give 10^6 viable cells/ml and incubated at 37°C. Two or 3 hours after onset of growth, smear preparations of each culture were made; the morphological change of bacterial cells was observed microscopically after single staining. The remainders were submitted to further cultivation to calculate the MIC of each antibiotic.

7. *In vitro* development of resistance.

S. aureus 209P and *E. coli* NIHJ were serially subcultured 16 times every day in nutrient broth (Eiken) containing increasing concentrations of ceftezole or a related antibiotic; each was inoculated at 37°C for 20 hours. The inoculation at each transfer was made from the most turbid culture.

8. Microbiological assay method for antibiotics.

Concentrations of antibiotics were determined by the agar-plate diffusion method using paper-discs. A 100-ml portion of melted nutrient agar was mixed with 0.1 ml of a spore suspension (10^9 spores/ml) of *Bacillus subtilis* ATCC 6633. Five milliliter portions of the mixture were poured in Petri dishes and solidified to make agar plates. Paper-discs (Toyo Roshi Co., diameter: 8 mm, "thick" type) were dipped in the standard or a test solution. After the excess solution had been removed, the discs were placed on the agar plates. The diameters of inhibitory zones were measured after incubation at 37°C for 20 hours and the amounts of drugs in the test samples were calculated from the standard curves.

9. Stabilities in liquid media and tissue homogenates.

Ceftezole and related antibiotics were dissolved individually in nutrient broth (Eiken, pH 7.0) to give concentrations of 10 μg/ml, which were kept at 5°C, 27°C and 37°C for 3 days. The residual activity in the medium was determined at one-day intervals by the paper-disc method.

To estimate the stability in tissue homogenates, male rats (S.D. strain, weighing about 150 g) were sacrificed by cervical dislocation and the blood, lungs, liver, spleen, kidneys and heart were removed. Serum was then separated from the blood and a 20% homogenate of each organ was prepared in 1/15 M phosphate buffered saline (pH 7.0). After 1 ml of a solution of 200 μg/ml of ceftezole or a related antibiotic was mixed with the homogenate or serum; the mixture was incubated at 37°C for 30 or 90 minutes. After incubation, 2 ml of 99% ethanol was added to the reaction mixture to stop the enzymic activity, and the residual antibacterial activity in the mixture was determined by the paper-disc method.

10. Determination of binding rate to serum protein.

The binding rates of ceftezole and related antibiotics to human, rat, mouse, rabbit and dog serum protein were determined by the equilibrium dialysis method. After one volume of each antibiotic solution (250, 500, 1,000 or 2,000 μg/ml) was added to nine volumes of serum, the mixture was incubated at 37°C for 60 minutes. Four ml of the resulting mixture were then put into a cellophane tube (Visking, diameter: 18 mm) and dialyzed against 20 ml of 1/15 M phosphate buffer (pH 7.4) at 4°C for 24 hours. The concentrations of antibiotics in the internal solution of the dialyzing tube were determined by the paper-disc method, and the binding rate to serum protein was calculated by the following formula:

$$\text{Binding rate } (\%) = \frac{\text{Total amounts added} - (\text{external solution conc.} \times \text{Total vol.})}{\text{Total amounts added}} \times 100$$

11. Experimental infections (intraperitoneal injection).

Male mice, ddY strain, weighing 22~25 g, were used; each experimental group consisted of seven animals. Each challenge strain was cultured at 37°C for 20 hours in HI broth containing 0.5% yeast extract. After appropriate dilution with 3% mucin (Wako Junyaku Co., Mucin gastric, W-1701) or

nutrient broth for *S. pneumoniae*, 0.4 ml doses of each diluted culture were inoculated intraperitoneally into mice. Ceftezole and other antibiotics were diluted twofold serially with sterilized physiological saline and each diluted solution was administered once subcutaneously into mice 1 hour after the challenge, or 3 times at 1-hour intervals from 1 hour after the challenge. The survival ratio of each experimental group was determined; the median effective dose (ED_{50}) was calculated by the method of BEHRENS-KÄRBER.

Virulence of challenge strains (LD_{50}) was estimated by the death rate of the infected mice. Each challenge strain prepared as described above was diluted 10-fold serially with 3% mucin or nutrient broth, and the diluted suspension was inoculated intraperitoneally into a group of 7 mice. The animals were observed for 7~10 days for the death rate and LD_{50} values were calculated by the method of BEHRENS-KÄRBER.

12. Experimental infections (subcutaneous injection).

Male mice, ddY strain, weighing 22~25 g, were used; each experimental group consisted of 5 mice. For the challenge, 6 strains of *S. aureus* were used. The experiments were carried out similarly as those described by TADOKORO[3], and OSONO *et al.*[4] Challenge strains were cultured on nutrient agar (Eiken) plates at 37°C for 24 hours. The bacterial cells were suspended in sterilized physiological saline to give a concentration of 10^9/ml, 0.1 ml of which was inoculated subcutaneously into the back of each mouse. Each antibiotic was administered twice subcutaneously in the infected mice 1 and 2 hours after the challenge. Forty-eight hours after the challenge, the skin of each animal at the inoculation site was excised and the diameters of abscesses formed were measured from the visceral side. Then the dose-response curves were drawn through the plots of the dose of each drug *versus* the mean diameter of the abscesses.

