Disposition of [14C]Aztreonam in Rats, Dogs, and Monkeys


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Aztreonam ([2S-[2α,3β(Z)]-3-[(2-amino-4-thiazolyl)-[(1-carboxy-1-methylethoxy)iminoo]acetyl]amino]-2-methyl-4-oxo-1-azetidinesulfonic acid; Fig. 1) is a new, synthetic, monomycyclic β-lactam antibiotic. It possesses potent antibacterial activity against a wide range of aerobic gram-negative organisms (5, 11). To provide information pertinent to clinical trials and toxicological studies, we investigated the disposition of aztreonam in rats, dogs, and monkeys after the parenteral administration of [14C]aztreonam.

MATERIALS AND METHODS

Compounds. [14C]Aztreonam (Fig. 1) was synthesized with a specific activity of 8.0 μCi/mg and a radiochemical purity of >96%, as determined by thin-layer chromatography and high-pressure liquid chromatography (HPLC). Solutions containing [14C]aztreonam at the desired specific activities were prepared by dissolving appropriate amounts of labeled and unlabeled aztreonam in water along with an equivalent amount of L-arginine. Both labeled and unlabeled aztreonam and the reference compound, SQ 26,992 (a potential metabolite and known degradation product of aztreonam), were synthesized at The Squibb Institute for Medical Research, Princeton, N.J. All other chemicals and reagents were obtained from commercial sources.

Animals. Male CD outbred albino rats (240 to 290 g), young adult male purebred beagles (9 to 11 kg), and female cynomolgus monkeys (Macaca fascicularis; about 2 kg) were used in this study. All animals were fasted overnight before dosing and for 8 h after dosing. Water was available ad libitum. Animals were housed individually in metabolic cages throughout the metabolic studies.

Drug administration. Groups of five male rats were given single 50-mg/kg doses of [14C]aztreonam intramuscularly (i.m.) in a thigh muscle (quadriceps femoris) or intravenously (i.v.) in the jugular vein. In addition, two unanesthetized male rats with cannulated bile ducts were given single 50-mg/kg doses of [14C]aztreonam i.m. Four male dogs and four female monkeys were each given single 25-mg/kg doses of [14C]aztreonam i.v. and either subcutaneously (s.c.) (dogs) or i.m. (monkeys). Doses administered i.v. were given into the cephalic or saphenous veins. Doses given s.c. (dogs) were administered into the dorsal side of the neck, and i.m. doses (monkeys) were administered into the quadriceps femoris. There was a washout period of 2 to 3 weeks between doses.

Sample collection. Urine from individual rats was collected quantitatively in ice-cooled containers for the sequential intervals ending 4, 8, 24, 48, 72, and 96 h after dosing. Feces were collected daily for 4 days. For rats with cannulated bile ducts, bile and urine were collected at frequent intervals for 24 h; feces were collected for 24 h.

Blood samples were withdrawn from the jugular veins of dogs at 0.08, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 48, 72, and 96 h after dosing; serum was prepared from each blood sample. Urine was collected in ice-cooled containers for the sequential intervals ending 1, 2, 3, 4, 6, 8, 24, 48, 72, and 96 h after dosing. Feces were collected daily for 4 days.

For monkeys, blood samples were withdrawn from the femoral artery at 0.08 (i.v. only), 0.25, 0.5, 1, 2, 3, 4, 6, 10, and 24 h after dosing; serum was prepared from each blood sample. Urine was collected in ice-cooled containers for the sequential intervals ending 4, 8, 24, 48, 72, and 96 h after dosing. Feces were collected daily for 4 days.

Additionally, in dogs and monkeys, extra blood samples were withdrawn at 0.25 and 2 h after dosing for the determination of serum protein binding. All samples were kept frozen until analyzed.

Preparation of control serum and urine samples. Control serum samples (dogs and monkeys) and urine samples (rats, dogs, and monkeys) were spiked with [12C]aztreonam at several different concentrations. These samples were stored frozen under the same conditions as those under which the study samples were stored and were analyzed at several time points during the storage period. Most of the samples collected after dosing were analyzed within 2 days of their collection.

Determination of protein binding. Serum samples obtained

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at 0.25 and 2 h after dosing were filtered through ultrafiltration, the cones of the filtration, and the concentration of binding was determined.  

