CEFAZOLIN, A NEW SEMISYNTHETIC CEPHALOSPORIN ANTIBIOTIC. II

IN VITRO AND *IN VIVO* ANTIMICROBIAL ACTIVITY

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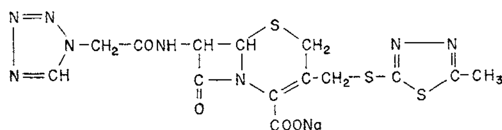
(Received for publication January 23, 1970)

Cefazolin is a new antibiotic derived from 7-aminocephalosporanic acid, having the structure of 7-[1-(1H)-tetrazolylacetamido]-3-[2-(5-methyl-1,3,4-thiadiazolyl)-thiomethyl]-Δ^3-cephem-4-carboxylic acid. This substance is a broad-spectrum antibiotic, active *in vitro* against most of Gram-positive and Gram-negative species of bacteria except for *Ps. aeruginosa*, and is also active against penicillinase-producing strains of *Staph. aureus*. The activity of cefazolin against fresh isolates of *E. coli* and *Kl. pneumoniae* seems superior to those of other antibiotics used in this study. The *in vitro* activity of cefazolin is little influenced by size of inoculum, presence of rabbit serum, or kinds or properties of test medium. The activity of cefazolin is apparently bactericidal against both Gram-positive and Gram-negative bacteria at or above the MIC levels. In experiments on the development of resistance *in vitro*, the MICs of cefazolin against *Staph. aureus* strain 209P and *E. coli* strain NIHJ increase slowly in a stepwise over a period of 17 transfers. The rate of resistance development of cefazolin is comparable with that of cepholoridine or ampicillin. Cefazolin is relatively stable to enzymes from *Staph. aureus* and *E. coli*, which readily inactivate ampicillin and benzyl penicillin. Cefazolin is not remarkably degraded by the tissue homogenates of rat, although cephalothin is degraded quite rapidly. Cefazolin, in the single subcutaneous dose, shows excellent protecting activity against experimental infections in mice infected with both PC-sensitive and resistant strains of *Staph. aureus*, and is also fairly effective against infections in mice with *Diplococcus pneumoniae*, *E. coli* and *Proteus mirabilis*.

Cefazolin (CEZ), with a chemical structure shown in Fig. 1, has the tetrazolylacetyl side-chain on the amino group, and the 5-methyl-thiadiazolylthiomethyl group on the 3-position of 7-aminocephalosporanic acid.

This substance was shown to be a bactericidal antibiotic with a wide range of activity against Gram-positive

Fig. 1. Chemical structure of sodium cefazolin



$C_{14}H_{13}O_4N_8S_3Na$ (M. W. 476.3)

Sodium 7-[1-(1 H)-tetrazolylacetamido]-3-[2-(5-methyl-1, 3, 4-thiadiazolyl)-thiomethyl]-Δ^3-cephem-4-carboxylate

and Gram-negative pathogens. Because of the structural similarity to other cephalosporin analogues, the *in vitro* activity of cefazolin was expected to overlap those of certain commercially available agents such as cephalothin and cephaloridine.

However, it was found that cefazolin is more active than related antibiotics against clinical isolates of Gram-negative rods, especially *Klebsiella pneumoniae* and *E. coli*, whereas the degree of serum-binding was estimated to be higher than that of cephaloridine.

The present paper deals with the results of the *in vitro* comparative evaluation of antimicrobial activities of cefazolin and the related antibiotics.

Materials and Methods

1. Antibiotics tested

The antibiotics used were: cefazolin (CEZ, 954 mcg/mg, Fujisawa Research Laboratories), cephalothin (CET, 971 mcg/mg, Eli Lilly and Co.), cephaloridine (CER, 988 mcg/mg, Glaxo Laboratories), cephalexin (CEX, 975 mcg/mg) and ampicillin (AB-PC, 840 mcg/mg, Beecham Research Laboratories). Test preparation of cephalexin was produced experimentally in Fujisawa Research Laboratories.

Sodium cefazolin is a white crystalline powder, freely soluble in water, and the pH of a 10 % solution is from 4.7 to 5.4.

2. Bacterial strains used

Standard strains stored in our laboratory were used in this study. The clinical isolates of various species of bacteria were supplied from several hospitals in Japan. Many of the test strains were conserved on heart infusion agar slopes, and subcultured at one-month intervals. Heart infusion agar with 10 % horse serum was used for strains of streptococci, pneumococci, and corynebacteria, ZEISSLER's blood-glucose agar for clostridia, DUBOS medium for mycobacteria, and GC-chocolate agar for *Neisseria gonorrhoeae*.

3. Method for estimation of antibacterial activity *in vitro*

In vitro antibacterial activity of cefazolin was determined by the two-fold agar-plate dilution method as described below.

