Omeprazole may exert both a bacteriostatic and a bacteriocidal effect on the growth of *Helicobacter pylori* (NCTC 11637) in vitro by inhibiting bacterial urease activity

F Mirshahi, G Fowler, A Patel, G Shaw

Abstract

**Aims**—To assess the potential antibacterial effect of omeprazole, a benzimidazole proton pump inhibitor, on the growth of *Helicobacter pylori* in vitro and to evaluate the effect of this compound on bacterial urease activity.

**Methods**—The growth of *H pylori* was observed in liquid culture in the presence and absence of omeprazole (0.8 mg/ml). Urease activity was evaluated in aliquots removed from two hour cultures by monitoring the initial change in absorbency at 560 nm in the presence of 0.02% phenol red.

**Results**—The minimum inhibitory concentration of omeprazole against *H pylori* was 0.8 mg/ml. The concentration of omeprazole required to inhibit growth was dependent on inoculum density: omeprazole (0.8 mg/ml) prevented growth from a 1 × 10⁶ cfu/ml inoculum, but not from the higher inocula of 10⁷ or 10⁸ cfu/ml. This is the first study to demonstrate that omeprazole exerts a bacteriocidal effect against low bacterial densities and a bacteriostatic effect when bacterial density is high. When used at the onset of growth, this concentration of omeprazole has a bacteriostatic effect after four hours, although it exerts a bacteriostatic effect when added to cultures after the exponential phase. Bacterial urease activity is competitively inhibited by omeprazole in a dose dependent manner.

**Conclusion**—The results suggest that omeprazole exerts both a bacteriocidal and a bacteriostatic effect against *H pylori* and competitively inhibits bacterial extracellular urease activity.

**Keywords:** *Helicobacter pylori;* urease; omeprazole

Peptic ulcer disease is a multifactorial pathological condition. The aetiological role played by acid hypersecretion and/or a failure of the mucosa to generate sufficient protective factors is well established. Since the discovery of a Gram negative, microaerophilic, curved bacillus in gastric biopsy specimens, there has been speculation about its role as an additional aetiological factor in peptic ulcer disease. This organism, *Campylobacter pylori* (now *Helicobacter pylori*) is now accepted as a major cause of chronic antral gastritis and gastric cancer.

In particular, *H pylori* may be responsible for peptic ulcer relapse.

Numerous strategies exist for the treatment of peptic ulcers. Possibly, the most promising agents are those with a dual mechanism of action. One such agent is the benzimidazole compound, omeprazole, which inhibits acid secretion and which may affect the growth of *H pylori*. Currently available evidence regarding the efficacy of omeprazole as a single agent therapy against *H pylori* is inconclusive. It has been suggested that omeprazole clears the antral mucosa of *H pylori* infection, although this is not accepted universally. A previous study suggested that omeprazole causes temporary suppression rather than true eradication of the organism.

Evidence derived from in vitro susceptibility studies is also inconclusive, suggesting both a bacteriostatic and a bacteriocidal effect for benzimidazole compounds against *H pylori*. Consequently, there is a need to elucidate more fully the nature of the effect of omeprazole on the growth of *H pylori* in vitro. Recent observations have suggested that the potent bacterial urease may represent a potential target for the benzimidazole proton pump inhibitors, and that inhibition of this enzyme may provide one explanation for the antibacterial properties of this group of compounds.

The present work reports our preliminary studies to evaluate the antibacterial properties of omeprazole when added to cultures of *H pylori* type strain NCTC 11637 at different stages of growth, in order to establish a possible bacteriocidal or a bacteriostatic effect of this anti-ulcer agent. Furthermore, we assess the effect of omeprazole on the extracellular urease activity of *H pylori*.

**Methods**

**BACTERIAL STRAINS**

*H pylori* strain NCTC 11637 used throughout this study was obtained from The Public Health Laboratory, Colindale, London, UK.

When required, inocula were derived by harvesting *H pylori* from 72 hour confluent brain-heart infusion agar medium plates in brain-heart infusion liquid media. The strength of the inoculum was determined using a Petroff-Hauser counting chamber with subsequent dilutions in brain-heart liquid media.

