Comparative Pharmacokinetics of Carumonam and Aztreonam in Mice, Rats, Rabbits, Dogs, and Cynomolgus Monkeys

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The pharmacokinetic properties of carumonam (AMA-1080, Ro 17-2301) (3S,4S)-3-[2-(2-ami
nothiazol-4-yl)-(Z)-2-carboxymethoxyiminacetamido]-4-carbamoyloxymethyl-2-azetidinone-1-
 sulfonic acid (see Fig. 1), has potent in vitro and in vivo activities against a wide range of aerobic gram-negative bacteria such as members of the family Enterobacteriaceae, Pseudomonas aeruginosa, and Haemophilus influenzae (4). Its activity is similar to that of the structurally related antibiotic aztreonam (12), but it shows stronger activity than aztreonam against Klebsiella oxytoca, Klebsiella pneumoniae, and Enterobacter cloacae (4). In the studies reported here, the pharmacokinetic properties of carumonam in experimental animals were compared with those of aztreonam (Fig. 1) (12).

(These results were presented in part at the 13th International Congress of Chemotherapy, Vienna, Austria, 1983.)

MATERIALS AND METHODS

Antibiotics. Carumonam and its metabolite, AMA-1294, were prepared in our division, and aztreonam was obtained from Hoffmann-La Roche, Inc., Nutley, N.J.

Animals. Five-week-old male Slc:ICR mice weighing 20 to 25 g, 7-week-old male Jcl:SD rats weighing 210 to 250 g, male New Zealand White rabbits weighing 2.5 to 3.5 kg, male and female beagle dogs weighing 10 to 15 kg, and female cynomolgus monkeys weighing 2.8 to 3.7 kg were used. They were deprived of feed for 16 to 18 h before the antibiotic was administered; water was given ad libitum.

Antibiotic administration. Just before use, each antibiotic was dissolved in a solution containing NaHCO3 equimolar to the antibiotic. A single dose of 20 mg of the antibiotic per kg of body weight was administered subcutaneously to mice (2 mg/ml, 0.1 ml/10 g) and intramuscularly to rats (10 mg/ml, 0.2 ml/100 g), rabbits (40 mg/ml, 0.5 ml/kg), dogs (100 mg/ml, 0.2 ml/kg), and monkeys (20 mg/ml per kg).

Specimens for antibiotic bioassay. Blood specimens collected on heparin were obtained from the axillary artery and vein of mice, rats, and rabbits that were anesthetized with ethyl ether and killed at each sampling period. Blood specimens of monkeys were collected successively from the femoral vein of the unanesthetized animals, and those of dogs were collected from the saphena and median veins of the unanesthetized animals. Plasma was separated by centrifugation at 2,000 × g for 10 min at 2°C.

After the animals were killed by bleeding, the lungs, liver, spleen, and kidneys were removed and washed with saline. The tissues were homogenized with the same part (wt/vol) of 0.1 M phosphate buffer (pH 7.0) and then homogenized again with 4 parts (vol/vol) of methanol. The homogenates were centrifuged (2,000 × g, 10 min), and the supernatant fluids were subjected to bioassay.

Urine specimens were collected from mice, rats, and...
monkeys in metabolism cages or with a urethral catheter in unanesthetized rabbits and dogs and anesthetized bile duct-cannulated rats and dogs. Bile specimens were collected from the common bile duct cannulated with polyethylene tubing in rats, rabbits, and dogs anesthetized with sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.). All specimens were assayed immediately after each experiment or within 2 days after storage at −80°C. The antibiotic was stable under these conditions.

**Antibiotic assays.** (i) **Microbiological assay.** Antibiotics in plasma, urine, and bile were assayed by the agar well method with *Escherichia coli* NIHJ as the test organism in sulbenicillin assay medium consisting of 0.6% Polypeptone (Daigo Nutritive Chemicals, Ltd.), 0.3% yeast extract (Daigo Nutritive Chemicals, Ltd.), 0.15% bonito meat extract (Wako Pure Chemical Industries, Ltd.), 0.1% glucose, and 1.2% agar (pH 6.5 after sterilization). The sensitivity of this method was about 0.1 µg/ml. The precision in terms of variation coefficient of replicate analyses (n = 8) was ±7.0%. There was good agreement between the results obtained by the bioassay and by high-pressure liquid chromatography. Antibiotics in the tissue extracts were assayed by the paper disk method with *Proteus rettgeri* ATCC 9250 as the test organism in MacConkey agar (Eiken Chemical Co., Ltd.). The detection limit of this method was about 0.03 µg/ml. The assay results from this method correlated well with those obtained by the above-mentioned agar well method with *Escherichia coli* NIHJ. The antibiotic concentrations in plasma were calculated from the standard curves of the drugs dissolved in normal plasma of the respective animals. Urine and bile specimens were diluted with 0.1 M phosphate buffer (pH 7.0). The antibiotic concentrations in the diluted specimens and supernatants of the tissue homogenates were calculated from the standard curves of the drugs dissolved in the buffer.

