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

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

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The protective activity of 3-deacetoxy-7-(α-amino-1-cyclohexenylacetamido) cephalosporanic acid (SCE-100) against experimental intraperitoneal infections in mice caused by several strains of Gram-positive and Gram-negative organisms, including penicillin resistant strains, was compared with that of cephalexin (CEX). Comparable protective effects by oral administration of SCE-100 and CEX were observed in mice infected with Gram-positive organisms, while in mice infected with Gram-negative organisms SCE-100 was less active than CEX. SCE-100 showed a protective effect in infections of mice with either penicillin G-resistant *Staphylococcus aureus* or ampicillin-resistant *Escherichia coli*. The oral ED_{50} of SCE-100 was similar to or slightly larger than the subcutaneous value.

3-Deacetoxy-7-(α-amino-1-cyclohexenylacetoamido) cephalosporanic acid (SCE-100) is a new semisynthetic cephalosporin, structurally analogous to cephalexin (CEX).[1] SCE-100 is similar to CEX in its *in vitro* antibacterial activity against Gram-positive organisms, but has slightly less activity[2] against Gram-negative organisms.

In vivo antibacterial activities of SCE-100 and CEX were studied comparatively with experimental infections in mice caused by representative Gram-positive and Gram-negative organisms.

Materials and Methods

Antibiotics: SCE-100, CEX and aminobenzyl penicillin (AB-PC) were prpeared in Takeda Chemical Industries, Ltd. The cephalosporins were dissolved in a small volume of 5% sodium bicarbonate and diluted with distilled water for parenteral administration. For oral administration, the cephalosporins were suspended in 0.2% sodium carboxymethylcellulose and ampicillin (AB-PC) was dissolved in distilled water.

Animals: Four weeks old male CF_1/H mice weighing 20~22 g were used in a group of five for each dosage level.

Protective test: The following organisms, such as *Staphylococcus aureus* 308A-1, *S. aureus* No. 61 (penicillin G-resistant clinical isolate), *Streptococcus pyogenes* E-14, *Streptococcus pneumoniae* type I, *Escherichia coli* O-111, *E. coli* No. 23 (ampicillin-resistant clinical isolate), *Klebsiella pneumoniae* DT and *Proteus vulgaris* IFO 3988 were used as challenge organisms. *S. pyogenes* and *S. pneumoniae* were cultured on Trypticase soy agar (BBL) supplemented with 10% bovine blood. Other organisms were cultured in Brain Heart Infusion (Difco).

Mice were infected intraperitoneally with 0.5 ml of bacterial suspensions. *S. pneumoniae* was suspended in Trypticase soy broth (BBL), and other organisms were suspended in 5% hog gastric mucin. The viable units in challenge doses were approximately 10^7/animal for *S. aureus*, 10^4/animal for *S. pyogenes*, 10/animal for *S. pneumoniae*, 10^8/animal for *E. coli* and *K. pneumoniae* and 10^5/animal

for *P. vulgaris*.

Mice infected with *S. pneumoniae* were treated with divided doses 3, 6 and 21 hours after infection. Mice infected with other organisms were treated with a single dose immediately after infection. Death of mice was recorded daily and the 50% effective dose (ED_{50}) was calculated from the survival rate of the animals on the 7th day after infection by the method of REED and MUENCH.[3]

Results

Protective Effect in Gram-Positive Bacterial Infections

The ED_{50} values of SCE-100 and CEX in mice infected with *S. aureus* 308A-1, *S. pyogenes* E-14 and *S. pneumoniae* type I are shown in Tables 1, 2 and 3. SCE-100 and CEX had similar protective effects in mice infected with these Gram-positive organisms, regardless of the administration route. The oral ED_{50} was equal or slightly larger than the subcutaneous one, and the smallest ED_{50} was observed on the intraperitoneal route, except when the animals were infected with *S. pneumoniae*. Furthermore, as shown in Table 4, oral administration of SCE-100 was effective in mice infected with

Table 1. Protective effect of SCE-100 and cephalexin against *Staphylococcus aureus* 308A-1 infection in mice

Cephalosporin	SCE-100				CEX			
MIC (μg/ml)	3.13				1.56			
Administration route	SC	IP	IV	PO	SC	IP	IV	PO
ED_{50} (mg/kg)	4.40	—	—	—	4.96	3.12	4.40	10.00
	3.12	2.47	5.25	7.02	3.41	2.40	—	—
	—	2.69	6.25	5.58	3.63	2.62	5.25	5.00
	—	2.47	10.87	7.76	6.25	2.26	—	8.86
	7.86	3.51	9.92	7.44	4.43	3.12	6.25	6.25
	7.44	—	7.86	6.25	5.04	—	6.25	9.61
Mean	5.70	2.78	8.03	6.81	4.62	2.70	5.54	7.94

Note: Mice were infected by the intraperitoneal injection of 10^7 viable units in 5% mucin. Antibiotic was administered as a single dose immediately after challenge.

