Comparison of the Inhibitory and Bactericidal Activity of Aztreonam and Amikacin Against Gram Negative Aerobic Bacilli

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ABSTRACT

Aztreonam has been compared both in vitro and in clinical trials to aminoglycosides in its activity against aerobic gram-negative bacteria. The inhibitory and killing abilities of aztreonam and amikacin were examined against five gram-negative bacillary strains. Time kill analysis was carried out at serum-achievable concentrations (25 μg per ml amikacin, 100 and 200 μg per ml aztreonam) and levels found three to five hours post-infusion (8 μg per ml amikacin and 25 μg per ml aztreonam). Amikacin killed all five strains faster than aztreonam at all the concentrations tested. Regrowth and the presence of persisters were observed in aztreonam-treated cultures. In the presence of amikacin, there was no detectable increase in cell mass, as measured by optical density. However, following aztreonam treatment, bacterial cell mass increased in the first two to three hours before decreasing. Long, filamentous cells were observed in aztreonam-containing cultures. Though amikacin and aztreonam are bactericidal drugs, prolonged bacterial survival, continued cell growth, regrowth, and persisters were observed only in aztreonam-treated cultures.

Introduction

Aztreonam (previously aztreonam and SQ 26,776) is a monocyclic β-lactam (monobactam). Its antibacterial spectrum is similar to that of the aminoglycosides, i.e., activity against gram-negative bacteria but not against anaerobic or gram-positive bacteria. The major indications for both aztreonam and aminoglycoside include treatment of infections caused by susceptible gram-negative bacteria. One desirable and clinical pertinent property of aminoglycosides is the rapid bactericidal effect of the class against susceptible bacteria. In this study, the inhibitory and bactericidal activity of aztreonam and amikacin were examined against five strains of gram-negative aerobic bacteria.
Methods

Five clinical isolates susceptible to aztreonam* and amikacin† were used (table I). Minimal inhibitory concentration (MIC) was determined by an agar dilution method in accordance with the procedure outlined by the National Committee for Clinical Laboratory Standards (NCCLS).6

Bactericidal activity was monitored by time-kill analysis. Exponential-phase cells were inoculated into prewarmed Mueller-Hinton broth‡ to a final inoculum of $5 \times 10^5$ to $5 \times 10^6$ cfu per ml. Freshly prepared antibiotics were added. Cultures were incubated at 35°C. Viability was determined at time intervals by plating samples and sample dilutions onto Mueller-Hinton agar with the spiral plater.§ Plates were incubated for 18 hours at 35°C. At each time point, a sample was removed and fixed in two percent formaldehyde. The cell lengths of bacteria in these samples were measured using an image analysis system.11

The growth curve patterns of neat and antibiotic-containing cultures were also determined using the Advantage System.¶ Two milliliters of each time-kill analysis culture were removed at the time of antibiotic addition and placed into a chamber of a multichamber research cuvette. Biomass in each culture was monitored and the growth curve plotted as optical density readings relative to the initial time point. All studies were performed in duplicate, done at various times.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/ml)</th>
<th>Amikacin</th>
<th>Aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli A20341</td>
<td>2</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae A15730</td>
<td>2</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae A15154</td>
<td>2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens A22243</td>
<td>2</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa A 9843</td>
<td>8</td>
<td>8.00</td>
<td></td>
</tr>
</tbody>
</table>

Results

Kill curves of the five strains are shown in figure 1 (A to E). The 100 and 200 µg per ml aztreonam concentrations represent, respectively, approximate peak serum levels after one g and two g intravenous infusions; 25 µg per ml aztreonam approximates the three to five hours post-infusion concentration.10 Similarly, an approximate peak post-infusion level of 25 µg per ml amikacin and four hour post-infusion concentration of 8 µg per ml amikacin were tested.5

Rapid killing was achieved with amikacin against all five gram-negative bacterial strains. By 0.5 hours of incubation in 25 µg per ml amikacin, there was >99.9 percent kill of all bacterial strains relative to the initial inoculum. At 8 µg per ml amikacin, this same level of killing was obtained by 0.5 hour against Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae, Escherichia coli, and by 1.5 hour against Serratia marcescens.

The bactericidal rate of aztreonam was much slower. The most rapid killing was obtained with Enterobacter cloacae and Escherichia coli, where 99.9 percent killing was achieved by 1.5 to two hours using 100 and 200 µg per ml aztreonam, and by 2.5 to three hours with 25 µg per ml aztreonam. With Serratia marcescens, a 3-log reduction resulted after 2.5
Figure 1. Time-kill studies comparing the response to amikacin and aztreonam of *Escherichia coli* (A), *Klebsiella pneumoniae* (B), *Enterobacter cloacae* (C), *Serratia marcescens* (D), and *Pseudomonas aeruginosa* (E). Antibiotic concentrations used were 25 µg per ml amikacin (○), 8 µg per ml amikacin (□), 200 µg per ml aztreonam (■), 100 µg per ml aztreonam (●), 25 µg per ml aztreonam (▲), and no antibiotic (△).

