3-DEACETOXY-7-(α-AMINO-1-CYCLOHEXENYLACETAMIDO) CEPHALOSPORANIC ACID (SCE-100), A NEW SEMISYNTHETIC CEPHALOSPORIN. I

COMPARATIVE *IN VITRO* ANTIBACTERIAL ACTIVITIES OF SCE-100 AND CEPHALEXIN (CEX)

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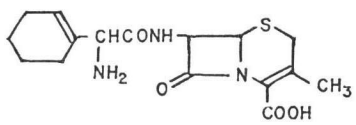
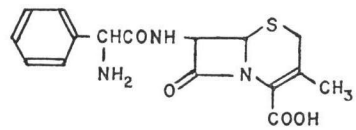
3-Deacetoxy-7-(α-amino-1-cyclohexenylacetamido) cephalosporanic acid (SCE-100) has a potent antibacterial activity against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration of SCE-100 is 0.2~12.5 μg/ml against Gram-positive bacteria including penicillin G-resistant *Staphylococcus aureus* and 6.25~100 μg/ml against Gram-negative bacteria. SCE-100 inhibits clinically isolated strains of penicillin G-resistant *S. aureus* and ampicillin-resistant *Escherichia coli*, as well as the corresponding sensitive strains. The activity of SCE-100 is enhanced by decreasing the inoculum size and is only slightly influenced by medium pH, difference of medium composition and the addition of horse serum. SCE-100 has bactericidal activity against *S. aureus* and *E. coli*. There is a stepwise development of *in vitro* bacterial resistance to SCE-100 by serial transfer of organisms into a liquid medium containing SCE-100. Cross resistance was observed between SCE-100 and other tested cephalosporins.

3-Deacetoxy-7-(α-amino-1-cyclohexenylacetamido) cephalosporanic acid (SCE-100) is a new semisynthetic cephalosporin,[1,2] whose chemical structure resembles cephalexin (CEX).[3] In SCE-100, 1-cyclohexenylglycine is introduced at position 7 instead of phenylglycine in CEX:



SCE-100



CEX

Preliminary *in vitro* and *in vivo* experiments revealed that SCE-100 is a potent antibiotic against Gram-positive and Gram-negative organisms. When administered orally, SCE-100 is effectively absorbed and distributed into various organs.

This report describes comparative *in vitro* studies on antibacterial properties of SCE-100 and CEX.

Materials and Methods

Antibiotics: SCE-100 and CEX were prepared in Takeda Chemical Industries, Ltd. Cephalothin (CET), cephaloridine (CER) and cefazolin (CEZ) were obtained from commercial sources.

SCE-100 and CEX were dissolved in a small volume of 5% sodium bicarbonate solution and diluted with distilled water. For other antibiotics, distilled water was used as a solvent.

Determination of minimum inhibitory concentration (MIC): The cultures were maintained on Trypticase soy agar (TSA; BBL) or TSA supplemented with 5% bovine blood (blood-TSA).

Clinical isolates of various bacterial species were kindly supplied by Miss Y. SHIMIZU, Central Clinical Laboratory, Osaka University Hospital.

The MIC of antibiotics was determined by the 2-fold serial dilution technique using TSA, blood-TSA or Trypticase soy broth (TSB; BBL) as test medium. One loopful of 10-fold diluted overnight broth containing approximately 10^8 viable units/ml was streaked on each assay plate. In the tube dilution technique, 0.1 ml of 1,000-fold diluted overnight culture was inoculated into 5 ml of TSB containing the antibiotic. The MIC is defined as the lowest concentration of antibiotics at which the visible growth of the test organism is completely prevented.

Determination of minimum bactericidal concentration (MBC): Inocula from culture tubes containing just the MIC or higher concentrations of antibiotics were transferred to TSA and incubated at 37°C for 48 hours. The MBC is defined as the lowest concentration of the antibiotic at which no visible growth on TSA medium is observed.