Table 2. Protective effect of SCE-100 and cephalexin against *Streptococcus pyogenes* E-14 infection in mice

Cephalosporin	SCE-100				CEX			
MIC (μg/ml)	0.39				0.78			
Administration route	SC	IP	IV	PO	SC	IP	IV	PO
ED_{50} (mg/kg)	0.39	0.32	0.55	1.00	0.73	—	0.63	—
	0.63	0.20	0.55	1.75	0.70	0.25	1.10	1.56
	1.10	0.46	1.10	2.20	1.39	0.70	2.02	1.56
	—	0.22	0.48	—	—	0.53	—	2.78
	0.78	0.35	0.70	1.33	0.70	0.29	1.25	—
	0.70	0.60	0.44	1.56	0.97	0.66	1.74	1.56
Mean	0.72	0.36	0.64	1.57	0.90	0.49	1.35	1.86

Note: Mice were infected by the intraperitoneal injection of 10^4 viable units in 5% mucin. Antibiotic was administered as a single dose immediately after challenge.

Table 3. Protective effect of SCE-100 and cephalexin against *Streptococcus pneumoniae* type I infection in mice

Cephalosporin	SCE-100			CEX		
MIC (μg/ml)	3.13			6.25		
Administration route	SC	IP	PO	SC	IP	PO
ED_{50} (mg/kg)	59.2	78.0	63.7	—	45.0	63.7
	28.1	—	—	—	—	40.0
	50.0	39.7	44.1	—	—	56.2
	35.5	—	—	70.9	—	70.9
	38.5	62.9	42.0	84.0	80.0	89.3
	35.7	—	32.5	70.4	80.0	89.3
	40.0	35.7	35.2	89.3	44.6	80.0
	56.2	60.2	34.5	84.0	70.9	56.2
Mean	42.9	55.3	42.0	79.7	64.1	68.2

Note: Mice were infected by the intraperitoneal injection of 10 viable units in TSB. Antibiotic was administered as three doses at 3, 6 and 21 hours after challenge. The figures indicate the ED_{50} value as a single dose.

Table 4. Protective effect of SCE-100 and cephalexin against *Staphylococcus aureus*, penicillin G resistant and sensitive, infection in mice

Challenge organism	*S. aureus* No. 61*			*S. aureus* 308A-1**		
Antibiotics	SCE-100	CEX	AB-PC	SCE-100	CEX	AB-PC
MIC (μg/ml)	12.5	6.25	>100	3.13	1.56	0.05
ED_{50} (mg/kg)	7.76	11.16	—	5.25	5.25	—
	12.50	17.24	>100	3.12	3.12	1.01
	10.00	17.73	>100	7.02	4.40	1.33
	14.94	11.16	>100	7.76	3.93	1.39
	—	10.50	>100	4.46	5.25	0.55
Mean	11.30	11.56	>100	5.52	4.39	1.07

Note: Mice were infected by the intraperitoneal injection of 10^7 viable units in 5% mucin. Antibiotic was administered as a single oral dose immediately after challenge.
* Penicillin G resistant.
** Penicillin G sensitive.

the clinically isolated penicillin G-resistant *S. aureus* No. 61. The resulting ED_{50} value was only 2 times larger than that in mice infected with penicillin G-sensitive *S. aureus*.

Protective Effect in Gram-Negative Bacterial Infections

The ED_{50} values of SCE-100 and CEX in mice infected with *E. coli* O-111, *K. pneumoniae* DT and *P. vulgaris* IFO 3988 are shown in Tables 5, 6 and 7. SCE-100 was not as protective as CEX in mice infected with Gram-negative organisms, and the ED_{50} values of this compound were 2 to 7 times larger than those of CEX. The protective effect of SCE-100 by oral administration was similar or somewhat smaller by use of other administration routes. It was further observed that SCE-100 protected mice infected with clinically isolated ampicillin-resistant *E. coli* No. 23. (Table 8).