Sample analysis. (i) Microbiological procedures. Aztreonam concentrations in selected serum and urine samples were determined by the agar diffusion method, with Escherichia coli SC 12,155 as the test organism (8). (ii) Extraction procedures for serum. Serum samples (2.0 ml) obtained up to 8 h after dosing in dogs and samples obtained at 0.5, 1, and 4 h after dosing in monkeys were extracted three times with 6 ml of acetonitrile (monkey) or methanol (dog). Samples of the combined extracts were analyzed for total radioactivity as described below. The remainder of each extract was filtered through glass wool and evaporated to dryness in vacuo at about 35°C. The residue was reconstituted in 0.5 to 0.6 ml of methanol, and the radioactive components (aztreonam and its metabolites) were quantified as described below. (iii) Liquid scintillation counting. The scintillation fluid of Anderson and McClure (1) was used to quantify radioactivity in all samples. PGM, a mixture that consisted of a saturated solution of sodium pyruvate in methanol, glacial acetic acid, and methanol in a ratio of 4:3:1 (vol/vol/vol), was used to neutralize the contents of the vials before they were counted. Individual samples were prepared as described below. All samples were counted in duplicate. Serum samples (0.1 or 0.5 ml) were digested in 0.5 to 2 ml of Soluene-350 (Packard Instrument Co., Downers Grove, Ill.), neutralized with 0.05 to 0.2 ml of PGM, and mixed with 15 ml of scintillation fluid. Samples reconstituted in methanol, the acetonitrile extracts of serum (1.0 ml) and protein-free filtrate (0.1 ml) were counted directly in 15 ml of scintillation fluid. Fecal samples were homogenized with about 2 to 3 volumes of water. A portion of the homogenate (0.2 g) was digested by shaking for about 48 h with 1 ml of Soluene-350. The digested sample was bleached with 1.0 ml of a 20% solution of benzoyl peroxide in toluene and mixed with 0.1 ml of PGM and 15 ml of scintillation fluid. Samples of urine (0.2 ml) were mixed with 1 ml of Soluene-350, 0.1 ml of PGM, and 15 ml of scintillation fluid. All samples were counted in either a model 3375 or a model 3380 Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.). Counting efficiency was determined with automatic external standardization and the use of previously prepared quench curves. Samples prepared from the chromatograms were counted in an Intertechnique model SL-4200 scintillation spectrometer (IN/US Service Corp., Fairfield, N.J.). External standardization, stored quench curve coefficients, and a computer program were used to calculate the percentage of radioactivity found in the different zones; the sum of the total radioactivity in all zones was taken to represent 100%, as described below.  

(iv) TLRC. For thin-layer radiochromatography (TLRC), aliquots of urine (50 μl) and the reconstituted extracts of serum (150 μl) were chromatographed on 0.25-mm silica gel GF plates in 1-butanol-ethyl acetate-acetic acid-water (1:1:1:1, vol/vol/vol/vol). Aztreonam and SQ 26,992 were used as reference compounds. Chromatograms were visualized under short-wavelength UV light. Each chromatogram was divided into six zones (see Fig. 3). All of the silica gel in each zone was scraped from the plate, mixed with 1 ml of water, and counted in 15 ml of scintillation fluid. Radioactivity in zone E represented unchanged aztreonam; radioactivity in zone D represented SQ 26,992; and radioactivity in zones A, B, C, and F represented unknown compounds. (v) HPLC. High pressure liquid chromatography (HPLC) of urine was carried out on a Partisil-10 M9 ODS-3 column (Whatman Inc., Clifton, N.J.), with the mobile phase consisting of 18% acetonitrile-82% aqueous tetrabutylammonium hydrogen sulfate (0.005 M, adjusted to pH 3.0 with 1.0 M dipotassium hydrogen sulfate; this buffered solution is hereafter referred to as 0.005 M TBA [pH 3]). Radioactivity in HPLC fractions was quantified as described above.  

(vi) Isolation and identification of the major metabolite in monkey urine. Urine (0 to 4 h) from three monkeys that had received 25-μg/kg i.m. and i.v. doses was combined, centrifuged, and concentrated in vacuo at room temperature to approximately half of its original volume. The entire concentrated urine was injected in 2-ml portions onto a Partisil-10 M9 ODS-3 column; a U6K injector (Waters Associates, Milford, Mass.) was used. The mobile phase was 20% acetonitrile-80% 0.005 M TBA (pH 3), and the flow rate was 4.0 ml/min. The column effluent was monitored by a Schoeffel SF770 UV detector (Kratos Inc., Westwood, N.J.). 

