Penetration of Aztreonam into Cerebrospinal Fluid and Brain of Noninfected Rabbits and Rabbits with Experimental Meningitis Caused by Pseudomonas aeruginosa

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This study examined the penetration of aztreonam into the cerebrospinal fluid (CSF) and brain in noninfected rabbits and rabbits with experimental meningitis caused by Pseudomonas aeruginosa. Animals received either 600 or 1,200 mg of aztreonam administered intravenously over 6 h. Aztreonam did not readily enter the CSF in the absence of meningitis. In noninfected animals, mean concentrations in the CSF ranged from 1.1 to 3.0 μg/ml with the 600-mg dose and from 2.3 to 4.7 μg/ml with the 1,200-mg dose. In contrast, mean concentrations of aztreonam in the CSF were significantly higher (P < 0.01) at each sampling time in rabbits with experimental meningitis caused by P. aeruginosa. They ranged from 10.2 to 14.6 μg/ml with the 600-mg dose and from 29 to 40 μg/ml with the 1,200-mg dose. Although concentrations in the brain measured at 6 h tended to be higher in infected rabbits, this difference was not statistically significant. Aztreonam therapy produced a substantial decline in CSF bacterium counts over 6 h: mean CSF counts decreased 2.4 log10 CFU/ml in the 600-mg dose group and 3.0 log10 CFU/ml in the 1,200-mg dose group. The results of this study suggest that aztreonam may be useful in the therapy of meningitis caused by P. aeruginosa.

Aztreonam is a synthetic monocyclic beta-lactam antimicrobial agent which belongs to the recently discovered monobactam family of antibiotics. It resists hydrolysis by the common chromosomally and plasmid-mediated beta-lactamases and demonstrates potent bactericidal activity against most pathogenic aerobic gram-negative bacteria. The activity of aztreonam against Pseudomonas aeruginosa prompted this evaluation of its use in the therapy of experimental pseudomonas meningitis (1, 16). Approximately 80% of clinical isolates are susceptible to concentrations ≤8 μg/ml. Although uncommon, meningitis caused by P. aeruginosa can be a formidable therapeutic problem (3, 6). This study was designed to evaluate the potential of aztreonam in this disease. It focused on the penetration of aztreonam into the cerebrospinal fluid (CSF) and brain in both noninfected rabbits and rabbits with experimental meningitis caused by P. aeruginosa and evaluated the effect of aztreonam therapy on CSF bacterium counts in infected animals.

(Rab.ch model. A total of 27 New Zealand white rabbits weighing 2 kg were studied by the basic methods described by Dacey and Sande (4). Meningitis was induced in 16 rabbits by intracisternal inoculation of 5.25 to 6.84 log10 CFU of P. aeruginosa by the method described by Scheld et al. (13). Five of these animals were not treated with antibiotics and served as controls. Within hours of inoculation, all animals developed acute meningitis. By 17 to 20 h after inoculation, when therapy was initiated, all rabbits manifested lethargy, fever (temperatures of >40°C), CSF leukocytosis (450 to 9,800 cells/mm3; 95% polymorphonuclear leukocytes), and CSF bacterium counts ranging from 4.0 to 8.9 log10 CFU/ml.

On the day of therapy, infected and noninfected rabbits were first anesthetized with 30 to 60 mg of sodium pentobarbital administered via the marginal ear vein. Femoral arterial and venous catheters (Innermedic polyethylene tubing 7420) were then inserted. The arterial catheter, whose patency was maintained with heparinized saline (10 U/ml), was used to obtain blood specimens for antibiotic assays. The venous catheter, whose patency was maintained by a continuous infusion of 0.9% saline (10 to 20 ml/h), was used to administer aztreonam and supplemental doses of sodium pentobarbital (<10 mg/kg per h) to maintain anesthesia throughout the 6-h experimental period. After catheter placement all rabbits were placed in a stereotaxic frame, and a spinal needle (25 gauge by ca. 9 cm) was positioned in the cisterna magna to obtain CSF specimens. All animals then received an intravenous loading dose of aztreonam, which was followed by continuous intravenous infusion for 6 h. Two doses of aztreonam were used for the infected and noninfected rabbits. Animals in the 600-mg dose group received a loading dose of 100 mg followed by a continuous infusion of 83.3 mg/h. Animals in the 1,200-mg dose group received a loading dose of 200 mg followed by a continuous infusion of 166.7 mg/h. During therapy blood specimens were collected at 1, 2, 3, 4, 5, and 6 h, and CSF specimens