If not otherwise specified, one loopful of an overnight culture of each test strain in Trypticase-soy broth (10^8 viable cells per ml) was streaked on heart infusion agar (HI-agar) containing graded concentrations of cefazolin, and the minimal inhibitory concentration (MIC) was expressed in terms of mcg/ml after incubation at 37°C for 20 hours, except for *Mycobacterium tuberculosis*, in which MIC was determined after 14 days of incubation. For the determination of the MICs against *Diplococcus pneumoniae*, *Streptococcus hemolyticus*, *Str. faecalis* and *Corynebacterium diphtheriae*, alteration was made by adding 10 % rabbit serum to the Trypticase-soy broth and 10 % defibrinated rabbit blood to the HI-agar medium. For *Clostridium perfringens* and *Neisseria gonorrhoeae*, ZEISSLER's medium and GC-chocolate agar were respectively used.

For observing the influence of the addition of serum to the test media on the antibacterial activity of cefazolin, the MICs against *Staph. aureus* 209P, *E. coli* NIHJ and *Kl. pneumoniae* 417 were determined, using HI-agar supplemented with rabbit serum at a concentration of 10, 25 and 50 % respectively, after incubation at 37°C for 20 hours, and the values obtained were compared with those in test media without serum.

To determine the influence of inoculum size, an overnight culture of each test strain in Trypticase-soy broth (10^8 viable cells per ml) was diluted to obtain the viable cell suspensions containing different numbers of viable cells ($10^4 \sim 10^7$/ml). One loopful of each cell suspension was streaked on HI-agar containing graded concentrations of cefazolin, and the MICs with the ranging inoculum sizes of the test organisms were determined

after incubation under the same condition, and compared each other.

To investigate the influence of different kinds of test media, the *in vitro* activity of cefazolin was determined using five kinds of agar media. Each one loopful of an overnight culture of the test strain was streaked on each of test agar media containing increasing levels of cefazolin and the MIC was determined after incubation under the same condition.

To investigate the influence of the reaction of test medium, the activity of cefazolin was determined using HI-agar with varying pH values. The MIC was determined under the same condition.

4. Bactericidal activity

Dilutions of cefazolin and cephaloridine were made in nutrient broth to give concentrations of one-fourth MIC, MIC and 4 times MIC against *Staph. aureus* 209P and *E. coli* NIHJ respectively. The media were inoculated with approximately 10^6 organisms per ml, incubated at 37°C (with shaking for initial 8 hours) and sampled for estimation of viable cell counts at 1, 3, 5, 8 and 24 hours. Each sample was diluted in sterile saline and one ml aliquots were placed in petri dishes, 20 ml of melted nutrient agar were added and mixed well. The plates were incubated overnight at 37°C and colonies produced at each time of sampling were counted.

5. Development of resistance *in vitro*

Both *Staph. aureus* 209P and *E. coli* NIHJ were serially subcultured daily seventeen times in nutrient broths containing increasing levels of each cefazolin, cephaloridine and ampicillin, respectively. The inoculation in each transfer was made from the tube showing visible growth and containing the highest antibiotic concentration.

6. Method for assay of drug

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

7. Stability in liquid media

The stabilities of cefazolin and related antibiotics were compared with each other by incorporating them in various liquid media and keeping at different temperatures. The antibiotics, dissolved in heart infusion, brain heart infusion, nutrient broth or Trypticase soy broth at a concentration of 10 mcg/ml, were kept for 3 days at temperature of 5°, 25° and 37°C. The residual activity in the media was determined at certain intervals by the disc method.

8. Sensitivity of cefazolin to bacterial enzymes

Phosphate buffer solutions (0.1 M) each containing a concentration of 500 mcg/ml of cefazolin, cephaloridine, cephalothin, or cephalexin, and equal volumes of BHI-broth cultures of *Staph. aureus* or *E. coli* were mixed in test tubes and kept with shaking at 37°C for 2 hours. Thereafter the enzymatic activity of the mixtures was terminated by boiling the mixtures for 1 minute. The residual activity of the antibiotics was determined by the disc method described above to estimate their degradation.

9. Stability to tissue homogenates

Male rats (Wistar strain, about 150 g), fasted for a day, were used as sources of tissue homogenates. Twenty percent homogenate was prepared from the small intestine, liver and kidney with KREBS-RINGER phosphate (pH 7.2), and from the stomach with saline of pH 2.0. Each 1 ml of cefazolin or other antibiotic solutions (200 mcg/ml) was incubated with each 1 ml of the homogenates for 30 or 90 minutes at 37°C. Enzymatic activity was terminated by adding an equal volume of 99 % ethanol to the incubation mixture.

The sample solutions obtained at 30 and 90 minutes were diluted with distilled water. The residual activity in the mixture was determined by the disc method.

