Metabolic Fate of SCE-129, a New Antipseudomonal Cephalosporin, After Parenteral Administration in Rats and Dogs

SHIGEHARU TANAYAMA,* KIYOSHI YOSHIDA, AND YOSHIO KANAI

Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan

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Disposition of [14C]SCE-129 was studied in rats and dogs after intramuscular (i.m.) or intravenous (i.v.) injection. In rats, the plasma level of i.m. [14C]SCE-129 attained a peak at 15 min after dosing and then declined with a half-life of 35 min, whereas the half-life after an i.v. dose was 26 min. The area under the plasma level curve within 2 h after the i.m. dose was 85% of that after the i.v. dose. Intramuscular injection of [14C]SCE-129 into dogs gave a peak plasma level at 30 min and a half-life of 60 min. In both rats and dogs, the plasma levels of [14C] were closely similar to those of the unchanged antibiotic, which was weakly bound to plasma protein. The rat tissue level of i.m. [14C]SCE-129 was maximum at 15 min, with the highest concentration in the kidney, followed by plasma, adrenal, lung, heart, thymus, gastrointestinal wall, and liver, and the lowest in the brain. The antibiotic barely entered erythrocytes of rats and dogs. Whole-body autoradiographic studies showed that i.m. [14C]SCE-129 scarcely crossed the rat placenta. [14C] was detected in the milk of rats given i.m. [14C]SCE-129. In both rats and dogs, almost all of the dose [14C] was excreted in urine within 24 h as the unaltered antibiotic, with only small amounts appearing in feces via bile. Thus, these findings evidenced a rapid absorption and wide distribution of i.m. SCE-129, followed by extensive renal elimination as the unaltered antibiotic.

SCE-129, 3-(4-carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfonylacetamido)-ceph-3-em-4-carboxylate monosodium salt, is active against Pseudomonas aeruginosa as well as against some gram-positive bacteria and shows a protective effect toward P. aeruginosa or Staphylococcus aureus infection in mice (19). It has also been reported that the antibiotic is quite stable in the hydrolysis by β-lactamases from various bacterial strains (12). Recent clinical studies have established the effectiveness of SCE-129 in the treatment of urinary tract infections, keratitis, sepsis, and respiratory tract infections without notable side effects.

The present study was undertaken to investigate the absorption, distribution, and excretion of [14C]-labeled SCE-129 in rats and dogs in an attempt to provide a pharmacokinetic basis for the pharmacological effects.

MATERIALS AND METHODS

Materials. [14C]-labeled SCE-129 (a mixture of D- and L-isomers), labeled at the carbonyl carbon in the sulphonylacetamido group (Fig. 1), was synthesized by Hayashi et al. (N. Hayashi, T. Toga, N. Tada, and T. Azuma, J. Labelled Compd. Radiopharm., in press) of the Radioisotope Laboratory of our Division. Two samples of specific radioactivities of 14.6 and 14.7 μCi/mg were used. Radiopurity (>90%) and chemical identity were checked by thin-layer electrophoresis and high-speed liquid chromatography (described below). Nonlabeled SCE-129 (D-isomer) was supplied by Y. Nomura of the Medicinal Research Laboratories of our Division.

Animals and drug administration. Animals used were male or female Wistar rats (inbred in the Drug Safety Research Laboratories of our Division) weighing 180 to 362 g and male beagles (CLEA Japan Inc., Tokyo, Japan) weighing 7.8 to 9.0 kg. They were maintained on laboratory chow (CE-2 for rats and CD-5 for dogs; CLEA Japan Inc.) and drinking water in temperature- and humidity-controlled rooms (26°C, 60%) with 12-h light-dark cycles for more than a week before use. [14C]SCE-129 diluted 3 to 10 times with the carrier (D-isomer) was dissolved in 0.9% NaCl solution for intramuscular (i.m.) or intravenous (i.v.) administration at a dose of 20 mg (26.8 to 98.7 μCi)/kg. We verified that there was no significant difference in the metabolic disposition between the D- and L-isomers. Namely, the D/L ratio of [14C]SCE-129 (1.24) in the 5-h urine of rats, which contained more than 85% of the
i.m. dose, was exactly the same as that of the labeled antibiotic administered (see Fig. 2A). A similar result was also obtained in dogs.