MATERIALS AND METHODS

Test organism. A clinical isolate of P. aeruginosa provided by W. Michael Scheld at the University of Virginia was used throughout this study. Its use in the rabbit meningitis model has been described previously (13, 14). The MIC and MBC of aztreonam for this strain, as determined in broth dilution susceptibility tests, was 8 μg/ml.

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were collected at 0, 2, 4, and 6 h. Bacterium counts were determined on CSF specimens from infected animals by serial 10-fold dilutions in 0.9% saline and tryptic soy agar pour plates. The entire rabbit brain was removed at the conclusion of the 6-h treatment period and rinsed once with 0.9% saline. Serum, CSF, and brain specimens were stored at -70°C until aztreonam assays were performed (within 7 days). Aztreonam concentrations were not affected by this means of storage within the 7-day period.

**Assays for aztreonam.** Concentrations of aztreonam in serum, CSF, and brain were determined by an agar well diffusion method with *Escherichia coli* SC12,155 as the indicator organism (10, 15). Assay plates (petri dishes, 100 by 15 mm) were made by adding 0.2 ml of an overnight tryptic soy broth culture of *E. coli* SC12,155 to 100 ml of antibiotic medium no. 1 (BBL Microbiology Systems, Cockeysville, Md.). Both plates and standard solutions of aztreonam were prepared on the day of use. No zone of inhibition was detected on these plates when wells were filled with serum or CSF or with supernatants of brain homogenates from noninfected and infected rabbits. Aztreonam standards for serum assays and serum specimens, when necessary, were diluted in pooled rabbit sera. CSF specimens were usually tested undiluted, but occasionally it was necessary to dilute them in 0.9% saline. Brain specimens were processed by the method described by Beam and Allen (2); aztreonam assays were performed on supernatants of brain homogenate. Aztreonam standard solutions for CSF and brain assays were prepared in 0.9% saline. Preliminary experiments indicated that standards prepared in noninfected and infected rabbit CSF samples and in supernatants from brain homogenates of noninfected and infected rabbits had zone diameters of inhibition that closely approximated (within ±5%) those of standards prepared in 0.9% saline. All serum, CSF, and supernatant specimens were tested in triplicate. Brain concentrations were corrected for blood contamination by the method of Lowry and Hastings (8). These microbiological assays detected aztreonam concentrations of 0.25 µg/ml in serum and CSF and approximately 0.50 µg/g in brain samples.

**Analysis of data.** Differences between experimental variables, i.e., concentration of aztreonam and number of bacteria in the CSF, in the noninfected and meningitis treatment groups at various sampling times were analyzed for statistical significance with the Wilcoxon rank sum test (7).

**RESULTS**

Although concentrations of aztreonam in the serum varied considerably from one animal to another, mean values were generally comparable over the 6-h treatment period in groups treated with the same dose (Table 1). In noninfected rabbits receiving the 600-mg dose, mean concentrations in serum ranged between the 98-µg/ml value measured at 3 h and the 111-µg/ml value measured at 4 h. In animals with experimental pseudomonas meningitis receiving the 600-mg dose, mean serum concentrations tended to be lower, ranging from 63 to 94 µg/ml. In noninfected rabbits that received the 1,200-mg dose, mean serum concentrations ranged between 149 µg/ml, measured at 6 h, and 259 µg/ml, measured at 2 h. Serum concentrations in the corresponding group with experimental meningitis ranged from 146 to 234 µg/ml.