10. Protein binding effect of cefazolin and related antibiotics

The degree of binding of cefazolin and other antibiotics to serum proteins was measured by ultra-filtration through Visking tubing (size: 8/32). Each 9 ml of fresh sera (man, dog, rabbit or rat) was mixed with 1 ml of 0.1 M phosphate buffer (pH 7.4) containing cefazolin or other antibiotics at 500 mcg/ml, then incubated at 37°C for 1 hour. After incubation, the mixtures were introduced into bags made from Visking tubing and centrifuged at $1,000 \times g$ for 30 minutes. The antibiotic activities present in protein-free ultra-filtrates were assayed by the disc method. As a control, the antibiotic solutions without serum were treated similarly. Unbound antibiotic was calculated as the ratio of the concentration in ultra-filtrate to the total concentration in serum.

11. Experimental infections in mice

Male albino mice of *dd*-strain, aged about 5 weeks and weighing 17~21 g, were used, and each experimental group consisted of 8 animals. Each challenge strain was cultured overnight in BHI-broth containing 10 % rabbit serum at 37°C.

In infection with *Staph. aureus*, 0.5 ml of the culture was injected intravenously, whereas, with strains of other bacterial species such as *D. pneumoniae*, *E. coli* and *P. mirabilis* the mice were challenged intraperitoneally with same doses. All test strains killed control groups of mice within 48 hours after challenge. Tested drugs were administered subcutaneously once at 1 hour after challenge.

The experimental animals were observed for death or survival for 2 weeks, and the results were calculated in terms of median effective doses (ED_{50}).

Results

1. Antimicrobial Spectrum of Cefazolin in Comparison with Related Antibiotics

The antimicrobial spectra of cefazolin and related antibiotics are summarized in Table 1. Cefazolin was active against both Gram-positive and Gram-negative bacteria, except for *Ps. aeruginosa* IAM-1095, *Pr. vulgaris* IAM-1025 and *Str. faecalis* 6733. Strains of *Staph. aureus* were slightly less susceptible to cefazolin than cephaloridine and ampicillin, while Gram-negative bacteria, as a whole, were slightly more susceptible to cefazolin than cephaloridine. From the results obtained, cefazolin appears to be a broad-spectrum antibiotic, the *in vitro* activity of which is nearly equal to cephaloridine.

2. Distribution of Susceptibility of Clinical Isolates to Cefazolin

Distribution of MICs of cefazolin and related antibiotics against the clinical isolates of several species is shown in Table 2. Experimental results revealed that cefazolin is slightly less potent than cephaloridine or cephalothin against 64 strains of *Staph. aureus*. None of the test strains of staphylococci were found highly resistant to cefazolin, although many of them were penicillinase-producers.

Out of the 42 clinical isolates of *E. coli*, 17 were highly sensitive, MICs being 1.56~3.13 mcg/ml, 23 were sensitive (6.25~12.5 mcg/ml), and only 2 were relatively resistant to cefazolin. Thus, the activity of cefazolin against clinical isolates of *E. coli* was nearly equal to cephaloridine and superior to those of the other antibiotics.

Of the 30 strains of *Pr. mirabilis*, 26 were suppressed by 3.13~12.5 mcg/ml of cefazolin, while 4 were resistant. Cefazolin appeared to be as effective *in vitro* as

Table 1. Antimicrobial spectra of cefazolin and related antibiotics

Organism	MIC (mcg/ml)				
	CEZ	CER	CET	CEX	AB-PC
Staph. aureus 209P	0.39	0.1	0.39	3.13	0.2
〃 〃 Newman	0.39	0.1	0.78	6.25	0.2
〃 〃 Terashima	0.78	0.2	0.78	12.5	0.39
〃 〃 Smith	0.39	0.1	0.39	3.13	0.2
**Strept. hemolyticus* S-23	0.2	0.05	0.1	1.56	0.05
* 〃 *faecalis* 6733	50	12.5	25	>100	1.56
**Dipl. pneumoniae* I	0.2	0.05	0.39	3.13	0.05
* 〃 〃 II	0.2	0.05	0.2	6.25	0.05
* 〃 〃 III	0.2	0.1	0.39	3.13	0.1
**Coryn. diphtheriae* P.W. 8	0.39	0.1	0.39	1.56	0.2
***Clost. perfringens* PB6K	1.56	3.13	3.13	25	3.13
****Mycob. tuberculosis* $H_{37}Rv$	12.5	6.25	25	50	50
B. subtilis ATCC-6633	0.39	0.025	0.05	0.39	0.39
Sarcina lutea PCI-1001	0.78	0.05	0.2	0.39	0.01
*****N. gonorrhoeae* Nakanishi	1.56	1.56	3.13	6.25	0.78
Salm. typhosa T-287	1.56	3.13	1.56	3.13	0.2
〃 〃 O-901	1.56	3.13	1.56	3.13	0.2
〃 *enteritidis*	6.25	12.5	12.5	25	3.13
Kl. pneumoniae ST-101	1.56	6.25	12.5	25	1.56
E. coli NIHJ	1.56	3.13	3.13	6.25	1.56
Sh. flexneri 2a	1.56	3.13	6.25	6.25	1.56
Sh. sonnei I	0.78	3.13	6.25	6.25	3.13
Pr. vulgaris IAM-1025	>100	50	100	100	>100
Ps. aeruginosa IAM-1095	>100	>100	>100	>100	>100

