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# SCE-963, a New Broad-Spectrum Cephalosporin: In Vitro and In Vivo Antibacterial Activities

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SCE-963 {7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[({1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl}thio)methyl]-ceph-3-em-4-carboxylic acid}, a new semisynthetic cephalosporin, showed excellent antibacterial activity against gram-positive and gram-negative bacteria, including *Haemophilus influenzae*, indole-positive *Proteus, Enterobacter* species, and *Citrobacter freundii*. The minimum inhibitory concentrations of SCE-963 against most strains of clinically isolated *Escherichia coli, Klebsiella pneumoniae, H. influenzae,* and *Proteus mirabilis* were within the range of 0.2 to 0.78 μg/ml. These activities were about 10 times more potent than those of cefazolin, cephaloridine, and cephalothin. Variations in pH, addition of horse serum, and type of growth medium had no significant effect on the activity of the cephalosporin, but the inoculum size elicited a considerable effect on the activity of β-lactamase-producing strains of bacteria. SCE-963 exerted bactericidal and bacteriolytic effects on *Staphylococcus aureus* and *E. coli*. The pronounced in vitro activity was reflected in the remarkable protection in mice infected with a wide range of gram-negative bacteria, such as *E. coli, K. pneumoniae, P. mirabilis, Proteus vulgaris, Proteus morganii,* and *Proteus rettgeri*. The protective effects of SCE-963 in mice infected with *E. coli, K. pneumoniae,* and *P. vulgaris* varied according to the challenge dose. The activity of SCE-963 was far more potent when the drug was administered parenterally rather than orally.

Cephalosporins are highly effective in the treatment of a wide variety of bacterial infections. All the congeners currently in clinical use, however, have certain limitations in their antibacterial spectra. Recently, infections caused by gram-negative rods which are unsusceptible to cephalosporins are increasing. A search for a new cephalosporin with pronounced potency and a broad spectrum of antibacterial activity was, therefore, undertaken in these laboratories.

SCE-963 {7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[({1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl}thio)methyl]-ceph-3-em-4-carboxylic acid} (Fig. 1) is a new semi-synthetic cephalosporin which was selected from a series of 7-aminothiazolacetamido cephalosporins. The compound, first synthesized by chemists of Takeda Chemical Industries, Ltd., has now been under collaborative development with Ciba-Geigy Ltd. in Japan and some other countries. The relationships between chemical structures and microbiological activities of some of these cephalosporins were reported by Numata et al. (M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, and T. Nishimura, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 17th, New York, N.Y., Abstr. no. 44, 1977). The present report describes the in vitro and in vivo antibacterial activity of SCE-963 in comparison with those of cefazolin, cephaloridine, and cephalothin.

## MATERIALS AND METHODS

**Antibiotics.** SCE-963 was prepared by Takeda Chemical Industries, Ltd. Cefazolin (Cefamezin; Fujisawa Pharmaceutical Co., Ltd., Osaka), cephaloridine (Keflodin; Shionogi & Co., Ltd., Osaka), and cephalothin (Keflin; Shionogi & Co., Ltd., Osaka) were obtained from commercial sources.

**Organisms.** Laboratory strains were maintained on Trypticase soy agar (TSA; BBL) or supplemented with 10% bovine blood (blood-TSA). Clinically isolated strains of bacteria were kindly supplied by several departments in medical schools and were maintained on Dorset egg medium (Nissui) at 4°C until use.

**Determination of MIC.** Organisms were transferred to TSA slants, blood-TSA slants, or Trypticase soy broth (TSB; BBL) and cultivated at 37°C overnight. Bacterial suspensions were prepared in a concentration of about $10^8$ or $10^6$ colony-forming units (CFU)/ml. For the agar dilution method, one loopful (2 mm in diameter) of a bacterial suspension ($10^8$ or

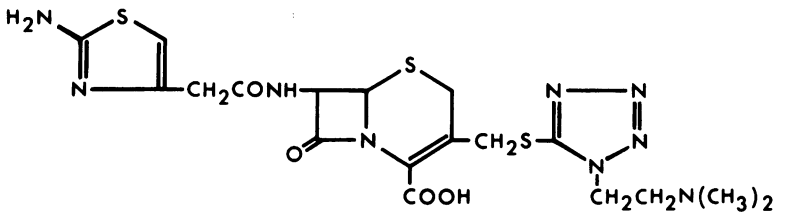

FIG. 1. *Chemical structure of SCE-963.*

$10^6$ CFU/ml) was streaked for a length of about 2 cm on TSA, blood-TSA, or MacConkey agar (Eiken) containing twofold serial dilutions of each antibiotic. For the broth dilution method, 0.1 ml of a suspension containing about $10^7$ CFU/ml was inoculated into 5 ml of TSB in the presence of increased concentrations of each antibiotic. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that prevented visible growth after overnight incubation at 37°C.

**Determination of MBC.** After the MIC was determined by the broth dilution method, a volume of 2 μl (calibrated loop) from each tube was subcultured onto an antibiotic-free TSA plate. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic preventing visual growth on the subculture plate after overnight incubation at 37°C.

**Bactericidal activity.** All experiments to determine the killing kinetics of SCE-963 and cefazolin were performed in TSB containing twofold serial dilutions of antibiotics, with incubation at 37°C. Inocula were prepared from overnight cultures in TSB to obtain a concentration of about $10^6$ CFU/ml. Samples were removed at various time intervals, and viable numbers (CFU) were determined by plate counts. Drug-free control media were inoculated and treated as in the drug-containing test.

**Bacteriolytic activity.** Turbidimetric studies were performed with a six-channel continuous turbidity monitoring device (Jouan biophotometer). Tubes were seeded with an overnight culture in TSB to give an inoculum of about $10^6$ CFU/ml. Various concentrations of antibiotics were added to actively growing cultures in TSB at a turbidity level of 30% of maximum.

**Development of resistance in vitro.** To generate resistance, the organisms were grown in TSB in the presence of twofold serial dilutions of each antibiotic. From the tube containing the highest concentration of antibiotic that allowed normal or nearly normal growth, successive transfers were made every 48 h into other series of tubes containing the same or higher concentrations.

**Protective test.** *Streptococcus pyogenes* and *Streptococcus pneumoniae* were cultured overnight on blood-TSA slants, and other organisms were cultured in brain heart infusion (Difco) overnight at 37°C. *S. aureus* E97 and *S. pneumoniae* type I were suspended in TSB, and other organisms were suspended in 5% mucin (Laboratories Division of Wilson Pharmaceutical and Chemical Co.). Four-week-old male Slc:ICR mice weighing 19 to 23 g were infected intraperitoneally with the organism suspended in 0.5 ml of the medium. The challenge doses of organisms were about 30 to 100 times the number of organisms required to kill 50% of the challenge-control mice. Groups of five mice at each dose level were given 0.2 ml of antibiotic. All experiments were repeated four to five times. The 50% effective dose ($ED_{50}$, milligrams per kilogram) was calculated by the probit method from the survival rates recorded 5 days after infection (6).