Collection of biological samples. Blood was taken from the tail vein or abdominal aorta in rats and from the cephalic vein in dogs. Radioactive carbon dioxide in the expired air of rats was absorbed in 5 M KOH (15). The spontaneous urine and feces were collected by using the usual metabolism cages equipped with urino-fecal separators. Bile samples of rats were obtained after cannulation of the common bile duct (16). Urine and bile samples were collected under freezing with solid carbon dioxide and kept as such until analyzed.

Analytical methods. (i) Measurement of radioactivity. Radioactivity was measured with a liquid scintillation counter (model LSC-683, Aloka Co., Ltd., Tokyo, Japan) with an automatic quenching monitor and an on-line digital computer (model JAC-2, Japan Radio Co. Ltd., Tokyo, Japan) for data processing. Radioactivity in plasma, urine, bile, milk, and homogenates of feces was counted directly in a toluene-phosphor mixture containing nonionic detergent (8). Blood radioactivity was determined in the same way after decolorizing with H2SO4 as described previously (18). Radioactivity in tissues was determined by using a sample oxidizer (model 306, Packard Instrument Co., Inc., Downers Grove, Ill.). Respiratory 14CO2 trapped in 5 M KOH was counted in a toluene-phosphor mixture (15) after regeneration and reabsorption into Hyamine 10-X solution (Packard Instrument Co., Inc.).

(ii) High-speed liquid chromatography. D- and L-isomers of [14C]SCE-129 were determined by high-speed liquid chromatography using a Nester/Faust apparatus (Nester/Faust Manufacturing Corp., Newark, N.J.) (Fig. 2A). Samples (5 μl) of the [14C]SCE-129 solution used for dosing or urine samples were submitted to high-speed liquid chromatography on a Zipax SAX column (100 cm by 2-mm ID; E. I. du Pont de Nemours & Co., Wilmington, Del.) eluted at a rate of 1 ml/min with a mixture of 0.1 M sodium acetate buffer-0.1 M NaCl solution (1:1, vol/vol; pH 4.5). The temperature of the column was 25°C. Fractions of 1 ml were collected for counting in the detergent-phosphor mixture. The compounds of interest were also monitored using a 254-nm-wavelength UV absorption detector (UVIDEC-100, Japan Spectroscopic Co. Ltd., Tokyo, Japan) connected to a Hitachi 056 recorder (Hitachi Ltd., Tokyo, Japan).

(iii) Thin-layer electrophoresis. [14C]SCE-129 in urine samples was quantitatively determined by high-voltage, thin-layer electrophoresis using a Fuji Riken apparatus (model MSF-6, Fuji Riken Co. Ltd., Tokyo, Japan) thermostatted at 0°C with electrophoresis coolant (Savant Instruments, Inc., Hicksville, N.Y.). The electrophoresis was carried out on precoated cellulose F plates (E. Merck AG, Darmstadt, Germany) in a 0.067 M phosphate buffer (Serensen, pH 6.2) for 45 min at 40 V/cm. [14C]SCE-129 on the dried electropherograms was located by UV absorption of the nonlabeled antibiotic added to the test samples as an internal standard. Figure 2B shows a typical radioelectropherogram of the 2-h urine of a rat given [14C]-SCE-129 i.m.

(iv) Measurement of antibacterial activity. Concentrations of SCE-129 in plasma and urine were determined by the agar well method, using P. aeruginosa NCTC 10490 as the test organism. The samples were diluted with 0.1 M potassium phosphate buffer (pH 7.0) to a final antibiotic concentration of <40 μg/ml. The calibration curve in each experiment was prepared using the respective [14C]-SCE-129 solution administered after appropriately diluting with the phosphate buffer. The antibacterial compounds were also examined by bioautography after thin-layer electrophoresis, using S. aureus 9P-9HS-9 as the test organism.

Distribution in erythrocytes. Radioactivity in whole blood and plasma was determined on the same blood samples obtained from rats and dogs given [14C]-SCE-129 i.m. The percentage of distribution of the antibiotic in erythrocytes was calculated by using the hematocrit value (18).

