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

COMPARATIVE STUDIES ON ABSORPTION, DISTRIBUTION AND EXCRETION OF SCE-100 AND CEPHALEXIN (CEX) IN LABORATORY ANIMALS

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3-Deacetoxy-7-(α-amino-1-cyclohexenylacetoamido) cephalosporanic acid (SCE-100) was compared to cephalexin with respect to absorption, tissue distribution, and urinary and biliary excretion in mice, rats, rabbits and dogs. The absorption of SCE-100 after oral administration is generally slower than that of cephalexin, but its disappearance from blood and tissues is delayed. The concentration of SCE-100 in the liver is distinctly higher than that of cephalexin. These properties of SCE-100 are most clearly manifested in rats. There is no significant difference in urinary excretion of the two cephalosporins in rabbits and dogs, while in rabbits, biliary excretion of SCE-100 is more intensive than that of cephalexin.

3-Deacetoxy-7-(α-amino-1-cyclohexenylacetamido) cephalosporanic acid (SCE-100) is a new semisynthetic cephalosporin which has a broad spectrum of antibacterial activity *in vitro* and *in vivo*.[1,2] This paper deals with comparative studies on the absorption, tissue distribution and excretion of SCE-100 and cephalexin (CEX) in laboratory animals.

Materials and Methods

Cephalosporins: SCE-100 and CEX were prepared in Takeda Chemical Industries, Ltd.

Animals: Male CF_1/H mice weighing 21~24 g, male Sprague Dawley/JCL rats weighing 250~380 g, female hybrid rabbits weighing 2.2~3.9 kg and female mongrel dogs weighing 7.4~10 kg were used.

Administration: Cephalosporins were administered at a dose of 100 mg/kg orally, and 20 mg/kg intravenously. Both cephalosporins were suspended in 0.2% carboxymethyl cellulose solution for oral use.

For intravenous administration, the drugs were dissolved in a small amount of 5% sodium bicarbonate, then diluted with sterile distilled water.

Cephalosporin suspensions of 10.5~12 mg/ml, 20 mg/ml and 40 mg/ml were given to mice, rats and rabbits at a dosage of 100 mg/kg through a stomach tube. A solution of 6 mg/ml was used for intravenous administration in rabbits. In dogs, a total dose of 100 mg/kg was administered in gelatin capsules. Rats and dogs were fasted overnight before use.

Measurement of cephalosporin concentrations: Heparinized blood samples were obtained at the selected time intervals as indicated in Table 1. Blood from mice and rats was obtained from incised axillary vessels under ether-anesthesia. Blood samples from 3 mice were pooled.

In rabbits and dogs, blood was drawn from the femoral vein or medial saphenous vein. The plasma was separated by centrifugation from heparinized blood samples and was used for the measurement of the antimicrobial activity. Lung, liver, spleen and kidney samples, collected from three

mice were combined and homogenized with M/15 phosphate buffer solution (PBS) (pH 7); 3-volumes for the liver, 4-volumes for the lung and kidney, and 7-volumes for the spleen. In rats and rabbits, each organ was homogenized with 9-volumes of PBS. The supernatant fluids obtained from the homogenates by centrifugation were used for the assay.

Rabbits that had received the drug orally were bled to death after the first and 8-hour experiments. Then urine samples were collected from their bladders. In experiments with intravenous drug administration, urine and bile samples were obtained from urethra-catheterized and bile duct-cannulated rabbits at selected time intervals under urethane anesthesia.

Urine samples of dogs housed in metabolism cages were collected over a 24-hour period.

The antibiotic concentration of each sample were measured by the cup-diffusion method using *Sarcina lutea* Mi 563232 as a test organism, and calculated on the basis of standard curves of the cephalosporins dissolved in the PBS.

Recovery test: Organs from three non-treated mice were combined together, and homogenized with PBS containing cephalosporin; 3-volumes for the liver, 4-volumes for the lung and kidney, and 7-volumes for the spleen. The tissues of rats and rabbits were homogenized with 9-volumes of PBS containing cephalosporin. The homogenates thus obtained were adjusted to cephalosporin concentrations of 10 and 100 μg/ml. The supernatants obtained by centrifugation were used for the measurement of antibiotic activity.