All plates were scraped in water and rechromatographed by preparative HPLC under the same conditions. The chromatogram obtained suggested the presence of a single radioactive compound, and the appropriate fractions were combined for further purification. The entire purification procedure is shown in Fig. 2. The isolated metabolite was subjected to 100-MHz Fourier Transform nuclear magnetic resonance spectroscopy (model XL-100; Varian Associates, Flornham Park, N.J.). Mass spectra of the purified metabolite and authentic SQ 26,992 were also obtained, with an AEI-MS902 spectrometer (Kratos, Inc.), after the preparation of their trimethyl-silyl derivatives with N,O-bis(trimethylsilyl) trifluoroacetamide.  

(vii) Isolation of metabolites from monkey urine for microbiological analysis. Urine was collected from three monkeys between 4 and 8 h after they had been given a single 25-μg/kg i.v. dose of [14C]aztreonam. This urine sample contained about 12% of the dose and was selected for the isolation of metabolites. An aliquot (2 ml) of the pooled urine sample
was injected onto a Whatman M9 ODS-3 column and eluted with the same mobile phase described above. Fractions (4 ml) were collected, and an aliquot from each fraction was mixed with scintillation fluid and counted. The procedure was repeated once more to accumulate enough of each metabolite for the microbiological assay. Fractions in each group containing the major metabolite or any of three relatively minor metabolites were combined. Tetrabutylammonium ion was removed by passing the solutions containing the metabolites through a small column of cation exchange resin (Bio-Rad AG50W-X2, K+ form). The removal of tetrabutylammonium ion was necessary because of its toxicity to the E. coli strain used in the microbiological assay. Fractions containing [14C]aztreonam and the mobile phase alone were treated similarly. These served as positive and negative controls, respectively. The metabolites and controls were bioassayed as described previously (8). On the basis of the specific activity of [14C]aztreonam administered, the solutions submitted for microbiological analysis contained from 2.7 to 5.0 µg of microbiological analysis per ml.

FIG. 2. Isolation of SQ 26,992 from monkey urine. MeOH is used to represent methanol.
The sensitivity of the microbiological assay for aztreonam in urine was 0.02 μg/mL.

(vii) Incubation of bile with Glusulase. Pooled 0- to 24-h bile (1 ml) from two rats was adjusted to a pH of 5.5 with 2 N acetic acid, mixed with 0.1 ml of Glusulase, a crude mixture of β-glucuronidase and sulfatase from Helix pomatia (Endo Laboratories, Garden City, N.Y.), and incubated at 37°C for 24 h. The enzymatic activity of Glusulase was verified by using phenolphthalein glucuronide and phenolphthalein disulfate as positive controls.

Calculation and analysis of data. The percentage of the administered dose excreted as unchanged aztreonam and its metabolites in urine was calculated by multiplying the fraction of total radioactivity present in a specific zone on the thin-layer chromatographic plate by the percentage of the radioactive dose excreted in the corresponding urine sample. Concentrations of unchanged aztreonam in serum were obtained by multiplying the concentrations of total radioactivity in serum (equivalents of aztreonam) by the fraction extracted into methanol and by the fraction of total radioactivity in the extract present as aztreonam (i.e., as determined by the fraction of radioactivity in zone E of the TLRC).

The percentage of radioactivity in serum bound to proteins was calculated by using the following equation: percent bound = [(concentration in serum − concentration in PFF)/ concentration in serum] × 100.

The areas under the serum concentration versus the time curves were calculated by the trapezoidal approximation method.

For one of the four monkeys, some urine, feces, or both were lost during the collection periods after i.m. and i.v. administration; as a result, the recoveries of the radioactive doses were only 40 and 55%, respectively. Hence, the excretion data and the urinary and fecal biotransformation data for this monkey were not included in the mean values.

All data in this report for which errors are reported are presented as the mean ± standard error.

RESULTS

Stability of aztreonam in urine and serum. On the basis of TLRC results, an average of about 95% of the radioactivity recovered from control rat, dog, and monkey urine to which [14C]aztreonam had been added was present as intact aztreonam. These stability standards were subjected to conditions identical to those under which the actual experimental samples had been collected and stored. The urinary data are reported uncorrected because recovery was almost quantitative.

In control dog serum, aztreonam underwent degradation to varying degrees (range 16 to 48%) under the storage conditions used for the samples, and a reliable correction factor could not be calculated. Therefore, the concentrations of aztreonam in dog serum are reported uncorrected and represent minimum values.