(ii) **Bioautography of active metabolites.** Plasma specimens were mixed with the same volume of methanol, and the supernatant fluids were separated by centrifugation. The supernatant fluids, urine, bile, and the standard antibiotic solutions were spotted on a thin-layer plate (Spotfilm silica gel; Tokyo Chemical Industry Co., Ltd.). After ascending development in the solvent CH3CN-H2O-CH3COOH-buffer (pH 7.0)-acetonitrile (88:12 [vol/vol]) (10), the active spots were detected by bioautography with *Proteus rettgeri* ATCC 9250 as the test organism on MacConkey agar. The sensitivity of this bioautography was about 0.02 µg as carumonam equivalent.

(iii) **High-pressure liquid chromatography.** The urine specimens were separated on a Nucleosil SC18 column (inner diameter, 15 by 0.4 cm; Machery-Nagel Co., Federal Republic of Germany) with the mobile phase consisting of 0.005 M tetrabutylammonium hydrogen sulfate (pH 3.0)-acetonitrile (88:12 [vol/vol]) (10). The flow rate was maintained at 0.8 ml/min. The eluate was monitored at 313 nm by using a UV detector (model 44; Waters Associates, Inc., Milford, Mass.). The detection limit for carumonam and its metabolite, AMA-1294, was 1.0 µg/ml (urine, ultrafiltrate, dialysate) with a precision of <2%.

**Protein binding.** The degree of protein binding of carumonam and aztreonam in serum was determined by the equilibrium dialysis technique with dialysis chambers (Diapack, model 4000; Konton AG, Zurich, Switzerland) (5). The initial concentration of the antibiotic in the protein-free side of the chamber was 20 µg/ml. The dialysis chambers were rotated slowly for 24 h at 4°C, and antibiotics in the protein-free chamber were assayed by high-pressure liquid chromatography (10). In addition, the binding was investigated by the ultrafiltration technique with the MPS-1 system (Amicon Corp., Lexington, Mass.) and a YMT membrane (2).

**Pharmacokinetic analysis.** Pharmacokinetic evaluation was performed by using the improved computer program reported by Ouchida et al. (9) and Mizuta and Tsubotani (8); the optimum values of parameters for the one- or two-compartment open model were determined by the iterative least-squares method.

The pharmacokinetic parameters for the antibiotics in mice, rats, and rabbits were calculated from the mean levels in plasma by the one-compartment open model which satisfactorily fitted their calculations; parameters for the anti-
FIG. 2. Concentrations of carumonam (●) and aztreonam (▲) in the plasma of mice, rats, rabbits, dogs, and monkeys after a single dose of 20 mg/kg (mean ± standard deviation). s.c., Subcutaneous injection; i.m., intramuscular injection.
otics in dogs and monkeys were calculated by the two-compartment open model, since their elimination-phase curves did not fit to the one-compartment open model.

The area under the plasma concentration-time curve (AUC) was calculated as follows: AUC = dose/keV (for one-compartment open model) and AUC = dose/βVp (for two-compartment open model), where keV is the elimination rate constant; β is the rate constant of elimination phase, and Vp and V are the apparent volumes of distribution in the body compartment and at β phase, respectively.

The AUC values were used to calculate the plasma clearance (CLp): CLp = keVV (for one-compartment open model) and CLp = βVp (for two-compartment open model).

The renal clearance (CLR) was obtained by the following equation: CLR = CLpUex, where Uex is the dose fraction (percent) excreted into urine.

