Bactericidal activity and synergy studies of proton pump inhibitors and antibiotics against *Helicobacter pylori* in *vitro*

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The bactericidal activity of the proton pump inhibitors omeprazole and lansoprazole alone and in combination with a \(\beta\)-lactam or macrolide antibiotic were investigated in *vitro*. Time–kill curves against *Helicobacter pylori* NCTC 11637 and a recent clinical isolate revealed significant concentration-dependent killing with all drugs other than amoxicillin. Combinations of proton pump inhibitor and erythromycin showed synergic activity. In contrast, proton pump inhibitor plus amoxicillin showed additive activity against the clinical isolate only. Bactericidal investigations of anti-helicobacter drugs *in vitro* may suggest optimum treatment strategies for this common infectious disease.

**Introduction**

Antimicrobial therapy is now commonly used to cure *Helicobacter pylori* infection. In its niche of gastric mucus, *H. pylori* is relatively protected from host antimicrobial defences. In order to cure this infection, antibiotics need to be bactericidal rather than bacteriostatic and able to kill every organism in the protected site. An analogy between the endocarditis model and *H. pylori* infection has been suggested for these reasons. As well as exhibiting good in-vitro activity against *H. pylori*, therapies need all or some of the following additional properties: good dispersion in the stomach, the ability to penetrate mucus, lack of acquired resistance and stability over a wide range of pH. Although single agents are effective in vitro at killing *H. pylori*, eradication of the organism in patients with single agents is poor. Combination therapy probably attacks the organism through different mechanisms of action producing at least additive or perhaps synergic anti-helicobacter effects. Recent data from Coudron et al.\(^2\) show that ampicillin is bactericidal against logarithmic phase culture of *H. pylori* whereas bismuth is active against stationary phase cultures using time–kill kinetic methods. The use of time–kill curves of *H. pylori* at achievable in-vivo drug concentrations measuring bactericidal activity over time (i.e. pharmacodynamics) may reveal differences in the effects of antimicrobial agents against *H. pylori* despite similar activities as measured by MIC. In this study, a \(\beta\)-lactam, amoxicillin, and a macrolide, erythromycin, were investigated with the proton pump inhibitors (PPIs) omeprazole or lansoprazole for bactericidal and synergic activity against *H. pylori*.

**Materials and methods**

**Bacteria**

Two strains of *H. pylori* were examined: *H. pylori* NCTC 11637 and a recent clinical isolate, M 98.

**MIC determinations**

Omeprazole and lansoprazole MICs were determined by agar dilution. Each drug was dissolved in dimethyl sulphoxide to make a stock solution of 10,000 mg/L. This was subsequently diluted in distilled water and added to Mueller–Hinton agar (BBL, Cockeysville, MD, USA) with 5% horse blood (MHA) to give plates with final concentrations of 1–256 mg/L for each PPI. One microlitre of a 1 MacFarland standard of each *H. pylori* strain in Brain Heart Infusion broth (Oxoid, Basingstoke, UK) was inoculated on to the plates, which had been dried at 35°C for 1 h using a Multipoint replicator (Mast, Liverpool, UK). This gave an organism density of \(7 \times 10^4\) cfu/mL. MHA plates with no drug were inoculated at the beginning and end of each set as growth control plates. All plates were incubated for 72 h at 35°C under microaerophilic conditions (Camp Gas Pak, Oxoid, Basingstoke, UK).

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A moxifloxacin and erythromycin MICs were tested by swabbing the 1 MacFarland broth suspensions of each H. pylori strain on to predried MHA plates. To each plate was placed a single E test strip (A B Biodisk, Solna, Sweden). All plates were incubated as for the agar dilution and the E tests were read according to manufacturer’s guidelines.

