Introduction

There now is overwhelming and convincing evidence that *Helicobacter pylori* is of major importance in the pathogenesis of gastric and duodenal ulcers. In addition, infection has been linked to the development of chronic active gastritis, gastric carcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma and possibly non-ulcer dyspepsia (NUD). Eradication of *H. pylori* is a challenge because of the unique niche it occupies in the gastric mucous layer of the stomach.

To date, there has been no consensus on the optimum treatment regimen for *H. pylori* infection. Only limited combinations of antimicrobials have been found to be successful for its eradication and many of these regimens contain metronidazole. Paromomycin, an oral non-absorbable aminoglycoside antibiotic, has activity against various gastrointestinal pathogens, including *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* spp. Attainment of high intra-luminal concentrations in the gastrointestinal tract suggests it is a sensible alternative to study for activity against *H. pylori*.

The in-vitro activities of paromomycin alone or in combination with metronidazole or metronidazole plus hydroxymetronidazole (2:1 ratio) were examined to determine whether activity is enhanced when these antimicrobials are used concurrently.

Materials and methods

Nineteen clinical isolates of *H. pylori* were obtained from the Microbiology Laboratory at the University of Illinois Medical Center (Chicago, IL, USA), Abbott Laboratories (Abbott Park, IL, USA) and Dr D. Y. Graham (Houston, TX, USA). The organisms were identified as *H. pylori* by Gram’s stain appearance and a positive urease test. The isolates were held frozen at –70°C in skim milk (Remel, Lenexa, KS, USA) and 17% glycerol (Fisher Scientific, Fairlawn, NJ, USA), subcultured once on to 5% sheep blood agar plates (Remel) and incubated at 37°C in 10% CO₂ for 4 days. Subculture was repeated once to ensure reliable growth.

Antimicrobials were obtained as powders and prepared as solutions on the day of use according to NCCLS guidelines or manufacturers’ recommendations. Paromomycin (aminosidine sulphate) was provided by Farmitalia Carlo Erba (Milan, Italy), metronidazole by Searle (Skokie, IL, USA) and hydroxymetronidazole by Abbott Laboratories (Abbott Park, IL, USA). The in-vitro activities of paromomycin and metronidazole alone or paromomycin and metronidazole plus hydroxymetronidazole (2:1 ratio) were studied against 19 *Helicobacter pylori* isolates using an in-vitro chequerboard technique. Partial synergy was demonstrated for the majority of isolates (11/19) for both combinations tested. When hydroxymetronidazole was added to the parent compound, the number of metronidazole-sensitive isolates demonstrating synergy increased to 5/12, compared with 1/12 for the combination that did not include the metabolite. In metronidazole-resistant isolates there was a shift from an additive effect to partial synergy for the combination containing hydroxymetronidazole. The in-vitro activity of paromomycin and the synergic effect that is achieved in combination with metronidazole and hydroxymetronidazole render paromomycin suitable for further investigation as a treatment option for *H. pylori* infection.
USA) and hydroxymetronidazole by Rhône-Poulenc Rorer (Alfortville, France). Sterile water was used as a solvent and diluent for paromomycin powder. Metronidazole and hydroxymetronidazole were dissolved in dimethylsulphoxide (DMSO) and diluted in sterile water. Metronidazole and hydroxymetronidazole were prepared in serial dilutions as separate solutions and then combined in a 2:1 concentration ratio. Molten medium (50°C) was added to the antimicrobial solution, the tubes were inverted three times and the contents poured into sterile Petri plates.

The medium used for MIC determinations and checkerboard titrations was Mueller–Hinton (Difco, Detroit, MI, USA) supplemented with 10% sterile defibrinated horse blood (Remel) at a neutral pH. Control and antimicrobial-containing plates were prepared 1 day before testing and 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 on to the agar plates containing antimicrobial agents with a Steers replicator (Craft Machine Inc., Chester, PA, USA), delivering 8 µL/spot to give 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.¹ MICs were determined for paromomycin, metronidazole and metronidazole/hydroxymetronidazole. All procedures were performed in duplicate. Isolates were determined to be resistant to metronidazole at an MIC >16 mg/L.

In order to quantitate the degree of synergy, the fractional inhibitory concentration (FIC) indices for both combinations were calculated according to Eliopoulos et al.² The FIC index was interpreted as follows: <0.5, synergy; 0.5–0.75, partial synergy; 0.75–1.0, additive effects; 1.0–4.0, indifference; and >4.0, antagonism.³

### Results

The range of MICs of paromomycin was 0.5–4 mg/L, with an MIC₉₀ of 2 mg/L. Metronidazole MICs were in the range 0.25–128 mg/L, with an MIC₉₀ of 64 mg/L. Seven of the 19 isolates demonstrated metronidazole resistance. The combination of metronidazole/hydroxymetronidazole achieved MICs of 0.25/0.125–64/32 mg/L, with an MIC₉₀ of 32/16 mg/L.

The results of synergy testing of paromomycin and metronidazole or metronidazole/hydroxymetronidazole for H. pylori are shown in Tables I and II.