**Statistical analysis.** The degree of significance between means was determined by the Student t test.

**RESULTS**

**Concentrations in plasma.** The concentrations of carumonam and aztreonam in the plasma of mice, rats, rabbits, dogs, and monkeys given a single dose each of 20 mg/kg are shown in Fig. 2. Values for the observed time of peak concentration in plasma (Tmax), the peak concentration in plasma (Cmax), the AUC (calculated from zero to the last time of measurement by the trapezoidal rule), the apparent elimination half-life (t1/2), and the CLp for carumonam and aztreonam are displayed in Table 1.

The Cmax of carumonam in the plasma of mice, rats, rabbits, and dogs was about 40 μg/ml, similar to that of aztreonam. The Cmax of carumonam for monkeys (68 μg/ml) was the same as that of aztreonam. The AUCs and t1/2s of carumonam for rodents were lower than those for other animals and were similar to those of aztreonam but were lower for rats. The CLp of carumonam for rodents (14 to 17 ml/min per kg) was higher than that for other animals (4.5 to 5.5 ml/min per kg). The two antibiotics showed similar CLps for all animals except the rats, for which the CLp of carumonam was greater than that of aztreonam.

**Distribution in tissue.** The concentrations of carumonam and aztreonam in tissues were examined at different times after 20 mg/kg was administered subcutaneously in mice and intramuscularly in rats. Tables 2 and 3. The concentrations...
in the tissues of rabbits, dogs, and cynomolgus monkeys were examined at 30 min after 20-mg/kg doses of the antibiotics were administered intramuscularly (Table 4). When the levels in tissue were compared 15 min after the drug was administered in rodents and 30 min after administration in other animals, the distribution patterns in the lungs and spleen were similar for all animal species tested with either of the antibiotics. The concentrations in the lungs and spleen were usually lower than the concentrations in plasma. The ratio of the concentration of carumonam in the lungs to that in plasma ranged from 0.27 (in monkeys) to 0.45 (in mice); the lung/plasma ratio of aztreonam ranged from 0.20 (in monkeys) to 0.32 (in mice). The ratio of the concentration of carumonam in the spleen to that in the plasma ranged from 0.05 (in monkeys) to 0.2 (in mice); the spleen/plasma ratio of aztreonam ranged from 0.05 (in monkeys) to 0.11 (in dogs). In contrast, the distribution in the kidneys and liver was different, depending upon the animal species and upon the antibiotic. The concentrations of both antibiotics in the kidneys were higher than the concentrations in plasma except for those concentrations in mice given aztreonam. The kidney/plasma ratios in mice, rats, rabbits, dogs, and monkeys were 1.75, 4.54, 5.52, 2.77, and 2.23, respectively, for carumonam and 0.79, 2.28, 3.01, 2.40, and 1.85, respectively, for aztreonam. The concentrations in the liver differed very much, depending upon the animal species and the antibiotic. The concentration of carumonam in the liver was always lower than that in the kidneys and was usually lower than that of aztreonam. The liver/plasma ratios in mice, rats, rabbits, dogs, and monkeys were 0.78, 1.96, 0.02, 0.94, and 0.29, respectively, for carumonam and 1.26, 1.36, 0.26, 0.89, and 2.99, respectively, for aztreonam. In mice and monkeys, the concentrations of aztreonam in the liver were higher than those in the kidneys.

**TABLE 4. Concentrations of carumonam and aztreonam in plasma and tissue of rabbits, dogs, and monkeys at 30 min after a single intramuscular administration of 20 mg/kg.**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Plasma or tissue</th>
<th>Carumonam</th>
<th>Aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit (n = 3)</td>
<td>Plasma</td>
<td>40.6 ± 7.7</td>
<td>43.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>13.1 ± 3.0</td>
<td>11.0 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.7 ± 0.7</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>224 ± 96.9</td>
<td>131 ± 24.5</td>
</tr>
<tr>
<td>Dog (n = 3 for carumonam; n = 2 for aztreonam)</td>
<td>Plasma</td>
<td>40.5 ± 3.3</td>
<td>48.3</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>14.2 ± 0.8</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>38.0 ± 15.3</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.4 ± 0.5</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>112 ± 16.9</td>
<td>116</td>
</tr>
<tr>
<td>Monkey (n = 3)</td>
<td>Plasma</td>
<td>66.8 ± 13.0</td>
<td>65.3 ± 27.9</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>18.2 ± 0.5</td>
<td>13.8 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>19.3 ± 13.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>195 ± 43.2</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>3.3 ± 3.7</td>
<td>3.4 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>149 ± 107</td>
<td>121 ± 68.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as mean ± SD (n = 3).
<sup>b</sup> ND, Not detected (<0.5 µg/ml).
<sup>c</sup> Significant difference between carumonam and aztreonam (P < 0.01).
Rat (n = 10)