Table 5. Protective effect of SCE-100 and cephalexin against *Escherichia coli* O-111 infection in mice

Cephalosporin	SCE-100			CEX		
MIC (μg/ml)	12.5			6.25		
Administration route	SC	IP	PO	SC	IP	PO
ED_{50} (mg/kg)	—	—	40.0	16.2	7.0	8.2
	—	—	17.2	5.3	10.8	8.1
	21.6	72.5	27.9	13.9	—	8.9
	11.2	—	17.6	—	—	5.5
	12.5	—	28.1	4.3	6.3	5.6
	29.8	72.5	17.6	12.5	8.1	9.3
	17.6	25.0	15.5	6.3	7.1	5.6
Mean	18.5	56.7	23.4	9.8	7.9	7.3

Note: Mice were infected by the intraperitoneal injection of 10^3 viable units in 5% mucin. Antibiotic was administered as a single dose immediately after challenge.

Table 6. Protective effect of SCE-100 and cephalexin against *Klebsiella pneumoniae* DT infection in mice

Cephalosporin	SCE-100			CEX		
MIC (μg/ml)	12.5			3.13		
Administration route	SC	IP	PO	SC	IP	PO
ED_{50} (mg/kg)	15.0	37.5	26.0	—	7.1	8.7
	8.8	29.1	25.0	—	—	5.6
	—	25.0	25.0	7.0	5.0	5.0
	14.0	25.0	29.6	3.9	3.5	6.3
	6.3	—	14.0	2.8	—	1.9
	9.7	9.6	25.0	3.5	—	3.1
	—	35.2	32.5	8.8	6.3	9.1
	20.0	27.9	25.0	10.1	3.9	7.4
Mean	12.3	27.0	25.3	6.0	5.2	5.9

Note: Mice were infected by the intraperitoneal injection of 10^3 viable units in 5% mucin. Antibiotic was administered as a single dose immediately after challenge.

Table 7. Protective effect of SCE-100 and cephalexin against *Proteus vulgaris* IFO 3988 infection in mice

Cephalosporin	SCE-100			CEX		
MIC (μg/ml)	50.0			25.0		
Administration route	SC	IP	PO	SC	IP	PO
ED_{50} (mg/kg)	96.4	—	112.5	32.1	67.8	29.8
	89.4	—	78.0	34.1	45.0	29.8
	112.4	89.3	112.4	29.8	—	31.3
	80.0	50.0	62.1	20.0	50.0	17.6
	62.9	85.5	89.3	25.0	37.9	18.1
Mean	88.2	74.9	90.9	28.2	50.2	25.3

Note: Mice were infected by the intraperitoneal injection of 10^5 viable units in 5% mucin. Antibiotic was administered as a single dose immediately after challenge.

Table 8. Protective effect of SCE-100 and cephalexin against *Escherichia coli*, ampicillin-resistant and sensitive infection in mice

Challenge organism	*E. coli* No. 23*			*E. coli* O-111**		
Antibiotics	SCE-100	CEX	AB-PC	SCE-100	CEX	AB-PC
MIC (μg/ml)	25.0	12.5	>100	12.5	6.25	3.13
ED_{50} (mg/kg)	25.0	9.61	>100	22.3	8.11	20.0
	44.6	8.86	>100	21.0	5.58	22.3
	28.0	8.80	>100	20.0	8.11	25.2
	20.0	8.80	>100	16.2	4.40	15.8
	22.3	8.56	>100	12.5	4.40	—
	28.0	8.93	>100	18.4	6.12	20.8

Note: Mice were infected by the intraperitoneal injection of 10^8 viable units in 5% mucin.
Antibiotic was administered as a single oral dose immediately after challenge.
* Ampicillin-resistant.
** Ampicillin-sensitive.

Discussion

The oral ED_{50} values of SCE-100 and CEX against infections caused by Gram-positive organisms were almost the same, irrespective of the difference of the MIC between SCE-100 and CEX. In mice infected with Gram-negative organisms, however, SCE-100 was somewhat less active than CEX, and, in general, the resulting ED_{50} values reflected the difference between the MIC values of the two cephalosporins. It was further noted that the ED_{50} values against infections with *S. aureus* and *S. pneumoniae* were significantly different, despite equal MIC-values for these two strains of bacteria.

References

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