### Table I. Summary of synergy results for paromomycin with metronidazole or metronidazole/hydroxymetronidazole against metronidazole-sensitive H. pylori

<table>
<thead>
<tr>
<th>FIC index</th>
<th>Interpretation</th>
<th>paromomycin + metronidazole</th>
<th>paromomycin + metronidazole/hydroxymetronidazole (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.5</td>
<td>synergy</td>
<td>1 (8)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>&gt;0.5–0.75</td>
<td>partial synergy</td>
<td>9 (75)</td>
<td>7 (58)</td>
</tr>
<tr>
<td>&gt;0.75–1.0</td>
<td>additive</td>
<td>2 (17)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;1.0–4.0</td>
<td>indifference</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>antagonism</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table II. Summary of synergy results for paromomycin (P) with metronidazole (M) or metronidazole/hydroxymetronidazole (M/HM) against metronidazole-resistant H. pylori

<table>
<thead>
<tr>
<th>FIC index</th>
<th>Interpretation</th>
<th>paromomycin + metronidazole</th>
<th>paromomycin + metronidazole/hydroxymetronidazole (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.5</td>
<td>synergy</td>
<td>2 (28.5)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>&gt;0.5–0.75</td>
<td>partial synergy</td>
<td>2 (28.5)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>&gt;0.75–1.0</td>
<td>additive</td>
<td>3 (43)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;1.0–4.0</td>
<td>indifference</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>antagonism</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussion

The treatment of *H. pylori* infection continues to evolve. Currently available treatment regimens are not always effective and eradication can be influenced by the presence of drug-resistant organisms. In addition, drug regimens are complex and can be associated with toxicity. To establish optimum treatment of this infection, investigation of new therapeutic agents is essential.

In a single published report, the MIC of paromomycin against *H. pylori* was reported to be in the range 0.5–4.0 mg/L. Oral paromomycin is not significantly absorbed and should achieve high concentrations within the gastrointestinal lumen, so exceeding the MIC for the organism at the site of infection. Its antibacterial activity *in vitro* has yet to be evaluated.

Metronidazole plays a key role in many available treatment regimens. The MIC of metronidazole against *H. pylori* has been reported to be in the range 0.25–64 mg/L. Hydroxymetronidazole, the major metabolite of metronidazole in humans, also has antibacterial activity. It has been suggested that in-vitro susceptibility to metronidazole may be underestimated if the active metabolite of metronidazole is not taken into account. For *H. pylori* the activity of hydroxymetronidazole is the same as, or within 1–2 dilutions of the parent compound in broth. We have also found (unpublished observation, J. M. Meyer, S. Ryu, S. L. Pendland, T. P. Kanyok & L. H. Danziger) that the MICs of hydroxymetronidazole are approximately 1–2 dilutions below the parent compound when tested against our isolates of *H. pylori* on solid media.

To determine more accurately the contribution of the metabolite to the activity of the parent compound for *H. pylori*, we studied metronidazole independently and in combination with hydroxymetronidazole. To simulate in-vivo conditions, a fixed 2:1 concentration ratio of metronidazole to hydroxymetronidazole was used, which is similar to the ratio that exists in serum after a single 500 mg oral dose.

Synergetic interactions between antimicrobials may contribute to the efficacy of selected treatment regimens. Synergy has been shown between metronidazole and bismuth subcitrate, amoxycillin and tetracycline. Hydroxymetronidazole alone has demonstrated synergy with metronidazole, amoxycillin and tetracycline. There have been no published reports of synergy studies using metronidazole and hydroxymetronidazole together with other compounds. We have previously studied clarithromycin in combination with its active metabolite, in an attempt to characterize the contribution of 14-hydroxyclarithromycin to synergy and the efficacy of treatment regimens containing clarithromycin.

In this investigation, partial synergy was demonstrated for the majority (11/19) of isolates when the combination of paromomycin and metronidazole alone or metronidazole plus hydroxymetronidazole was tested. When metronidazole was combined with paromomycin, three of the 19 isolates (16%) demonstrated synergy. Addition of hydroxymetronidazole to the parent compound increased the number to seven (37%). No antagonism was seen with either combination.

For metronidazole-sensitive strains, only one of 12 isolates demonstrated synergy to the combination of metronidazole and paromomycin. Addition of hydroxymetronidazole to the parent compound increased the number of isolates demonstrating synergy (Table I) to five. When metronidazole resistant strains were tested, there was a trend towards an increased number of isolates demonstrating partial synergy when the metabolite was added. The small number of isolates studied limits any conclusions from this data.

Methods for performing susceptibility and synergy testing of *H. pylori* have not been standardized. A breakpoint of 16 mg/L for metronidazole was selected based on correlation with clinical failure. The agar dilution method for synergy testing on Mueller–Hinton medium supplemented with 10% defibrinated horse blood is preferred in our laboratory, since it has consistently supported reliable growth and reproducible results. This choice of medium was recently corroborated by other investigators who found it superior to other media in its ability to support growth of *H. pylori* both semiquantitatively and qualitatively.

Paromomycin has been shown in this investigation to demonstrate substantial in-vitro activity against *H. pylori*. It appears to have synergistic activity with metronidazole against both metronidazole sensitive and resistant isolates, enhanced by the addition of hydroxymetronidazole. Paromomycin warrants further investigation as a potential therapeutic agent for treatment of *H. pylori* infection.

Acknowledgement

This work was presented as a poster at the 1997 Annual Meeting of the American College of Clinical Pharmacy (AACP), 9–12 November 1997, Phoenix, Arizona, USA.

References

J. M. Meyer et al.


Received 20 April 1998; returned 15 June 1998; revised 22 July 1998; accepted 13 October 1998