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# In Vitro Antibacterial Activity of E-0702, a New Semisynthetic Cephalosporin

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E-0702 is an antipseudomonal cephalosporin derivative which has a broad spectrum of antibacterial activity against clinical isolates of gram-positive and gram-negative bacteria. Its in vitro antibacterial activities were less than those of cefoperazone and cefotaxime against *Staphylococcus aureus* and *Staphylococcus epidermidis*, but were significantly high against gram-negative bacteria including the *Pseudomonas* group. It was most characteristic that E-0702 showed high antibacterial activity against *Pseudomonas aeruginosa*. E-0702 was relatively stable to inactivation by plasmid-mediated penicillinases and cephalosporinases produced by gram-negative bacteria.

Many new cephalosporins with a broad antibacterial spectrum have been developed in the past few years. Several of these antibiotics have been in clinical use for the treatment of a wide range of bacterial infections. E-0702, (6*R*, 7*R*)-3-[(1 - carboxymethyl - 1*H* - tetrazol - 5 - yl) - thiomethyl]-7-[(2*R*)-2-(6,7-dihydroxy-4-oxo-4*H*-1 - benzopyran - 3 - carboxamido) - 2 - (4 - hydroxyphenyl) - acetamido] - 8 - oxo - 5 - thia - 1 - azabicyclo[4.2.0]oct - 2 - ene - 2 - carboxylic acid disodium salt (Fig. 1), is a new antipseudomonal cephalosporin derivative with a broad antibacterial spectrum, developed by Eisai Co., Ltd., Tokyo, Japan. The current report summarizes the results of studies of the in vitro antibacterial activity and β-lactamase stability of this new cephalosporin with clinical isolates stocked in this laboratory.

## MATERIALS AND METHODS

**Antibiotics.** E-0702 was synthesized in the research laboratories of Eisai Co. As comparative compounds, cefoperazone (CFP) and cefotaxime (CTX) were supplied by Toyama Chemical Co., Japan, and Hoechst Japan Ltd., Japan, respectively. Cephaloridine, cefazolin, and penicillin G (PCG) were obtained commercially.

**Organisms.** The strains of gram-positive and gram-negative bacteria used in this study were isolated from clinical materials in Japan between 1979 and 1981 and have been maintained since isolation in the Laboratory of Drug Resistance in Bacteria, Gunma University, Japan.

**Media.** Sensitivity test (ST) agar, ST broth (Nissui Seiyaku Co., Japan), and nutrient agar containing bromothymol blue and lactose were used. For the dilution of cell suspensions, buffered saline containing 0.01% gelatin (1) was used.

**Determination of MICs.** Minimal inhibitory concentration (MIC) was determined by an agar dilution method. Twofold serial antibiotic dilutions were made in ST agar, ST agar containing 5% defibrinated horse blood for *Streptococcus pyogenes*, or chocolate ST agar for *Neisseria gonorrhoeae*. The test organism was grown at 37°C overnight in ST broth used for preculture. ST broth containing 0.3% $KNO_3$ was used for *Pseudomonas* strains. The strains of *N. gonorrhoeae* were grown on chocolate ST agar, and colonies were suspended in buffered saline–0.01% gelatin just before dilution. Overnight cultures of test strains were diluted in buffered saline–0.01% gelatin to a final concentration of about $10^6$ colony-forming units (CFU) per ml, and one loopful (about 0.005 ml) of each bacterial suspension was inoculated on agar plates with an inoculator (Microplanter; Sakuma Seisakusho, Ltd., Tokyo). Plates were incubated for 18 h at 37°C. *N. gonorrhoeae* was incubated in $CO_2$ for 48 h at 37°C. The MIC was defined as the lowest concentration of antibiotic that allowed no visible growth.

**Bactericidal activity.** An overnight culture in ST broth at 37°C was diluted in the same fresh broth to about $10^3$ CFU/ml and incubated with shaking at 37°C. After 2 h of incubation, antibiotics were added to the cultures. Samples were taken at appropriate time intervals, immediately diluted in broth, and plated at several dilutions on bromothymol blue agar. The numbers of colonies were counted after 18 h of incubation at 37°C.

**Effect of inoculum size on MICs and MBCs.** Each test organism was grown overnight in ST broth at 37°C. Tenfold-diluted cultures were inoculated in ST broth containing twofold serial dilutions of a drug. MICs were read after incubation at 37°C for 18 h. One loopful (about 0.01 ml) of each culture tube in the MIC test series was spotted onto drug-free bromothymol blue agar plates. After incubation at 37°C for 18 h, the minimal bactericidal concentrations (MBCs) of antibiotic were estimated as the lowest concentrations that did not allow the formation of colonies.