Hours with 200 µg per ml aztreonam and 4.5 hours with 25 and 100 µg per ml aztreonam. Though this level of *Klebsiella pneumoniae* killing was reached by 1.5 hours with 100 and 200 µg per ml aztreonam, regrowth occurred by six hours of incubation. With *Pseudomonas aeruginosa*, 99.9 percent kill was never achieved with aztreonam treatment. Despite the decrease in viable counts of aztreonam-containing cultures, biomass continued to increase for all five strains during the first two to three hours of aztreonam treatment (figure 2 A to E). Only after two to three hours did cell mass decrease. On the other hand, growth immediately ceased with addition of amikacin; there was no change in cell mass following addition of amikacin.

Long filaments in aztreonam-treated cultures probably account for increased biomass in spite of decreased viability. In table II are listed the average cell length in cultures containing no antibiotic, amikacin, or aztreonam. There was no difference in cell length between amikacin-treated cells and cells from growth control cultures. By three hours of incubation, aztreonam-treated cells reached three to 14 times the length of nontreated cells. Longer filaments were observed in cultures containing lower concentrations of aztreonam.

**Discussion**

Amikacin and aztreonam are both active against a broad spectrum of gram-negative aerobic bacilli. Though both antibiotics are bactericidal, aztreonam showed substantially delayed killing compared to amikacin. In addition, *Pseudomonas aeruginosa* did not decrease more than three logs in the presence of aztreonam and *Klebsiella pneumoniae* regrew within six hours following exposure to aztreonam. The presence of persisters in aztreonam-treated *Pseudomonas aeruginosa* cultures has been reported. The highest aztreonam concentrations used in that study were 2.5 and 5 µg per ml. We observed *Pseudomonas aeruginosa* persisters even at 100 and 200 µg per ml peak concentra-
Figure 2. Growth curves of *Escherichia coli* (A), *Klebsiella pneumoniae* (B), *Enterobacter cloacae* (C), *Serratia marcescens* (D), and *Pseudomonas aeruginosa* (E) in the presence of amikacin and aztreonam. Growth curve lines represent no antibiotic (1), 8 \( \mu \)g per ml amikacin (2), 25 \( \mu \)g per ml amikacin (3), 25 \( \mu \)g per ml aztreonam (4), 100 \( \mu \)g per ml aztreonam (5), and 200 \( \mu \)g per ml aztreonam (6).

Our results agree with previous data reporting rapid killing of *Escherichia coli* by aztreonam.\(^{11}\) However, as with our strains, prolonged survival was also noted for *Klebsiella pneumoniae*, *Serratia marcescens*, and *Proteus mirabilis* in that study and regrowth was also observed in aztreonam-treated *Klebsiella pneumoniae* and *Proteus mirabilis* cultures.\(^{11}\) These *in vitro* observations may reflect the 40 percent failure rate of aztreonam to eradicate *Pseudomonas aeruginosa* *in vivo*.\(^{3,13}\) The results from this study also show that despite the lower aztreonam MIC for three of the five strains tested, amikacin exhibited a faster rate of killing.

Long filaments were observed in aztreonam-treated cultures, these filaments were longer in cultures containing lower concentrations of aztreonam. This filamentous growth will not result in increased viable counts, thus the viable counts shown for the first three hours following exposure to aztreonam yield underestimates of the growth inhibitory activity of aztreonam for these strains. The filamentous cells which result from inhibition of penicillin-binding protein 3 (PBP 3) in *E. coli* or its equivalents in the other strains,\(^{11,12}\) are inhibited from forming septa\(^4\) but continued to be metabolically capable of producing the various endotoxins and exotoxins of these bacteria.

Prolonged bacterial survival, continued growth of cells, regrowth, and the presence of persisters observed in aztreonam-containing cultures are not observed with amikacin-treated cells. As a general rule, bactericidal antibiotics are desired in the treatment of serious and deep-seated infections. Whether the delayed killing by aztreonam observed *in vitro* is clinically significant awaits results from more extensive experience with aztreonam in serious infections.

Careful usage of aminoglycosides such as amikacin should continue to be important in therapy of severe infections due to gram-negative aerobic bacilli. When used in combination with \( \beta \)-lactams in treatment of *Pseudomonas aeruginosa*, staphylococcal and enterococcal
ACTIVITY OF AZTREONAM AND AMIKACIN

**Average Cell Length Measured at Three Hours of Incubation in Cultures Containing No Antibiotics, Amikacin, or Aztreonam**

**TABLE II**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No Antibiotic</th>
<th>Amikacin 8 µg/ml</th>
<th>Amikacin 25 µg/ml</th>
<th>Aztreonam 25 µg/ml</th>
<th>Aztreonam 100 µg/ml</th>
<th>Aztreonam 200 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.9</td>
<td>3.6</td>
<td>3.7</td>
<td>14.4</td>
<td>13.6</td>
<td>9.4</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>3.9</td>
<td>3.6</td>
<td>4.3</td>
<td>22.7</td>
<td>14.7</td>
<td>11.5</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>3.5</td>
<td>4.8</td>
<td>3.4</td>
<td>34.1</td>
<td>16.1</td>
<td>22.8</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>2.7</td>
<td>2.8</td>
<td>3.4</td>
<td>38.6</td>
<td>18.5</td>
<td>17.2</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3.0</td>
<td>2.4</td>
<td>1.1</td>
<td>40.2</td>
<td>35.8</td>
<td>27.7</td>
</tr>
</tbody>
</table>

* The average cell length is estimated from measurements taken from approximately 30 cells.

Infections they offer the benefits of dual therapy and avoid the controversy of dual β-lactam therapy.9,14,15

**References**