Mouse (n = 10)

Dog (n = 4 for carumonam; n = 3 for aztreonam)

Monkey (n = 4)

Time (h) 0-8 8-24
Concn (µg/ml) 432 ± 63.5 2.4 ± 1.3
Excretion (%) 65.5 ± 5.2 1.0 ± 0.8

Time (h) 0-1 1-2 2-4 4-6 6-8 8-24
Concn (µg/ml) 2,130 ± 1,250 4,090 ± 2,680 2,300 ± 764 1,530 ± 269 808 ± 156 257 ± 246
Excretion (%) 16.5 ± 7.4 22.1 ± 6.0 12.4 ± 3.3 12.7 ± 3.3 3.7 ± 1.6 5.4 ± 1.3

Time (h) 0-1 1-2 2-4 4-6 6-8 8-24
Concn (µg/ml) 7,370 ± 1,880 3,680 ± 1,580 1,850 ± 229 357 ± 93.6 1,020 ± 376 5.9 ± 2.5
Excretion (%) 28.4 ± 0.3 11.0 ± 0.9 10.2 ± 4.7 1.6 ± 0.5 65.7 ± 7.8 0.6 ± 0.3

Time (h) 0-1 1-2 2-4 4-6 6-8 8-24
Concn (µg/ml) 7.9 ± 2.4 9.5 ± 8.1 933 ± 685 149 ± 82.9 665 ± 387 3.8 ± 1.5
Excretion (%) 187 ± 15.3 19.4 ± 2.9 3.4 ± 2.0 0.8 ± 0.3 38.4 ± 8.9 0.3 ± 0.1

* See the text for administration route. All values are expressed as means ± SD. The difference between carumonam and aztreonam was significant (P < 0.001) for mice, rats, dogs, and monkeys.

** Values are expressed as means ± SD. The difference between carumonam and aztreonam was significant for rats and dogs (P < 0.01) and for rabbits (P < 0.001).

** ND, Not detected (<0.5 µg/ml).

Table 5. Concentrations in urine and excretion of carumonam and aztreonam in animals after a single 20-mg/kg administration

Table 6. Biliary concentrations and excretion of carumonam and aztreonam in rats, rabbits, and dogs after a single intramuscular administration of 20 mg/kg

Discussion

Carumonam and aztreonam are N-sulfonated monocyclic β-lactam antibiotics closely related in chemical structure and antibacterial activity; aztreonam is already used clinically and carumonam is under phase III clinical trials. Both are very active against a wide range of gram-negative bacteria, but carumonam is more active than aztreonam against some members of the family Enterobacteriaceae such as K. oxytoca, K. pneumoniae, and Enterobacter cloacae. In the present report, the pharmacokinetic properties of the two antibiotics have been compared in mice, rats, rabbits, dogs, and cynomolgus monkeys.

Carumonam and aztreonam were readily absorbed and
penetrated well into tissues. The levels of both antibiotics in the plasma, kidneys, liver, and lungs attained after a 20-mg/kg dose was administered subcutaneously in mice and intramuscularly in other species reached more than 10 μg/ml or μg/g. This level is the concentration of carumonam sufficient to inhibit more than 90% of clinical isolates of most species of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. This fact suggests that carumonam is effective against infection caused by most gram-negative bacteria in the above-mentioned tissues. The effectiveness of carumonam against septicemia and urinary tract and respiratory tract infections has been demonstrated in model infections and in the phase II trials (unpublished data).

Carumonam and aztreonam have similar pharmacokinetics in plasma but differ slightly in distribution in tissues and excretion. In the plasma, the *T*<sub>max</sub>, *C*<sub>max</sub>, AUC, *t*<sub>1/2</sub>, and CL<sub>p</sub> values of the two antibiotics were similar except those obtained for rats, in whose plasma carumonam was eliminated faster and consequently showed a lower AUC than aztreonam. Although the two antibiotics had similar plasma pharmacokinetics in most species, their binding rates to serum proteins were different. The protein-binding rate of carumonam in the sera of animal species and humans was 1.9 to 4.2 times lower than that of aztreonam.