**Susceptibility to β-lactamase.** Chromosomally mediated and plasmid-mediated β-lactamases used in this study were purified by methods described previously

TABLE 1. In vitro antibacterial activities of E-0702 and other cephalosporins

| Organism (no. of strains) | Antibiotic | Range of MIC (μg/ml) | $MIC_{50}$ (μg/ml)[a] | $MIC_{90}$ (μg/ml)[a] |
|---|---|---|---|---|
| *Staphylococcus aureus* (100) | E-0702 | 3.13–12.5 | 4.15 | 5.80 |
| | CFP | 0.4–3.13 | 0.97 | 1.50 |
| | CTX | 0.4–3.13 | 0.61 | 1.25 |
| *S. epidermidis* (50) | E-0702 | 1.56–50 | 7.00 | 28.0 |
| | CFP | 0.4–50 | 1.47 | 9.50 |
| | CTX | 0.8–>100 | 1.38 | 12.5 |
| *Neisseria gonorrhoeae* (53) | E-0702 | 0.025–1.56 | 0.09 | 0.50 |
| | CFP | 0.006–0.2 | 0.02 | 0.07 |
| | CTX | ≦0.003–0.1 | 0.006 | 0.04 |
| *Escherichia coli* (200) | E-0702 | ≦0.003–3.13 | 0.01 | 0.08 |
| | CFP | 0.025–100 | 0.16 | 2.10 |
| | CTX | 0.0125–0.4 | 0.03 | 0.04 |
| *Klebsiella pneumoniae* (100) | E-0702 | 0.006–3.13 | 0.02 | 0.19 |
| | CFP | 0.1–25 | 0.34 | 2.70 |
| | CTX | 0.0125–0.4 | 0.03 | 0.07 |
| *Enterobacter cloacae* (100) | E-0702 | 0.025–100 | 0.27 | 2.20 |
| | CFP | 0.05–>100 | 0.37 | 17.5 |
| | CTX | 0.025–>100 | 0.11 | 8.70 |
| *Serratia marcescens* (100) | E-0702 | 0.0125–>100 | 0.06 | 56.0 |
| | CFP | 0.05–>100 | 0.88 | 38.2 |
| | CTX | 0.05–12.5 | 0.22 | 2.30 |
| *Proteus mirabilis* (50) | E-0702 | 0.025–6.25 | 0.05 | 0.57 |
| | CFP | 0.4–3.13 | 0.82 | 1.56 |
| | CTX | 0.0125–0.2 | 0.02 | 0.04 |
| *P. vulgaris* (50) | E-0702 | 0.025–50 | 0.08 | 0.80 |
| | CFP | 0.2–25 | 1.00 | 2.35 |
| | CTX | 0.0125–3.13 | 0.02 | 0.04 |
| *P. morganii* (50) | E-0702 | 0.05–12.5 | 0.14 | 1.18 |
| | CFP | 0.2–25 | 0.92 | 5.00 |
| | CTX | 0.0125–6.25 | 0.02 | 0.20 |
| *P. rettgeri* (25) | E-0702 | 0.025–12.5 | 0.06 | 0.24 |
| | CFP | 0.1–25 | 0.64 | 7.40 |
| | CTX | 0.025–0.2 | 0.02 | 0.09 |
| *P. inconstans* (21) | E-0702 | 0.0125–0.8 | 0.02 | 0.39 |
| | CFP | 0.2–25 | 1.38 | 6.25 |
| | CTX | 0.0125–0.8 | 0.05 | 0.20 |
| *Citrobacter freundii* (50) | E-0702 | 0.003–25 | 0.006 | 0.40 |
| | CFP | 0.05–>100 | 0.10 | 17.0 |
| | CTX | 0.05–100 | 0.18 | 25.0 |
| *Salmonella* spp. (50) | E-0702 | ≦0.0008–0.025 | 0.001 | 0.005 |
| | CFP | 0.2–3.13 | 0.43 | 1.00 |
| | CTX | 0.0125–0.4 | 0.06 | 0.16 |
| *Acinetobacter calcoaceticus* (100) | E-0702 | 0.1–3.13 | 0.29 | 0.98 |
| | CFP | 3.13–>100 | 26.7 | 75.0 |
| | CTX | 0.4–50 | 7.45 | 20.0 |
| *Pseudomonas aeruginosa* (200) | E-0702 | 0.025–6.25 | 0.09 | 0.45 |
| | CFP | 0.8–100 | 3.30 | 8.80 |
| | CTX | 0.4–>100 | 10.0 | 24.5 |

