In Vitro Activity of Gentamicin and Minocycline Alone and in Combination Against Bacteria Associated with Intra-Abdominal Sepsis

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The minimal inhibitory concentrations of gentamicin and minocycline alone and in combination were determined by a broth microdilution method for 100 aerobic, facultative, and anaerobic isolates representative of pathogens recovered from patients with intra-abdominal sepsis. Gentamicin inhibited all strains of Klebsiella, Enterobacter, and Pseudomonas aeruginosa in concentrations of 0.4 to 3.1 μg/ml and all strains of Escherichia coli and Proteus mirabilis in concentrations of 0.8 to 12.5 μg/ml. Whereas minocycline did not consistently inhibit these organisms in concentrations of 1.6 μg or less/ml, it did act synergistically with gentamicin against 43% of the Enterobacteriaceae tested in clinically achievable concentrations; significant synergy was most common with E. coli (60%). Minocycline inhibited 62% of Bacteroides fragilis, 71% of Clostridium, 40% of anaerobic cocci, and 40% of enterococci tested in concentrations of 1.6 μg or less/ml. Whereas gentamicin rarely inhibited these organisms in concentrations of 6.2 μg or less/ml, it did act synergistically with minocycline against 20% of B. fragilis, 67% of Clostridium, 22% of anaerobic cocci, and 22% of enterococci (which had minimal inhibitory concentrations of minocycline within the range tested) at clinically achievable concentrations. Although only four (13%) of the 30 isolates resistant to both gentamicin and minocycline alone were inhibited by clinically achievable concentrations of the combination, the observed synergy, particularly against strains of E. coli, was considered to be of potential clinical usefulness. Antagonism between gentamicin and minocycline was not observed at the concentrations tested.

Intra-abdominal sepsis associated with gastrointestinal or gynecological disease is usually mixed in etiology. The most frequently isolated organisms include gram-negative aerobic and facultative bacilli, enterococci, and anaerobes (3). Gentamicin has a broad spectrum of activity against gram-negative aerobic and facultative bacilli. Minocycline is active against some of the same organisms, some strains of enterococci, and many anaerobic bacteria (R. J. Fass, D. E. Ruiz, W. G. Gardner, and C. A. Rotlie, Arch. Intern. Med., in press). Because the two antibiotics have complementary spectra of activity, which together include many of the bacterial species associated with intra-abdominal sepsis, they might be used in combination to treat such mixed infections.

The present study was designed to determine the in vitro activity of gentamicin and minocycline alone and in combination against a variety of aerobic, facultative, and anaerobic bacterial pathogens commonly associated with mixed intra-abdominal sepsis.

MATERIALS AND METHODS

Bacteria. One hundred clinical isolates from patients in University Hospitals, Columbus, Ohio, were studied. They included: 20 strains of Escherichia coli, 5 strains of Klebsiella, 5 strains of Enterobacter, 5 strains of Proteus mirabilis, 5 strains of Pseudomonas aeruginosa, 10 strains of enterococcus, 26 strains of Bacteroides fragilis, 14 strains of Clostridium (including 6 strains of C. perfringens and 8 strains of other Clostridium species), and 10 strains of anaerobic cocci (including 4 strains of Peptococcus, 4 strains of Peptostreptococcus, and 2 strains of Veillonella). The isolates tested had known susceptibility patterns prior to their selection, and a disproportionate number of minocycline-resistant strains were included to obtain sufficient data on this group of organisms (Table 1).

Antibiotics. Commercially available laboratory standards were dissolved in sterile distilled water. Working solutions with concentrations of 100 and 50 μg of gentamicin and 50 and 25 μg of minocycline per ml were stored at −20 C until used.

Susceptibility tests. Minimal inhibitory concentrations (MICs) of gentamicin and minocycline alone and in combinations were determined using a
broth microdilution method (2). Serial twofold dilutions of each antibiotic were made in duplicate to test each antibiotic alone and in a checkerboard fashion so that 121 different combinations of concentrations ranging from 25 to 0.02 μg/ml for gentamicin and 12.5 to 0.01 μg/ml for minocycline were tested. For gentamicin, MICs of 6.2 μg or less/ml were considered to signify susceptibility. For minocycline, MICs of 1.6 μg or less/ml were considered to signify susceptibility. The combined action of gentamicin and minocycline was considered synergistic when the MIC of each drug in combination was one-fourth or less of the MIC of each respective drug when tested alone. Synergy was considered to be of potential clinical significance only if the MICs of gentamicin and minocycline in combination were 6.2 μg or less and 1.6 μg or less/ml, respectively. The combination was considered antagonistic when the MIC of each drug in combination was four or more times the MIC of each respective drug when tested alone. When MICs of each drug in combination were not both at least fourfold different from MICs when tested alone, the combination was considered to be indifferent, since twofold (one concentration) variations of MICs are commonly observed with serial dilution tests.

