In Vitro Synergy Testing of Clarithromycin and 14-Hydroxyclarithromycin with Amoxicillin or Bismuth Subsalicylate against *Helicobacter pylori*

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The activity of clarithromycin–14-hydroxyclarithromycin (2:1 ratio) and bismuth subsalicylate or amoxicillin against *Helicobacter pylori* was determined by the checkerboard technique in vitro. Clarithromycin–14-hydroxyclarithromycin and amoxicillin resulted in additive effects in 7 of 22 isolates, compared to 14 of 22 isolates when bismuth subsalicylate was substituted for amoxicillin. Synergy was not demonstrated and is probably not responsible for the clinical success of treatment combinations containing clarithromycin.

After a more than a decade of research and controversy, it has been determined that *Helicobacter pylori* is the main cause of duodenal and gastric ulcers and that infected patients with ulcers should be treated with antimicrobial regimens (9, 11). Only limited combinations of antimicrobials achieve eradication rates greater than 90%. The reason for success of certain regimens but not others is not completely understood, but synergism between certain agents may contribute to increased eradication rates. In vitro studies have shown bismuth subsalicylate to be synergistic with multiple antimicrobial agents (12). Clarithromycin has demonstrated additive effects in vitro with amoxicillin, erythromycin, metronidazole, and omeprazole (1).

We examined the in vitro activity of clarithromycin–14-hydroxyclarithromycin (2:1 ratio) and amoxicillin or bismuth subsalicylate to determine if the in vivo success of clinical treatment regimens can be explained by synergism between these antimicrobials.

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Clinical isolates of *H. pylori* were obtained from the Microbiology Laboratory at the University of Illinois Medical Center (Chicago), Abbott Laboratories (Abbott Park, Ill.), and D. Y. Graham (Houston, Tex.). One American Type Culture Collection (Rockville, Md.) control strain was used (ATCC 43504). The organisms were identified as *H. pylori* by Gram staining and a positive urease test. The isolates were held at 70°C in skim milk (Remel, Lenexa, Kans.) and 17% glycerol (Fisher Scientific, Fairlawn, N.J.) and subcultured once after 4 days to ensure reliable growth.

Antimicrobials were obtained as powders and prepared in solution form on the day of use. Clarithromycin and 14-hydroxyclarithromycin were provided by Abbott Laboratories, and bismuth subsalicylate was provided by Proctor and Gamble (Greenville, S.C.). Amoxicillin was purchased from the United States Pharmacopeia (Rockville, Md.). Clarithromycin and 14-hydroxyclarithromycin powder were dissolved in methanol and diluted in phosphate buffer (pH 6.5). Sterile water was used as a solvent and diluent for bismuth subsalicylate, and phosphate buffer (pH 6.0) was both the solvent and the diluent for amoxicillin. Clarithromycin and 14-hydroxyclarithromycin were prepared separately in serial dilutions and then combined in a 2:1 concentration ratio. Molten medium (50°C) was added to the antimicrobial solution(s), the tubes were inverted three times, and the mixture was poured into sterile petri plates.

The organisms were grown up on 5% sheep blood agar plates (Remel) and incubated at 37°C in a 10% CO₂ atmosphere for 4 days. The medium for MIC determinations and checkerboard titration was Mueller-Hinton (Difco, Detroit, Mich.) supplemented with 10% sterile defibrinated horse blood (Remel) at a neutral pH. Control plates, medium without antimicrobials, and antimicrobial-containing plates were prepared 1 day prior to testing and kept refrigerated.

The inocula were prepared by suspending organisms in sterile tryptic soy broth (Remel) and adjusting the turbidity to that of a 2.0 McFarland standard (10⁶ CFU/ml by prior colony count of a representative strain). The organisms were inoculated onto the agar plates containing antimicrobial agents with a Steers replicator (Craft Machine Inc., Chester, Pa.), delivering 8 µl per spot for a final inoculum of 10⁴ CFU/spot. Plates were incubated at 37°C in 10% CO₂ and examined for growth after 5 days.

The MIC was defined as the lowest concentration of antibiotic at which no visible growth or only a faint haze occurred (8). MICs were determined for clarithromycin–14-hydroxyclarithromycin, bismuth subsalicylate, and amoxicillin. All procedures were performed in duplicate. Synergy between clarithromycin–14-hydroxyclarithromycin and bismuth subsalicylate or amoxicillin was determined by the agar dilution checkerboard technique. For quantitation of synergism, the fractional inhibitory concentration (FIC) indices for both combinations were calculated as described by Eliopoulos et al. (2). The FIC index was interpreted as follows: ≤0.5, synergy; >0.5 to 1.0, additive effects; >1.0 to 4.0, indifference; and >4.0, antagonism.