MIC for each challenge strain was determined by inoculating one loopful each of bacterial suspensions of 10^8/ml (without dilution) and of 10^6/ml (100-fold dilution).

Results

1. Antimicrobial Spectrum

The antimicrobial spectra of ceftezole and related antibiotics are summarized in Table 1.

Ceftezole was highly active against a wide range of both gram-positive and gram-negative bacteria, with the exception of *P. aeruginosa*, *S. marcescens*, *P. vulgaris* and *P. morganii*. Its antimicrobial spectrum was very similar to that of cefazolin.

2. Susceptibilities of Clinical Isolates

MIC values of ceftezole and related antibiotics for clinical isolates of several species is shown in Table 2. Susceptibilities of 48 clinical isolates of *S. aureus* to ceftezole showed a peak at 0.20 μg/ml and no resistant strain with an MIC of 3.13 μg/ml or above was observed. The activity of ceftezole was slightly higher than those of cefazolin and cephalothin, and lower than that of cephaloridine. Ceftezole also showed high activity against strains resistant to benzylpenicillin. Distribution of MIC values of ceftezole against 51 strains of *E. coli* and 46 strains of *Klebsiella* spp. showed peaks at 0.78 and 1.56 μg/ml, respectively. The growth of most strains was inhibited at 3.13 μg/ml or below, and no strain resistant to 100 μg/ml or above was observed. The activity of ceftezole was nearly the same as that of cefazolin and higher than those of cephaloridine and cephalothin.

The distribution of MIC values of ceftezole against 92 strains of *P. mirabilis* peaked at 3.13 μg/ml, and only four strains were resistant with MIC values of 50 μg/ml or above. The activity of ceftezole was apparently higher than that of cephaloridine and equal to or slightly higher than those of cefazolin and cephalothin.

There were some strains resistant to cephaloridine at 100 μg/ml or above among the clinical iso-

Table 1. Antibacterial spectra of ceftezole and related antibiotics

Organism		MIC (μg/ml)			
		CTZ	CEZ	CER	CET
Staphylococcus aureus	209P, JC-1	0.10	0.10	<0.013	0.10
S. aureus	EQP-3	0.78	0.78	0.20	0.39
S. aureus	Terajima	0.20	0.20	<0.025	0.20
S. epidermidis	ATCC-12228	0.20	0.20	0.05	0.10
S. epidermidis	I	0.39	0.39	0.20	0.20
S. epidermidis	II	0.39	0.39	0.20	0.20
**Streptococcus pyogenes*	Cook	0.39	0.20	0.05	0.20
**S. pyogenes*	J-12	0.39	0.20	0.025	0.39
**S. pneumoniae*	1	0.20	0.20	0.05	0.20
**S. pneumoniae*	2	0.39	0.20	0.05	0.20
**Corynebacterium diphtheriae*	N-90	0.39	0.39	0.10	0.20
**C. diphtheriae*	TC-7	0.78	0.78	0.20	0.78
**C. diphtheriae*	Wakabayashi	0.39	0.39	0.10	0.20
Bacillus subtilis	ATCC-6633	0.10	0.20	<0.025	<0.025
Sarcina lutea	PCI-1001	0.39	0.39	0.025	0.10
***Neisseria gonorrhoeae*	No. 9	0.31	0.31	1.25	0.075
***N. meningitidis*	GA	0.62	0.62	2.5	0.15
***Hemophilus influenzae*	9321 (type A)	6.25	3.13	3.13	1.56
**Brucella suis*	TO-1	12.5	25	12.5	6.25
Salmonella paratyphi A	TO-1	1.56	1.56	3.13	1.56
S. paratyphi B	TO-1	3.13	3.13	0.78	6.25
S. typhi	Tanaka	1.56	1.56	3.13	0.78
S. enteritidis	M-11	1.56	1.56	3.13	3.13
Shigella flexneri 2a	TO-1	1.56	1.56	3.13	6.25
S. flexneri 2b	TO-1	1.56	1.56	1.56	3.13
S. sonnei	SS-1	1.56	1.56	3.13	6.25
Escherichia coli	NIHJ JC-2	1.56	1.56	6.25	12.5
E. coli	Denken	1.56	1.56	1.56	1.56
E. coli	Hasegawa (TC^r)	0.78	0.78	1.56	3.13
Klebsiella pneumoniae	K1-15	1.56	1.56	3.13	3.13
Vibrio cholerae	El Tor 2	6.25	6.25	12.5	1.56
V. cholerae	El Tor 3	3.13	3.13	25	1.56
V. cholerae	classical 1	3.13	3.13	12.5	0.78
V. cholerae	classical 2	3.13	3.13	12.5	0.78
Proteus mirabilis	24	3.13	6.25	6.25	6.25
P. vulgaris	1	100	200	>200	200
P. morganii	4	25	50	200	>200
Serratia marcescens	SM-1	>200	>200	>200	>200
Pseudomonas aeruginosa	Ps-1	>200	>200	>200	>200