Aztreonam concentrations in the CSF were significantly higher in rabbits with experimental meningitis (Table 2). In the 600-mg dose groups, mean concentrations of aztreonam in the CSF ranged from 1.1 to 3.0 µg/ml in noninfected rabbits and from 10.2 to 14.6 µg/ml in rabbits with experimental meningitis. Differences between these two groups were statistically significant at 2 h (P < 0.01), 4 h (P < 0.01), and 6 h (P < 0.01). In the 1,200-mg dose groups, mean concentrations of aztreonam in the CSF ranged from 2.3 to 4.7 µg/ml in noninfected rabbits, whereas in rabbits with

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**TABLE 1. Concentrations of aztreonam in the serum in the four treatment groups**

<table>
<thead>
<tr>
<th>Dose (mg) and group (no. of rabbits)</th>
<th>Mean concn in serum ± SD (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Noninfected rabbits (6)</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>Infected rabbits (6)</td>
<td>63 ± 19</td>
</tr>
<tr>
<td>1,200</td>
<td></td>
</tr>
<tr>
<td>Noninfected rabbits (5)</td>
<td>246 ± 42</td>
</tr>
<tr>
<td>Infected rabbits (5)</td>
<td>176 ± 29</td>
</tr>
</tbody>
</table>

* Serum concentration not determined for one animal in this group.

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**TABLE 2. Concentrations of aztreonam in the CSF and brain in the four treatment groups**

<table>
<thead>
<tr>
<th>Dose (mg) and group (no. of rabbits)</th>
<th>Mean concn ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Noninfected rabbits (6)</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Infected rabbits (6)</td>
<td>10.2 ± 3.1*</td>
</tr>
<tr>
<td>1,200</td>
<td></td>
</tr>
<tr>
<td>Noninfected rabbits (5)</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Infected rabbits (5)</td>
<td>29.0 ± 7.8</td>
</tr>
</tbody>
</table>

* Undetectable for two animals in this group.

* Not determined for one animal in this group.

* Undetectable for one animal in this group.
meningitis they ranged from 29.0 to 40.0 μg/ml. Differences between these groups were also highly significant at each sampling time. When the concentration in the CSF at each sampling time was expressed as a percentage of the concurrent concentration in serum, the mean values averaged 1 to 4% for noninfected rabbits and 15 to 30% for infected rabbits.

Concentrations of aztreonam in brain tissue tended to be higher in rabbits with meningitis, but the difference did not achieve statistical significance. In noninfected rabbits treated with 600 mg of aztreonam, the mean brain concentration was 0.8 μg/g; in their counterparts with meningitis it was 1.7 μg/g. With the 1,200-mg dose, the mean was 1.5 μg/g for the noninfected group and 2.6 μg/g for the meningitis group. Brain concentrations varied considerably from one animal to another, and values for the infected and noninfected groups frequently overlapped.

Aztreonam therapy significantly lowered the number of bacteria in the CSF during the 6-h treatment period (Table 3). In untreated rabbits with experimental meningitis caused by P. aeruginosa, mean CSF counts increased from 5.53 to 6.11 log_{10} CFU/ml. In contrast, mean CSF counts in the 600-mg dose group decreased from 5.88 to 3.48 log_{10} CFU/ml. The counts observed in this treatment group differed significantly from those in untreated animals at the 6-h sampling time (P < 0.01). In the 1,200-mg dose group, mean counts decreased from 5.52 to 2.50 CFU/ml. Counts in this group differed significantly from those in the untreated group at 4 h (P < 0.01) and at 6-h (P < 0.01). The number of bacteria in the CSF in the 1,200-mg dose group did not differ significantly from that in the 600-mg dose group at any sampling time.