Bactericidal activity: The killing activity of the antibiotics was determined by the plate count technique. An overnight broth culture was diluted with TSB to approximately 10^6 viable units/ml, and the antibiotics were added to give a final concentration of 1/8, 1/4, 1 and 4-fold MIC of each cephalosporin. The growth of test organisms in the broth with or without addition of the antibiotics was followed by plating aliquots at various time intervals.

Resistance development: The development of *in vitro* resistance to the cephalosporin was studied with *S. aureus* FDA 209P and *E. coli* NIHJ JC-1. After 48 hours of incubation, inoculum from the culture tube containing the highest concentration of cephalosporin where growth equivalent to the control tube (without antibiotic) was observed, was transferred to the next series of tubes containing identical or higher levels of antibiotic.

Results

The Antibacterial Spectrum

The antibacterial activities of SCE-100 and CEX against representative Gram-positive and Gram-negative organisms are summarized in Table 1 The MIC of SCE-100 is 0.2~12.5 μg/ml against Gram-positive organisms including Penicillin G-resistant *S. aureus*, and 6.25~

Fig. 1. Sensitivity of clinically isolated *S. aureus* to SCE-100 and cephalexin.

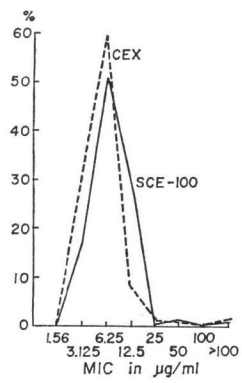


Fig. 2. Sensitivity of clinically isolated *E. coli* to SCE-100 and cephalexin.

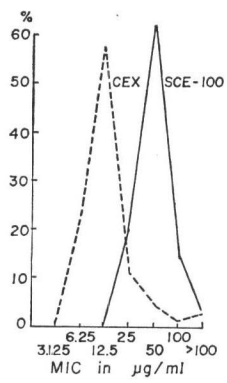


Fig. 3. Sensitivity of clinically isolated *Proteus* spp. to SCE-100 and cephalexin.