Medium: Heart infusion agar
Inoculum size: 1 loopful of 10^6 cells/ml
*: blood agar (10% horse blood)
**: GC agar containing 2% hemoglobin
***: chocolate agar (10% horse blood)
Drugs: CTZ (ceftezole), CEZ (cefazolin), CER (cephaloridine), CET (cephalothin).

lates of *P. mirabilis*. These strains were highly resistant to ampicillin with MIC values of 200 μg/ml or above. However, these strains were susceptible to ceftezole and cefazolin (Figs. 2 and 3).

Nearly all 24 isolates of *P. vulgaris* were highly resistant to ceftezole, cefazolin, cephaloridine and cephalothin with MIC values higher than 100 μg/ml. A total of 34 strains of *P. morganii, P. rettgeri* and

Table 2. Distribution of susceptibilities of clinical isolates to ceftezole and related antibiotics

Organism	Anti-biotic	MIC (μg/ml)												
		≤0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
S. aureus 48 strains	CTZ		1	22	13	11	1							
	CEZ			11	21	15		1						
	CER	11	18	17	1		1							
	CET			16	29	2		1						
	PC G*	11	1		12	8		2		3	4	6	1	
E. coli 51 strains	CTZ					25	21			3		2		
	CEZ					10	34	2		3	1	1		
	CER						1	38	3	4	2	2		1
	CET							8	27	10	1	2		3
	ABPC**						6	25	9				1	10
Klebsiella spp. 46 strains	CTZ					4	32	8			2			
	CEZ						35	3	6		2			
	CER						3	26	12	1	2		2	
	CET					2	8	23	9	2		2		
	ABPC								2	8	14	14	3	5
P. mirabilis 92 strains	CTZ						4	65	13	6		1	2	1
	CEZ						1	38	39	10			1	3
	CER						2	2	66	3		3	3	13
	CET					2	6	34	29	6	14			1
	ABPC				3	30	29	8	2	1	3			16
P. vulgaris 24 strains	CTZ							1			1	1	4	17
	CEZ								1			1	1	21
	CER								1	1			2	20
	CET							1	1		1	1	1	19
	ABPC						2			1	1	2	2	16
P. morganii *P. rettgeri* *P. inconstans* 34 strains	CTZ				2	3	2	1		3	4	6	6	7
	CEZ				2	3	2	1	2	2	3	4	4	11
	CER								2	2	1	2	7	20
	CET					1		2	3	1	3	1	3	20
	ABPC				1	2	2	4	5	3	7	5		5
Citrobacter spp. 42 strains	CTZ						6	6	7	4	2	3	3	11
	CEZ						3	4	11	4	1	1	3	15
	CER							1	3		3	8	7	20
	CET							1		12	10	6	1	12
	ABPC						2	9	14		1			16

Inoculum size: 1 loopful of 10^6 cells/ml

*: Benzylpenicillin, unit/ml

**: Ampicillin

P. inconstans, and 48 strains of *Citrobacter* spp. showed widely different susceptibilities to ceftezole.

3. Influences of Various Experimental Conditions on Activity *In Vitro*

Influences of inoculum sizes, pH values of the test medium, presence of human serum and test media on the activities of ceftezole and cefazolin *in vitro* were examined with *S. aureus* 209P and *E. coli* NIHJ as test organisms. The results are shown in Table 3.

With different inoculum sizes of *S. aureus* 209P, MIC values of ceftezole and cefazolin hardly fluctuated. With a large inoculum size of *E. coli* NIHJ, the MIC value of ceftezole tended to become higher than that of cefazolin.

Both ceftezole and cefazolin were more active at acidic pH than at alkaline pH.

Addition of human serum even at 40% to the test medium did not change the MIC value of ceftezole against *S. aureus* 209P, whereas it tended to increase the MIC value of cefazolin.

The MIC values of ceftezole fluctuated slightly in different test media and this tendency was also observed with cefazolin.

4. Bactericidal Activity

The bactericidal activity of ceftezole was compared with that of cefazolin with *S. aureus* 209P

Fig. 2. Correlation of antimicrobial activity between cephaloridine and ampicillin.