Merrik et al. (7) have reported that β-lactam antibiotics with higher protein-binding capacities in serum have longer half-lives in serum, but others (3, 14, 15) could not demonstrate such a correlation, as was the case in this study with carumonam and aztreonam.

Both antibiotics had high levels in the kidneys, levels that were greater (*P < 0.05*) than the respective levels in plasma for most species tested; the levels attained by carumonam in mice and rats were higher (*P < 0.05*) than those attained by aztreonam; the distribution patterns of the two antibiotics in the tissues of dogs were very similar. In contrast, the levels of aztreonam in the livers of mice, rats, rabbits, and monkeys were higher (*P < 0.01*) than those of carumonam. The levels of carumonam in the liver were usually lower (*P < 0.05*) than those in the kidneys, but the levels of aztreonam in the liver were comparable to or even higher (*P < 0.05*) than those in the kidneys of mice, rats, and monkeys. Thus, carumonam and aztreonam differ with respect to penetrating into the kidneys and liver. This difference was reflected in recoveries in the urine and bile: the urinary recovery of carumonam (66.5, 61.5, and 66.3% in mice, rats, and monkeys, respectively) was greater (*P < 0.001*) than that of aztreonam (49.7, 44.8, and 38.7%, respectively). For dogs, the urinary recovery of aztreonam (69.3%) was higher (*P < 0.001*) than that of carumonam (51.8%). For rabbits, the urinary recovery of the two antibiotics into the liver was poor, and the urinary recoveries of carumonam (72.8%) and aztreonam (81.0%) were the highest among the species tested. The urinary recoveries of carumonam and aztreonam from humans have been reported to be usually higher than those from animals: 85.1 ± 3.2% for carumonam (13) and 74 ± 3% for aztreonam (11) within 24 h after intravenous administration of 1,000 mg to healthy subjects. The biliary recovery of carumonam (4.1% from rats and less than 1% from rabbits and dogs) was lower than that of aztreonam (19.1% from rats and around 1% from rabbits and dogs); this difference might be related to the differences in the levels of the two antibiotics in the liver. It is also presumed that the poor penetration of carumonam into the hepatobiliary system compensatingly increases its penetration into the kidneys and excretion in the urine.

Though carumonam and aztreonam showed different distribution patterns in the kidneys and liver, their levels in the lungs and spleen were similar regardless of the animal species examined. The pharmacokinetic properties of aztreonam in animals observed in the present report are comparable with those reported previously (1, 6).

No spots other than those of carumonam could be detected in the urine of mice, rats, dogs, and monkeys examined by thin-layer chromatography followed by bioautography. However, high-pressure liquid chromatography revealed that the open-ring β-lactam metabolite of carumonam is formed in the urine of monkeys. The metabolite was also detected in the urine and bile of rats, dogs, and monkeys given aztreonam (6). Thus, the hydrolytic opening of the β-lactam ring seems to be a phenomenon common to these N-sulfonated monocyclic β-lactam antibiotics.

In conclusion, the pharmacokinetic properties of carumonam are generally similar to those of the structurally related aztreonam. The two antibiotics differ slightly in protein binding in serum, in levels in the kidneys and liver, and in urinary and biliary recovery rates. The clinical trials that are being carried out will reveal whether these differences observed in animals are reflected in the clinical effectiveness and the side effects of these two structurally related N-sulfonated monocyclic β-lactam antibiotics.

ACKNOWLEDGMENTS

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LITERATURE CITED


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<thead>
<tr>
<th>Serum source</th>
<th>Carumonam (%)</th>
<th>Aztreonam (%)</th>
</tr>
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<tbody>
<tr>
<td>Mouse</td>
<td>20</td>
<td>84</td>
</tr>
<tr>
<td>Rat</td>
<td>36</td>
<td>85</td>
</tr>
<tr>
<td>Dog</td>
<td>21</td>
<td>55</td>
</tr>
<tr>
<td>Monkey</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Human</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td>HSA*</td>
<td>34</td>
<td>66</td>
</tr>
</tbody>
</table>

* HSA, 4% human serum albumin solution.