## RESULTS

**Antibacterial spectrum.** The spectrum of antibacterial activity of SCE-963 against gram-positive and gram-negative organisms is shown in Table 1, compared with those of cefazolin, cephaloridine, and cephalothin. Several laboratory strains of gram-positive bacteria were inhibited by similar concentrations of SCE-963, cefazolin, and cephalothin. Cephaloridine was slightly more active. Against strains of gram-negative bacteria, including indole-positive *Proteus*, SCE-963 was more active than cefazolin, cephaloridine, and cephalothin. The growth of *Pseudomonas aeruginosa* was not inhibited by SCE-963 or the reference cephalosporins.

**Activity against clinical isolates.** The antibacterial activity of SCE-963 against clinical isolates was compared with those of cefazolin, cephaloridine, and cephalothin. The activity of SCE-963 against *S. aureus* was similar to or slightly less than those of cefazolin and cephalothin. Against *S. pyogenes*, SCE-963 showed slightly more potent activity than cefazolin and cephalothin. The activity of SCE-963 against both species was apparently inferior to that of cephaloridine. The activity of SCE-963 against several gram-negative bacteria was excellent. Most strains of *Escherichia coli, Klebsiella pneumoniae, Haemophilus influenzae*, and *Proteus mirabilis* were inhibited by 0.78 μg or less of SCE-963 per ml. Against these species, SCE-963 was about 10 times more potent than other cephalosporins. SCE-963 inhibited the growth of half or more strains of *Proteus vulgaris, Proteus rettgeri, Proteus morganii, Enterobacter cloacae*, and *Citrobacter freundii* at a concentration of 12.5 μg or less per ml, which was lower than the plasma level clinically obtainable (I. Naka-

yama, H. Iwamoto, S. Iwai, T. Kawabe, H. Mizuashi, and S. Ishiyama, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 17th, New York, N.Y., Abstr. no. 46, 1977; N. Yamaguchi, K. Tsuchiya, T. Yamamoto, T. Sakai, and K. Shimizu, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 17th, New York, N.Y., Abstr. no. 47, 1977). The MICs of reference cephalosporins against these strains were 50 μg or higher per ml, except cefazolin inhibited the growth of most strains of *P. rettgeri* at a concentration of 25 μg or less per ml. Although the MICs of SCE-963 against some strains of *Serratia marcescens* were lower than 12.5 μg/ml, SCE-963 was ineffective against most strains of *S. marcescens* and *Acinetobacter calcoaceticus* (Table 2). Clinical isolates of *E. coli* and *K. pneumoniae* which were not inhibited by concentrations higher than 50 μg of cefazolin per ml with an inoculum of $10^8$ CFU/ml were classified arbitrarily as resistant. These strains were also resistant to cephaloridine and cephalothin. The MICs of SCE-963 against most strains of cefazolin-resistant *E. coli* and *K. pneumoniae* ranged from 1.56 to 25 μg/ml with the heavy inoculum and from 0.2 to 3.13 μg/ml with the light inoculum (Table 3).

**Effect of inoculum size, medium pH, horse serum, and culture medium.** The MICs of SCE-963 were determined under various conditions. Changes in inoculum size had no significant effect on the activity of SCE-963, or on that of cefazolin, against the majority of strains tested. However, the activity of SCE-963 against *E. coli* T-7 and *P. morganii* IFO 3168, β-lactamase-producing organisms, was reduced by increasing the size of the inoculum from $10^4$ to $10^8$ CFU/ml. The MIC of SCE-963 was slightly lower at an acidic pH than at an alkaline pH. The MICs were little affected by the addition of 10 to 50% horse serum to the medium or by varing the culture medium (Table 4).

**Development of resistance in vitro.** The resistance of *S. aureus* FDA 209 P to SCE-963 was gradually acquired, as with that to cefazolin. After transfer 20, the MICs of SCE-963 and cefazolin were 6.25 and 12.5 μg/ml, respectively. Repeated exposure of *E. coli* NIHJ JC-2 to SCE-963 also produced a stepwise increase in resistance, similar to that found with cefazolin. After

TABLE 1. *Antibacterial spectra of SCE-963, cefazolin, cephaloridine, and cephalothin*

| Organism[a] (no. of strains) | | Medium[b] | MIC (μg/ml) | | | |
|---|---|---|---|---|---|---|
| | | | SCE-963 | Cefazolin | Cephaloridine | Cephalothin |
| *Staphylococcus aureus* | (3) | TSA | 0.39 | 0.39 | 0.05 | 0.2 |
| *Streptococcus pyogenes* | (4) | Blood-TSA | 0.05 | 0.05 | 0.013 | 0.1 |
| *S. mitior* | (1) | Blood-TSA | 0.78 | 0.39 | 0.05 | 0.39 |
| *S. faecium* | (1) | Blood-TSA | >100 | >100 | 12.5 | 100 |
| *S. pneumoniae* | (3) | Blood-TSA | 0.05–0.2 | 0.05–0.2 | 0.025–0.05 | 0.05–0.2 |
| *Corynebacterium diphtheriae* | (1) | Blood-TSA | 0.05 | 0.05 | 0.025 | 0.05 |
| *Bacillus subtilis* | (1) | TSA | 0.2 | 0.2 | 0.05 | 0.025 |
| *Escherichia coli* | (6) | TSA | 0.05–0.2 | 1.56–3.13 | 3.13–6.25 | 1.56–12.5 |
| *Klebsiella pneumoniae* | (1) | TSA | 0.1 | 1.56 | 3.13 | 1.56 |
| *Salmonella paratyphi* | (1) | TSA | 0.2 | 1.56 | 3.13 | 6.25 |
| *S. schottmuelleri* | (1) | TSA | 0.1 | 1.56 | 3.13 | 1.56 |
| *S. hirschfeldii* | (1) | TSA | 0.2 | 1.56 | 3.13 | 3.13 |
| *S. typhi* | (2) | TSA | 0.05–0.2 | 0.78–1.56 | 1.56–3.13 | 0.78–3.13 |
| *S. typhimurium* | (1) | TSA | 0.2 | 1.56 | 3.13 | 1.56 |
| *Shigella dysenteriae* | (1) | TSA | 0.05 | 0.78 | 1.56 | 1.56 |
| *S. flexneri* | (2) | TSA | 0.1 | 1.56–3.13 | 6.25 | 3.13–6.25 |
| *S. sonnei* | (1) | TSA | 0.1 | 0.78 | 3.13 | 6.25 |
| *Haemophilus influenzae* | (7) | CA | 0.78 | 12.5–50 | 12.5–25 | 6.25–12.5 |
| *Proteus mirabilis* | (2) | MCA | 3.13–12.5 | 50 | 50 | 50–100 |
| *P. vulgaris* | (4) | MCA | 0.2–25 | 6.25–100 | 6.25–>100 | 1.56–50 |
| *P. morganii* | (2) | MCA | 0.39–100 | 100–>100 | 100–>100 | 100–>100 |
| *P. rettgeri* | (1) | MCA | 0.05 | 1.56 | 1.56 | 1.56 |
| *Serratia marcescens* | (1) | TSA | >100 | >100 | >100 | >100 |
| *Vibrio cholerae* | (1) | TSA | 0.78 | 6.25 | 0.78 | 1.56 |
| *Pseudomonas aeruginosa* | (5) | TSA | >100 | >100 | >100 | >100 |