The MICs and synergy testing results for clarithromycin–14-hydroxyclarithromycin and amoxicillin or bismuth subsalicylate against *H. pylori* are shown in Tables 1 and 2. The MICs of clarithromycin and 14-hydroxyclarithromycin have been reported to be in the ranges of 0.004 to 0.1 µg/ml (1, 4, 7) and 0.03 to 0.12 µg/ml (1), respectively. Amoxicillin has been reported to have an MIC in the range of 0.004 to 0.25 µg/ml (1, 3), and the MIC of bismuth subsalicylate is between 8 and 128 µg/ml (13). Our MIC results are in general agreement with those obtained by other investigators in experiments conducted at a neutral pH.

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The major metabolite of clarithromycin, in humans, is 14-hydroxyclarithromycin. We have found (unpublished observation) that the MICs of 14-hydroxyclarithromycin are approximately 1 to 2 dilutions above those of clarithromycin for our isolates of *H. pylori*. This is in agreement with the work of Cederbrant et al. (1).

Clarithromycin has been shown to be synergistic with 14-hydroxyclarithromycin against *Haemophilus influenzae* in vitro (5), and partial synergy or additive effects against *Enterococcus faecalis*, staphylococci, and *Legionella* spp. have been observed (6, 10). For *H. pylori*, clarithromycin and 14-hydroxyclarithromycin demonstrated additive effects (1).

In our investigation, 14-hydroxyclarithromycin was added to the parent compound to evaluate activity of the combination against *H. pylori*. Clarithromycin alone plus amoxicillin has previously demonstrated additive effects (1), but by including the active metabolite of clarithromycin we attempted to more accurately simulate in vivo conditions. A fixed 2:1 concentration ratio of clarithromycin to 14-hydroxyclarithromycin, which is similar to the 2:1-to-4:1 ratio which exists in serum (5), was used.

When clarithromycin–14-hydroxyclarithromycin was combined with amoxicillin, additive effects were observed for 7 isolates (32%) and indifference was observed for 15. Substitution of bismuth subsalicylate for amoxicillin resulted in additive effects for 14 isolates (64%) and indifference for 8. Synergy was not observed with either combination.

The National Committee for Clinical Laboratory Standards has not standardized susceptibility testing for *H. pylori*. The agar dilution method has been used in most published studies; however, the testing method and type of medium used are highly variable (1, 4, 7, 13). The agar dilution method on Mueller-Hinton medium supplemented with 10% defibrinated horse blood was found in our laboratory to support reliable and reproducible results.

Our results showed that the clarithromycin–14-hydroxyclarithromycin combination achieved additive effects with bismuth subsalicylate and indifference with amoxicillin for the majority of isolates tested. Therefore, the in vivo efficacy of antimicrobial regimens containing clarithromycin and amoxicillin or bismuth subsalicylate does not appear to be explained by synergistic interactions of these agents.

REFERENCES


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**TABLE 1. Susceptibilities of 22 *H. pylori* strains to amoxicillin, bismuth subsalicylate, and clarithromycin–14-hydroxyclarithromycin**

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>MIC (µg/ml)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>0.125</td>
<td>0.0039–0.25</td>
</tr>
<tr>
<td>Bismuth subsalicylate</td>
<td>64</td>
<td>8–64</td>
</tr>
<tr>
<td>Clarithromycin–14-hydroxyclarithromycin</td>
<td>0.0625/0.03125</td>
<td>0.0156/0.0078–0.125/0.0625</td>
</tr>
</tbody>
</table>

*MIC at which 90% of the isolates are inhibited.*

**TABLE 2. Summary of synergy results for clarithromycin–14-hydroxyclarithromycin with amoxicillin or bismuth subsalicylate against *H. pylori***

<table>
<thead>
<tr>
<th>FIC index</th>
<th>Interpretation</th>
<th>No. (%) of isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-14-HC + A</td>
<td>C-14-HC + BSS</td>
</tr>
<tr>
<td>≤0.5</td>
<td>Synergy</td>
<td>0</td>
</tr>
<tr>
<td>&gt;0.5–0.75</td>
<td>Additive</td>
<td>6 (27)</td>
</tr>
<tr>
<td>&gt;0.75–1.0</td>
<td>Additive</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>&gt;1.0–2.0</td>
<td>Indifference</td>
<td>14 (64)</td>
</tr>
<tr>
<td>&gt;2.0–4.0</td>
<td>Indifference</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>Antagonism</td>
<td>0</td>
</tr>
</tbody>
</table>

* C-14-HC, clarithromycin–14-hydroxyclarithromycin; A, amoxicillin; BSS, bismuth subsalicylate.