**Bactericidal assay**

To investigate possible synergic activity between PPIs and antibiotics, combinations of PPI and antibiotic at 1 × MIC were compared with 1 × MIC of each agent alone with respect to bactericidal activity. A modification of methods of Coudron et al. and NCCLS document M26-T were used. Our previous studies had found that Wilkins-Chalgren anaerobe broth (Oxoid, Basingstoke, UK) supplemented with 0.5% (2,6-di-O-methyl)-β-cyclodextrin (WCC) gave optimal growth of H. pylori in broth culture. Test organisms were subcultured twice in WCC to ensure good growth. Growth was checked by turbidity and viability was checked by examining a drop of broth culture under a phase contrast microscope (×40 objective) for motile bacilli. One millilitre of fresh overnight broth culture was added to 9 mL of WCC medium with and without antibiotics. Serial ten-fold dilutions of these broths were made immediately in phosphate-buffered saline (PBS) to represent the initial inoculum. Four 20 μL drops of each dilution were placed on to a Columbia agar (BBL, Cockeysville, MD, USA) with 5% horse blood (HBA) plate and incubated in a 5% CO₂ incubator at 35°C for 7 days. The inoculated WCC broths were placed in an anaerobic jar with a microaerobic gas generating kit (Camp Gas Pak, Oxoid, UK) and placed on a 120 rpm rotator in a 35°C warm room. At regular intervals (e.g. 2 h, 4 h, 24 h) after inoculation, the WCC broths were serially diluted in PBS and inoculated on to HBA to determine the viable counts as cfu/mL at each time period. Colony counts were performed at several dilutions at each time point but only the dilution giving a readable count of <50 colonies per drop were recorded. The limit of detection for the assay was 13 cfu/mL.

**Discussion**

Dual therapies consisting of a PPI and antibiotic are being used successfully to cure patients of their H. pylori infection. Neither PPI nor a single antibiotic alone seems to be clinically effective. These drugs may act together synergistically or additively to kill H. pylori rapidly. A part from the direct antimicrobial action of PPI/antibiotic combinations, there may be important pharmacokinetic and pharmacodynamic considerations with either or both drugs. Omeprazole has recently been shown to facilitate secretion of clarithromycin into the gastric mucosa and increase its bioavailability. Knowledge of pharmacodynamic characteristics of drugs can improve treatment outcomes. Craig & Ebert stated that if a drug exhibits major concentration-dependent killing, the goal of a dosing regimen would be to maximize drug concentrations. Aministration of larger doses would increase peak drug concentrations and enhance bactericidal activity. If a drug produces more time-dependent killing, the goal of a dosing regimen would be to maximize antimicrobial exposure. High concentrations of such drugs would not kill bacteria any faster, therefore the duration of time for which drug concentrations exceed the MIC would be expected to be the major determinant of efficacy with such drugs. Because proton pump inhibitors when used as monotherapy against H. pylori suppress but do not eradicate the organism, it was thought that these agents were bacteriostatic in nature. Both PPIs were clearly bactericidal in this in vitro study. In this study, concentration-dependent killing of H. pylori was demonstrated with omeprazole, lansopra-
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The most active single agent was erythromycin with a 4 log₁₀ decrease in viable count after 2 h incubation at 5 × MIC or greater (Figure 4). In support of this study macrolides have achieved the highest reported eradication rate to date of any monotherapy. However, the development of resistance to macrolides has been reported in about 20% of patients after treatment, either as monotherapy or in combination with a PPI. A moxycillin did not show concentration-dependent killing although amoxycillin/PPI activity showed strain variation. A clinical trial of PPI/amoxycillin coadministration with increased infrequency of amoxycillin dosing on H. pylori eradication seems warranted. The in-vitro experiments in this study for PPI and amoxycillin alone and in combination showed some strain variation. The type strain NCTC 11637 was killed more slowly with PPI/amoxycillin combinations than with amoxycillin alone, but for the recent clinical isolate there was an additive effect of PPI and amoxycillin on the rate of organism killing. Clinical trials with PPI and amoxycillin have produced eradication rates of up to 80% with a simple and well tolerated regimen which probably improves patient compliance. The major advantage of PPI/amoxycillin dual therapy is the lack of antimicrobial resistance selection and hence treatment can be repeated, although penicillin allergy precludes its use in some individuals. Treatment of H. pylori infection and peptic ulcer disease with PPI/macrolide is now commonly being used with eradication rates of up to 80% in patients with macrolide sensitive strains. Omeprazole 40 mg once daily and clarithromycin 500 mg tid both for 2 weeks is the most common dual therapy. Previous in-vitro studies of macrolide activity have shown that all are active against H. pylori with MICs of between 0.06 mg/L and 1 mg/L and that spontaneous resistance development is rare at <1 × 10⁻⁹ to 1 × 10⁻⁷. Few data exist on the use of erythromycin in combination with other drugs for the treatment of H. pylori infection. An early study by Goodwin et al. showed that erythromycin 500 mg qid for 2 weeks and bismuth subcitrate 120 mg qid for 2 weeks achieved clearance of the organism in 65% of subjects. In this present study, erythromycin with a proton pump inhibitor was rapidly bactericidal for H. pylori in vitro, though a recent study has shown that the combination of lansoprazole with macrolides against H. pylori in vitro is generally additive. The use of erythromycin in a treatment regimen would have a cost advantage to patients being much cheaper than the newer macrolides but is known to be associated with higher side-effect rates. Erythromycin has infrequently been used for H. pylori treatment because of its lability in acid. Since PPIs are very effective antisecretory agents and raise intragastric pH, coadministration of these drugs would protect the erythromycin from acid degradation. Proton pump inhibitors are bactericidal against H. pylori in vitro and have complex mechanisms of action including antibacterial and antisecretory activity. Clinical trials are required to examine the effectiveness of dual therapies such as PPI and erythromycin and of various dosing schedules to maximize the eradication of H. pylori from infected individuals.