[a] Inoculum size: one loopful of bacterial suspension ($10^8$ CFU/ml).
[b] MAC, MacConkey agar (Eiken); CA, chocolate aga. (beef extract agar supplemented with 10% horse blood).

TABLE 2. *Susceptibility of clinical isolates to SCE-963, cefazolin, caphaloridine, and cephalothin*

| Organism | Cephalosporin | No. inhibited at MIC (μg/ml)[a] of: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤0.025 | 0.05 | 0.1 | 0.2 | 0.39 | 0.78 | 1.56 | 3.13 | 6.25 | 12.5 | 25 | 50 | 100 | >100 |
| *S. aureus*[b] (53 strains) | SCE-963 | | | | | 24 | 29 | | | | | | | | |
| | Cefazolin | | | | 7 | 32 | 14 | | | | | | | | |
| | Cephaloridine | 11 | 27 | 15 | | | | | | | | | | | |
| | Cephalothin | | | | 28 | 25 | | | | | | | | | |
| *S. pyogenes*[c] | SCE-963 | 28 | 11 | | | | | | | | | | | | |
| (39 strains) | Cefazolin | | 22 | 17 | | | | | | | | | | | |
| | Cephaloridine | 39 | | | | | | | | | | | | | |
| | Cephalothin | | 15 | 25 | | | | | | | | | | | |
| *E. coli*[b] (98 strains) | SCE-963 | | 9 | 40 | 44 | 5 | | | | | | | | | |
| | Cefazolin | | | | | | 9 | 64 | 13 | 12 | | | | | |
| | Cephaloridine | | | | | | 1 | 14 | 58 | 10 | 9 | 6 | | | |
| | Cephalothin | | | | | | | 2 | 10 | 37 | 30 | 18 | 1 | | |
| *K. pneumoniae*[b] (89 | SCE-963 | | | 18 | 56 | 11 | 3 | 1 | | | | | | | |
| strains) | Cefazolin | | | | | | 3 | 62 | 19 | 4 | 1 | | | | |
| | Cephaloridine | | | | | | | 2 | 59 | 14 | 12 | 2 | | | |
| | Cephalothin | | | | | | | 17 | 43 | 19 | 9 | | 1 | | |
| *H. influenzae*[d] (64 | SCE-963 | | | | | 24 | 40 | | | | | | | | |
| strains) | Cefazolin | | | | | | | | 11 | 2 | 23 | 25 | 3 | | |
| | Cephaloridine | | | | | | | 1 | 12 | 10 | 29 | 12 | | | |
| | Cephalothin | | | | | | 2 | 13 | 5 | 20 | 22 | 2 | | | |
| *P. mirabilis*[b] (52 | SCE-963 | | | | 30 | 18 | 3 | 1 | | | | | | | |
| strains) | Cefazolin | | | | | | | | 36 | 15 | | | | 1 | |
| | Cephaloridine | | | | | | | | 7 | 41 | 3 | | | | 1 |
| | Cephalothin | | | | | | | 9 | 27 | 11 | 4 | | | | 1 |
| *P. vulgaris*[b] (52 strains) | SCE-963 | | | | 1 | 1 | 2 | 3 | 7 | 8 | 4 | 13 | 7 | 2 | 4 |
| | Cefazolin | | | | | | | | | | 1 | | 1 | 10 | 40 |
| | Cephaloridine | | | | | | | | | 1 | | | | 2 | 49 |
| | Cephalothin | | | | | | | | | 1 | | | 1 | 4 | 46 |
| *P. rettgeri*[b] (44 strains) | SCE-963 | 11 | 10 | 6 | 4 | 4 | 4 | | 2 | 1 | 1 | | 1 | | |
| | Cefazolin | | | | 3 | | 1 | 4 | 11 | 6 | 7 | 2 | 4 | | |
| | Cephaloridine | | | | | | | 2 | | 2 | 3 | 16 | 7 | 5 | 9 |
| | Cephalothin | | | | | | | 2 | | | 1 | 2 | 12 | 9 | 18 |
| *P. morganii*[b] (54 | SCE-963 | | | 1 | 8 | 12 | 13 | 5 | 4 | 1 | 7 | 2 | 1 | | |
| strains) | Cefazolin | | | | | | | | | 1 | 2 | | | 3 | 48 |
| | Cephaloridine | | | | | | | | | | | | 1 | | 53 |
| | Cephalothin | | | | | | | | | | 1 | | | | 53 |
| *E. cloacae*[b] (54 strains) | SCE-963 | | | | 2 | 10 | 10 | 10 | 4 | 2 | 2 | | 2 | 1 | 11 |
| | Cefazolin | | | | | | | | | 1 | 3 | | 3 | 3 | 44 |
| | Cephaloridine | | | | | | | | | | | 3 | 1 | 2 | 48 |
| | Cephalothin | | | | | | | | | | | 1 | 3 | 1 | 49 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. freundii*[b] (52 strains) | SCE-963 | 2 | 5 | 14 | 3 | 4 | | 1 | | 2 | 13 | 8 | |
| | Cefazolin | | | | | 1 | | 3 | 2 | 6 | 9 | 31 | |
| | Cephaloridine | | | | | | | 2 | | 3 | 9 | 38 | |
| | Cephalothin | | | | | | | 1 | 2 | 9 | 11 | 29 | |
| *S. marcescens*[b] (59 strains) | SCE-693 | | | | 3 | 12 | 4 | 1 | 7 | 6 | 2 | 25 | |
| | Cefazolin | | | | | | | | | | | 59 | |
| | Cephaloridine | | | | | | | | 2 | | | 57 | |
| | Cephalothin | | | | | | | | | | | 59 | |
| *A. calcoaceticus*[b] (36 strains) | SCE-963 | | | | | | | 1 | 9 | 12 | 9 | 5 | |
| | Cefazolin | | | | | | | | | | 4 | 32 | |
| | Cephaloridine | | | | | | | | 2 | 2 | 9 | 23 | |
| | Cephalothin | | | | | | | | | | 2 | 34 | |

[a] Inoculum size: one loopful of bacterial suspension ($10^6$ CFU/ml).
[b] Medium: TSA.
[c] Medium: blood-TSA.
[d] Medium: chocolate agar (see Table 1).