Results

Recovery Test

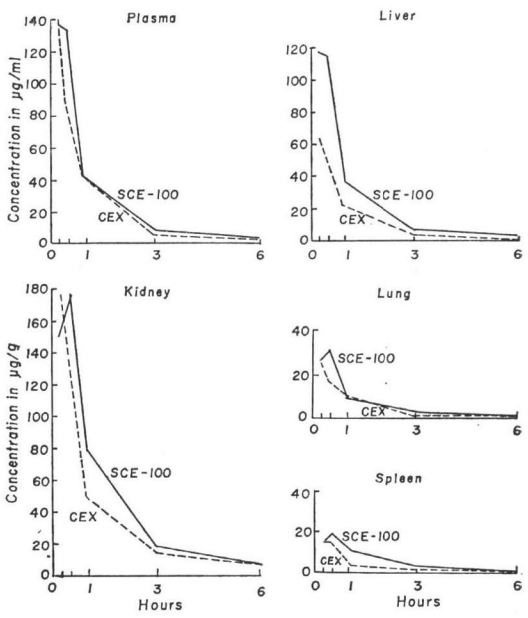
As shown in Table 1, the mean recoveries of SCE-100 and CEX from various tissue homogenates were more than 80 percent. There were no significant differences in the recoveries between the two concentrations of SCE-100 and of CEX, or among the various tissues of the animals tested.

Animal Experiments

Mice

The results of 6 separate experiments are summarized in Table 2 and Fig. 1. Plasma levels reached the peak 15 minutes after oral administration of SCE-100. Thereafter the plasma levels gradually declined. Tissue levels in various organs attained the peak 15 to 30 minutes after administration. The concentration of SCE-100 in the liver was higher than that of CEX, although comparable peak concentrations of both cephalosporins were observed in the plasma and other tissues.

Fig. 1. Plasma and tissue levels of SCE-100 and cephalexin after single oral doses of 100 mg/kg in mice.



Rats

As shown in Table 3 and Fig. 2, there was a marked difference in the distribution pattern between SCE-100 and CEX. When SCE-100 was given orally, plasma levels reached a peak 1 hour after administration. Thereafter, a gradual decline of the plasma levels was observed until 15 hours after administration, and a demon-

Table 1. Recovery of SCE-100 and CEX from mixtures of the cephalosporins with tissue homogenates

	Exp. No.	Recovery (%)											
		Mice*				Rat				Rabbit			
		SCE-100		CEX		SCE-100		CEX		SCE-100		CEX	
		10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml	10 μg/ml	100 μg/ml
Plasma	1	97.9	106.9	65.5	82.0	92.4	104.8	103.8	81.4	109.7	97.6	81.9	83.2
	2	100.0	101.9	93.6	107.0	102.6	104.8	96.2	100.0	101.9	105.6	91.9	100.0
	3	112.0	100.0	101.5	105.2	97.4	100.8	95.9	114.9	102.6	81.7	105.3	95.7
	mean	103.3	102.6	86.9	98.2	97.5	103.5	98.6	98.8	104.7	94.9	93.0	92.9
Lung	1	95.7	92.2	80.9	108.0	86.0	89.7	98.1	82.2	95.1	97.6	77.6	85.6
	2	86.0	98.1	108.6	98.1	104.3	100.0	104.0	100.0	98.1	105.6	100.9	100.0
	3	108.0	103.0	89.2	122.6	97.4	113.0	102.0	92.6	96.9	83.3	103.4	95.7
	mean	96.6	97.8	92.9	109.6	95.9	100.9	101.4	91.6	96.7	95.5	94.0	93.8
Liver	1	93.2	100.0	77.3	93.0	83.0	85.4	88.6	75.0	95.1	100.0	80.2	92.0
	2	91.2	101.9	97.6	102.8	104.3	94.4	108.4	109.6	99.1	103.7	93.8	101.9
	3	112.0	119.0	92.3	118.0	90.4	106.6	95.9	92.6	89.7	83.3	98.9	94.0
	mean	98.8	106.9	89.1	104.6	92.6	95.5	97.6	92.4	94.6	95.7	91.0	96.0
Kidney	1	83.0	78.4	77.3	102.0	88.4	84.5	99.0	87.9	95.1	97.6	75.0	71.2
	2	93.0	106.5	101.6	104.1	107.0	102.7	96.2	95.7	102.8	100.9	83.0	102.9
	3	102.0	102.6	85.4	116.9	99.1	100.8	89.9	96.8	102.6	85.0	92.0	89.4
	mean	92.7	95.8	88.1	107.7	98.2	96.0	95.0	93.5	100.2	94.5	83.3	87.8
Spleen	1	109.0	86.7	84.5	128.0	92.4	93.2	91.5	116.0	102.4	102.4	91.4	84.8
	2	92.1	100.0	92.6	100.9	100.0	98.4	107.3	106.4	105.6	105.6	100.9	99.0
	3	102.0	98.5	85.4	101.5	93.9	113.1	105.1	109.6	89.7	80.0	98.6	92.6
	mean	101.0	95.1	87.5	110.1	95.4	101.6	101.3	110.7	99.2	96.0	97.0	92.1