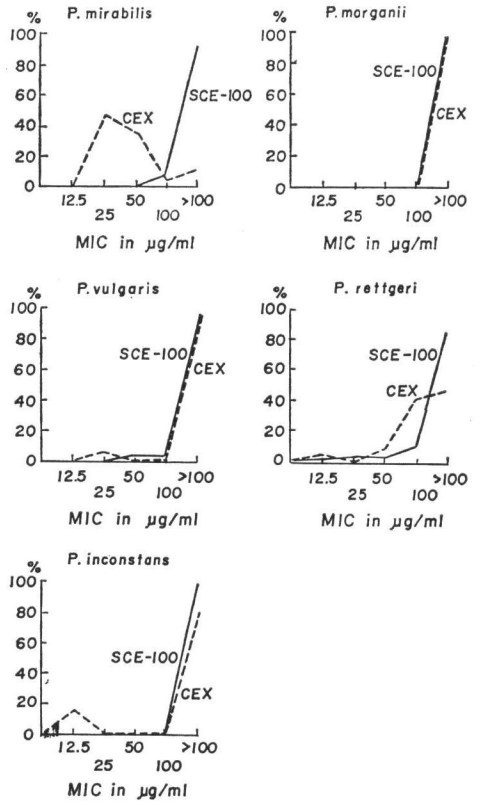


Table 1. The antibacterial spectrum of SCE-100 and cephalexin

Organism	Medium	MIC*	
		SCE-100	CEX
Staphylococcus aureus FDA 209P	Trypticase soy agar	1.56	1.56
Staphylococcus aureus 308A-1	〃	3.13	1.56
Staphylococcus aureus 1840	〃	12.5	12.5
Streptococcus pyogenes E-14	Trypticase soy agar +10% bovine blood	0.39	0.78
Streptococcus pyogenes Dick	〃	0.78	0.78
Streptococcus pyogenes S-8	〃	0.78	0.78
Streptococcus pyogenes NY-5	〃	0.39	0.78
Streptococcus mitior America	〃	12.5	25.0
Streptococcus pneumoniae Type I	〃	3.13	6.25
Streptococcus pneumoniae Type II	〃	1.56	1.56
Streptococcus pneumoniae Type III	〃	1.56	3.13
Corynebacterium diphtheriae Toronto	〃	0.195	0.39
Bacillus subtilis PCI-219	Trypticase soy agar	0.39	0.78
Shigella flexneri EW-10	〃	25.0	12.5
Shigella flexneri EW-40	〃	25.0	12.5
Shigella dysenteriae EW-1	〃	12.5	6.25
Shigella sonnei EW-33	〃	25.0	6.25
Salmonella paratyphi A	〃	50.0	12.5
Salmonella schottmuelleri	〃	25.0	6.25
Salmonella hirschfeldii	〃	25.0	6.25
Salmonella typhi Boxhill-58	〃	25.0	6.25
Salmonella typhi Watson	〃	25.0	6.25
Salmonella typhimurium	〃	25.0	6.25
Escherichia coli NIHJ JC-1	〃	50.0	12.5
Escherichia coli Umezawa	〃	12.5	6.25
Escherichia coli K-12	〃	50.0	12.5
Escherichia coli O-78	〃	50.0	12.5
Escherichia coli O-111	〃	12.5	6.25
Vibrio cholerae Inaba	〃	6.25	6.25
Klebsiella pneumoniae DT	〃	12.5	3.13
Proteus vulgaris IFO 3851	〃	50.0	25.0
Proteus morganii IFO 3168	〃	>100	>100
Proteus mirabilis IFO 3849	〃	>100	25.0
Proteus OX-19	〃	100	50.0
Proteus OX-K	〃	50	12.5
Pseudomonas aeruginosa sp.	〃	>100	>100
Pseudomonas aeruginosa U 31	〃	>100	>100
Pseudomonas aeruginosa N 18	〃	>100	>100
Pseudomonas aeruginosa D 363	〃	>100	>100
Pseudomonas aeruginosa P 8	〃	>100	>100
Pseudomonas aeruginosa No. 10	〃	>100	>100
Candida albicans	〃	>100	>100

* Minimum inhibitory concentration in μg/ml.

100 μg/ml against Gram-negative organisms except *Proteus morganii*, *P. mirabilis* and *Pseudomonas aeruginosa*. The antibacterial activity of SCE-100 against Gram-positive organisms was comparable to that of CEX. However, SCE-100 seemed to be a little less active against Gram-negative organisms than CEX. Both cephalosporins showed no antibacterial activity against *Candida albicans*.

Antibacterial Activity against Clinical Strains of Bacteria

The antibacterial activity of SCE-100 and CEX against clinical isolates of *S. aureus*, *E. coli* and *Proteus* is shown in Figs. 1, 2 and 3. The MIC of SCE-100 against 99 strains of *S. aureus* including penicillin G-resistant strains ranged from 3.13 to 12.5 μg/ml. Of these strains 52% were inhibited at a concentration of 6.25 μg/ml. A similar distribution pattern of MIC was observed for CEX (Fig. 1). Sixty-three percent of 102 strains of *E. coli* including ampicillin-resistant strains were inhibited by 50 μg of SCE-100/ml, whereas 57.8% of the isolates were inhibited by 12.5 μg of CEX/ml (Fig. 2). With *P. mirabilis*, the majority of 79 tested strains were resistant to at least 100 μg of SCE-100/ml, whereas CEX inhibited over 80% of these strains at concentrations ranging from 25 to 50 μg/ml. For a large number of strains of other *Proteus* spp., the activity of SCE-100 was similar to or slightly lower than that of CEX (Fig. 3).