P. mirabilis 92 strains
r (correlation coefficient) = 0.93

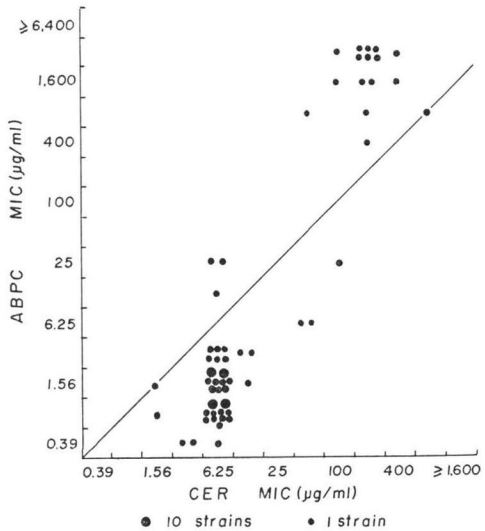


Fig. 3. Correlation of antimicrobial activity between ceftezole and ampicillin.

P. mirabilis 92 strains
r (correlation coefficient) = 0.50

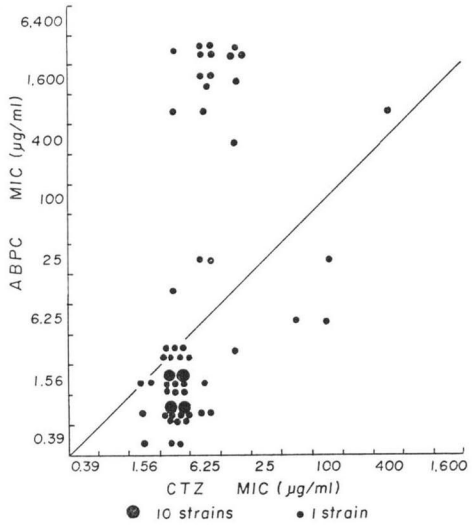


Table 3. Influence of various factors on activities of ceftezole and cefazolin

Factor		MIC (μg/ml)			
		S. aureus 209P		*E. coli* NIHJ	
		CTZ	CEZ	CTZ	CEZ
Inoculum size (cells/ml)	8.6×10^5	0.2	0.2	12.5	6.25
	8.6×10^4	0.1	0.1	3.13	6.25
	8.6×10^3	0.1	0.1	1.56	3.13
	8.6×10^2	0.1	0.1	1.56	3.13
	8.6×10^1	0.05	0.05	1.56	3.13
pH*	5	—	—	0.78	0.78
	6	0.05	0.05	1.56	1.56
	7	0.1	0.1	1.56	3.13
	8	0.1	0.1	3.13	3.13
	9	0.2	0.2	6.25	6.25
Human** serum	0 %	0.2	0.2	6.25	6.25
	10	0.2	0.2	6.25	6.25
	20	0.2	0.39	—	—
	30	0.2	0.39	—	—
	40	0.2	0.39	—	—
Media*	NB	0.05	0.1	3.13	3.13
	HIB	0.1	0.1	1.56	3.13
	BHIB	0.025	0.05	1.56	1.56
	TSB	0.025	0.05	1.56	3.13

Medium: Heart infusion broth,
HB; Nutrient broth (Eiken), HIB; Heart infusion broth (BBL), BHIB; Brain-heart infusion broth (Eiken), TSB; Trypto soy broth (Eiken)
Inoculum size: *10^3 cells/ml, **_S. aureus_ 10^4 cells/ml _E. coli_ 10^5 cells/ml

and *E. coli* NIHJ, with the results shown in Figs. 4 and 5. When *S. aureus* 209P grown in nutrient broth was exposed to ceftezole at half the MIC, bacterial growth was suppressed for 12 hours; thereafter the viable cell count increased gradually. At a concentration equal to the MIC value, the viable cell count decreased for 12 hours but increased thereafter. At 2 or 4 times the MIC value, the viable cell count decreased markedly during 24-hour incubation. When the experiments with *E. coli* NIHJ

Fig. 4. Bactericidal effects of ceftezole and cefazolin against *S. aureus* 209P.