Plasma protein binding. Binding of [14C]SCE-129 to the plasma protein of rats and dogs was determined by the equilibrium dialysis method. One milliliter of plasma containing 10 to 100 μg of the labeled antibiotic was placed in Visking dialysis bags (Kebo A.B., Stockholm, Sweden) and dialyzed in 2 ml of a mixture of physiological saline-0.155 M sodium phosphate buffer (29.2, vol/vol; pH 7.4) for 24 h at 4°C.

Whole-body autoradiography. Whole-body sections (40-μm thickness) of male rats were prepared at 5 and 15 min and 1, 6, and 24 h after i.m. injection of [14C]SCE-129 by the method of Ullberg (20) and exposed to X-ray film (industrial type, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 25 days at 4°C.
Placental transfer of $[^{14}C]$SCE-129 was also studied by whole-body autoradiography in pregnant rats on day 20 of gestation.

Lacteal excretion. Milk was collected at 2, 4, and 6 h after i.m. administration of $[^{14}C]$SCE-129 on days 14 to 21 after parturition, as described elsewhere (17).

RESULTS

Plasma levels. Plasma levels of radioactivity and antibacterial activity were studied after i.m. or i.v. injection of $[^{14}C]$SCE-129 into rats and dogs (Fig. 3 and 4). In rats given the labeled antibiotic i.m., the plasma level of radioactivity peaked at 15 min (38.7 µg-equivalents of SCE-129 per ml) after dosing and then declined with a half-life of 35 min (Fig. 3). After i.v. injection into rats, the plasma radioactivity decreased, with half-lives of 11 min (0 to 5 min, distribution phase) and 26 min (5 to 60 min, elimination phase). The areas under the plasma level versus the time curve within 2 h after i.m. and i.v. injections were 40.6 and 47.5 µ × h per ml, respectively (i.m./i.v. ratio, 0.85). The plasma level after i.m. administration to dogs reached a peak at 30 min (48.9 µg-equivalents of SCE-129 per ml), followed by a decline with a half-life of 60 min (Fig. 3).

In both rats and dogs, plasma levels of the unchanged antibiotic estimated by bioassay were not significantly different ($P > 0.05$) from the levels of radioactivity within 1 h after dosing (Fig. 4).

Distribution. (i) Distribution in erythrocytes. At 15, 60, and 120 min after i.m. injection of $[^{14}C]$SCE-129 into rats and dogs, more than 90% of the blood radioactivity was present in the plasma fraction. This finding indicated that the antibiotic could barely enter erythrocytes of these animals.

(ii) Tissue levels. Tissue distribution of i.m. $[^{14}C]$SCE-129 was preliminarily studied in rats by whole-body autoradiography. The results showed that radioactive concentrations in tissues generally attained a peak at 15 min, with much higher concentrations in the kidney, and thereafter declined rapidly to low levels at 24 h. Based on these findings, tissue levels of radioactivity were then estimated at 15 min, 2 h, and 24 h after i.m. administration of $[^{14}C]$SCE-129 to rats (Table 1). At 15 min after dosing, the radioactivity was the highest in the kidney, followed by plasma, adrenal, lung, heart, thymus, gastrointestinal wall, and liver, and the lowest in the brain. The radioactivities in most tissues decreased to very low levels within 24 h except in the kidney, in which a significant amount of radioactivity still remained.

(iii) Placental transfer. Placental transfer of the antibiotic was studied in pregnant rats

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**Fig. 3.** Plasma levels of radioactivity after i.m. or i.v. administration of $[^{14}C]$SCE-129 to rats and dogs. Rats (average body weight, 226 g) and dogs (average body weight, 8.3 kg) were given the labeled antibiotic i.m. or i.v. at a dose of 20 mg (28.7 to 61.7 µCi)/kg. Plasma levels are expressed as the mean ± standard deviation of three animals in each experiment. Apparent half-lives, shown in parentheses, were calculated using linear regression analysis.

**Fig. 4.** Comparison of radioactivity and unchanged SCE-129 in plasma of rats and dogs given the $^{14}$C-labeled antibiotic i.m. Rats (average body weight, 202 g) and dogs (average body weight, 8.3 kg) were given the labeled antibiotic i.m. at a dose of 20 mg (31.5 to 59.1 µCi)/kg. The concentration of SCE-129 was determined by bioassay. Data are the mean ± standard deviation of three animals in each experiment. Symbols: open bars, radioactivity; striped bars, antibacterial activity.
using the whole-body autoradiographic technique (Fig. 5). At any time after i.m. injection of \([^{14}C]\)SCE-129, no detectable amount of radioactivity was present in the fetus, showing that the antibiotic could barely cross the placenta. The distribution pattern of radioactivity in maternal tissues was similar to that in male rats (Table 1).