In monkey serum, for which acetonitrile was used for extraction, concentrations of aztreonam were corrected for low recovery. Subsequent experiments showed that, whereas only 64% of added [14C]aztreonam was extracted from control serum with acetonitrile, essentially 100% was recovered by methanol extraction.

Excretion in urine, feces, and bile of rats. In 96 h, the total recoveries of the radioactive doses in urine and feces after i.m. and i.v. administration to rats were 96.0 ± 3.0 and 101.3 ± 4.4%, respectively. Excretion in urine accounted for 70.5 ± 4.7% (i.m.) and 68.3 ± 2.4% (i.v.). The excretion in urine occurred rapidly, as was evidenced by the recovery of 57.3 ± 7.2% (i.m.) and 62.6 ± 2.8% (i.v.) of the radioactive doses in the first 4 h. Excretion in feces (0 to 96 h) accounted for 25.5 ± 2.1 and 33.0 ± 2.5% of the dose after i.m. and i.v. administrations, respectively. This result strongly suggests that an appreciable amount of the dose was excreted by the biliary route. In two unanesthetized rats with cannulated bile ducts, 12.9 and 16.4% of single 50-mg/kg, i.m. doses of [14C]aztreonam were recovered in bile in 24 h and 79.6 and 77.1% were recovered in urine; for both rats, only about 2% of the dose was recovered in feces in 0 to 24 h.

Biotransformation profiles in urine and bile of rats. Biotransformation profiles of radioactivity in urine were essentially identical after i.m. and i.v. administrations. On the basis of TLRC results, unchanged aztreonam accounted for 82.8 ± 1.1% (i.m.) and 84.7 ± 0.7% (i.v.) of the radioactivity in 0- to 24-h urine (57% of the i.m. or i.v. dose). The identity of SQ 26,992, the compound which results from hydrolysis of the β-lactam ring, as a metabolite of aztreonam was confirmed in monkeys (see below). SQ 26,992 accounted for 14.3 ± 1.0% (i.m.) and 12.8 ± 0.6% (i.v.) of the urinary radioactivity (9 to 10% of the dose). The remainder of the radioactivity in the urine of rats (about 2% of the dose) was present as minor, unidentified components. The biotransformation profile for the urine collected from rats with cannulated bile ducts, 12.9 and 16.4% of single 50-mg/kg, i.m. doses of [14C]aztreonam were essentially the same as that for the urine of intact rats. In bile, a different profile was observed; 39.6 ± 5.1% of the radioactivity (about 6% of the dose) was present as an unknown metabolite(s) which appeared in zone C of the chromatogram (Fig. 3). 51.3 ± 4.5% (about 8% of the dose) as aztreonam (zone E), 4.7 ± 0.0% (about 1% of the dose) as SQ 26,992 (zone D), and 4.5 ± 0.6% (about 1% of the dose) as zones A, B, and F combined.

Incubation of bile with Glusulase did not change the thin-layer chromatographic pattern of the radioactivity, indicating that the unknown metabolite fraction did not contain enzymatically hydrolyzable glucuronide or sulfate conjugates.

Excretion in urine and feces of dogs. By 96 h after i.v. and

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**FIG. 3.** Schematic diagram of a thin-layer chromatogram illustrating the TLRC procedure.
subcutaneous (s.c.) administration of [14C]aztreonam (25 mg/kg) to four dogs, means of 92.6 ± 1.8 and 99.4 ± 2.6% of the respective doses were recovered in the excreta. Excretion in urine averaged 86.0 ± 1.7% of the i.v. dose and 90.2 ± 2.4% of the s.c. dose; fecal excretion averaged 6.6 ± 1.0% (i.v.) and 9.2 ± 0.9% (s.c.). During the first 2 h after dosing, excretion occurred at a faster rate after i.v. (67%) than after s.c. (24%) administration. This result appeared to be due to the relatively slow release of [14C]aztreonam from the site of s.c. injection.

**Biotransformation profiles in urine of dogs.** Biotransformation profiles of [14C]aztreonam in the urine of dogs were very similar after i.v. and s.c. administrations (Table 1). On the basis of results of TLC analysis of individual urine samples from each dog, 85 to 86% of the radioactivity in 0- to 24-h urine was present as aztreonam and 10 to 11% was present as SQ 26,992. The remaining 4% of the radioactivity was present as unknown compounds. In sequential urine samples after i.v. or s.c. administration, the relative amount of aztreonam decreased, whereas that of SQ 26,992 increased, with time (Table 1). The biotransformation profile, based on the analysis of individually pooled 0- to 24-h urine prepared from each of the four dogs, was in excellent agreement with the cumulative 0- to 24-h values shown in Table 1. After both i.v. and s.c. administrations, the biotransformation profiles obtained by TLC and HPLC of 0- to 24-h pooled urine of all four dogs were in good agreement.