**RESULTS**

The in vitro susceptibilities of the 100 clinical isolates tested to gentamicin and minocycline alone and in combinations are shown in Fig. 1 and 2. Gentamicin inhibited all strains of *Klebsiella, Enterobacter*, and *P. aeruginosa* in concentrations of 3.1 μg or less/ml and 80% of *E. coli* were considered to be susceptible. For minocycline, MICs of 1.6 μg or less/ml were considered to signify susceptibility. For minocycline, MICs of 1.6 μg or less/ml were considered to signify susceptibility. The combined action of gentamicin and minocycline was considered synergistic when the MIC of each drug in combination was one-fourth or less of the MIC of each respective drug when tested alone. Synergy was considered to be of potential clinical significance only if the MICs of gentamicin and minocycline in combination were 6.2 μg or less and 1.6 μg or less/ml, respectively. The combination was considered antagonistic when the MIC of each drug in combination was four or more times the MIC of each respective drug when tested alone. When MICs of each drug in combination were not both at least fourfold different from MICs when tested alone, the combination was considered to be indifferent, since twofold (one concentration) variations of MICs are commonly observed with serial dilution tests.

**TABLE 1. Percentage of clinical isolates from University Hospitals** and of isolates selected for the present study that were susceptible to gentamicin (MIC, 6.2 μg or less/ml) and minocycline (MIC, 1.6 μg or less/ml)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Gentamicin</th>
<th>Minocycline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>University Hospitals</td>
<td>Present study</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>87</td>
<td>85</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>89</td>
<td>60</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Enterococci</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. fragilis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Anaerobic cocci</td>
<td>24</td>
<td>20</td>
</tr>
</tbody>
</table>

* Fass et al., in press.

**FIG. 1. Susceptibility of Enterobacteriaceae and *P. aeruginosa* to gentamicin and minocycline alone and in combinations. Symbols: ●, gentamicin and minocycline alone; □, nonsynergistic combinations; □, synergistic combinations. MICs of gentamicin that were 6.2 μg or less/ml and MICs of minocycline that were 1.6 μg or less/ml were considered to indicate susceptibility.
coli and P. mirabilis in concentrations of 6.2 μg or less/ml; enterococci and anaerobes were usually more resistant. Minocycline inhibited 60% of anaerobes, 50% of E. coli, and 40% of enterococci in concentrations of 1.6 μg or less/ml; Klebsiella, Enterobacter, P. mirabilis, and P. aeruginosa were usually resistant to those concentrations. Most isolates were either susceptible to only one drug or to neither drug; only 13 isolates, including 10 strains of E. coli and 1 strain each of Klebsiella, Clostridium, and Peptostreptococcus, were susceptible to both drugs.

The presence or absence of synergy could not be determined for 1 strain of enterococcus, 11 strains of B. fragilis, 8 strains of Clostridium, and 1 strain of Peptostreptococcus, which had MICs of minocycline that were 0.02 μg or less/ml because the lowest concentration tested was 0.01 μg/ml. Among the other strains tested, the combination was synergistic for 24 (69%) of the 35 Enterobacteriaceae, none of the 5 P. aeruginosa, 2 (22%) of the 9 enterococci, 3 (20%) of the 15 B. fragilis, 4 (67%) of the 6 Clostridium, and 2 (22%) of the 9 anaerobic cocci. Synergy was considered to be of potential clinical significance for 12 (60%) of the E. coli, 3 (30%) of the Klebsiella and Enterobacter, 1 (11%) of the enterococci, 2 (13%) of the B. fragilis, 4 (67%) of the Clostridium, and 2 (22%) of the anaerobic cocci. There was no evidence of antagonism within the range of concentrations tested.

**DISCUSSION**

In the present study 88% of the Enterobacteriaceae and P. aeruginosa, 40% of the enterococci, and 62% of the anaerobes tested were susceptible to gentamicin or minocycline or both. Among the 70 susceptible isolates, 21 (primarily B. fragilis and Clostridium) were inhibited by 0.02 μg or less of minocycline per ml, and the presence or absence of synergy could not be determined. Significant synergy was demonstrated for 20 (41%) of the remaining susceptible isolates and for 4 (13%) of the 30 resistant isolates. Significant synergy was most commonly observed among strains of E. coli and Clostridium; two isolates of each were resistant to both antibiotics alone but susceptible to the combination. There were no instances of antagonism.

On the basis of the in vitro data, the treatment of mixed intra-abdominal sepsis with gentamicin and minocycline would seem reasona-
Minocycline is active against a higher percentage of anaerobes, particularly *B. fragilis*, than is tetracycline (1, 4–6; Fass et al., in press) and, in the present study, acted synergistically with gentamicin against a number of strains tested, particularly *E. coli* and *Clostridium*. Since *E. coli* is the most common gram-negative facultative pathogen isolated from patients with intra-abdominal sepsis and is not consistently susceptible to the concentrations of gentamicin achieved after recommended dosage (Fass et al., in press), this synergy could be of considerable clinical significance. The combination of gentamicin and minocycline would not be expected to be ideal, however, since a significant number of organisms, particularly enterococci, *B. fragilis*, and anaerobic cocci, which were resistant to both drugs alone, were usually resistant to the combination as well.

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**LITERATURE CITED**