TABLE 3. *Susceptibility of cefazolin-resistant isolates of E. coli and K. pneumoniae to SCE-963, cefazolin, cephaloridine, and cephalothin*

| Organism | Inoculum size (CFU/ml) | Antibiotic | No. of isolates susceptible at MIC (μg/ml) of:[a] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.2 | 0.39 | 0.78 | 1.56 | 3.13 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | 400 | 800 | >800 |
| *E. coli*[b] (7 strains) | $10^8$ | SCE-963 | | | | 1 | 1 | 1 | 2 | 1 | | 1 | | | | |
| | | Cefazolin | | | | | | | | | 1 | 4 | | 1 | 1 | |
| | | Cephaloridine | | | | | | | | | | | 3 | 2 | 1 | 1 |
| | | Cephalothin | | | | | | | | | | | 4 | 2 | | 1 |
| | $10^6$ | SCE-963 | 3 | 1 | | 2 | | | 1 | | | | | | | |
| | | Cefazolin | | | | | | 3 | 1 | 2 | | 1 | | | | |
| | | Cephaloridine | | | | | | | | 2 | 3 | 1 | 1 | | | |
| | | Cephalothin | | | | | | | 1 | 3 | | 2 | | | | 1 |
| *K. pneumoniae*[b] (31 strains) | $10^8$ | SCE-963 | | | | | | 4 | 7 | 12 | 1 | 3 | 2 | 2 | | |
| | | Cefazolin | | | | | | | | | | 2 | 6 | 3 | 14 | 6 |
| | | Cephaloridine | | | | | | | | | 1 | 2 | | 7 | 11 | 10 |
| | | Cephalothin | | | | | | | | | | | 4 | 9 | 12 | 6 |
| | $10^6$ | SCE-963 | | 2 | 17 | 3 | 3 | 1 | 1 | 2 | 1 | 1 | | | | |
| | | Cefazolin | | | | | | | 1 | 12 | 2 | 3 | 5 | 5 | 2 | 1 |
| | | Cephaloridine | | | | | | | | 2 | 7 | 17 | 2 | 1 | | 2 |
| | | Cephalothin | | | | | | | | | 14 | 8 | 3 | 2 | 2 | 2 |

[a] Inoculum size: one loopful of bacterial suspension.
[b] Medium: TSA.

TABLE 4. *Effect of various factors on the antibacterial activity of SCE-963*

| Factor | MIC (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* FDA 209 P | *E. coli* NIHJ JC-2 | *E. coli* T-7 | *K. pneumoniae* DT | *P. mirabilis* IFO 3849 | *P. morganii* IFO 3168 |
| Inoculum size[a] | | | | | | |
| $10^4$ | 0.2 | 0.1 | 0.39 | 0.05 | 0.2 | 0.1 |
| $10^5$ | 0.2 | 0.1 | 0.78 | 0.1 | 0.2 | 0.1 |
| $10^6$ | 0.2 | 0.1 | 1.56 | 0.1 | 0.39 | 0.2 |
| $10^7$ | 0.39 | 0.2 | 3.13 | 0.2 | 0.78 | 0.39 |
| $10^8$ | 0.39 | 0.2 | 25 | 0.2 | 1.56 | 25 |
| Medium pH[b] | | | | | | |
| 6 | 0.2 | 0.39 | 3.13 | 0.1 | 0.78 | 12.5 |
| 7 | 0.39 | 0.2 | 25 | 0.2 | 1.56 | 25 |
| 8 | 0.39 | 0.39 | 25 | 0.2 | 1.56 | 12.5 |
| 9 | 0.39 | 0.78 | 12.5 | 0.2 | 1.56 | 6.25 |
| Horse serum (%)[c] | | | | | | |
| 0 | 0.2 | 0.78 | 12.5 | 0.1 | 0.78 | 6.25 |
| 10 | 0.2 | 0.78 | 12.5 | 0.2 | 0.39 | 6.25 |
| 20 | 0.2 | 1.56 | 12.5 | 0.2 | 0.39 | 6.25 |
| 50 | 0.2 | 0.39 | 25 | 0.2 | 0.39 | 6.25 |
| Medium[d] | | | | | | |
| TSA | 0.39 | 0.2 | 25 | 0.2 | 1.56 | 25 |
| NA | 0.2 | 0.2 | 12.5 | 0.2 | 1.56 | 25 |
| MH | 0.2 | 0.39 | 12.5 | 0.2 | 1.56 | 100 |
| HI | 0.39 | 0.2 | 50 | 0.2 | 1.56 | 100 |
| BHI | 0.78 | 0.2 | 25 | 0.39 | 3.13 | 25 |

[a] Inoculum size: one loopful of bacterial suspension; medium: TSA.
[b] Inoculum size: one loopful of bacterial suspension ($10^8$ CFU/ml); medium: TSA.
[c] Inoculum size: 0.1 ml of bacterial suspension ($10^6$ CFU/ml); medium: TSB.
[d] Inoculum size: one loopful of bacterial suspension ($10^8$ CFU/ml). NA, Nutrient agar (Eiken); MH, Mueller-Hinton medium (Eiken); HI, heart infusion agar (Eiken); BHI, brain heart infusion agar (Eiken).

transfer 20, the MICs of SCE-963 and cefazolin reached 12.5 and 100 μg/ml, respectively.

**Cross-resistance.** The resistant mutants of *S. aureus* FDA 209 P and *E. coli* NIHJ JC-2 which developed in vitro to SCE-963 and cefazolin showed low susceptibility to SCE-963, cefazolin, cephaloridine, and cephalothin, but no increase in β-lactamase activities was detected in the crude enzyme preparations from these strains.

**Correlation of MIC and MBC.** The MBCs of SCE-963 against strains of *K. pneumoniae* and *P. mirabilis* were the same or only two times higher than the MICs, and the MBCs of SCE-963 against half of the strains of *E. coli* and against most strains of *P. rettgeri* ranged from the same to eight times higher than the MICs. However, the MBCs of SCE-963 against the remaining strains of *E. coli* and some strains of *P. rettgeri* were much higher than the MICs. Correlation between the MIC and the MBC of cefazolin against these strains showed the same trend as with SCE-963 (Table 5).