Pooled 0- to 24-h urine samples from individual dogs were analyzed for unchanged aztreonam by TLC and microbiological procedures. The mean values for the percentage of the dose excreted as aztreonam in 0 to 24 h were 73.1 ± 1.7% (radioassay) versus 76.9 ± 4.0% (bioassay) for i.v. administration; the corresponding values for s.c. administration were 75.3 ± 3.5 and 79.5 ± 5.7%. The differences between the assay methods were not statistically significant (P > 0.05).

**Concentrations and biotransformation profiles in dog serum.** Maximum concentrations of total radioactivity in serum after s.c. administration were attained between 0.5 and 1.0 h after dosing (time to maximum concentration in serum, 0.9 ± 0.1 h) and ranged from 33 to 36 μg/ml (35.0 ± 0.7 μg/ml). From 1 to 96 h after s.c. administration, concentrations in serum remained consistently higher than after i.v. administration. The apparent elimination half-life (t1/2b) of total radioactivity in serum between 1 and 5 h after i.v. administration was 0.9 ± 0.02 h; the apparent t1/2b between 2 and 6 h after s.c. administration was 1.3 ± 0.04 h. The higher concentrations in serum after the first hour and apparently longer t1/2b after s.c. administration indicated the relatively slower absorption of the dose from the s.c. injection site. The difference between 0- to 96-h values for area under the serum concentration versus time curve (in microgram · hours per milliliter) obtained for i.v. (149 ± 5.0) and s.c. (168 ± 21) administrations was not statistically significant (P > 0.05).

Concentrations of unchanged aztreonam in serum, up to 8 h after i.v. or s.c. administration, appeared to follow the same time course as total radioactivity (Fig. 4). Maximum concentrations of unchanged aztreonam in serum after s.c. administration ranged from 13 to 19 μg/ml (16.9 ± 1.4 μg/ml) and were attained between 0.5 and 1.0 h (time to maximum concentration in serum, 0.9 ± 0.1 h) after dosing. Between 1 and 6 h, the concentrations of unchanged aztreonam were considerably higher after s.c. than i.v. administration (Fig. 4), probably owing to the relatively slower absorption of the s.c. dose from the injection site. The values for area under the serum concentration versus time curve (0 to 8 h), 43.6 ± 2.7 and 48.6 ± 4.3 μg · h/ml for i.v. and s.c. routes, respectively, were not significantly different (P > 0.05) and were approximately 44% of the corresponding values for total radioactivity. The apparent t1/2b of aztreonam in serum after i.v. administration was estimated to be 0.7 ± 0.01 h. After s.c. administration, the t1/2b averaged about 1.3 h in two of the dogs, but the t1/2b could not be calculated for the other two dogs because the semilogarithmic plots of concentration in serum versus time were not linear. The longer apparent t1/2b after s.c. administration actually reflected slow entry of part of the s.c. dose into the circulation.

In serum, most of the radioactivity not accounted for as aztreonam was unextractable with methanol; still smaller amounts were also present as SQ 26,992 and unknown extractable compounds.

The binding of total radioactivity to serum proteins was determined at 0.25 and 2.0 h. No differences in binding were observed between 0.25 and 2.0 h; the average values were 28.0 ± 2.6% after i.v. administration and 33.5 ± 3.0% after s.c. administration. The differences in protein binding between the two routes were not statistically significant (P > 0.05).

**Excretion in urine and feces of monkeys.** At 0 to 96 h, 85.5 ± 3.4 and 89.9 ± 5.1% of the dose was recovered in the excreta after i.m. and i.v. administration, respectively; ex-

