Postantibiotic Effects and Bactericidal Activities of Clarithromycin–14-Hydroxy-Clarithromycin, versus Those of Amoxicillin-Clavulanate, against Anaerobes

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The bactericidal activities and postantibiotic effects (PAE) of clarithromycin–14-hydroxy-clarithromycin and amoxicillin-clavulanate against Bacteroides fragilis and Peptostreptococcus anaerobius were determined. A concentration of twice the MIC resulted in bactericidal activity against four of four and three of four organisms at 24 h with clarithromycin–14-hydroxy-clarithromycin and amoxicillin-clavulanate, respectively. The PAE of clarithromycin–14-hydroxy-clarithromycin was 1.44 to 3.20 h, compared to the less than 1 h of amoxicillin-clavulanate. Clarithromycin–14-hydroxy-clarithromycin possesses good activity against susceptible anaerobes.

In recent years, interest in macrolides has been renewed due to the extended spectrum of activity of newer agents such as clarithromycin. Unlike that of erythromycin, the metabolism of clarithromycin further enhances its activity by forming an active metabolite, 14-hydroxy-clarithromycin (6, 8; S. J. Martin, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E–59). The 14-hydroxy metabolite has demonstrated in vitro activity similar to that of the parent drug against Bacteroides fragilis and other anaerobes (5). The purpose of this study was to evaluate the bactericidal activity and postantibiotic effect (PAE) of the combination of clarithromycin and its 14-hydroxy metabolite against common anaerobic pathogens. Amoxicillin-clavulanate was used as a control agent due to its excellent activity against anaerobes.

Clarithromycin and 14-hydroxy-clarithromycin (Abbott Laboratories, North Chicago, Ill.) were prepared in accordance with the manufacturer’s recommendations. The macrolides were combined in a 3:1 ratio of parent to metabolite for susceptibility, time-kill, and PAE testing. Amoxicillin and clavulanate (United States Pharmacopeia, Rockville, Md.) were prepared as described by the National Committee for Clinical Laboratory Standards (9).

Two clinical strains each of B. fragilis (BF 2800 and BF 3181) and Peptostreptococcus anaerobius (PA 2612 and PA 3871) were tested. Each bacterial suspension at log-phase growth was diluted in prereduced Wilkins-Chalgren broth (Oxoid-Unipath, Ogdensburg, N.Y.) to obtain a final inoculum of 3.105 CFU/ml for MIC and time-kill studies. The broth was incubated for 1 h on a shaking platform in a 35°C anaerobic incubator. At the end of the 1-h exposure period, the antibiotics were removed by repeated washing (three times). Viable counts were performed at this time and every hour thereafter until the broth became cloudy. PAE was defined as the time required for the count to increase 3-log10 above the count observed immediately after drug removal and completion of the same procedure used on the test culture for drug removal, respectively. The Mann-Whitney test

<table>
<thead>
<tr>
<th>Strain</th>
<th>MICs (µg/ml) of:</th>
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<tbody>
<tr>
<td></td>
<td>Amoxicillin-clavulanate</td>
</tr>
<tr>
<td><em>B. fragilis BF 2800</em></td>
<td>0.35/0.117</td>
</tr>
<tr>
<td><em>B. fragilis BF 3181</em></td>
<td>1.5/0.5</td>
</tr>
<tr>
<td><em>P. anaerobius PA 2612</em></td>
<td>0.38/0.13</td>
</tr>
<tr>
<td><em>P. anaerobius PA 3871</em></td>
<td>3/1</td>
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</tbody>
</table>

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TABLE 2. PAEs of amoxicillin-clavulanate and clarithromycin–14-hydroxy-clarithromycin against anaerobes

<table>
<thead>
<tr>
<th>Organism</th>
<th>PAE (h) of:</th>
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<tbody>
<tr>
<td></td>
<td>Amoxicillin-clavulanate</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>0.03–0.39</td>
</tr>
<tr>
<td>P. anaerobius</td>
<td>0.56–1.00</td>
</tr>
</tbody>
</table>

a Statistically significantly different at the 5% level using Mann-Whitney tests comparing PAEs obtained with amoxicillin-clavulanate and clarithromycin–14-hydroxy-clarithromycin.

was used to compare significant differences in duration of PAE, with a P value of 0.05 considered significant.

The MIC results for the four anaerobes are presented in Table 1. The B. fragilis and P. anaerobius strains were susceptible to both antibiotics. The PAE data are presented in Table 2. The PAE of clarithromycin–14-hydroxy-clarithromycin was longer than that of amoxicillin-clavulanate against all four organisms. Since the killing curves obtained were similar for all four anaerobes, a representative time-kill curve of strain BF 3181 is presented in Fig. 1. A clarithromycin-metabolite concentration equal to the MIC was bacteriostatic for all of the organisms, while a concentration of twice the MIC was bactericidal. Unlike the macrolide combination, amoxicillin-clavulanate demonstrated bactericidal activity against the B. fragilis strains at both the MIC and twice the MIC. One strain of P. anaerobius (PA 3871) was more resistant to amoxicillin-clavulanate than were the other anaerobes. For this isolate, a concentration of twice the MIC did not result in a reduction in viable counts after 24 h of incubation. Amoxicillin-clavulanate was bactericidal at twice the MIC against the other P. anaerobius strain (PA 2612).

Macrolides are assumed to have minimal activity against anaerobic organisms. However, the high reported MIC for 90% of the strains tested may be a reflection of limitations dictated by in vitro conditions. Several studies have reported that acidic medium created by a CO2-enriched environment results in elevated MICs of erythromycin, clarithromycin, or azithromycin (2–4, 10, 11). The anaerobic conditions (5% hydrogen, 5% carbon dioxide, 90% nitrogen) used in this study lowered the pH of the Wilkins-Chalgren broth used from 7.12 to 6.76 after 24 h of incubation. Although these four anaerobic strains were still susceptible to both antibiotics under these conditions, an anaerobic environment may falsely underestimate the antianaerobic activity of macrolides.

Visual inspection reveals that the rate of killing of the two B. fragilis strains was faster with amoxicillin-clavulanate than with the macrolides. However, both antibiotics demonstrated complete killing at 24 h when tested at a concentration of twice the MIC. Clarithromycin–14-hydroxy-clarithromycin showed good kill kinetics against both strains of P. anaerobius. However, amoxicillin-clavulanate was only bacteriostatic against one of the Peptostreptococcus strains (PA 3871). Additional testing with this isolate revealed MBCs of 16 and 5.3 μg/ml, which explains the lack of bactericidal activity at the highest concentrations tested (6/2 μg/ml).

A PAE of approximately 1.5 to 3 h was observed for clarithromycin–14-hydroxy-clarithromycin against the B. fragilis and P. anaerobius isolates. A search of the literature revealed no published reports on the PAE of macrolides against anaerobes. The presence of a PAE with clarithromycin and its metabolite may be clinically important when the drug concentration between doses decreases below the MIC of the antibiotic for these pathogens. The PAE of amoxicillin-clavulanate was less than 1 h against the four anaerobic strains, which is in agreement with the results obtained by other investigators (W. Craig and H. Mattie; Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 147). Our data indicate that clarithromycin–14-hydroxy-clarithromycin possesses good bactericidal activity against susceptible anaerobes. Further in vitro and in vivo studies are needed to further define the role of the newer macrolides in the treatment of antianaerobic or polymicrobial (aerobic-anaerobic) infections.

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REFERENCES