**Bactericidal activity.** The bactericidal activity of SCE-963 against *S. aureus* FDA 209 P was slightly less than that of cefazolin. An apparent decrease in the number of living bacteria was observed at concentrations of 0.39 μg of SCE-963 and 0.2 μg of cefazolin per ml, which were the MICs determined by the broth dilution assay with this strain. The activity of SCE-963 against *E. coli* NIHJ JC-2 was greater than that of cefazolin. At a concentration of 0.78 μg of SCE-963 per ml, an apparent decrease in the number of living bacteria was observed, and no regrowth was observed during incubation for 9 h. The same effect was observed with cefazolin at a concentration of 6.25 μg/ml (Fig. 2).

**Bacteriolytic activity.** SCE-963 showed bacteriolytic activity against *S. aureus* FDA 209 P and *E. coli* NIHJ JC-2 at concentrations higher than 0.39 and 1.56 μg/ml, respectively. Bacteriolytic activity of cefazolin was observed at a concentration of 0.2 μg/ml with *S. aureus* and 1.56 μg/ml with *E. coli* (Fig. 3).

**Protective effect of SCE-963.** The protective effects of SCE-963 in mice infected intraperitoneally with several strains of gram-positive and gram-negative bacteria were compared with those of cefazolin and cephalothin (Table 6). In mice infected with *S. aureus* and *S. pneumoniae* the protective effect of SCE-963 was less than that of cefazolin, whereas the activity of SCE-963 in mice infected with *S. pyogenes* was greater

TABLE 5. *MIC[a] and MBC[b] of SCE-963 and cefazolin against clinical isolates of E. coli, K. pneumoniae, P. mirabilis, and P. rettgeri*

| Organism | SCE-963 | | Cefazolin | |
|---|---|---|---|---|
| | MIC (μg/ml) | MBC (μg/ml) | MIC (μg/ml) | MBC (μg/ml) |
| *E. coli* | 0.2 | 0.2 | 1.56 | 1.56 |
| | 0.2 | 0.78 | 3.13 | 6.25 |
| | 0.39 | 0.39 | 3.13 | 12.5 |
| | 0.39 | 3.13 | 3.13 | 25 |
| | 0.78 | 6.25 | 3.13 | 50 |
| | 0.78 | 25 | 6.25 | 12.5 |
| | 0.78 | 100 | 12.5 | >100 |
| | 0.78 | 100 | 12.5 | >100 |
| | 1.56 | 25 | 12.5 | >100 |
| | 1.56 | 100 | 50 | >100 |
| *K. pneumoniae* | 0.39 | 0.39 | 1.56 | 3.13 |
| | 0.39 | 0.39 | 1.56 | 6.25 |
| | 0.78 | 0.78 | 3.13 | 3.13 |
| | 0.78 | 0.78 | 3.13 | 25 |
| | 0.78 | 1.56 | 6.25 | 6.25 |
| | 0.78 | 3.13 | 6.25 | 6.25 |
| | 1.56 | 3.13 | 6.25 | 6.25 |
| | 3.13 | 3.13 | 25 | 25 |
| | 3.13 | 3.13 | 25 | 25 |
| | 6.25 | 6.25 | 50 | 50 |
| *P. mirabilis* | 0.39 | 0.39 | 6.25 | 12.5 |
| | 0.39 | 0.39 | 6.25 | 12.5 |
| | 0.39 | 0.39 | 6.25 | 50 |
| | 0.39 | 0.78 | 12.5 | 12.5 |
| | 0.39 | 0.78 | 12.5 | 12.5 |
| | 0.78 | 0.78 | 12.5 | 100 |
| | 0.78 | 3.13 | 25 | 25 |
| | 3.13 | 3.13 | 25 | 25 |
| | 3.13 | 3.13 | 50 | 50 |
| | 12.5 | 12.5 | 100 | 100 |
| *P. rettgeri* | 0.05 | 0.1 | 3.13 | 3.13 |
| | 0.1 | 0.39 | 6.25 | 25 |
| | 0.1 | 0.39 | 12.5 | 100 |
| | 0.2 | 0.78 | 12.5 | >100 |
| | 0.2 | 0.78 | 12.5 | >100 |
| | 0.39 | 1.56 | 25 | >100 |
| | 0.78 | 1.56 | 25 | >100 |
| | 0.78 | 50 | 25 | >100 |
| | 1.56 | 100 | 25 | >100 |
| | 3.13 | 3.13 | >100 | >100 |

[a] Inoculum size: 0.1 ml of bacterial suspension ($10^7$ CFU/ml); medium: TSB.

[b] Inoculum size: 2 μl of the culture; subculture medium: TSA.

than that of cefazolin. The activity of SCE-963 in mice infected with gram-positive bacteria was superior to that of cephalothin. In mice infected with gram-negative bacteria the protective effect of SCE-963 was 3 to 27 times greater than that of cefazolin and 3 to >200 times greater than that of cephalothin. The protective effect of SCE-963 in mice infected with strains of *P. vulgaris* and *P. mirabilis* correlated well with the MIC of SCE-963 measured with the light inoculum.

**Effect of challenge dose on the protective test.** In mice infected with *E. coli* O-111, a similar protective effect was observed in mice infected with 1 minimal lethal dose (the minimum number of organisms required to kill all untreated mice) and 10 minimal lethal doses. In mice infected with *E. coli* O-111 whose challenge dose was increased from 10 minimal lethal doses ($10^6$ CFU/mouse) to 1,000 minimal lethal doses ($10^8$ CFU/mouse), the 50% effective doses of SCE-963 and cefazolin changed from 0.185 to 36.2 and from 2.46 to 60.7 mg/kg, respectively. In mice infected with *E. coli* T-7, *K. pneumoniae* DT, and *P. vulgaris* GN 4712, the 50% effective doses of SCE-963 and cefazolin increased about 2.7 and 2.4 times, respectively, as the challenge dose increased every 10 times (Table 7).

**Effect of administration route on the protective test.** SCE-963 was similarly effective when administered by the subcutaneous, intravenous, or intraperitoneal route in mice infected with several strains of gram-positive and gram-negative bacteria. The protective activity of SCE-963 on oral administration was inferior to that on parenteral administration (Table 8).

**Plasma level.** The plasma level of SCE-963 in mice after subcutaneous administration of 0.625 to 40 mg/kg was examined. The peak concentration in plasma was observed 5 to 15 min after administration, and thereafter the concentrations declined rapidly. The peak and duration of plasma levels increased as the dose increased (Fig. 4).