Heart infusion broth, shaking culture

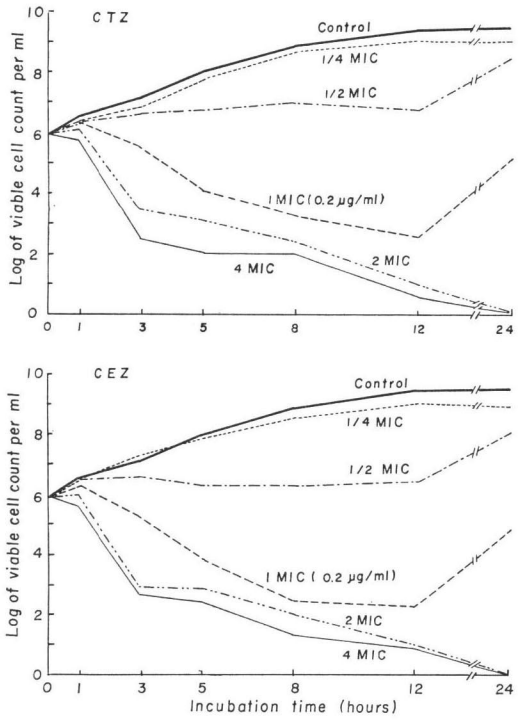
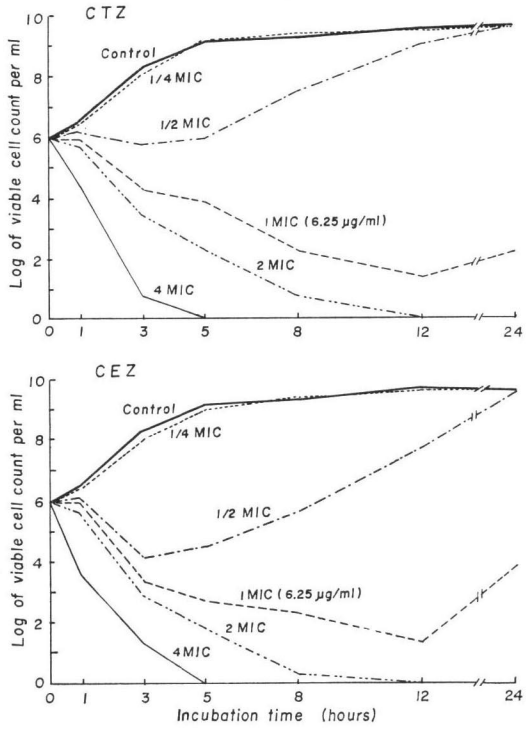


Fig. 5. Bactericidal effects of ceftezole and cefazolin against *E. coli* NIHJ.

Heart infusion broth, shaking culture



were carried out under the same conditions as those with *S. aureus* 209P, similar results were obtained. However, at 4 times the MIC, ceftezole manifested a high bactericidal activity and no viable cells were detected after 5 hours.

It was concluded that the bactericidal activities of ceftezole and cefazolin are essentially equivalent.

5. Morphological Changes of Bacterial Cells Induced by Ceftezole and Other Drugs

Morphological changes of *E. coli* NIHJ grown in HI broth containing twofold serial concentrations of ceftezole were compared with those induced by related antibiotics. The results are shown in Fig. 6. Ceftezole at concentrations in a range of one-fourth to one MIC induced filamentation of bacterial cells. The concentration range of ceftezole causing such morphological changes was nearly the same as that of cefazolin, although that of cephaloridine was a little narrower. However, cephalexin at concentrations in a remarkably wide range centering around the MIC value induced filamentous cells.

6. Development of Resistance *In Vitro*

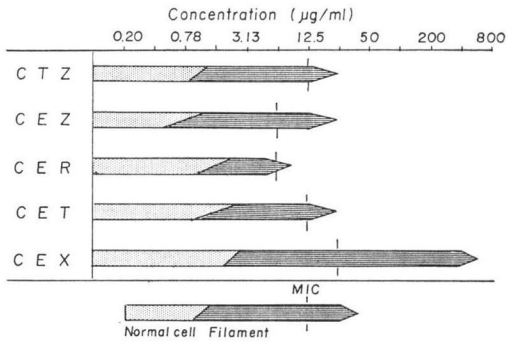
The development of resistance during serial transfers of *S. aureus* 209P and *E. coli* NIHJ in broth containing increasing amounts of ceftezole was compared with that with related antibiotics, as shown in Fig. 7.

In *E. coli* NIHJ, the acquired resistance to ceftezole after 16 serial transfers was very similar to that to cefazolin, and lower than those to ampicillin, cephalothin and cephaloridine.

The resistance of *S. aureus* 209P increased very slowly and the differences in resistance to the dif-

ferent antibiotics tested were not clear after 16 serial transfers.

Fig. 6. Morphological changes of cells of *E. coli* NIHJ induced by ceftezole and related antibiotics.
Medium: Heart infusion broth
Inoculum size: 10^6 cells/ml
Incubation time: 37°C, 2~3 hours.



7. Stabilities in Liquid Medium and in Tissue Homogenate

As shown in Fig. 8, the stability of ceftezole in nutrient broth was found to be substantially equal to that of cefazolin and higher than those of cephaloridine and cephalothin. About 80% of the initial activity was maintained after incubating ceftezole at 37°C for 3 days.

The results of stability tests of ceftezole and related antibiotics in rat serum and tissue homogenates are shown in Fig. 9. Little or no decrease in activity was observed after incubating ceftezole, cefazolin or cephaloridine in rat serum or in a homogenate of liver, lungs, spleen, kidneys or heart at 37°C for 90 minutes. In contrast, the activity of cephalothin decreased remarkably in rat tissue homogenate.

8. Binding to Serum Protein

Binding rates of ceftezole and related antibiotics to human and various animal serum proteins were estimated by the equilibrium dialysis method in cellophane tubes. The results are shown in Table 4.

There were considerable differences in binding rates to serum protein of different animal species. At 50 μg/ml of ceftezole, the binding rates to human serum and the sera of various animals were as follows: human 69%, rat 70%, mouse 53%, rabbit 83% and dog 25%. From these results, the binding rate of ceftezole was similar or slightly higher than that of cephalothin and higher than that of cephaloridine. However, the binding rate of ceftezole was apparently lower than that of cefazolin. In dog serum, the binding rate of all antibiotics was remarkably low and almost no difference was observed among the drugs.