**MEDIA**

Brain-heart infusion medium was used throughout this study. Solid medium contained 0.25% (wt/vol) yeast extract, 10% (vol/vol)
Table 1 Effect of omeprazole on the growth of Helicobacter pylori derived from a starting inoculum of 1 × 10^7 cfu/ml

<table>
<thead>
<tr>
<th>Omeprazole concentration (mg/cm^3)</th>
<th>cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>8.9 (1.4) × 10^6</td>
</tr>
<tr>
<td>0.2</td>
<td>6.8 (0.8) × 10^6</td>
</tr>
<tr>
<td>0.4</td>
<td>4.5 (1.1) × 10^6*</td>
</tr>
<tr>
<td>0.8</td>
<td>&lt; 1 × 10^4*</td>
</tr>
<tr>
<td>1.0</td>
<td>&lt; 1 × 10^4*</td>
</tr>
</tbody>
</table>

Helicobacter pylori was cultured at 37°C for five days in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂ on brain-heart infusion agar containing 10% laked horse blood, 0.25% yeast extract, 0.4% supplement SR147E, and a range of omeprazole concentrations.

Values of colony forming units (cfu/ml) are means (SEM) of triplicate determinations.

*Comparison of confidence limits showed there to be a significant difference (p < 0.01) between these values.

Table 2 Effect of bacterial density on the inhibitory effect of omeprazole (0.8 mg/ml) against Helicobacter pylori

<table>
<thead>
<tr>
<th>Inoculum density (cfu/ml)</th>
<th>Solvent alone</th>
<th>Solvent + omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^6</td>
<td>8.7 (1.1) × 10^6</td>
<td>&lt; 1 × 10^4</td>
</tr>
<tr>
<td>1 × 10^7</td>
<td>&gt; 1 × 10^6</td>
<td>2.1 (0.5) × 10^5</td>
</tr>
<tr>
<td>1 × 10^8</td>
<td>&gt; 1 × 10^6</td>
<td>2.5 (3.3) × 10^5</td>
</tr>
</tbody>
</table>

Helicobacter pylori was cultured at 37°C for five days in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂ on brain-heart infusion agar containing 10% laked horse blood, 0.25% yeast extract, 0.4% supplement SR147E, and a range of omeprazole concentrations.

Values of colony forming units (cfu/ml) are means (SEM) of triplicate determinations.

Determined by one way analysis of variance.

EFFECT OF BACTERIAL DENSITY ON THE IN VITRO EFFICACY OF OMEPRAZOLE

A 100 µl aliquot derived from each of the starting inocula (1 × 10^3, 1 × 10^4, and 1 × 10^5 cfu/ml) of omeprazole. Solvent (2% vol/vol DMSO) alone was added to control agar.

Once inoculated, all plates were incubated for five days at 37°C in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂, and viability was determined by colony counts.

THE EFFECT OF OMEPRAZOLE ON THE GROWTH OF H PYLORI

An H pylori inoculum was prepared (~ 1 × 10^6 cfu/ml) in brain-heart infusion broth and 1 ml was added to two sets of triplicate flasks, one set containing 50 ml of the same medium plus omeprazole (0.8 mg/ml, previously shown to be the minimum inhibitory concentration, pH 6.9), and one set of control flasks containing medium and solvent only (total volume 50 ml, pH 6.9). Following inoculation, each flask was incubated at 37°C in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂, with continuous agitation (100 rpm, G25 incubator shaker; New Brunswick Scientific, Edison, New Jersey, USA). Samples (100 µl) were removed from both sets of broth cultures after two and four hours incubation and then at regular 12 hour intervals for five days to establish viability. Additional samples (1 ml) were removed after two hours to determine urease activity and at 36 hour intervals to measure pH. To determine the number of viable bacteria at each time point, serial dilutions of each
aliquot were made in sterile saline (0.9% NaCl) and 100 µl added to the surface of brain-heart infusion agar medium. These plates were incubated for five days at 37 °C in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂ and the growth rate of *H. pylori* in the presence and absence of omeprazole (0.8 mg/ml) was established. Duplicate experiments were performed and, because two way analysis of variance showed no significant difference between experiments, the data are presented as mean (SEM) of six determinations. A significant difference (p < 0.05) between test and control data was determined by one way analysis of variance.