## DISCUSSION

SCE-963 showed a broad spectrum of antibacterial activity. Its activity against gram-negative bacteria was about 10 times greater than that of cefazolin, cephaloridine or cephalothin. One of the most important properties was that SCE-963 showed excellent activity against *H. influenzae*. The MICs of SCE-963 against most strains of *H. influenzae* were lower than 0.78 μg/ml and similar to those of ampicillin (8). SCE-963 was active against cefazolin-resistant strains of *E. coli* and *K. pneumoniae* but less effective than against cefazolin-susceptible strains. Moreover, with the light inoculum, SCE-963 showed high activity against *P. vulgaris, P. morganii, Enterobacter* species, and *C. freundii*. The activity of SCE-963 against these strains may be explained by the stability of SCE-963 to hydrolysis by β-lactamases from these strains as well as by improved accessibility of the drug to

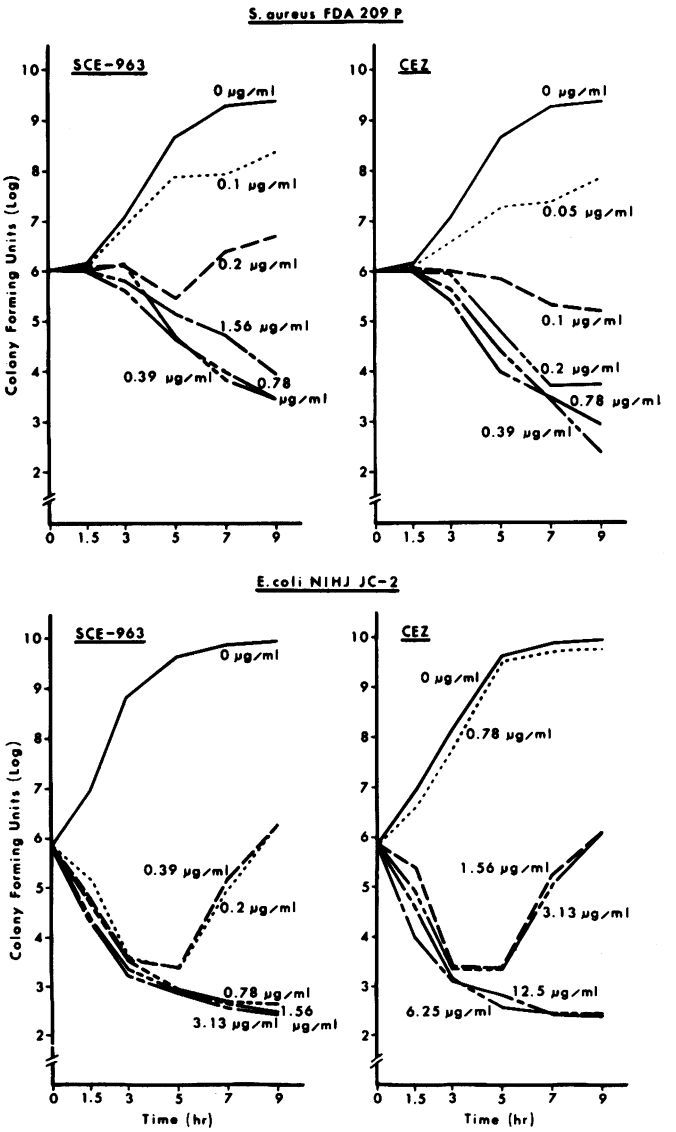


FIG. 2. *Bactericidal effect of SCE-963 and cefazolin on S. aureus FDA 209 P and E. coli NIHJ JC-2.*

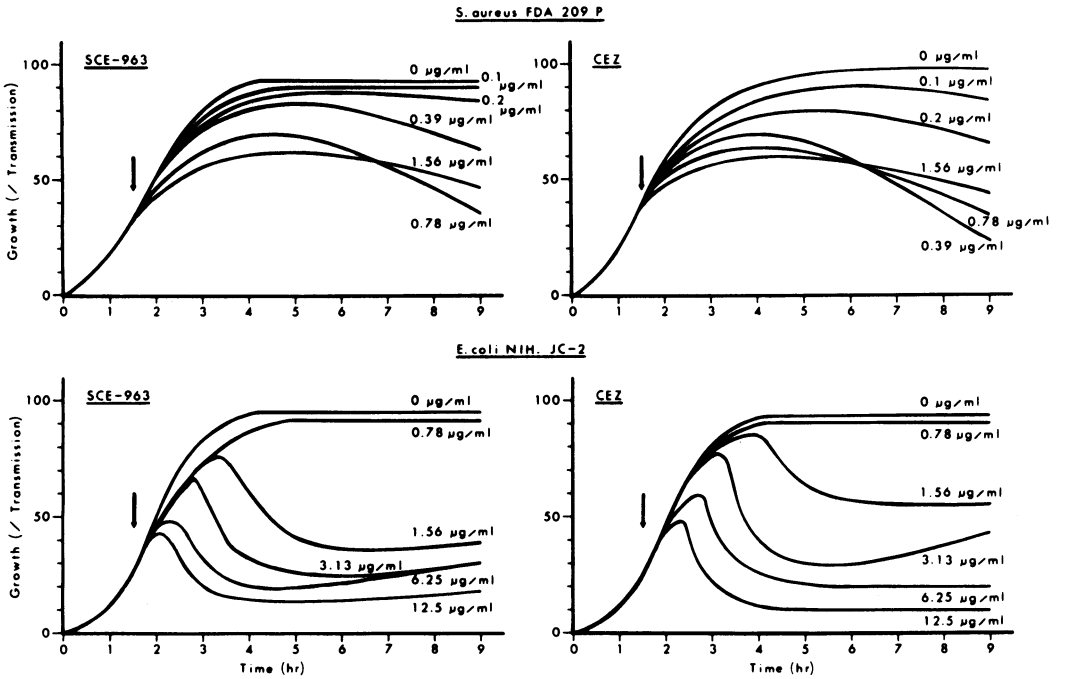


FIG. 3. *Bacteriolytic effect of SCE-963 and cefazolin on S. aureus FDA 209 P and E. coli NIHJ JC-2.*

target sites, because the growth inhibition of any β-lactamase-producing organisms by a β-lactam antibiotic is thought to be the outcome of complex steps involving the rate of penetration of the drug to the active sites, inhibition of vital processes, and the rate of breakdown of the drug (7). Indeed, SCE-963 was more stable to hydrolysis by β-lactamase from these strains than cefazolin (unpublished data).