Fig. 7. Development of resistance to ceftezole and related antibiotics *in vitro*.

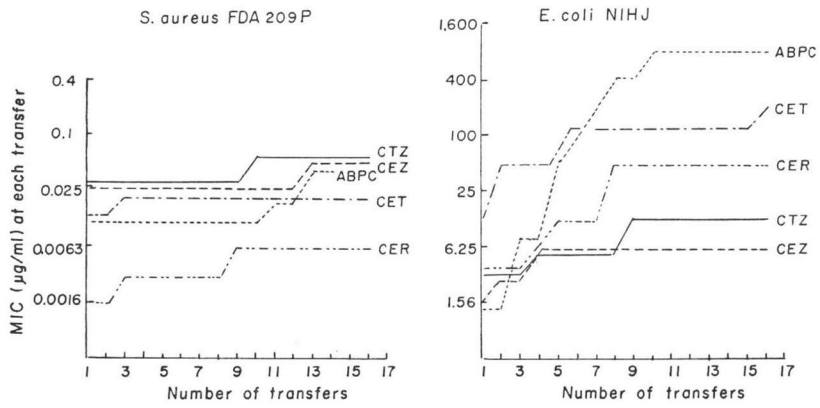


Fig. 8. Stability of ceftezole and related antibiotics in nutrient broth (pH 7.0).

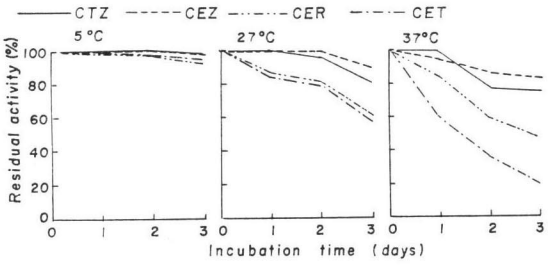
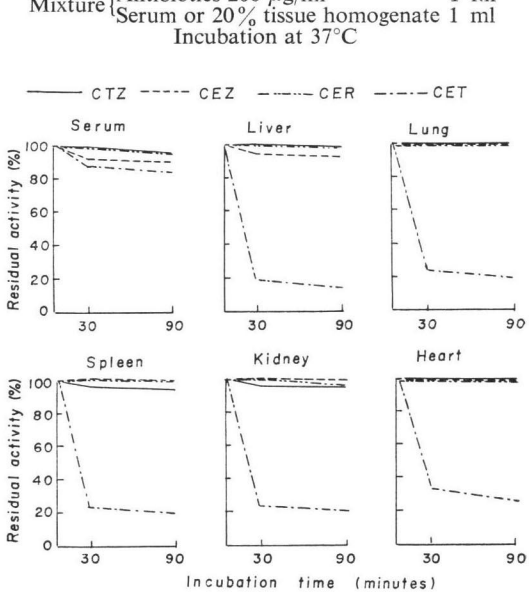


Fig. 9. Stability of ceftezole and related antibiotics in rat serum and tissue homogenates.



9. Protecting Effects against Experimental Infections in Mice Challenged Intraperitoneally

Ceftezole and the related antibiotics were administered subcutaneously once or three times to mice challenged intraperitoneally with a strain of *S. pyogenes*, *S. pneumoniae*, *E. coli*, *K. pneumoniae* or *P. mirabilis*. Individual protective effects of the drugs were expressed in ED_{50} values as shown in Table 5.

Generally, ceftezole, cefazolin and cephaloridine were highly efficacious against the various experimental infections; however, cephalothin was less active than the former drugs. Among ceftezole, cefazolin and cephaloridine, cephaloridine was particularly efficacious for treatment of infections with *S. pyogenes* or *S. pneumoniae*. No appreciable difference was noted among the three antibiotics against

Table 4. Extent of protein binding of ceftezole and related antibiotics in sera of different animal species (Equilibrium dialysis)

Species	Strain	Antibiotic conc. (μg/ml)	Binding rate (%)			
			CTZ	CEZ	CER	CET
Human	adults (male)	25	72	86	38	60
		50	69	83	37	58
		100	67	84	35	55
		200	56	81	36	52
Rat	Wistar-Imamichi (male)	25	73	90	30	68
		50	70	89	30	64
		100	65	88	23	60
		200	45	70	20	56
Mouse	IVCS (female)	25	57	76	18	66
		50	53	75	16	63
		100	49	74	14	60
		200	39	65	12	57
Rabbit	Japanese white (male)	25	86	92	25	80
		50	83	90	27	78
		100	79	91	18	76
		200	72	86	18	74
Dog	Beagle (male)	25	34	32	28	30
		50	25	25	25	22
		100	15	22	20	18
		200	8	17	14	12

One volume of an antibiotic solution was added to 9 volumes of serum. After incubation at 37°C for 60 minutes, the mixture was dialyzed against 1/15 M phosphate buffer (pH 7.4) at 4°C for 24 hours.