Plasma protein binding. In vitro studies using \([^{14}C]\)SCE-129 (10 to 100 µg/ml) showed that 26 and 18% of the antibiotic were bound to the plasma protein of rats and dogs, respectively (Table 2).

Excretion. (i) Urinary and fecal excretion. After i.m. or i.v. administration of \([^{14}C]\)SCE-129 to rats and dogs, elimination of radioactivity was almost complete within 24 h, with much more radioactivity appearing in urine than in feces (Table 3). In both animals, more than 83% of the dose was excreted in the first 5-h urine. No detectable amount of radioactivity was excreted in the expired air of rats.

As evidenced by thin-layer electrophoresis, urinary radioactivity was derived mostly from the unaltered antibiotic in both rats and dogs (Fig. 6). It was confirmed by bioautography that SCE-129 was the only component with antibacterial activity in the 24-h urine of these animals. In addition, urinary contents of SCE-129 estimated by bioassay were fairly consistent with those quantified by thin-layer electrophoresis (Fig. 6). All of these findings indicated that the largest part of the dosed SCE-129 was eliminated unchanged into urine.

(ii) Biliary excretion. Biliary excretion of the radioactive antibiotic was studied in biliary-cannulated rats (Table 3). In 24 h, only 4.1% of the dose was excreted in bile after i.m. injection. Fecal elimination of radioactivity in the biliary-cannulated rats was much less than that in the intact rats, indicating that the fecal radioactivity in the latter animals was due to hepato-biliary excretion.

Lacteal excretion. Radioactivity was detectable in rat milk after i.m. administration of \([^{14}C]\)-SCE-129 (Table 4).

**DISCUSSION**

The present studies have clarified the metabolic fate of SCE-129 in rats and dogs. SCE-129 injected i.m. is readily absorbed by these animals, as evidenced by a rapid appearance of the antibiotic in the circulation (Fig. 3). The rate of bioavailability of SCE-129 was calculated to be 85% from the ratio of the area under the plasma level curves for the i.m. and i.v. doses (Fig. 3). A quantitative absorption of the i.m. antibiotic was, however, shown by the urinary and fecal recoveries, which amounted to almost 100% of the administered dose in both rats and dogs (Table 3). The rate of absorption of SCE-129, like that of other cephalosporins (10), seems to be somewhat slower in dogs than in rats, as suggested by the longer time needed to reach the peak plasma level and the slower disappearance from the plasma in dogs (Fig. 3).

A marked species variation has been noted in the plasma protein binding of cephalosporins (10, 11). SCE-129 is 26 and 18% bound in rats and dogs, respectively, the binding being much less extensive than that of cepazolin, cephalothin, cephaloridine, or ceftezole even in rats (10). As is generally accepted, plasma protein binding may affect the biological activities or metabolism of a drug. However, a study by Tsuchiya et al. (19) found no significant change in the antibacterial activity of SCE-129 toward either *P. aeruginosa* or *S. aureus* after the addition of horse serum. Gillette (1) has calculated that protein binding effectively lowers the free concentration of a drug in the body only when more than 80% of the drug is bound to the plasma protein. It is, therefore, unlikely that plasma protein binding significantly affects metabolic disposition of SCE-129.

SCE-129 is widely distributed in rat tissues (Table 1). The only tissue in which the concentration of the antibiotic is higher than in plasma is the kidney. During the first 2 h after dosing, the renal concentration of SCE-129 is higher than the minimum inhibitory concentrations.
against *P. aeruginosa* and certain gram-positive bacteria (19). Significant levels of the antibiotic were also demonstrated in other tissues, including adrenal, lung, heart, gastrointestinal wall, thymus, and liver. Many studies have appeared concerning the placental transfer of cephalosporins. Namely, cefazolin (3), cephapirin (6), and cefaclor (18) are reported to cross the placenta of rats or mice poorly, whereas a rapid transference of cephalothin (5) from the maternal blood supply to the fetus is demonstrated in humans. The present study indicates that SCE-129 is scarcely transferred into the rat fetus (Fig. 5). It was also demonstrated that the concentration of SCE-129 in rat milk is very low (Table 4).