* added 10% serum ** ZEISSLERS's glucose blood agar
*** DUBOS medium **** GC chocolate agar

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

		MIC (mcg/ml) ≧ 100 ~ 50 ~ 25 ~ 12.5 ~ 6.25 ~ 3.13 ~ 1.56 ~ 0.78 ~ 0.1 ~ ≦ 0.05						
		≧ 100	50 ~ 25	12.5 ~ 6.25	6.25 ~ 3.13	3.13 ~ 1.56	0.78 ~ 0.1	≦ 0.05
Staph. aureus 64 strains	CEZ				13		51	
	CER		1		18		20	25
	CET						62	2
	CEX		31		33			
	AB-PC	14	18		21		10	1
E. coli 42 strains	CEZ		2	23		17		
	CER		3	37		2		
	CET	14	27	1				
	CEX	39	2	1				
	AB-PC	1	18	23				
Pr. mirabilis 30 strains	CEZ	3	1	11		15		
	CER	2	9	19				
	CET	3	5	22				
	CEX	10	20					
	AB-PC	2		5		21	2	
Kl. pneumoniae 33 strains	CEZ	2	4	8		19		
	CER	4	17	10		2		
	CET	4	23	6				
	CEX	24	7	2				
	AB-PC	33						

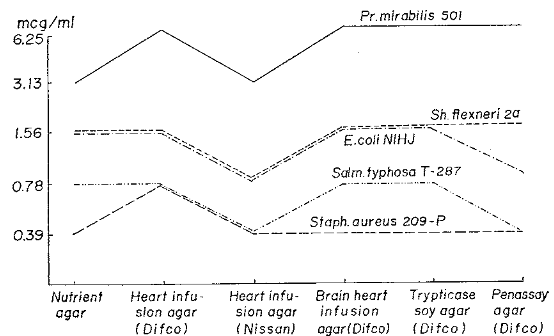
Each figure indicates number of strains which showed the appropriate MIC.

the other antibiotics included in this study, and more effective than cephalexin, which was inferior in activity to other antibiotics.

Of the 33 strains of *Kl. pneumoniae*, 19 were suppressed by 1.56~3.13 mcg/ml of cefazolin, 8 by 6.25~12.5 mcg/ml, 4 by 25~50 mcg/ml, and 2 were highly resistant. In contrast, the majority of the strains was highly resistant to cephalexin or ampicillin, and more than half was moderately or highly resistant to cephalothin and cephaloridine. The results indicate that cefazolin is more effective against *Kl. pneumoniae* than other antibiotics used.

Fig. 2. MIC values of cefazolin on various kinds of agar media

Inoculum suspension: 10^8 viable cells per ml
Incubation: 37°C, 20 hours



3. Influence of Various Experimental Conditions on the *in vitro* Activity of Cefazolin

(1) Kinds of test media: The fluctuation of MICs of 5 test strains on different kinds of media is illustrated in Fig. 2. No significant difference in MICs was noted by the media used in this experiment.

(2) Effect of reaction of test medium on the activity of cefazolin: The activity of cefazolin against *Staph. aureus* 209P depended to a certain extent on the reaction of the test medium. Cefazolin exerted its maximal antibacterial effect at pH 5.0, quite in accordance with cephaloridine and cephalothin. Such a marked difference in MICs by pH of test medium was not observed in *E. coli* NIHJ and *Kl. pneumoniae* 417 (Table 3).

(3) Effect of inoculum size on the activity of cefazolin: The MICs of cefazolin were estimated with varying size of inocula (Table 4). A decrease in size of the inoculum enhanced to some extent the activity of cefazolin against *E. coli* NIHJ. This tendency was also noted in other antibiotics. However, in the strains of *Staph. aureus* 209P and *Kl. pneumoniae* 417, the MICs were hardly influenced by the size of inoculum. On the other hand, the MICs of cephaloridine, cephalothin and cephalexin were

Table 3. Effect of pH of the test medium on the antibacterial activity of cefazolin

Organism	pH of the test medium	MIC (mcg/ml)				
		CEZ	CER	CET	CEX	AB-PC
Staph. aureus 209P	5	0.05	0.012	0.025	0.78	0.05
	6	0.2	0.05	0.2	3.13	0.1
	7	0.39	0.1	0.39	3.13	0.2
	8	0.39	0.1	0.39	6.25	0.2
	9	0.78	0.1	0.39	6.25	0.2
E. coli NIHJ	5	0.78	3.13	3.13	50	0.78
	6	1.56	3.13	3.13	12.5	0.78
	7	1.56	3.13	3.13	6.25	1.56
	8	3.13	3.13	1.56	12.5	1.56
	9	3.13	3.13	1.56	25	1.56
Kl. pneumoniae 417	5	3.13	25	12.5	>100	>100
	6	3.13	12.5	12.5	100	>100
	7	3.13	12.5	25	100	>100
	8	6.25	12.5	25	100	>100
	9	12.5	25	50	100	>100

Medium: Heart infusion agar
Inoculum suspension: 10^8 variable cells per ml

considerably influenced by the size of inoculum when *Kl. pneumoniae* 417 was used.