* Organs from three non-treated mice were combined.

Table 2. Plasma and tissue levels of SCE-100 and cephalexin after a single oral doses of 100 mg/kg in mice

Cephalosporin	Tissue	Mean concentration (and range) in μg/ml or g				
		1/4 hr.	1/2 hr.	1 hr.	3 hr.	6 hr.
SCE-100 (n*=6)	Plasma	139.1 (96.2~189.9)	131.3 (63.7~249.7)	42.6 (22.8~ 52.0)	7.2 (3.8~ 11.4)	2.9 (1.0~5.6)
	Lung	27.1 (22.0~ 34.7)	30.3 (16.0~ 50.8)	10.5 (6.5~ 15.6)	1.8 (0~ 2.4)	0.3 (0~1.0)
	Liver	117.4 (74.1~181.7)	115.8 (76.2~170.2)	36.7 (18.8~ 50.5)	8.7 (5.7~ 12.2)	3.9 (1.8~ 8.7)
	Spleen	15.8 (13.3~ 17.9)	18.5 (7.7~ 28.0)	10.8 (3.9~ 19.3)	2.4 (0~ 8.7)	0 (0)
	Kidney	151.8 (101.8~217.1)	178.0 (67.0~329.0)	74.8 (30.8~103.2)	17.8 (5.7~ 26.5)	6.6 (2.3~ 10.8)
Cephalexin (n=6)	Plasma	143.6 (58.5~226.4)	89.8 (54.7~169.4)	42.2 (18.9~ 66.2)	5.3 (3.3~10.8)	1.8 (0~ 2.7)
	Lung	25.8 (17.8~ 31.2)	17.9 (10.3~ 21.4)	9.0 (6.2~ 13.9)	0.6 (0~ 1.7)	0.3 (0~ 1.6)
	Liver	63.8 (28.3~ 95.1)	49.5 (28.5~ 74.1)	22.3 (10.5~ 57.4)	4.4 (2.1~ 6.6)	1.3 (0~ 3.7)
	Spleen	15.8 (10.3~ 18.5)	14.2 (9.1~ 21.8)	4.85 (0~ 11.3)	0.7 (0~ 3.7)	0 (0)
	Kidney	175.4 (133.2~212.4)	120.0 (90.5~200.3)	49.3 (20.3~ 79.2)	14.7 (0~ 23.0)	6.6 (0~ 9.6)

* Number of tests.

Fig. 2. Plasma and tissue concentrations of SCE-100 and cephalexin after single oral doses of 100 mg/kg in rats.

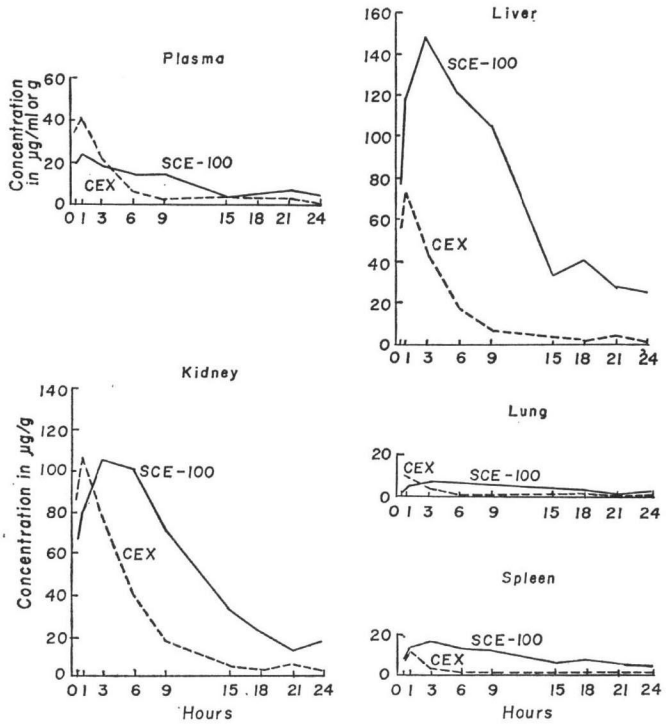


Table 3. Plasma and tissue levels of SCE-100 and cephalexin after single oral doses of 100 mg/kg in rats