Bactericidal activity of SCE-963 against *E. coli* NIHJ JC-2 was greater than that of cefazolin, but the bacteriolytic activity was similar to that of cefazolin. These results were also confirmed morphologically (unpublished data). It has been reported that the protective activity of β-lactam antibiotics which have the same MIC against the challenge organism reflects more directly bactericidal and bacteriolytic activity than does the MIC (3, 4, 11). CGP 9000 {7-[D-2-amino-2-(1,4-cyclohexadienyl)-acetamido]-3-methyl-3-cephem-4-carboxylic acid} has MICs almost equal to those of cephalexin but more rapid and more intensive bactericidal activity than cephalexin. The protective activity of CGP 9000 in experimental infection in mice is greater than that of cephalexin, although the plasma levels after oral administration of both CGP 9000 and cephalexin are similar. Amoxicillin and ampicillin have the same antibacterial activity against several organisms, and in mice infected intraperitoneally with *E. coli* the superior protective activity of amoxicillin to that of ampicillin is observed. The phenomenon may be explained by the greater bactericidal and bacteriolytic activities of amoxicillin in vivo. SCE-963 has about 10 times greater growth-inhibitory activity (MIC) and bactericidal activity than cefazolin, and both SCE-963 and cefazolin have the same bacteriolytic activity. In mice infected with several species of gram-negative organisms, SCE-963 has about a three times greater protective effect than cefazolin. On the other hand, cefsulodin {3-(4-carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt} did not show bacteriolytic activity and has about 10 times stronger MIC and bactericidal activities against *P. aeruginosa* than carbenicillin and sulbenicillin. The protective activity in mice of cefsulodin was about 30 times more potent than those of carbenicilin and sulbenicillin (5, 9). It has been reported that the bactericidal activity does not indicate the bacteriolytic activity (1, 2, 10). These findings suggest that the growth-inhibitory activity (MIC) and the bactericidal activity of a β-lactam antibiotic in vitro would reflect the antibacterial activity in vivo, and morphological changes and the bacteriolytic activity would produce further modifications of the antibacterial activity of the drug in vivo.

TABLE 6. *Comparative protective effect of SCE-963, cefazolin and cephalothin against experimental intraperitoneal infection in mice*[a]

| Organism | Challenge dose (CFU/animal) | $ED_{50}$ (mg/kg)[b] | | |
|---|---|---|---|---|
| | | SCE-963 | Cefazolin | Cephalothin |
| *S. aureus* 308 A-1 | $10^8$ | 6.41(4.83–8.58) | 1.36(1.06–1.77) | 19.6(13.0–28.8) |
| | | 0.39[c] | 0.2 | 0.1 |
| *S. aureus* E 97 | $10^9$ | 2.42(1.90–3.01) | 0.445(0.298–0.603) | 5.3(3.93–6.95) |
| | | 0.78 | 0.39 | 0.2 |
| *S. pyogenes* E 14 | $10^3$ | 0.175(0.143–0.216) | 0.318(0.248–0.403) | 1.32(0.91–1.86) |
| | | 0.05 | 0.1 | 0.1 |
| *S. pneumoniae* type I | $10^2$ | 12.9(9.61–19.4) | 2.78(0.79–4.43) | 93.0(69.3–123) |
| | | 0.02 | 0.1 | 0.2 |
| *E. coli* O-111 | $10^5$ | 0.0607(0.0476–0.0758) | 1.84(1.57–2.26) | 13.1(9.13–17.6) |
| | | 0.05 | 1.56 | 1.56 |
| *E. coli* T-7 | $10^2$ | 5.63(3.73–7.68) | 48.9(39.2–61.0) | >800 |
| | | 0.78 | 25 | 100 |
| *K. pneumoniae* DT | $10^2$ | 6.74(4.96–13.3) | 15.2(11.2–22.2) | 108(78.9–149) |
| | | 0.1 | 1.56 | 1.56 |
| *P. mirabilis* IFO 3849 | $10^6$ | 6.56(4.29–9.15) | 26.4(22.2–31.5) | 95.8(71.4–122) |
| | | 1.56 | 25 | 25 |
| *P. mirabilis* GN 4330 | $10^5$ | 6.77(5.40–8.39) | 12.9(10.6–15.6) | 31.6(23.4–41.7) |
| | | 3.13 | 6.25 | 12.5 |
| *P. mirabilis* GN 4336 | $10^4$ | 1.81(1.23–2.49) | 10.6(9.03–12.5) | 26.5(20.4–33.8) |
| | | 1.56 | 12.5 | 25 |
| *P. mirabilis* GN 4355 | $10^3$ | 1.43(0.89–2.08) | 5.02(3.86–6.59) | 9.25(6.90–12.4) |
| | | 1.56 | 12.5 | 12.5 |
| *P. vulgaris* IFO 3988 | $10^6$ | 1.11(0.71–1.50) | 3.48(2.56–4.51) | 2.75(0.48–4.63) |
| | | 0.2 | 6.25 | 3.13 |
| *P. vulgaris* GN 4422 | $10^3$ | 36.1(26.4–48.4) | 83.0(70.6–98.1) | 145(122–173) |
| | | 3.13 | 50 | >100 |
| *P. vulgaris* GN 4712 | $10^1$ | 27.6(16.6–38.7) | 89.3(64.9–119) | 276(214–355) |
| | | 1.56 | 100 | 100 |
| *P. vulgaris* TN 237 | $10^4$ | 52.0(38.7–67.1) | 162(131–204) | 344(267–454) |
| | | 3.13 | 100 | 100 |
| *P. morganii* IFO 3168 | $10^5$ | 10.9(7.33–14.9) | 75.7(59.5–95.2) | >800 |
| | | 0.78 | >100 | >100 |
| *P. morganii* GN 4757 | $10^6$ | 9.27(7.42–11.7) | 80.9(66.2–97.1) | >800 |
| | | 1.56 | >100 | >100 |
| *P. morganii* GN 4794 | $10^2$ | 6.54(4.57–9.33) | 64.3(53.6–77.0) | >800 |
| | | 3.13 | >100 | >100 |
| *P. morganii* TN 373 | $10^4$ | 67.5(49.5–118) | 167(129–210) | >800 |
| | | 0.39 | >100 | >100 |
| *P. rettgeri* TN 338 | $10^4$ | 0.157(0.106–0.214) | 1.72(1.17–2.28) | 69.3(48.5–92.6) |

[a] *S. aureus* E. 97 and *S. pneumonia* type I were suspended in TSB, and other organisms were suspended in 5% mucin. Mice were infected intraperitoneally with test organism in 0.5 ml of medium.

[b] In mice infected with *S. pneumoniae*, antibiotics were administered subcutaneously at 0 and 4 h after infection. In mice infected with other organisms, antibiotics were administered subcutaneously at 0 h after infection. $ED_{50}$ (50% effective dose) values were calculated by the probit method. Number in parentheses indicates 95% confidence limits.