A parallel study was carried out with omeprazole (0.8 mg/ml) added to the test flasks after three days of bacterial growth, that is, after the onset of exponential growth.

**EFFECT OF OMEPRAZOLE ON THE UREASE ACTIVITY OF *H. PYLORI***

Bacterial cells were removed from a two hour liquid culture grown in the presence and absence of omeprazole (0–0.8 mg/ml) and washed twice in phosphate buffer (50 mM, pH 6.8). This washed, whole cell preparation was used as a source of enzyme throughout. A reaction mixture containing 0.02% phenol red and urea (0–6 mM) was prepared in 50 mM phosphate buffer (pH 6.8) and the reaction was started by the addition of 50 µl of the washed, whole cell preparation. Urease activity was determined at 43°C by monitoring the initial change in absorbency at 560 nm against a jack bean urease standard (12 500 µM units/g). One micromolar unit of enzyme activity will liberate 1.0 µmol of ammonium/minute. The protein content of the urease preparation was determined using the method of Lowry and colleagues.¹²

**Results**

**DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION OF OMEPRAZOLE**

In this study, the minimum inhibitory concentration of omeprazole against *H. pylori* was established as 0.8 mg/ml (table 1).

In addition, the concentration of omeprazole required to inhibit growth was shown to be dependent on inoculum density because omeprazole (0.8 mg/ml) prevented growth from a 1 × 10⁶ cfu/ml inoculum, but not from the higher inocula of 10⁷ or 10⁸ cfu/ml (table 2).

**EFFECT OF OMEPRAZOLE ON THE GROWTH OF *H. PYLORI***

Under the standard growth conditions used throughout this study, *H. pylori* typically had a growth rate of −0.14 (0.01) cfu/hour and a cell generation time of 4.8 (0.5) hours. Other workers have reported cell generation times for *H pylori* when grown in supplemented brucella broth of 3.6 hours¹³ and 2.7 hours.¹⁴ Growth kinetics of *H pylori* in the presence and absence of omeprazole were evaluated in brain-heart infusion broth with a relatively small starting inoculum (~ 1 × 10⁶ cfu/ml). Figure 1 shows a representative growth pattern in which there is an immediate onset of exponential growth under control conditions. When grown in the presence of omeprazole (0.8 mg/ml), *H. pylori* has a similar initial growth pattern, although exponential growth was only maintained for four hours before the microbial population quickly declined (fig 1).
When omeprazole was added to the growing culture at 72 hours, a consistent although not significant reduction in growth was observed (fig 2).

**EFFECT OF OMEPRAZOLE ON THE UREASE ACTIVITY OF \textit{H pylori}\**

Under the conditions used in this assay, \textit{H pylori} urease activity exhibits hyperbolic kinetics with respect to urea concentration (fig 3). The rate of urea degradation was proportional to the concentration of urea, up to 0.1 mM (fig 3), and was constant over the incubation period for at least 20 minutes. At all the substrate concentrations tested, the urease activity of bacteria grown in the presence of omeprazole was lower than that found in control bacteria (fig 3). At concentrations of urea above 1 mM, this reduction is significant (p < 0.05). Lineweaver-Burk analysis of the data (fig 4) indicated that control urease activity exhibited a Km of 0.28 mM, which was increased to 2.9 mM in bacteria grown in the presence of omeprazole (0.8 mg/ml). The extent of this inhibition is proportional to omeprazole concentration up to 0.4 mg/ml (fig 5). The Ki value for urea degradation in the presence of omeprazole was calculated to be 0.25 mM.