Figure 1. Effect of lansoprazole at 1 × MIC, 5 × MIC and 10 × MIC on H. pylori NCTC 11637. Control; lansoprazole 8 mg/L; lansoprazole 40 mg/L; lansoprazole 80 mg/L.
Figure 2. Effect of omeprazole at 1 × MIC, 5 × MIC and 10 × MIC on *H. pylori* NCTC 11637. ◆, Control; ■, omeprazole 16 mg/L; ▲, omeprazole 80 mg/L; ○, omeprazole 160 mg/L.

Figure 3. Effect of amoxycillin at 1 × MIC, 5 × MIC and 10 × MIC on *H. pylori* NCTC 11637. ◆, Control; ■, amoxycillin 0.015 mg/L; ▲, amoxycillin 0.075 mg/L; ○, amoxycillin 0.15 mg/L.
**Figure 4.** Effect of erythromycin at 1 × MIC, 5 × MIC and 10 × MIC on *H. pylori* NCTC 11637. ◆, Control; ■, erythromycin 0.005 mg/L; ▲, erythromycin 0.025 mg/L; ●, erythromycin 0.05 mg/L.

**Figure 5.** Effect of proton pump inhibitor and amoxycillin both at 1 × MIC on *H. pylori* NCTC 11637. ◆, Control; ■, amoxycillin 0.015 mg/L; ▲, omeprazole 16 mg/L; ○, amoxycillin 0.015 mg/L + omeprazole 16 mg/L; ●, amoxycillin 0.015 mg/L + lansoprazole 8 mg/L; □, lansoprazole 8 mg/L.
Figure 6. Effect of proton pump inhibitor and amoxycillin at 1 × MIC against patient M98 H. pylori isolate. ◆, Control; ■, amoxycillin 0.015 mg/L; ▲, amoxycillin 0.015 mg/L + omeprazole 16 mg/L; ○, omeprazole 16 mg/L; □, lansoprazole 8 mg/L; ●, amoxycillin 0.015 mg/L + lansoprazole 8 mg/L.

Figure 7. Effect of proton pump inhibitor and erythromycin both at 1 × MIC on H. pylori NCTC 11637. ◆, Control; ●, erythromycin 0.005 mg/L; ▲, omeprazole 16 mg/L; ■, erythromycin 0.005 mg/L + omeprazole 16 mg/L; ○, erythromycin 0.005 mg/L + lansoprazole 8 mg/L; □, lansoprazole 8 mg/L.
References


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