Influence of Inoculum Size, Medium pH, Difference in Medium Composition and Addition of Serum

Various factors that affect the activity of both cephalosporins against *S. aureus*, *E. coli*, *K. pneumoniae* and *P. vulgaris* were studied. As shown in Table 2, an increase of the MIC paralleled the

Table 2. Effect of inoculum size on the activity of SCE-100 and cephalexin

Organism	Minimum inhibitory concentration (μg/ml)									
	SCE-100					CEX				
	10^8	10^7	10^6	10^5	10^4	10^8	10^7	10^6	10^5	10^4
S. aureus FDA 209P	3.13	1.56	0.78	0.78	0.19	3.13	0.78	0.78	0.39	0.39
S. aureus 308A-1	3.13	1.56	1.56	1.56	0.78	3.13	1.56	0.78	0.78	0.78
E. coli Umezawa	25	25	25	25	12.5	12.5	6.25	6.25	6.25	6.25
E. coli NIHJ JC-1	50	25	25	25	25	6.25	6.25	6.25	6.25	6.25
K. pneumoniae DT	12.5	12.5	12.5	6.25	6.25	3.13	3.13	1.56	1.56	1.56
P. vulgaris IFO 3851	50	25	12.5	12.5	12.5	25	12.5	6.25	6.25	6.25

Inoculum size: A loopful of bacterial suspension ($10^4 \sim 10^5$ cells/ml).
Medium: Trypticase soy agar.

increase in inoculum size. As indicated in Tables 3 and 4, the MIC of both antibiotic compounds was not significantly influenced by the medium pH (from 6 to 9), and the type of media. Furthermore, the addition of 10~50% horse serum to TSB medium hardly influenced the activity of both cephalosporins (Table 5).

Bactericidal Activity

The MBC and MIC values of SCE-100 and CEX against 3 strains of *S. aureus*, 2 of *E. coli*, 1 of *P. vulgaris* and *K. pneumoniae* were determined. As shown in Table 6, there was only a small difference between the MBC and MIC values. The bactericidal effects of SCE-100 and CEX

Table 3. Effect of medium pH on the activity of SCE-100 and cephalexin

Organism	Minimum inhibitory concentration (μg/ml)							
	SCE-100				CEX			
	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
S. aureus FDA 209P	3.13	3.13	3.13	3.13	1.56	3.13	3.13	3.13
S. aureus 308A-1	3.13	3.13	6.25	6.25	1.56	3.13	3.13	3.13
E. coli Umezawa	100	25	25	25	25	12.5	6.25	12.5
E. coli NIHJ JC-1	50	50	50	100	12.5	6.25	12.5	25
K. pneumoniae DT	25	12.5	12.5	25	6.25	6.25	6.25	12.5
P. vulgaris IFO 3851	100	50	25	25	50	50	25	25

Inoculum size: A loopful of bacterial suspension (approximately 10^5/ml).
Medium: Trypticase soy agar.

Table 4. Effect of various media on the activity of SCE-100 and cephalexin

Organism	Minimum inhibitory concentration (μg/ml)									
	SCE-100					CEX				
	TSA*	MH*	HI*	NA*	BHI*	TSA	MH	HI	NA	BHI
S. aureus FDA 209P	3.13	1.56	1.56	1.56	1.56	1.56	1.56	1.56	0.78	1.56
S. aureus 308A-1	3.13	3.13	3.13	1.56	3.13	3.13	3.13	3.13	0.78	3.13
E. coli Umezawa	25	25	50	50	50	12.5	12.5	12.5	12.5	12.5
E. coli NIHJ JC-1	25	50	50	50	50	12.5	12.5	12.5	12.5	12.5
K. pneumoniae DT	12.5	12.5	25	25	25	3.13	6.25	6.25	6.25	6.25
P. vulgaris IFO 3851	50	50	50	50	50	25.0	50	25	50	25

* TSA: Trypticase soy agar. MH: MUELLER-HINTON agar .HI: Heart infusion agar. NA: Nutrient agar. BHI: Brain heart infusion agar.