TABLE 1—*Continued*

| Organism (no. of strains) | Antibiotic | Range of MIC (μg/ml) | $MIC_{50}$ (μg/ml)[a] | $MIC_{90}$ (μg/ml)[a] |
|---|---|---|---|---|
| *P. cepacia* (50) | E-0702 | 0.0125–>100 | 1.26 | 37.5 |
| | CFP | 3.13–>100 | 21.0 | 94.0 |
| | CTX | 1.56–>100 | 10.8 | 42.5 |
| *P. maltophilia* (50) | E-0702 | 0.0125–100 | 0.39 | 19.8 |
| | CFP | 1.56–>100 | 8.50 | 44.5 |
| | CTX | 3.13–>100 | 31.5 | >100 |

[a] $MIC_{50}$ and $MIC_{90}$, Concentrations required to inhibit the growth of 50 and 90%, respectively, of the organisms used.

(3–8, 10, 13–17). β-Lactamase activity was determined by the spectrophotometric method, as described previously (11, 12). The reaction mixture consisted of 3 ml of a 100 μM substrate solution in 50 mM phosphate buffer (pH 7.0) and 50 μl of enzyme solution. Substrate specificity was expressed as the relative rate of hydrolysis of the five antibiotics, taking the absolute rate of PCG or cephaloridine hydrolysis as 100.

## RESULTS

**Antibacterial activity.** The in vitro activity of E-0702 against gram-positive and gram-negative bacteria was compared with those of CFP and CTX (Table 1). Against both *Staphylococcus aureus* and *Staphylococcus epidermidis*, E-0702 was less active than other antibiotics. On the other hand, E-0702 was more active than CFP against gram-negative bacteria except *N. gonorrhoeae*. However, 90% of *N. gonorrhoeae* strains were inhibited by 0.5 μg of E-0702 per ml. The concentrations of E-0702 required to inhibit the growth of 90% of organisms of *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter cloacae* were 0.08, 0.19, and 2.20 μg/ml, respectively, indicating that E-0702 was 10- to 25-fold more potent than CFP and similar to CTX. E-0702 was also highly active against *Serratia marcescens, Proteus mirabilis*, and indole-positive *Proteus* species. The activity of E-0702 against *S. marcescens* and *Proteus inconstans* was greater than that of CFP and similar to that of CTX. Against *P. mirabilis, Proteus morganii, Proteus vulgaris*, and *Proteus rettgeri*, E-0702 was some 10-fold more active than CFP but slightly less active than CTX. E-0702 was the most active compound tested against *Citrobacter freundii* and *Salmonella* species.

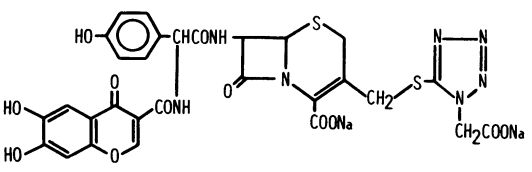


FIG. 1. Chemical structure of E-0702.

The activity of E-0702 against *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* was remarkable: 90% of the strains of these species were inhibited by E-0702 at concentrations of 0.45 and 0.98 μg/ml, respectively. It was found that E-0702 was also the most effective compound against the other *Pseudomonas* species.

**Bactericidal activity.** The bactericidal activities of E-0702 were compared with those of CFP against representative strains of *S. marcescens* and *P. aeruginosa* (Fig. 2). Against *S. marcescens* IAM1184, E-0702 and CFP were bactericidal at concentrations of 0.8 and 1.56 μg/ml, respectively. Against *P. aeruginosa* PAO1, however, E-0702 displayed a much higher bacte-

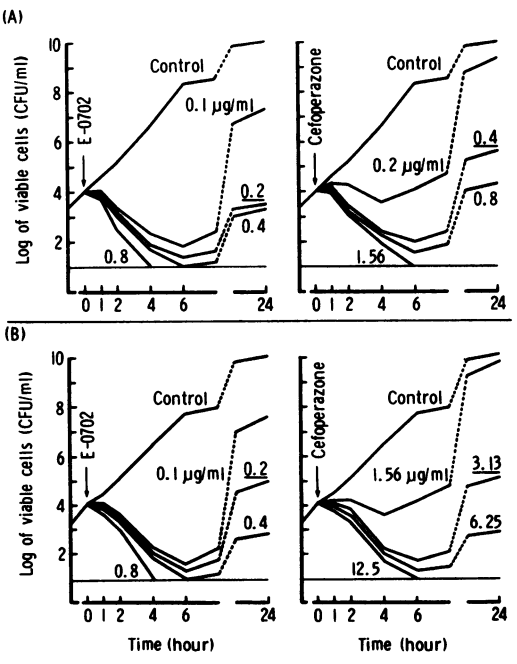


FIG. 2. Bactericidal activity of E-0702 and CFP. (A) *S. marcescens* IAM1184. (B) *P. aeruginosa* PAO1. Underlined numerals indicate the MIC (in micrograms per milliliter) of each drug.