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**TABLE I. Excretion of total radioactivity, unchanged aztreonam, and metabolite SQ 26,992 in urine of dogs after i.v. and s.c. administrations of single 25-mg/kg doses of [14C] aztreonam**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Total radioactivity (% of dose)</th>
<th>Relative % in urine</th>
<th>Total radioactivity (% of dose)</th>
<th>Relative % in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aztreonam</td>
<td>SQ 26,992</td>
<td>Aztreonam</td>
<td>SQ 26,992</td>
</tr>
<tr>
<td>0–1</td>
<td>38.4 ± 11.1</td>
<td>92.2 ± 0.7</td>
<td>5.0 ± 0.4</td>
<td>7.4 ± 2.0</td>
</tr>
<tr>
<td>1–2</td>
<td>28.9 ± 10.1</td>
<td>90.2 ± 2.2</td>
<td>5.7 ± 1.7</td>
<td>17.1 ± 3.7</td>
</tr>
<tr>
<td>2–3</td>
<td>6.4 ± 0.6</td>
<td>84.0 ± 2.3</td>
<td>10.1 ± 1.0</td>
<td>28.5 ± 5.5</td>
</tr>
<tr>
<td>3–4</td>
<td>4.0 ± 1.0</td>
<td>80.6 ± 1.4</td>
<td>13.6 ± 1.2</td>
<td>14.9 ± 1.4</td>
</tr>
<tr>
<td>4–6</td>
<td>3.3 ± 0.8</td>
<td>67.6 ± 0.7</td>
<td>24.7 ± 1.6</td>
<td>10.5 ± 1.0</td>
</tr>
<tr>
<td>6–8</td>
<td>1.3 ± 0.1</td>
<td>21.1 ± 2.2</td>
<td>69.2 ± 2.1</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>8–24</td>
<td>2.8 ± 0.5</td>
<td>11.1 ± 3.7</td>
<td>81.8 ± 8.0</td>
<td>8.3 ± 4.0</td>
</tr>
<tr>
<td>Cumulative (0–24)</td>
<td>85.2 ± 1.63</td>
<td>86.1 ± 1.1</td>
<td>10.0 ± 0.5</td>
<td>89.1 ± 2.5</td>
</tr>
</tbody>
</table>

*All values are mean ± standard error for four dogs.*
cretion in urine accounted for 42.9 ± 1.8% (i.m.) and 43.9 ± 6.1% (i.v.) and excretion in feces accounted for 42.6 ± 4.7% (i.m.) and 46.0 ± 2.6% (i.v.). In the first 4 h, 23.3 ± 6.6% (i.m.) and 24.8 ± 4.7% (i.v.) of the dose was excreted in urine. Total recovery of the dose in the excreta was incomplete, owing at least in part to slow excretion; during the 72- to 96-h period, an average of about 2% of the dose was excreted, regardless of the route of administration.

Excretion in feces indicated substantial biliary excretion (at least 43% of the dose) of the radioactivity after administration of [14C]aztreonam by both routes.

**Biotransformation profile in urine of monkeys.** On the basis of results of TLRC analysis of individual urine samples, unchanged aztreonam accounted for 78.2 ± 4.0% (i.m.) and 77.0 ± 0.7% (i.v.) of the total radioactivity in 0- to 24-h urine. For both routes of administration, the relative amount of unchanged aztreonam in urine decreased from about 88% in 0- to 4-h urine to about 25 to 30% in 8- to 24-h urine. Most of the remainder of the radioactivity was associated with SQ 26,992. On the basis of results of TLRC and HPLC analyses of pooled 0- to 24-h urine, SQ 26,992 accounted for 14 to 15% (i.m.) and 12 to 13% (i.v.) of the radioactivity in urine. SQ 26,992 and three other unidentified metabolites that were isolated from urine were tested and found to be devoid of antimicrobial activity. With the HPLC system used, the retention times of aztreonam, SQ 26,992, and the three other metabolites were about 24, 10, 8, 7, and 5 min, respectively. The shorter retention times indicate that the three unknown metabolites were more polar than was aztreonam or SQ 26,992.

Radioassay and microbiological assay results for the amount of unchanged aztreonam excreted in urine were in excellent agreement (Table 2); the coefficient of determination ($r^2$) was 0.98, and the slope of the curve was about 1.0.

**Biotransformation profile in feces of monkeys.** Biotransformation profiles in fecal samples from all monkeys after i.m. or i.v. administration were similar. SQ 26,992 was the major radioactive compound, accounting for 46.6 ± 3.4% (i.m.) and 46.4 ± 3.3% (i.v.) of the radioactivity in 0- to 72-h feces, unchanged aztreonam for 10.7 ± 1.1% (i.m.) and 17.0 ± 3.5% (i.v.), and unknown radioactive compounds for the remainder. The stability of aztreonam in feces and during the extraction and TLRC procedures was very good, as was indicated by the recovery of 93 to 95% of the aztreonam added to control fecal homogenates.