Cephalosporin	Tissue	Mean concentration (and range) in μg/ml or g								
		1/2 hr.	1 hr.	3 hr.	6 hr.	9 hr.	15 hr.	18 hr.	21 hr.	24 hr.
SCE-100 ($n^*=6$)	Plasma	19.4 (13.1~23.1)	23.4 (13.4~39.3)	19.0 (6.9~31.4)	14.4 (3.6~29.7)	14.1 (3.1~26.9)	3.6 (0~5.4)	3.9 (3.8~3.9)	5.5 (2.3~10.1)	3.6 (1.5~4.9)
	Lung	4.04 (2.5~5.8)	4.87 (3.8~7.7)	5.73 (4.4~7.0)	5.37 (3.3~7.6)	4.60 (1.6~7.7)	2.66 (0~4.7)	2.8 (0~7.3)	0.7 (0~1.4)	1.1 (0~1.6)
	Liver	77.3 (59.1~112.1)	120.0 (60.6~207.0)	149.8 (104.0~252.5)	120.8 (70.7~171.7)	104.5 (46.9~169.7)	31.8 (13.1~53.5)	39.0 (23.2~63.6)	26.5 (13.9~43.9)	24.0 (6.1~36.4)
	Spleen	9.5 (6.6~13.5)	13.1 (6.4~20.7)	15.1 (9.4~19.1)	12.8 (6.4~18.8)	11.8 (2.8~19.0)	5.7 (0~9.1)	6.4 (2.7~11.8)	3.8 (1.9~5.7)	3.3 (0~5.1)
	Kidney	65.2 (49.2~92.2)	67.0 (29.9~118.3)	105.1 (73.5~127.0)	99.0 (63.8~137.0)	71.0 (24.8~110.2)	31.6 (13.2~45.5)	22.2 (14.3~33.4)	12.8 (7.7~16.7)	17.3 (4.4~28.0)
	Brain	0	0	0	0	0	0	0	0	0
Cephalexin (n=6)	Plasma	32.6 (19.6~56.7)	40.1 (16.8~62.3)	20.6 (11.6~32.6)	6.1 (4.0~9.2)	2.1 (0~4.2)	1.0 (0.3~1.5)	1.2 (0~2.4)	1.7 (0~3.2)	0
	Lung	9.89 (5.9~23.0)	8.64 (6.2~12.8)	4.06 (0~5.9)	0.65 (0~2.5)	0	0	0	0	0
	Liver	55.5 (43.0~59.5)	75.8 (57.5~111.5)	44.7 (35.5~55.0)	17.2 (0~30.3)	7.1 (0~11.6)	2.2 (0~4.7)	1.2 (0~4.6)	3.4 (0~5.2)	0
	Spleen	7.50 (1.6~27.9)	9.50 (2.4~29.8)	2.60 (0~3.6)	0.30 (0~2.1)	0	0	0	0	0
	Kidney	85.0 (59.3~118.6)	109.4 (91.6~125.6)	77.4 (44.0~147.9)	41.2 (28.8~70.4)	17.9 (9.8~21.8)	5.6 (0~13.5)	4.9 (0~8.8)	6.1 (0~9.6)	2.3 (0~3.7)
	Brain	0	0	0	0	0	0	0	0	0

* Number of rats used.

strable concentration of the cephalosporin was still observed at 24 hours after administration. Tissue levels of SCE-100 in various organs attained their peaks 3 hours after administration. Remarkably, the peak level of SCE-100 in the liver was nearly two times higher than that of CEX. Plasma and tissue levels of CEX reached a peak during 30 minutes to 1 hour after oral administration. The disappearance of CEX from plasma or various tissues was more rapid than that of SCE-100, and no antibacterial activity was found in any tissues except in the kidney 24 hours after administration.