Some cephalosporins, such as cephaloglycin (13), cephalothin (2, 4), cephapirin (6), and cephacetrile (8), have been shown to be metabolized by deacetylation at the C3 methyl position, yielding deacetyl cephalosporins. With cephalosporin (14), hydrolysis of the amide linkage to form 2-phenylglycine has also been reported. These metabolic degradations, however, are not the case for SCE-129, since our study in rats and dogs using bioassay and thin-layer electrophoresis showed that no significant amounts of metabolites are present in the plasma and urine (Fig. 4 and 6). Thus, it seems that SCE-129 hardly underwent biotransformation in the body and was excreted as such exclusively in urine, as described below.

Extensive renal excretion is a property shared by most cephalosporins and is reported to be carried out by the processes of glomerular filtration and tubular secretion (9). SCE-129 is also removed from rats and dogs mainly through renal excretion (Table 3). In both animals, fecal

### Table 2. Plasma protein binding of [14C]SCE-129 in rats and dogs

<table>
<thead>
<tr>
<th>Plasma concn of SCE-129 (µg/ml)</th>
<th>Binding (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rats</td>
</tr>
<tr>
<td>10</td>
<td>25.9</td>
</tr>
<tr>
<td>50</td>
<td>25.5</td>
</tr>
<tr>
<td>100</td>
<td>25.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Binding was estimated by the equilibrium dialysis method, using 1 ml of plasma containing the labeled antibiotic.

### Table 3. Cumulative excretion of radioactivity in urine, feces, and bile after i.m. or i.v. administration of [14C]SCE-129 to rats and dogs<sup>a</sup>

<table>
<thead>
<tr>
<th>Species</th>
<th>Route of administration</th>
<th>Time after dosage (h)</th>
<th>Urine</th>
<th>Feces</th>
<th>Bile</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>i.m.</td>
<td>5</td>
<td>83.2 ± 0.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>94.4 ± 5.5</td>
<td>4.8 ± 1.7</td>
<td>ND</td>
<td>99.2 ± 3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>95.5 ± 5.1</td>
<td>6.1 ± 1.8</td>
<td>ND</td>
<td>101.7 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>5</td>
<td>83.1 ± 0.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>90.5 ± 0.8</td>
<td>6.2 ± 0.9</td>
<td>ND</td>
<td>96.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>91.9 ± 0.9</td>
<td>7.2 ± 1.2</td>
<td>ND</td>
<td>99.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>i.m.</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>3.9 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>88.8 ± 1.1</td>
<td>0.2 ± 0.3</td>
<td>4.1 ± 0.6</td>
<td>93.2 ± 0.8</td>
</tr>
<tr>
<td>Dog</td>
<td>i.m.</td>
<td>5</td>
<td>85.3 ± 6.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>96.5 ± 5.0</td>
<td>1.2 ± 0.4</td>
<td>ND</td>
<td>97.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>97.7 ± 5.1</td>
<td>1.4 ± 0.4</td>
<td>ND</td>
<td>99.2 ± 5.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Rats (average body weight, 211 g) and dogs (average body weight, 8.3 kg) were given the labeled antibiotic i.m. or i.v. at a dose of 20 mg (28.7 to 60.3 µCi/kg). Data are the mean ± standard deviation of three animals in each experiment. ND, Not determined.

![Fig. 6. Comparison of radioactivity and unchanged SCE-129 in urine of rats and dogs given the 14C-labeled antibiotic i.m. The rats and dogs used for the experiment in Table 3 were used. Excretions of radioactivity (open bar) and unchanged SCE-129, estimated by bioassay (striped bar) or thin-layer electrophoresis (stippled bar), are expressed as the mean ± standard deviation of three animals.](http://aac.asm.org/)
excretion via bile is only a minor process in the elimination of the antibiotic.

In conclusion, a rapid absorption of SCE-129 followed by extensive urinary excretion as the unchanged form seems to support the excellent clinical efficacy of the antibiotic in the treatment of urinary tract infections.

ACKNOWLEDGMENTS

We are deeply indebted to N. Hayashi for a supply of the labeled antibiotic and to T. Fugono and R. Maeda for the microbiological assays.

LITERATURE CITED