**DISCUSSION**

Aztreonam, like other beta-lactam antibiotics, does not readily enter the CSF in the absence of meningeal inflammation. In the presence of meningitis, however, aztreonam crosses the blood-CSF barrier more readily, and therapeutic concentrations for susceptible pathogens are achievable. In the present study concentrations of aztreonam in the CSF averaged only 1 to 4% of concurrent concentrations in the serum in noninfected rabbits, whereas in rabbits with experimental meningitis caused by P. aeruginosa, they averaged 15 to 30%. These penetration data for meningitis are similar to those reported for other rabbit models. McCracken et al. reported that mean aztreonam concentrations in the CSF expressed as a percentage of mean serum concentration were 15.4 and 24.9% in rabbits with experimental *Haemophilus influenzae* type b and *Escherichia coli* K1 meningitis, respectively, receiving a continuous infusion of aztreonam for 9 h (9). Similarly, Scheld et al. reported mean penetration values of 22.9% in rabbits with experimental *H. influenzae* and *E. coli* meningitis (12). Results from human studies parallel those obtained in these experimental models. Duma and his associates measured CSF concentrations ranging from 0.14 to 1.97 μg/ml in 25 patients with noninflamed meninges who had received a 2-g intravenous dose of aztreonam 1 to 9 h before CSF collection (5). Peak serum concentrations in these patients ranged from 100 to 216 μg/ml. From these data a penetration value of 1.5% was calculated from the area under the curve values for CSF and serum concentrations. In contrast, these investigators measured CSF concentrations that were fourfold higher in nine patients with inflamed meninges: CSF concentrations ranged from 0.16 to 8.11 μg/ml at 0.9 to 4.3 h after a 2-g intravenous dose of aztreonam. Therefore, both clinical and experimental observations provide a similar description of the penetration of aztreonam into CSF in the presence and absence of meningeal inflammation.

Aztreonam does not readily enter brain parenchyma, even in the presence of inflammation. In the present study mean concentrations in the brain averaged about 1% of concurrent mean concentrations in the serum in noninfected rabbits and about 2% in rabbits with experimental meningitis caused by *P. aeruginosa*. The differences were not statistically significant in either dose group. Of interest, these penetration results mirror those reported in studies on rats with experimental cerebritis caused by *E. coli* (11).

Aztreonam therapy produced a bactericidal effect in rabbits with experimental meningitis caused by *P. aeruginosa*. The number of bacteria in the CSF declined 2 to 3 log_{10} CFU/ml over 6 h in the two aztreonam treatment groups. These results contrast sharply with those achieved with other antibiotics in this experimental model. With the same model, Scheld et al. found that therapy with gentamicin, tobramycin, amikacin, carbenicillin, mezlocillin, or piperacillin for 8 h did not produce a mean reduction in CSF counts exceeding 1 log_{10} CFU/ml despite the presence of antibiotic concentrations in the CSF in excess of the in vitro MIC (13, 14; W. M. Scheld, W. J. Kelly, and M. A. Sande, Program Abstr. 19th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 749, 1979). Ticarcillin therapy for 8 h produced a 1.09 log_{10} CFU/ml mean reduction, and azlocillin produced a 2.1 log_{10} CFU/ml mean reduction in CSF counts. Thus, the bacteriological results obtained with aztreonam in the present study are superior to those obtained by single-agent therapy with other beta-lactam agents or aminoglycoside antibiotics. Moreover, therapy with aztreonam alone yielded bacteriological results comparable to those obtained with azlocillin-tobramycin or azlocillin-amikacin combination therapy in these other studies (14).

In conclusion, the bacteriological response to aztreonam therapy observed in experimental meningitis caused by *P. aeruginosa* coupled with in vitro susceptibility and penetration data suggests that aztreonam may be useful in the treatment of meningitis caused by this organism. Further study appears to be warranted. Since pseudomonas infections are rarely treated with single agents, it is noteworthy that the combination of aztreonam and tobramycin demonstrates synergistic inhibitory and bactericidal activity against some strains of *P. aeruginosa* in vitro (1).

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LITERATURE CITED