Rabbits

The plasma levels after oral and intravenous doses of both cephalosporins are summarized in Table 4. At an oral dose of 100 mg/kg of SCE-100, peak plasma levels were attained 1

Fig. 3. Plasma levels of SCE-100 and cephalexin after single doses in rabbits.

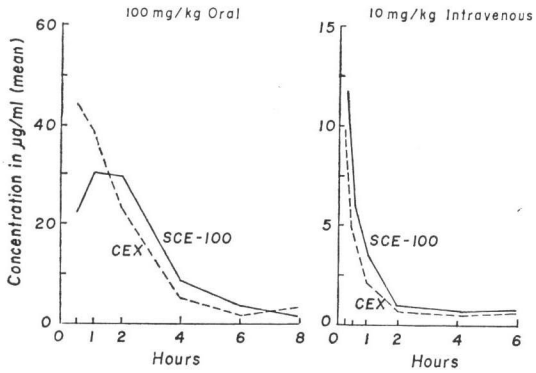


Fig. 4. Plasma levels of SCE-100 and cephalexin after single oral doses of 100 mg/kg in dogs

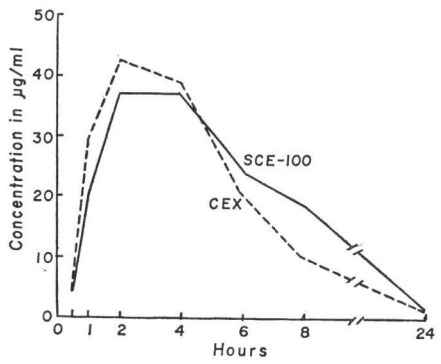


Table 4. Plasma levels and urinary- and biliary-excretion of SCE-100 and cephalexin after a single doses in rabbits

Administration route	Cephalosporin	Dose (mg/kg)	Mean plasma concentration (and range) in μg/ml						Mean excretion, % (range)	
			1/4 hr.	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	Urine	Bile
P.O.	SCE-100 (n*=6)	100	—	22.4 (7.1~48.8)	30.6 (15.2~42.0)	29.8 (14.8~46.4)	9.07 (7.2~11.4)	4.02 (1.6~9.0)	1.47 (0.81~2.38)	50.7+ (30.8~62.9)	—
	Cephalexin (n=4)	100	—	44.0 (33.0~52.0)	39.0 (34.4~46.4)	23.4 (15.3~38.6)	4.92 (2.1~11.0)	2.36 (0.74~4.75)	2.84 (0.58~5.46)	42.2+ (32.3~51.2)	—
I.V.	SCE-100 (n=4)	10	11.7 (10.4~13.8)	6.48 (4.9~9.0)	3.55 (2.45~4.9)	0.96 (0.64~1.60)	0.60 (0.36~0.88)	0.67 (0.27~1.37)	—	58.8++ (39.8~73.1)	3.2++ (2.12~4.09)
	Cephalexin (n=3)	10	9.93 (7.2~15.4)	4.75 (2.9~6.8)	2.13 (0.79~3.85)	0.63 (0.45~0.78)	0.50 (0.33~0.78)	0.56 (0.22~1.45)	—	57.8++ (35.3~71.8)	1.5++ (0.49~1.99)

— Not tested. * Number of rabbits used. + Within 8 hours. ++ Within 6 hours.

hour after administration. When a single intravenous dose of 10 mg/kg of SCE-100 was given to anesthetized rabbits, peak plasma levels 15 minutes after administration were followed by a rapid decline (Fig. 3). The tissue levels of SCE-100 were measured 1 and 8 hours after oral administration (Table 5). A particularly high concentrations of SCE-100 were found in the kidney. The antibiotic levels of other organs were observed in descending order in the liver, lung and spleen.

Table 5. Tissue levels of SCE-100 and cephalexin after a single oral doses of 100 mg/kg in rabbits

Cephalosporin	Tissue	Mean concentration (and range) in μg/g	
		1 hr. (n*=3)	8 hr. (n=3)
SCE-100	Lung	7.17 (4.31~9.64)	0.33 (0~1.64)
	Liver	28.2 (13.4~40.9)	3.45 (1.35~6.51)
	Spleen	4.42 (2.55~5.98)	0
	Kidney	227.5 (129.7~329.3)	65.1 (40.9~114.8)
Cephalexin	Lung	10.8 (3.97~18.0)	0
	Liver	21.3 (12.5~26.4)	2.12 (0~4.24)
	Spleen	4.23 (2.04~5.97)	0
	Kidney	344.6 (236.8~488.4)	89.4 (78.2~190.3)

* Number of rabbits used.