These results indicate that the activity of cefazolin is only slightly dependent upon the size of inoculum.

(4) Effect of the addition of serum to the test medium on the activity of cefazolin: The effect of the addition of serum to the test medium on the antibacterial activity of cefazolin and other antibiotics was evaluated by determining MIC in HI-agar containing various levels of rabbit serum (Table 5). Cefazolin showed a slight reduction in activity under the presence of serum of 25~50 %. With cephaloridine and ampicillin, on the other hand, the effect of serum was negligible. This indicates that cefazolin is bound to the serum proteins. The precise extent of the binding was determined by an ultra-filtration method, as described later in this paper.

Table 4. Effect of inoculum size on the antibacterial activity of cefazolin

Organism	Viable cell counts of inocula suspensions	MIC (mcg/ml)				
		CEZ	CER	CET	CEX	AB-PC
Staph. aureus 209P	10^4/ml	0.2	0.025	0.1	0.78	0.05
	10^5	0.39	0.05	0.2	1.56	0.05
	10^6	0.39	0.05	0.2	1.56	0.1
	10^7	0.39	0.1	0.39	3.13	0.1
	10^8	0.39	0.1	0.39	3.13	0.2
E. coli NIHJ	10^4/ml	0.39	0.78	0.39	1.56	0.2
	10^5	0.78	1.56	0.78	3.13	0.39
	10^6	1.56	1.56	1.56	3.13	0.39
	10^7	1.56	1.56	1.56	6.25	0.78
	10^8	1.56	3.13	3.13	6.25	1.56
Kl. pneumoniae 417	10^4/ml	1.56	1.56	3.13	3.13	25
	10^5	1.56	3.13	3.13	3.13	25
	10^6	1.56	3.13	3.13	6.25	25
	10^7	1.56	6.25	6.25	12.5	100
	10^8	3.13	12.5	25	100	>100

Medium: Heart infusion agar

Table 5. Effect of the addition of serum to the test medium on the antibacterial activity of cefazolin

Organism	Serum (%)	MIC (mcg/ml)				
		CEZ	CER	CET	CEX	AB-PC
Staph. aureus 209P	0	0.39	0.1	0.39	3.13	0.2
	10	0.78	0.1	0.78	6.25	0.2
	25	1.56	0.1	0.78	6.25	0.39
	50	1.56	0.1	1.56	12.5	0.39
E. coli NIHJ	0	1.56	3.13	3.13	6.25	1.56
	10	3.13	3.13	3.13	6.25	1.56
	25	3.13	3.13	3.13	6.25	1.56
	50	6.25	3.13	6.25	6.25	1.56
Kl. pneumoniae 417	0	3.13	12.5	25	100	>100
	10	3.13	12.5	25	100	>100
	25	6.25	12.5	25	100	>100
	50	6.25	12.5	50	100	>100

Medium: Heart infusion agar
Serum: Rabbit serum
Inoculum suspension: 10^8 viable cells per ml

4. Bactericidal Activity

The bactericidal activity of cefazolin was compared with that of cephaloridine using *Staph. aureus* 209P and *E. coli* NIHJ (Fig. 3).

When *Staph. aureus* 209P grown in nutrient broth was exposed to cefazolin at the levels of MIC or 4 times MIC, the viable cell count decreased markedly during 24-hour incubation. At one-fourth MIC, the viable cell count decreased in initial stage but increased from 8-hour incubation onward. These results with cefazolin are quite in accordance with those of cephaloridine in which an excellent bactericidal activity against this strain is manifest.

A series of experiment with *E. coli* NIHJ was carried out under the same condition with that of *Staph. aureus* 209P. As with cephaloridine, no noticeable bactericidal

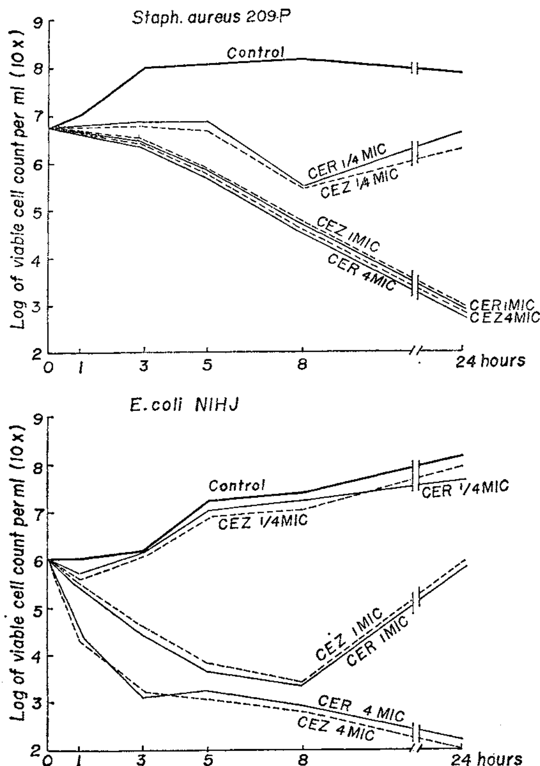


Fig. 3. Bactericidal activity of cefazolin and cephaloridine.