TABLE 2. Bacteriostatic and bactericidal activities of E-0702 at various inoculum sizes

| Organism | Inoculum size (CFU) | E-0702 | | CFP | | CTX | |
|---|---|---|---|---|---|---|---|
| | | MIC (μg/ml) | MBC (μg/ml) | MIC (μg/ml) | MBC (μg/ml) | MIC (μg/ml) | MBC (μg/ml) |
| *E. coli* NIHJ JC-2 | $10^8$ | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |
| | $10^7$ | 3.13 | 3.13 | 3.13 | 3.13 | 1.56 | 3.13 |
| | $10^6$ | 0.025 | 0.05 | 0.4 | 0.8 | 0.1 | 0.1 |
| | $10^5$ | 0.013 | 0.013 | 0.2 | 0.2 | 0.1 | 0.1 |
| | $10^4$ | ≦0.003 | 0.006 | 0.1 | 0.1 | 0.1 | 0.1 |
| | $10^3$ | ≦0.003 | ≦0.003 | 0.1 | 0.1 | 0.1 | 0.1 |
| | $10^2$ | ≦0.003 | ≦0.003 | 0.1 | 0.1 | 0.1 | 0.1 |
| *K. pneumoniae* PCI-602 | $10^8$ | 6.25 | 6.25 | 50 | 50 | 0.4 | 0.4 |
| | $10^7$ | 1.56 | 1.56 | 12.5 | 12.5 | 0.2 | 0.2 |
| | $10^6$ | 0.2 | 0.4 | 0.2 | 0.2 | 0.013 | 0.025 |
| | $10^5$ | 0.013 | 0.025 | 0.1 | 0.2 | 0.006 | 0.006 |
| | $10^4$ | ≦0.003 | ≦0.003 | 0.05 | 0.05 | 0.006 | 0.006 |
| | $10^3$ | ≦0.003 | ≦0.003 | 0.025 | 0.025 | ≦0.003 | ≦0.003 |
| | $10^2$ | ≦0.003 | ≦0.003 | 0.013 | 0.013 | ≦0.003 | ≦0.003 |
| *S. marcescens* IAM1184 | $10^8$ | 200 | 800 | 400 | >800 | 100 | 400 |
| | $10^7$ | 50 | 200 | 100 | 400 | 50 | 200 |
| | $10^6$ | 6.25 | 12.5 | 6.25 | 25 | 0.8 | 3.13 |
| | $10^5$ | 0.4 | 0.8 | 1.56 | 3.13 | 0.4 | 0.8 |
| | $10^4$ | 0.1 | 0.4 | 0.8 | 3.13 | 0.2 | 0.4 |
| | $10^3$ | 0.1 | 0.2 | 0.8 | 1.56 | 0.2 | 0.4 |
| | $10^2$ | 0.05 | 0.05 | 0.4 | 0.4 | 0.1 | 0.1 |
| *P. aeruginosa* PAO1 | $10^8$ | >800 | >800 | >800 | >800 | >800 | >800 |
| | $10^7$ | 800 | >800 | >800 | >800 | 800 | >800 |
| | $10^6$ | 50 | 800 | 100 | 800 | 100 | 800 |
| | $10^5$ | 0.1 | 0.8 | 6.25 | 50 | 12.5 | 50 |
| | $10^4$ | 0.05 | 0.4 | 3.13 | 6.25 | 12.5 | 25 |
| | $10^3$ | 0.025 | 0.2 | 3.13 | 6.25 | 12.5 | 12.5 |
| | $10^2$ | 0.006 | 0.05 | 3.13 | 3.13 | 6.25 | 12.5 |

ricidal activity than did CFP.