[c] MIC (micrograms per milliliter) was determined with a bacterial suspension of $10^6$ CFU/ml.

TABLE 7. *Effect of challenge dose on 50% effective dose ($ED_{50}$) of SCE-963 and cefazolin against experimental intraperitoneal infection in mice*[a]

| Challenge dose (CFU/animal) | $ED_{50}$ (mg/kg)[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* O-111 | | *E. coli* T-7[c] | | *K. pneumoniae* DT | | *P. vulgaris* GN 4712 | |
| | SCE-963 | Cefazolin | SCE-963 | Cefazolin | SCE-963 | Cefazolin | SCE-963 | Cefazolin |
| $10^1$ | | | | | 0.81 (0.41–1.21) | 3.27 (1.72–4.48) | 27.6 (16.6–38.7) | 89.3 (64.9–119) |
| $10^2$ | | | 1.20 (0.96–1.45) | 26.1 (16.6–32.9) | 2.39 (1.69–3.15) | 12.6 (9.58–16.3) | 72.6 (45.8–232) | 133 (108–175) |
| $10^3$ | | | 2.16 (1.66–2.76) | 43.4 (30.1–56.8) | 5.62 (4.36–7.48) | 24.4 (16.8–34.2) | 108 (73.9–204) | 198 (161–244) |
| $10^4$ | | | 11.1 (6.30–15.6) | 107 (85.7–135) | 11.7 (8.86–16.4) | 40.6 (30.1–59.8) | 312 (196–529) | 449 (355–603) |
| $10^5$ | 0.054 (0.040–0.070) | 1.10 (1.90–1.33) | 60.4 (35.7–79.5) | 342 (255–470) | 20.0 (15.0–28.5) | 101 (68.1–175) | 632 (444–1,260) | 761 (527–2,360) |
| $10^6$ | 0.119 (0.006–0.191) | 2.28 (1.83–2.79) | 124 (92.6–163) | 660 (518–999) | 50.2 (34.9–66.9) | >560 | >800 | >800 |
| $10^7$ | 1.95 (1.26–2.63) | 10.6 (8.21–13.5) | 143 (106–179) | >800 | 98.3 (75.6–133) | >800 | | |
| $10^8$ | 34.5 (23.6–46.9) | 54.3 (41.0–69.1) | 660 (518–999) | >800 | 225 (152–367) | >800 | | |

[a] Mice were infected intraperitoneally with test organism in 0.5 ml of 5% mucin.
[b] Antibiotics were administered at 0 h after infection. $ED_{50}$ values were calculated by the probit method. Number in parentheses indicates 95% confidence limits.
[c] Cefazolin-resistant strain.

TABLE 8. *Effect of administration route on 50% effective dose ($ED_{50}$) of SCE-963 against experimental intraperitoneal infection in mice*[a]

| Organism | Challenge dose (CFU/animal) | $ED_{50}$ (mg/kg)[b] | | | |
|---|---|---|---|---|---|
| | | Subcutaneous | Intravenous | Intraperitoneal | Oral |
| *S. aureus* 308 A-1 | $10^8$ | 6.41 (4.83–8.58) | 5.19 (4.06–6.54) | 3.72 (2.63–5.13) | 24.5 (20.8–28.9) |
| *S. pyogenes* E-14 | $10^3$ | 0.175 (0.143–0.216) | 0.33 (0.244–0.456) | 0.0757 (0.0569–0.104) | 4.53 (3.96–5.17) |
| *S. pneumoniae* type I | $10^2$ | 44.0 (33.5–57.1) | 60.6 (45.6–81.3) | 30.9 (21.4–42.5) | 57.2 (45.1–72.8) |
| *E. coli* O-111 | $10^5$ | 0.0607 (0.0476–0.0758) | 0.113 (0.0261–0.795) | 0.0436 (0.0247–0.0729) | 1.22 (0.99–1.49) |
| *E. coli* T-7[c] | $10^2$ | 5.63 (3.73–7.86) | 9.65 (7.0–12.5) | 4.86 (3.75–6.27) | 42.3 (33.3–51.7) |
| *K. pneumoniae* DT | $10^2$ | 6.74 (4.96–13.3) | 5.53 (3.99–9.29) | 2.11 (1.58–3.15) | 6.83 (5.58–8.21) |
| *P. mirabilis* IFO 3849 | $10^6$ | 6.56 (4.29–9.15) | 7.75 (5.62–10.0) | 3.40 (2.43–4.50) | 13.4 (11.4–15.9) |
| *P. vulgaris* IFO 3988 | $10^6$ | 1.11 (0.714–1.49) | 1.33 (0.995—1.92) | 0.434 (0.223–0.695) | 3.53 (2.97–4.18) |
| *P. morganii* IFO 3168 | $10^5$ | 10.9 (7.33–14.9) | 10.5 (6.97–15.8) | 4.56 (3.42–5.85) | 14.3 (9.68–19.1) |
| *P. rettgeri* TN 338 | $10^4$ | 0.157 (0.106–0.214) | 0.413 (0.294–0.565) | 0.173 (0.054–0.274) | 0.764 (0.623–0.923) |

[a] *S. pneumoniae* type I was suspended in TSB, and other organisms were suspended in 5% mucin. Mice were infected intraperitoneally with test organism in 0.5 ml of medium.

[b] Antibiotics were administered at 0 h after infection. $ED_{50}$ values were calculated by the probit method. Number in parentheses indicates 95% confidence limits.

[c] Cefazolin-resistant strain.

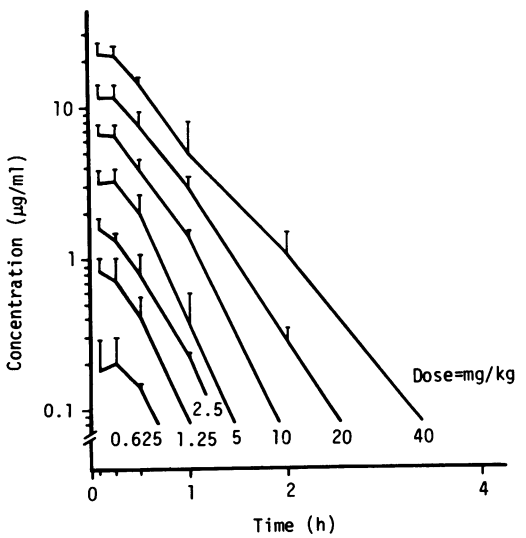


FIG. 4. *Plasma level of SCE-963 after a single subcutaneous administration in mice. Groups of five mice at each point were used.*

ACKNOWLEDGMENTS

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