**Concentrations in monkey serum.** The concentrations of

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**TABLE 2. Comparison of radioassay and microbiological assay for unchanged aztreonam in urine of monkeys after i.m. and i.v. administrations of single 25-mg/kg doses of [14C]aztreonam**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>% of dose excreted in urine after$^a$</th>
<th>i.m. administration</th>
<th>Aztreonam</th>
<th>i.v. administration</th>
<th>Aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total radioactivity</td>
<td>Radioassay$^b$</td>
<td>Bioassay$^b$</td>
<td>Total radioactivity</td>
<td>Radioassay$^b$</td>
</tr>
<tr>
<td>0–4</td>
<td>23.3 ± 6.6</td>
<td>19.9 ± 5.1</td>
<td>20.6 ± 5.2</td>
<td>24.8 ± 4.7</td>
<td>22.1 ± 5.2</td>
</tr>
<tr>
<td>4–8</td>
<td>11.6 ± 3.8</td>
<td>9.0 ± 3.5</td>
<td>10.0 ± 4.5</td>
<td>11.8 ± 3.3</td>
<td>9.1 ± 2.9</td>
</tr>
</tbody>
</table>

$^a$ All values are mean ± standard error for three monkeys.

$^b$ Not significantly different ($P > 0.05$).
TABLE 3. Concentrations of total radioactivity and aztreonam in sera of monkeys after i.m. and i.v. administrations of single 25-mg/kg doses of $[^{14}C]$aztreonam

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>i.m. administration</th>
<th>i.v. administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total radioactivity*</td>
<td>Aztreonam Radioassay*</td>
</tr>
<tr>
<td>0.08</td>
<td>66.6 ± 6.1</td>
<td>45.9 ± 4.8</td>
</tr>
<tr>
<td>0.25</td>
<td>54.7 ± 2.9</td>
<td>26.4 ± 2.0</td>
</tr>
<tr>
<td>0.5</td>
<td>37.0 ± 3.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>17.4 ± 2.2</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>2.0</td>
<td>8.2 ± 1.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>3.0</td>
<td>4.8 ± 0.5</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>4.0</td>
<td>3.0 ± 0.5</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>6.0</td>
<td>2.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>10.0</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

* All values are mean ± standard error for four monkeys.

* Expressed as aztreonam equivalents.

* Not significantly different ($P > 0.05$).

total radioactivity in serum after i.m. and i.v. administration of $[^{14}C]$aztreonam were similar between 0.5 and 24 h (Table 3). The concentrations of unchanged aztreonam in serum, determined at 0.5, 1.0, and 4.0 h after dosing, were also similar for the two routes of administration (Table 3). The total antimicrobial activity in these serum samples, expressed as aztreonam equivalents, was in excellent agreement with aztreonam concentrations determined by the radioassay procedure (Table 3). The coefficient of determination ($r^2$) between the radioassay and bioassay values was 0.98, and the slope of the curve was about 1.0.

Serum protein binding of total radioactivity at 0.25 h after dosing was 49.1 ± 1.1% (i.m.) and 49.3 ± 1.1% (i.v.); at 2.0 h, it was 55.8 ± 2.8% (i.m.) and 59.4 ± 2.7% (i.v.).

Isolation and Identification of SQ 26,992 from Monkey Urine. SQ 26,992 was isolated from 0- to 4-h pooled urine of three monkeys by HPLC as previously described. Total radioactivity in this sample was equivalent to about 5 mg of aztreonam. The HPLC eluate corresponding to SQ 26,992 contained 10.6% of the total radioactivity recovered from the column; the recovery of the radioactivity injected into the column was essentially quantitative. Thus, based on the specific activity of $[^{14}C]$aztreonam, about 0.5 mg of SQ 26,992 was isolated from the urine.

Although the presence of chemical impurities was evident, the proton nuclear magnetic resonance spectrum of the isolated metabolite clearly showed the characteristic peaks corresponding to the three methyl groups and the thiazole proton of SQ 26,992. The mass spectrum of the metabolite was also found to be consistent with the structure of SQ 26,992. Spectroscopic and chromatographic data thus confirmed the identity of SQ 26,992 as the most prominent metabolite of aztreonam in monkey urine.