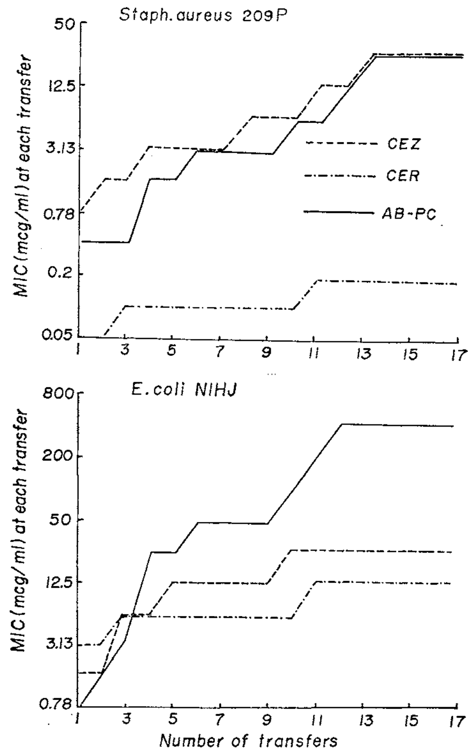


Fig. 4. Rate of development of resistance to cefazolin and other antibiotics over 17 serial transfers.

activity was noted with one-fourth MIC of cefazolin. With the MIC, the viable cell count decreased slowly in the period of 8-hour incubation, followed by a marked increase. With 4 times MIC, the viable cell count decreased rapidly and no recovery of viable cells was obtained after 24-hour incubation.

These experimental results revealed that the bactericidal activity of cefazolin is approximately equal to that of cephaloridine.

5. Development of Resistance *in vitro*

Experiments were carried out, as described above, with cefazolin, cephaloridine and ampicillin in order to compare the rates of resistance development. The results are summarized in Fig. 4.

In the case of *Staph. aureus* 209P, the MIC of cefazolin increased slowly from 0.78 mcg/ml to 25 mcg/ml over a period of 17 serial transfers. This pattern of resistance development was quite similar to that of ampicillin. Only a slight increase of resistance was found with cephaloridine.

In the case of *E. coli* NIHJ, the MIC of cefazolin increased slowly from 1.56 mcg/ml to 25 mcg/ml over a period of 17 serial transfers. The pattern of resistance development for cefazolin was nearly similar to that of cephaloridine and relatively slower than ampicillin.

These results indicate that, under the above-mentioned conditions, the resistance development to the test substance took place in relatively small steps without any

Fig. 5. Stability of cefazolin and other antibiotics in nutrient broth at pH 7.0 (10 mcg/ml).

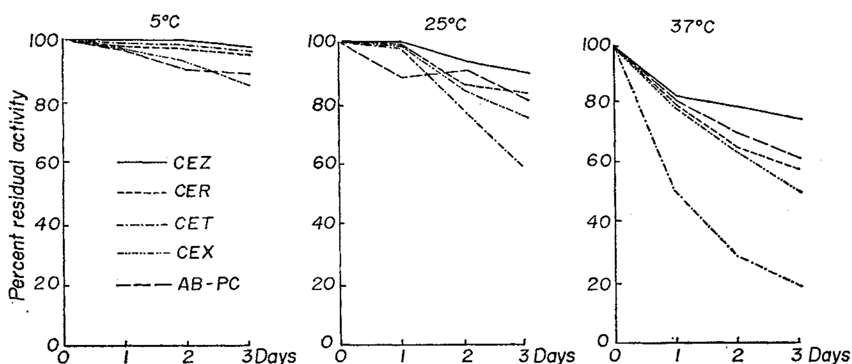


Fig. 6. Sensitivity of cefazolin and other antibiotics to bacterial enzymes. (% degraded)
Staphylococcus 10 strains
E. coli 5 strains

Cephaloridine in 0.1 M phosphate buffer pH 6.5, 500 mcg/ml 1 ml
Broth culture 1 ml
kept at 37℃, 2 hours

