In-vitro effects of a combination of antipseudomonal antibiotics against multi-drug resistant *Pseudomonas aeruginosa*

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We evaluated the in-vitro effects of various combinations of five types of widely used antipseudomonal antibiotics (piperacillin, meropenem, ceftazidime, aztreonam and amikacin) against six *Pseudomonas aeruginosa* strains that were resistant to each of these antibiotics. Among two-drug combinations, the combinations of two \(\beta\)-lactam antibiotics inhibited growth of one to three *P. aeruginosa* strains, while those of one \(\beta\)-lactam antibiotic and amikacin inhibited growth of two to four strains. Among three-drug combinations, the combinations of three \(\beta\)-lactam antibiotics inhibited growth of four to five strains, and those of two \(\beta\)-lactam antibiotics and amikacin inhibited growth of five strains. These results suggest the potential usefulness of a combination of two \(\beta\)-lactam antibiotics and amikacin or that of three \(\beta\)-lactam antibiotics in treating multi-drug resistant *P. aeruginosa* infections.

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The antibiotic concentration was set as follows: piperacillin, 64 mg/L; meropenem, 8 mg/L; ceftazidime, 16 mg/L; aztreonam, 16 mg/L; amikacin, 4 mg/L. The presence or absence of growth on Sensitivity Disc Agar-N was determined by a method similar to that used for the measurement of MIC.

Data analysis

The effects against the six P. aeruginosa strains were compared (between the combinations of two drugs and those of three drugs, and between the combinations of three drugs not including amikacin and three drugs including amikacin) by the chi-square test, using the number of growth-inhibited strains as a variable.

Results

A total number of approximately 7000 P. aeruginosa strains were isolated from patients over a 3 year period at the five hospitals that were requested to provide multi-drug resistant P. aeruginosa isolates. The isolation incidence of multi-drug resistant P. aeruginosa strains that were resistant to all five of the drugs (piperacillin, meropenem, ceftazidime, aztreonam and amikacin) was approximately 0.08% (6/7000).

The Table shows the MICs of the five types of drugs used for the six multi-drug resistant P. aeruginosa strains, and the presence or absence of growth inhibition by the combination of two or three drugs among the five drugs. P. aeruginosa Nos 1 and 3 were obtained from patients treated in the same hospital, but four other isolates were obtained, respectively, from patients treated in four different hospitals. The combinations of two β-lactam antibiotics inhibited growth of one to three strains. The combinations of one β-lactam antibiotic and amikacin inhibited growth of two to four strains. Among the combinations of three β-lactam antibiotics, piperacillin + meropenem + ceftazidime and piperacillin + meropenem + aztreonam inhibited growth of four strains, while piperacillin + ceftazidime + aztreonam and meropenem + ceftazidime + aztreonam inhibited growth of five strains. The combinations of two β-lactam drugs and amikacin also inhibited growth of five strains.

Statistical comparison of the combination effects of antibiotics against the strains used showed a significant difference in the number of growth-inhibited strains between the combinations of two drugs and those of three drugs (P < 0.01). However, no difference was observed between the combinations of three drugs not including amikacin and those of three drugs including amikacin.

Discussion

The isolation incidence of multi-drug resistant P. aeruginosa strains may still be low in Japan. However, since multi-drug resistant P. aeruginosa strains that are resistant to all widely used antipseudomonal antibiotics have appeared, the establishment of treatment methods for P. aeruginosa infection is of major importance. The studies of antibiotic combinations should be carried out now rather than when it is too late. Breakpoints used for all antibiotics tested except amikacin were according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria. However, in Japan, the administration dose of amikacin is 200–400 mg/day (in one to two divided doses) in adults, which is lower than that used in Western countries. Therefore, our own criteria were used for amikacin.

In this study, the combinations of two β-lactam antibiotics and amikacin were moderately effective against multi-drug resistant P. aeruginosa. Roussel-D elvallez et al., who performed a 24 h time–kill study, also showed potentiation effects of a three-drug combination of imipenem + (ticarcillin + clavulanic acid) + amikacin on multi-drug resistant P. aeruginosa. Therefore, the combination of two β-lactam antibiotics and amikacin is worth considering for multi-drug resistant P. aeruginosa infection. In some patients receiving nephrotoxic drugs such as amphotericin B, cisplatin or cyclosporin, since administration of amikacin is inappropriate, piperacillin + ceftazidime + aztreonam or meropenem + ceftazidime + aztreonam may be effective.

In this study, the three-drug combinations of two β-lactam antibiotics and amikacin was ineffective against one of the six multi-drug resistant P. aeruginosa isolates. Even when the amikacin concentration was increased from 4 to 32 mg/L, these three-drug combinations were ineffective against this strain (data not shown). A future question may be what combinations of antibiotics should be used for such multi-drug resistant P. aeruginosa strains?

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References

Table. MICs (mg/L) of five antipseudomonal antibiotics and inhibitory effects of these combinations against six strains of multi-drug resistant P. aeruginosa

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>P. aeruginosa N.o.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MICs (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>64</td>
<td>32</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>64</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>32</td>
<td>128</td>
<td></td>
</tr>
</tbody>
</table>

Growth after exposure of
- Piperacillin + meropenem: _b_ + + +
- Piperacillin + ceftazidime: + + + + +
- Piperacillin + aztreonam: + + + + +
- Meropenem + ceftazidime: + - + + +
- Meropenem + aztreonam: + - + + +
- Ceftazidime + aztreonam: + - + + +
- Piperacillin + amikacin: - + + + +
- Meropenem + amikacin: + - - + +
- Ceftazidime + amikacin: - - - + +
- Aztreonam + amikacin: + - - + +
- Piperacillin + meropenem + ceftazidime: - - - + +
- Piperacillin + meropenem + aztreonam: - - - - +
- Piperacillin + ceftazidime + aztreonam: - - - - -
- Meropenem + ceftazidime + aztreonam: - - - - -
- Piperacillin + meropenem + amikacin: - - - - -
- Piperacillin + ceftazidime + amikacin: - - - - -
- Piperacillin + aztreonam + amikacin: - - - - -
- Meropenem + aztreonam + amikacin: - - - - -
- Meropenem + aztreonam + amikacin: - - - - -

*a*Piperacillin, 64 mg/L; meropenem, 8 mg/L; ceftazidime, 16 mg/L; aztreonam, 16 mg/L; amikacin, 4 mg/L.
*b*Absence of growth.
*c*Presence of growth.


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