**Effect of inoculum size on MICs and MBCs.** The effect of inoculum size on the MICs and MBCs of E-0702 was studied with four species, with the inocula varying between $10^2$ and $10^8$ CFU (Table 2). A large difference in the values occurred between $10^7$ and $10^6$ CFU in *E. coli* NIHJ JC-2 and *K. pneumoniae* PCI-602 and between $10^7$ and $10^5$ CFU in *S. marcescens* IAM1184 and *P. aeruginosa* PAO1, and the values became lower as the inoculum decreased. The MBCs of all the antibiotics were almost identical to the MICs against *E. coli* and *K. pneumoniae*, but 2- to 16-fold higher against *S. marcescens* and *P. aeruginosa*.

**Susceptibility to β-lactamase.** The maximum rates of hydrolysis ($V_{max}$) of the five cephalosporins and PCG are presented in Table 3. E-0702 was stable to various types of cephalosporinases produced by *E. coli* (7), *E. cloacae* (8), *C. freundii* (15), *P. aeruginosa* (10), and *P. rettgeri* (6). The compound also showed a high stability to penicillinase (PCase) types II (2) and III (oxacillin-hydrolyzing PCase; 17) and type IV (carbenicillin-hydrolyzing PCase; 14). However, E-0702 was partially hydrolyzed by PCase type I (TEM-type PCase; 3), *P. morganii* cephalosporinase (16), and the cefuroxime-hydrolyzing β-lactamases (9) produced by *P. vulgaris* (5), *Pseudomonas cepacia* (4), and *Bacteroides fragilis* (13). The stability of E-0702 to β-lactamase was almost the same as that of CFP.

## DISCUSSION

E-0702 is a new parenteral cephalosporin with a broad spectrum of antibacterial activity. This study shows that, with the exception of *N. gonorrhoeae*, E-0702 is more active than CFP against gram-negative bacteria isolated from clinical specimens. A notable characteristic of E-0702 is that this compound has a marked advantage over the other cephalosporins with respect to activity against *P. aeruginosa*, being 20- to 110-fold more active than CFP and CTX. Furthermore, E-0702 also has remarkable activity in vitro against *Salmonella* species, *C. freundii*, and *A. calcoaceticus*. E-0702 is less active in vitro against staphylococci than against gram-negative bacteria.

TABLE 3. Stability of E-0702 to β-lactamases produced by gram-negative bacteria

| Organism | Type of β-lactamase | Relative rate of hydrolysis[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | CER | E-0702 | CFP | CTX | CEZ | PCG |
| *E. coli* W3630(Rms212) | PCase type I | 120 | 54 | 42 | <1 | 13 | 100 |
| *E. coli* W3630(Rms213) | PCase type II | 30 | <1 | 1 | 3 | 3 | 100 |
| *E. coli* W3630(Rte16) | PCase type III | 68 | 3 | 7 | <1 | <1 | 100 |
| *P. aeruginosa* MI4259(Rms139) | PCase type IV | 15 | <1 | <1 | <1 | 3 | 100 |
| *E. coli* GN5482 | CSase | 100 | <1 | 4 | <1 | 311 | 63 |
| *E. cloacae* GN7471 | CSase | 100 | <1 | 1 | <1 | 100 | 12 |
| *C. freundii* GN346 | CSase | 100 | <1 | 2 | <1 | 116 | 3 |
| *P. aeruginosa* GN10362 | CSase | 100 | <1 | 5 | <1 | 222 | 29 |
| *P. rettgeri* GN4430 | CSase | 100 | <1 | <1 | <1 | 99 | 8 |
| *P. morganii* GN5407 | CSase | 100 | 20 | 1 | <1 | 20 | 16 |
| *S. marcescens* GN10857 | CSase | 100 | 11 | 3 | <1 | 198 | 3 |
| *P. cepacia* GN11164 | CXase | 100 | 153 | 10 | 174 | 156 | 161 |
| *P. vulgaris* GN7919 | CXase | 100 | 50 | 15 | 29 | 387 | 20 |
| *B. fragilis* GN11478 | CXase | 100 | 20 | 8 | 7 | 60 | 50 |

[a] Hydrolysis of each substrate by PCase, cephalosporinase (CSase), and cefuroxime-hydrolyzing β-lactamase (CXase) is expressed as a relative rate of hydrolysis, taking the absolute rate of PCG or cephaloridine (CER) hydrolysis as 100. CEZ, Cefazolin.

Like CFP, E-0702 is relatively stable to various types of β-lactamase and has an inoculum effect in vitro against *E. coli, K. pneumoniae, S. marcescens*, and *P. aeruginosa*.

Thus, E-0702 has exceptional antibacterial properties which may have clinical significance if pharmacokinetic and toxicological studies demonstrate adequate levels and safety.

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