Urinary excretion of SCE-100 after oral and intravenous administrations was 50.7 and 58.8% of the given dose, respectively (Table 4). In addition, 3.2% of intravenous SCE-100 was excreted in the bile.

The plasma level of CEX reached its peak 30 minutes after oral administration. The urinary excretion of CEX was similar to that of SCE-100, while biliary excretion of the former was about half of the latter.

Dogs

Plasma levels and urinary excretion after oral dosing of SCE-100 and CEX are shown in Table 6. Plasma levels of SCE-100 attained the peak 2 hours after administration (Fig. 4). Urinary excretion of SCE-100 within 24 hours after administration was 31.5% of the given dose. Similar plasma levels and urinary excretion were observed in dogs given CEX.

Table 6. Plasma levels and urinary-excretion of SCE-100 and cephalexin after a single oral doses of 100 mg/kg in dogs

Cephalosporin	Mean plasma concentration (and range) in μg/ml							Mean urinary excretion % (range)
	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	24 hr.	
SCE-100 (n*=4)	3.98 (0.4~ 7.8)	19.9 (5.0~ 34.0)	37.1 (17.4~ 53.2)	37.1 (32.8~ 40.0)	24.3 (18.4~ 35.0)	18.8 (9.0~ 35.0)	1.21 (0.89~ 1.65)	31.55 (17.48~ 45.51)
Cephalexin (n=4)	5.21 (1.15~ 8.2)	29.2 (7.5~ 65.0)	42.3 (21.2~ 66.8)	38.7 (23.4~ 50.0)	20.5 (9.7~ 31.0)	10.2 (4.8~ 16.8)	0.93 (0.18~ 2.2)	31.20 (11.06~ 56.98)

* Number of dogs used.

Discussion

Previous studies[1,2] have shown SCE-100 to possess similar or somewhat lower *in vitro* and

in vivo activities than CEX. In this study it was found that when SCE-100 was administered orally, the drug was absorbed by the gastro-intestinal tract and distributed to serveral organs as similar as CEX. High concentrations of SCE-100 were detected in the liver and kidney, and the levels in these tissues were generally higher than the plasma levels. The antibiotic levels in the lung and spleen, however, were lower than the plasma levels. Similar distribution patterns were observed in animals to which CEX was administered, and the results coincided with those by other researchers.[3~5]

Generally, SCE-100 was absorbed more slowly than CEX and a slower disappearance of the antibiotic activity from blood and various organs was observed. Among the animals examined the earliest and highest peak level of SCE-100 was attained in mice. SULLIVAN *et al.*[6] also observed the difference between mice and rats with respect to the absorption rate of CEX from the gastro-intestinal tract. Marked differences in absorption, and distribution patterns between the cephalosporins were observed in rats. The peak level of SCE-100 in the liver was approximately two times higher than that of CEX, and the concentration of the former was still detectable in the liver 24 hours after administration.

The biliary excretion of SCE-100 in rabbits was nearly twice that of CEX. Although the excretion of both cephalosporins into bile was much less than urinary excretion, the concentration of SCE-100 in bile was higher than the *in vitro* minimum inhibitory concentration of SCE-100 against several pathogenic organisms.

The urinary excretion of SCE-100 after oral and intravenous doses in rabbits was similar. Furthermore, oral doses of SCE-100 and CEX in rabbits and dogs showed a similar degree of urinary excretion. These findings suggest that SCE-100 as well as CEX are well absorbed by the gastro-intestinal tract and are mainly excreted by the urine. SULLIVAN *et al.*[6] also demonstrated that CEX was eliminated in an unchanged chemical form into the urine of rats. Preliminary experiments with SCE-100 revealed that there were no active metabolites in the urine specimens of rats after oral administration. In addition, preliminary one-month oral toxicity studies in rats showed that SCE-100 is as low in toxicity as CEX (unpublished data). These observations suggest that SCE-100 might be useful as an oral cephalosporin. More detailed comparative studies with SCE-100 and CEX must be conducted to determine the site of absorption from the gastro-intestinal tract and the affinity to various tissues. It would be interesting further to investigate the therapeutic effectiveness of SCE-100 against a localized infection in specific organs of animals.

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