Table 5. Efficacy of ceftezole and related antibiotics for the treatment of experimental infections in mice

Strain	MIC (μg/ml)				Challenge dose/mouse		Times of administration	ED_{50} (mg/mouse)			
	CTZ	CEZ	CER	CET	cells/mouse	$\times LD_{50}$		CTZ	CEZ	CER	CET
S. pyogenes J-12	0.04	0.04	0.02	—	4×10^2	87	1	0.17	0.11	0.04	—
S. pyogenes J-13	0.08	0.08	0.02	—	4×10^2	86	1	0.21	0.09	0.04	—
S. pneumoniae TO-1	0.08	0.08	0.02	0.08	2×10^2	23	1	0.10	0.05	0.03	1.28
							3	0.02	0.01	0.01	0.10
E. coli 11	1.56	1.56	1.56	1.56	4×10^1	24	1	0.12	0.07	0.10	0.77
							3	0.07	0.09	0.06	0.32
E. coli 49	1.56	3.12	6.25	6.25	4×10^2	120	1	0.36	0.19	0.18	>4.0
							3	0.13	0.13	0.12	0.79
E. coli 54	3.12	6.25	3.12	—	4×10^2	118	1	0.12	0.10	0.08	—
K. pneumoniae 3K25	1.56	1.56	3.12	3.12	4×10^1	12	1	0.28	0.22	0.24	1.28
							3	0.18	0.16	0.16	0.79
K. pneumoniae 15C	1.56	1.56	3.12	—	4×10^3	24	1	0.10	0.06	0.06	—
P. mirabilis 9′	3.12	6.25	6.25	6.25	4×10^5	447	1	0.22	0.24	0.35	0.95
							3	0.20	0.21	0.20	0.29
P. mirabilis 1287	3.12	6.25	6.25	—	4×10^3	33	1	0.35	0.46	0.18	—
P. mirabilis JU-453	6.25	12.5	200	12.5	4×10^4	32	1	0.95	0.78	>9.0	1.52
							3	0.31	0.27	2.40	0.78

Animals: Mice, ddY, male, 22~25 g, 7 mice/group
Infection: Challenged intraperitoneally with a bacterial suspension in 3% gastric mucin or nutrient broth culture
Therapy: Subcutaneous administration
Once: 1 hour after the challenge
Three times: starting 1 hour after the challenge, at 1-hour intervals (ED_{50}: mg/mouse $\times$ 3)

the infections caused by the gram-negative rods. Ceftezole was somewhat less efficacious than either cefazolin or cephaloridine by single administration, but was similar in efficacy to the other two antibiotics when administered in three doses.

In addition, cephaloridine had poor efficacy against the infection with *P. mirabilis* JU-453, a highly resistant strain with a MIC of 200 μg/ml, although both ceftezole and cefazolin exhibited good efficacy.

10. Suppressive Effects against Experimental Abscess Formation in Mice Challenged Subcutaneously

Six clinical isolates of *S. aureus* with the ability to form subcutaneous abscesses were used for challenge. Ceftezole given in two divided doses was compared with cefazolin, cephalothin and ampicillin for suppressive activity. Abscess formation was induced by subcutaneous inoculation. The results are shown in Fig. 10.

Although the effects of each drug varied slightly depending on challenge strain, ceftezole and cefazolin appeared to be more effective than cephalothin. Ampicillin was highly effective against infections with susceptible strains (215 and JU-39) but demonstrated little or no efficacy against infections either with strains of low resistance at large inoculum sizes (JU-48 and JU-38) or with highly resistant strains (JU-5 and JU-33).

Fig. 10. Efficacy of ceftezole and related antibiotics for the treatment of subcutaneous abscess formation in mice.

Mice (ddY, male, 22~25 g) were inoculated subcutaneously with 10^8 cells/mouse of each strain of *S. aureus*.

Each antibiotic was injected subcutaneously at 1 and 2 hours after infection into 4 groups of mice (5 mice/group).

Abscess formation was observed at 48 hours after infection.