DISCUSSION

In rats and dogs, the majority of a parenteral dose of $[^{14}C]$aztreonam was eliminated by the renal route; in monkeys, the elimination was about equally divided between urine and feces. The ratio of urinary to fecal excretion was of the order dog > rat > monkey. Biliary elimination, which was suggested by fecal excretion, was confirmed in bile-cannulated rats. In healthy human subjects, the urinary excretion of unchanged aztreonam accounted for 68% of an i.m. or i.v. dose ranging from 125 to 4,000 mg (10). In another study with human subjects who received 500 mg i.v. doses of $[^{14}C]$aztreonam, about 66% of the dose was excreted as unchanged aztreonam in urine; the excretion of total radioactivity in urine and feces was 76 and 12%, respectively, after i.v. administration and 78 and 15%, respectively, after i.m. administration (9). Thus, the renal route of elimination was favored in rats, dogs, and humans, but not in monkeys. In the three species in the present study, as well as in the previous study in humans, unchanged aztreonam was the major component of total radioactivity in urine. The prominent metabolite (SQ 26,992) resulting from hydrolysis of the β-lactam ring accounted for about 5 to 10% of the dose in rats, dogs, or monkeys and about 7% in humans (9); the urinary biotransformation profile in the four species is summarized in Table 4. In bile (rats and feces (monkeys), metabolites, including SQ 26,992, were present in larger amounts than in urine.

Good agreement between the results obtained by TLRC and microbiological assay procedures for concentrations of aztreonam in serum (monkeys) and urine (dogs and monkeys) indicated that SQ 26,992 and any other metabolites were devoid of any significant antimicrobial activity. This indication was confirmed when SQ 26,992 and three unknown minor metabolites isolated from monkey urine were shown to lack any detectable antimicrobial activity.

The apparent $t_{1/2b}$ of aztreonam in the sera of dogs (0.7 h)

TABLE 4. Mean distribution of aztreonam and its metabolites in urine after parenteral administration of $[^{14}C]$aztreonam

<table>
<thead>
<tr>
<th>Species (dose)</th>
<th>% of total radioactivity in urine after administration of $[^{14}C]$aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (50 mg/kg)</td>
<td>Aztreonam SQ 26,992 Unidentified</td>
</tr>
<tr>
<td>Dog (25 mg/kg)</td>
<td>85                                  13       2      83                        14       3</td>
</tr>
<tr>
<td>Monkey (25 mg/kg)</td>
<td>77                                   13      10      78                        14       7</td>
</tr>
<tr>
<td>Man (500 mg)</td>
<td>87                                   9        4      85                        10       5</td>
</tr>
</tbody>
</table>

* Radioactivity was measured in 0- to 24-h urine for dogs, rats, and monkeys and in 0- to 144-h urine for humans.

* An s.c. dose was given to dogs, and an i.m. dose was given to rats and monkeys.

* Data are from reference 9; mean weight of subjects was 73.3 kg.
after i.v. administration was considerably shorter than those (~1.7 to 1.9 h) reported for human subjects after i.v. doses of aztreonam (125 to 4,000 mg) or [14C]aztreonam (9, 10). The serum t1/2B for unchanged aztreonam was not determined in rats and monkeys, but the serum t1/2B of total radioactivity (representing aztreonam, SQ 26,992, and other minor metabolites) in monkeys was about 1 h. In humans, SQ 26,992, the primary metabolite of aztreonam, is eliminated at a considerably slower rate than aztreonam (9). If SQ 26,992 is eliminated at a slower rate in monkeys as well, then the t1/2B of aztreonam in monkey serum may be no longer than 1 h.

Serum protein binding of total radioactivity was greater in monkeys (49 to 59%) than in dogs (28 to 35%). For both species, the serum protein binding was less than that of total radioactivity found in humans (71%) (9). The species differences in protein binding did not seem to be due to differences in serum concentrations, because the latter were similar in all three species.

The metabolism and disposition of β-lactam antibiotics such as penicillins (3), cefotaxime (2, 6), and cefatrizine (4) have recently been studied in animals and humans. For various penicillins, 82 to 100% of an i.m. dose administered to humans is excreted in urine, predominantly as unmetabolized penicillin, and 2 to 15% of the dose is excreted as penicilloic acids. It has also been noted that the penicilloic acids are more slowly excreted in urine than the parent drug. Both Reeves et al. (6) and Chamberlain et al. (2) have shown that appreciable amounts of products of β-lactam ring hydrolysis are excreted in urine after the administration of cefotaxime to humans, rats, and dogs. Again, the open-ring metabolites appear to have longer t1/2B than the parent drug. Although the metabolites of cefatrizine (4) were not identified, they were shown to be inactive; 80% of the drug-related material excreted in urine was intact cefatrizine. Thus, although aztreonam has a novel structure and spectrum of activity, it has characteristics with regard to disposition and metabolism that are similar to those of other β-lactam antibiotics.

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LITERATURE CITED