Table 5. Effect of horse serum in medium on the activity of SCE-100 and cephalexin

Organism	Minimum inhibitory concentration(μg/ml)							
	SCE-100				CEX			
	0%	10%	20%	50%	0%	10%	20%	50%
S. aureus FDA 209P	3.13	3.13	3.13	3.13	1.56	1.56	1.56	1.56
S. aureus 308A-1	6.25	12.5	12.5	12.5	3.13	6.25	6.25	12.5
E. coli Umezawa	50	50	50	25	25	25	12.5	12.5
E. coli NIHJ JC-1	100	50	50	25	25	12.5	12.5	12.5
K. pneumoniae DT	25	25	25	25	6.25	6.25	6.25	6.25
P. vulgaris IFO 3851	100	100	100	100	50	50	50	50

Inoculum size: 0.1 ml bacterial suspension (approximately 10^5/ml).
Medium: Trypticase soy broth.

against *S. aureus* FDA 209P and *E. coli* NIHJ JC-1 grown in TSB were studied more precisely. As shown in Figs. 4 and 5, both compounds produced a considerable decrease of viable units of these strains at the concentrations of MIC and 4-fold MIC during 8 hours of incubation. There was no substantial difference between the bactericidal effects of both cephalosporins.

Fig. 4. Bactericidal activity of SCE-100 and cephalexin on *S. aureus* FDA 209P.

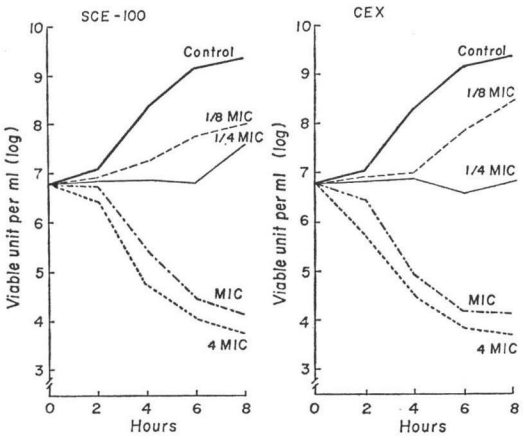


Fig. 5. Bactericidal activity of SCE-100 and cephalexin on *E. coli* NIHJ JC-1

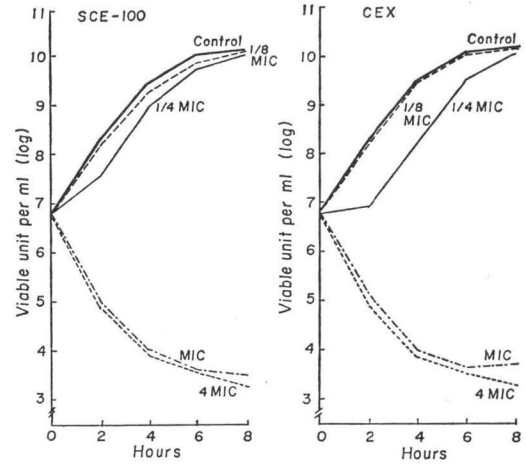


Fig. 6. Patterns of development of resistance of *S. aureus* FDA 209P to SCE-100 and cephalexin.

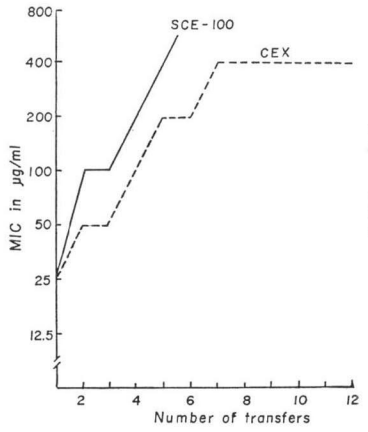


Fig. 7. Patterns of development of resistance of *E. coli* NIHJ JC-1 to SCE-100 and cephalexin.