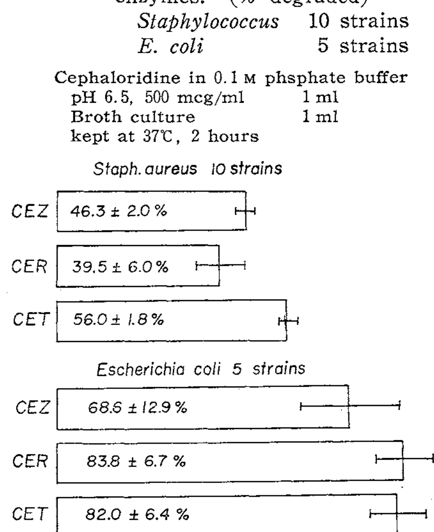
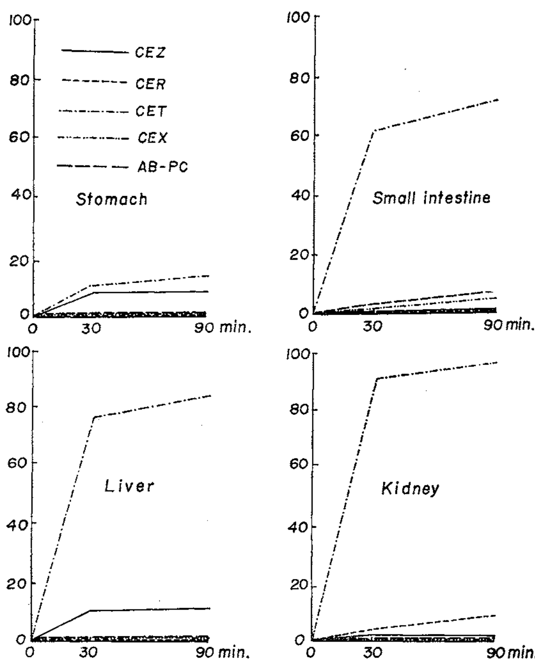


Fig. 7. Stability of cefazolin and other antibiotics to rats' tissue homogenates.

Cephalosporins or penicillins 200 mcg/ml 1 ml
20% Tissue homogenate 1 ml
Incubation at 37℃



significant differences in the rates.

6. Stability of Cefazolin in Liquid Medium

The stabilities of cefazolin and related antibiotics in nutrient broth at different temperatures are illustrated in Fig. 5.

At 5°C, no significant decrease in activity was noted over a period of 3 days. At room temperature (25°C), about 10 % decrease in potency was shown with cefazolin after 3 days, whereas more rapid degradation was observed in cephalothin. At 37°C, cefazolin was most stable among the tested antibiotics, only 20 % degradation being noted after 3 days.

The similar tendency was found in other liquid media (HI, BHI and Trypticase soy broth). From these results, cefazolin appears to be relatively stable in conventional test media.

7. Sensitivity of Cefazolin to Bacterial Enzymes

Enzymatic degradation of cefazolin and other antibiotics was studied using overnight broth cultures of 10 strains of *Staph. aureus* and 5 strains of *E. coli* as the enzyme sources (Fig. 6).

In the case of *Staph. aureus*, under the test condition, 500 mcg of ampicillin or benzyl penicillin were completely degraded by all the cultures within 2-hour incubation. However, cefazolin was partially degraded (40~60 %) as were cephalothin and cephaloridine under the same condition. Ampicillin and benzyl penicillin were also substantially degraded by the cultures of *E. coli* used. Cefazolin was substantially degraded by the cultures of 2 strains of *E. coli*, and was partially (44~50 %) degraded by the cultures of the remaining 3 strains. No apparent differences were recorded in the enzymatic degradation between cefazolin and other cephalosporins.

8. Stability to Tissue Homogenates

As shown in Fig. 7, cephalothin rapidly lost its activity in the presence of tissue homogenates of rats except for the stomach, and inactivation was most remarkable with kidney, in which about 90 % of the initial amount was inactivated even with only 30-minute incubation. On the contrary, with all the homogenates tested the activity of cefazolin remained unimpaired, 90 % or more remaining unchanged after 90-minute incubation. Similar results were obtained with other antibiotics except cephalothin. From these results, cefazolin appears to be stable to enzymatic attacks by tissue homogenates.

9. Protein-binding Effect of Cefazolin

Table 6 shows the degree

Table 6. Extent of binding of cefazolin and other antibiotics in sera of different species

Sera	Bound antibiotics (%)					
	CEZ	CER	CET	CEX	PC-G	MDI-PC
Human	74	31	79	12	41	95
Dog	20	11	50	3	8	92
Rabbit	90	29	85	15	43	96
Rat	91	11	73	5	6	93

Serum 9 volumes, antibiotic (500 mcg/ml) 1 volume : 37°C, 1 hour. Ultra filtration with centrifugation.