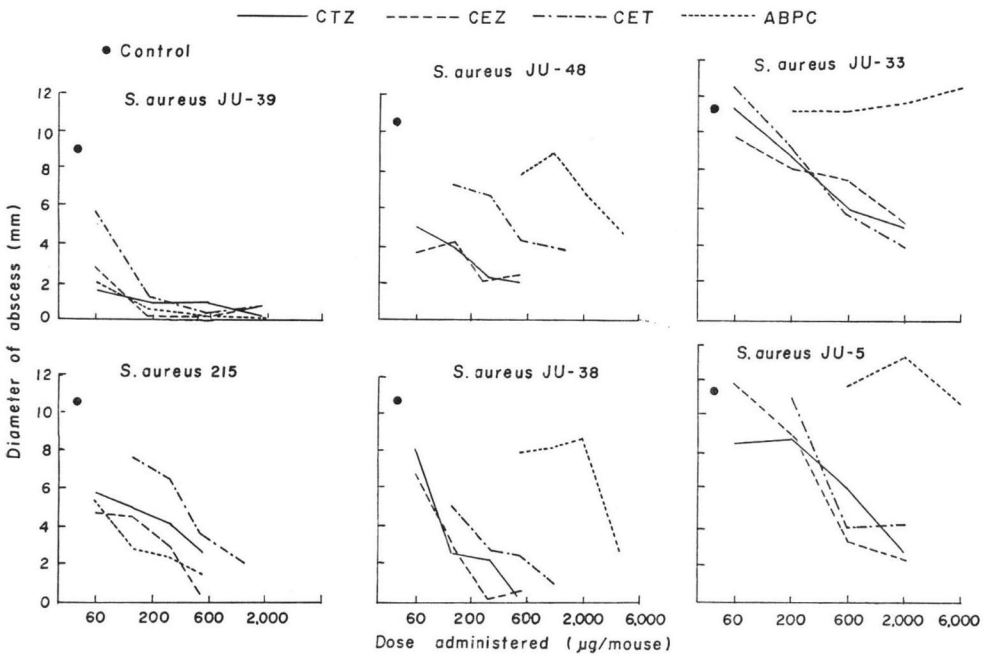


Table 6. Antibacterial activity of ceftezole and related antibiotics *in vitro* against *S. aureus*. Effect of inoculum size on MIC.

Test strain	Inoculum size (1 loopful of cells/ml)	MIC (μg/ml)			
		CTZ	CEZ	CET	ABPC
S. aureus JU-39	10^8	0.16	0.31	0.16	0.08
	10^6	0.16	0.16	0.16	0.08
S. aureus 215	10^8	0.31	0.31	0.31	0.16
	10^6	0.16	0.31	0.16	0.08
S. aureus JU-48	18^8	0.31	0.31	0.31	2.50
	10^6	0.16	0.16	0.16	0.62
S. aureus JU-38	10^8	0.62	0.62	0.31	5
	10^6	0.16	0.31	0.16	0.62
S. aureus JU-33	10^8	0.62	0.62	0.31	125
	10^6	0.31	0.31	0.16	1.25
S. aureus JU-5	10^8	0.62	0.62	0.31	250
	10^6	0.31	0.31	0.16	1.25

Discussion

The antibacterial spectrum of ceftezole including its activity *in vitro* against clinical isolates was similar to that of cefazolin. The activity of ceftezole against gram-positive bacteria was somewhat lower than that of cephaloridine. However, against gram-negative rods, especially *E. coli*, *Klebsiella* spp. and *P. mirabilis*, the activity of ceftezole was higher than those of cephaloridine and cephalothin.

It was found that clinical isolates of *P. mirabilis* highly resistant to ampicillin were also resistant to cephaloridine. However, these strains were susceptible to both ceftezole and cefazolin. The resistance of these isolates was derived from R-plasmids (unpublished data). Therefore, it is indicated that R-plasmid-bearing strains of gram-negative rods have not acquired resistance to ceftezole as yet.

NEHASHI *et al.*[5] studied the stability of ceftezole against various β-lactamases and found that ceftezole was highly stable against penicillinase-type β-lactamase of *S. aureus*, and that it was also only minimally hydrolyzed by penicillinase-type β-lactamase which was mediated by conjugative R-plasmids of gram-negative rods. However, they found that ceftezole and other cephalosporin C antibiotics were easily hydrolyzed by the chromosomally originated cephalosporinase type β-lactamase of *Enterobacter cloacae*, a strain highly resistant to ceftezole.

The bactericidal activity of ceftezole was found to be nearly the same as that of cefazolin. Both ceftezole and cefazolin were highly stable in test media and in rat tissue homogenates.

The binding of cefazolin to human serum protein was considerably higher than that of cephaloridine. However, binding of ceftezole was lower than that of cefazolin, and equivalent to that of cephalothin.

In experimental intraperitoneal infections in mice with gram-positive bacteria or gram-negative rods, the protective effects of ceftezole by single administration were slightly lower than those of cefazolin, but ceftezole was as effective as cefazolin when administered in three doses. As will be reported in a subsequent paper,[6] the blood levels of ceftezole were lower than those obtained with cefazolin, and the retention time of the former was also slightly shorter than that of the latter. Therefore, maximum efficacy may not be obtained by a single administration of ceftezole in those experiments where efficacy is determined by septic death; it is believed that the activity *in vitro* would be reflected *in vivo* if repeated administrations of the antibiotic are made.

The suppression by ceftezole of subcutaneous abscess formation by *S. aureus* was similar to that of cefazolin. Clinical effectiveness of ceftezole against local infections can be expected as the antibiotic is distributed uniformly throughout the various tissue of animals.[6]

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