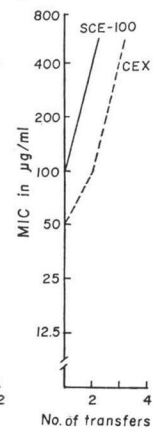


Table 6. Bacteriostatic and bactericidal concentration of SCE-100 and cephalexin

Organism		SCE-100	CEX
S. aureus FDA 209P	MIC*	1.56	1.56
	MBC**	3.13	3.13
S. aureus 308A-1	MIC	3.13	3.13
	MBC	3.13	3.13
S. aureus Heatley	MIC	3.13	3.13
	MBC	3.13	3.13
E. coli Umezawa	MIC	50	12.5
	MBC	50	12.5
E. coli NIHJ JC-1	MIC	100	12.5
	MBC	100	12.5
P. vulgaris IFO 3988	MIC	100	12.5
	MBC	100	25
K. pneumoniae DT	MIC	25	3.13
	MBC	25	6.25

Inoculum size: 0.1 ml of bacterial suspension (10^8 viable units/ml).
* Minimum inhibitory concentration in μg/ml.
** Minimum bactericidal concentration in μg/ml.

Resistance Development

As shown in Fig. 6, the rates and degrees of development of resistance to SCE-100 and to CEX by *S. aureus* FDA 209P were quite similar. After 5 and 7 transfers the organisms showed visible growth in the presence of 400 μg/ml of SCE-100 or CEX. The development of resistance by *E. coli* NIHJ JC-1 to SCE-100 and CEX appeared early. Thus, after 2～3 transfers, the MIC values of both cephalosporins increased to more than 400 μg/ml (Fig. 7). As shown in Table 7, mutual cross resistance to four cephalosporins could be observed.

Discussion

In the present comparative studies it was found that the antibacterial spectrum of SCE-100, and its antibacterial *in vitro* activities against Gram-positive organisms including clinically isolated

Table 7. Cross resistance test among SCE-100, cephalexin, cephalothin, cephaloridine and cefazolin

Organism	MIC in μg/ml				
	SCE-100	CEX	CET	CER	CEZ
S. aureus FDA 209P (parent)	3.13	3.13	0.2	0.1	0.39
R-SCE-100*	>400	>400	12.5	1.56	50
R-CEX**	>400	>400	25.0	3.13	50
R-CER#	>400	>400	>400	>400	>400
R-CEZ##	>400	>400	200	100	200
E. coli NIHJ JC-1 (parent)	50	12.5	12.5	6.25	3.13
R-SCE-100	>400	>400	>400	200	200
R-CEX	>400	>400	>400	200	400
R-CET	>400	>400	>400	>400	>400
R-CER	>400	>400	>400	>400	>400
R-CEZ	>400	>400	>400	50	>400

* SCE-100 resistant. # Cephaloridine-resistant.
** Cephalexin-resistant. ## Cefazolin-resistant.

penicillin G-resistant strains of *S. aureus* are similar to those of CEX, although, to some extent, SCE-100 is less active than CEX against Gram-negative organisms. SCE-100 was bactericidal at the concentration of the MIC. The behavior of SCE-100 to inoculum size, medium pH, difference in medium composition and addition of serum was similar to that of CEX. Other investigators[3~6] have observed *in vitro*, that CEX is bctericidal at a concentration equal to or 2~4 time greater than the MIC, and that the medium and presence of serum do not affect activity, although the antibacterial activity is enhanced by a decrease in inoculum size and in the medium pH below 5.

In view of the similarity in chemical structure and *in vitro* activity of both cephalosporins, further studies on SCE-100 as an oral cephalosporin are planned.

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