Table 7. Protecting effect of cefazolin against experimental infections in mice

Organism	Strain	MIC (mcg/ml)			ED_{50} (s.c.)		
		CEZ	CER	AB-PC	CEZ	CER	AB-PC
Staph. aureus	226	3.13	3.13	100	<0.09	0.28	1.8
	T-5	1.25	1.25	50	1.78	0.19	>6.0
	STP	0.31	0.1	≦0.05	0.53	0.13	0.53
	204	3.13	3.13	>100	<0.09	<0.09	0.26
	213	1.56	3.13	>100	0.18	0.30	0.75
	235	6.25	6.25	>100	0.55	0.12	2.59
Dip. pneumoniae III		0.2	0.1	0.1	2.73	0.39	0.58
E. coli	T-16	3.13	6.25	6.25	0.44	0.29	0.48
	T-15	3.13	6.25	6.25	2.15	1.11	1.31
	2-E-5	12.5	6.25	12.5	0.56	<0.2	0.34
	2-E-6	6.25	6.25	25	3.02	<0.2	1.54
	2-E-7	12.5	6.25	12.5	3.42	4.06	1.64
	312	3.13	12.5	6.25	3.63	1.11	0.93
	323	12.5	50	100	0.78	3.15	0.88
	324	3.13	6.25	6.25	3.60	≧12.0	8.51
Pr. mirabilis	519	6.25	6.25	3.13	5.20	2.20	6.30
	522	6.25	12.5	3.13	3.60	5.10	8.60
	524	3.13	3.13	0.78	4.28	1.55	0.40
	523	6.25	6.25	1.56	2.31	1.27	1.05

Eight mice (*dd*-strain, 17~21g) of each group were challenged intravenously (*Staph. aureus*) or intraperitoneally (*Dipl. pneumoniae, E. coli, Pr. mirabilis*) with 0.5 ml of overnight culture ($2\sim3\times10^8$/ml).

The antibiotics were administered subcutaneously once 1 hour after the challenge.

ED_{50} was expressed in terms of mg/mouse.

of binding of cefazolin and other antibiotics to serum proteins from different animal species. The binding of each tested antibiotic to serum proteins depended to a great extent upon the species of animals except for dicloxacillin. Such tendency was especially remarkable in cefazolin, the extent being only 20 % of the initial amount in dog serum, 74 % in human and 90 % in rabbit serum. In general, it seems likely that the binding of cefazolin was higher than cephaloridine and cephalexin. However, as stated above, no marked decrease was noted in *in vitro* activity of cefazolin, even when rabbit serum, which showed the highest rate of binding, was added to HI-agar at a concentration of 50 %. These results are suggestive of reversibility of the antibiotic-protein binding.

10. Protecting Effect in Experimental Mice Infections

Six strains of *Staph. aureus*, 8 strains of *E. coli*, 4 strains of *Pr. mirabilis* and one strain of *Dipl. pneumoniae* type III were used as challenge organisms. MICs and ED_{50} of each strain are summarized in Table 7.

MICs of all test strains of *Staph. aureus* to cefazolin and cephaloridine were nearly equal, whereas, all except for one strain (STP) were highly resistant to ampicillin.

In experimental infections, the protecting activities of both cefazolin and cephaloridine were found nearly equal, parallel to *in vitro* activities, values of ED_{50} being smaller than ampicillin. However, considerable high protecting effects were observed with ampicillin in some of the highly ampicillin-resistant strains.

As for the experimental infections with *E. coli*, no distinct difference of protecting effects was noted among tested strains. In infections with *Dipl. pneumoniae* and *Pr. mirabilis*, the protecting action of cefazolin was somewhat lower than the other two antibiotics.

Discussion

As is quite well known, cephaloridine is the most useful drug among the broad-spectrum antibiotics[2,3] so far available because of the facts that its *in vitro* activity is very high, that it is quite stable in *in vivo* as well as against inactivating enzymes produced by bacteria,[4,5,6] and is of very low toxicity except for an ability to damage the kidney of the rabbit.[7,8,9] Cephalothin[10,11,12] seems somewhat inferior to cephaloridine, since this substance is not so stable within the human body, being easily converted into a desacetyl-compound of relatively lower activity, although its toxicity is lower than cephaloridine.[13,14]

The *in vitro* activity of cefazolin seems quite comparable to that of cephaloridine, and definitely higher than those of other cephalosporin C derivatives.[15,16,17] The activity of this substance is considerably stable not only against inactivating enzymes of bacterial origin but also against various tissue homogenates. Various experimental conditions such as kinds and reaction of test media, size of inoculum and addition of serum to the test medium caused only slight fluctuation of MIC.

The protecting action of cefazolin against experimental infections in mice with *Staph. aureus* and *E. coli* was also established to be similar to that of cephaloridine,[18,19] values of ED_{50} being parallel to *in vitro* activities. These results indicate that protein-binding of cefazolin is not so firm as to reduce the antimicrobial action *in vivo*[20,21].

Unpublished data[22] in our laboratory revealed that this substance is of very low toxicity, quite well absorbed into serum and various tissues, when given intramuscularly

to experimental animals and human volunteers, and more than 80 % of the given dose is excreted unchanged in the urine within 10 hours after administration. The detailed results of toxicity, absorption and excretion of this substance will be reported in forthcoming papers. These results strongly suggest that this substance merits clinical trials.

Acknowledgement

We thank Dr. H. NAKANO and Dr. S. KUMADA for encouragement. We are indebted to Dr. T. TAKANO and Dr. K. KARIYONE for supply of cephalosporin antibiotics and for helpful suggestions.

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