**Discussion**

By investigating the effect of omeprazole on the growth of \textit{H pylori} NCTC 11637 in vitro, this study was able to test for a direct action of omeprazole on \textit{H pylori}, rather than an antibacterial action mediated by a reduction in acid secretion. In this study, we have established that the effect of omeprazole on \textit{H pylori}\ is concentration dependent. This concentration dependent effect of omeprazole is particularly significant because we cannot be certain of the drug concentration within the gastric/duodenal microenvironment occupied by \textit{H pylori}. Furthermore, we have established the minimum inhibitory concentration of omeprazole against \textit{H pylori} to be 0.8 mg/ml. This result suggests that omeprazole at 0.8 mg/ml is capable of exerting a direct antibacterial action on this strain of \textit{H pylori} in vivo. This effect of omeprazole is not mediated by changes in the pH of the medium, which remained neutral throughout the culture period, thereby suggesting that the drug was not acid activated. \textit{Helicobacter pylori} grows well at this pH because it is adapted to live below the mucous layer, where the pH is also approximately neutral.\textsuperscript{15} \textit{Helicobacter pylori} is known to be capable of replication over the pH range 6.9–8.0.\textsuperscript{16} Other studies\textsuperscript{10, 17} have suggested lower minimum inhibitory concentrations for omeprazole against \textit{H pylori}, although direct comparison is difficult because the bacteriocidal effect of omeprazole in vivo is influenced by both the pH of the medium and the content of fetal calf serum.\textsuperscript{18}

This antibacterial effect of omeprazole is dependent on bacterial density because omeprazole (0.8 mg/ml) fails to reduce growth completely when inocula of $1 \times 10^6$ cfu/ml and above are used. This study suggests that when challenged with a small inoculum ($1 \times 10^6$ cfu/ml or below), omeprazole (0.8 mg/ml) has a bacteriocidal effect on the growth of \textit{H pylori}, which takes effect in four hours. The data presented in this study are supported by other workers,\textsuperscript{16} who have demonstrated a bacteriocidal action of the proton pump inhibitor lansoprazole when used against \textit{H pylori} NCTC 11637 in vitro at a concentration of 0.1 mg/ml.

**Figure 4** Lineweaver-Burk plot. The relation between 1/urease activity and 1/substrate concentration. Data in the presence (open symbols) and absence (closed symbols) of omeprazole (0.8 mg/ml) are presented as the mean of six determinations.

**Figure 5** The relation between urease activity and omeprazole concentration over the range 0–0.8 mg/ml and at a concentration of 2 mM urea. Data are presented as mean (SEM) of six determinations.
Furthermore, a bacteriostatic effect of both lansoprazole and omeprazole has also been demonstrated when used at lower concentrations in vitro, or against high density growth, as in this study. When added to established cultures of \textit{H pylori}, omeprazole only reduces, although not significantly, the microbial population. To our knowledge this is the first study to show a density dependent dual effect of omeprazole.

It is our opinion that omeprazole exerts a bacteriocidal effect on \textit{H pylori} when used either at raised concentrations or against a small number of organisms. These circumstances may be present within the gastric/duodenal mucosa during the early stages of an infection or as a consequence of combined antibiotic therapy. Therefore, under such circumstances, omeprazole might be able to eradicate the organism in vivo. Alternatively, omeprazole may exert a bacteriostatic effect, as suggested in this study and by others, when used against large numbers of organisms, as would be present in more chronic pathological conditions or against established infections. Under these circumstances, omeprazole might only suppress the growth of the organism and not effect complete eradication, thereby providing one possible explanation for peptic ulcer relapse.

The effect of proton pump inhibitors on the urease activity of \textit{H pylori} has been studied extensively, although kinetic data are sparse. In this study, we have shown that omeprazole competitively inhibits the extracellular urease enzyme of \textit{H pylori} in a dose dependent manner. Urease activity is inhibited by 78% in the presence of 0.8 mg/ml omeprazole. Although this enzyme is known to be essential for bacterial colonisation of the gastric mucosa in vivo, and is therefore a potential target for antibacterial agents, the fact that the growth of urease negative mutants of \textit{H pylori} is also inhibited by omeprazole indicates that the enzyme itself is not a lethal target for this drug in vitro and other potential targets must exist. It is likely that the omeprazole mediated inhibition of urease may render the organism susceptible to other agents. Thus, the effects of omeprazole on bacterial growth in vitro may be independent of the ability of this benzimidazole compound to inhibit urease activity. In this study, we have presented data on the effect of omeprazole on the growth of the type strain NCTC 11637 at neutral pH in vitro and suggest that this proton pump inhibitor may exert both a bacteriostatic and a bacteriocidal effect. However, because different clinical isolates of \textit{H pylori} are effected to differing extents by proton pump inhibitors, it is difficult to extrapolate these observations to other strains of this organism.

However, it is our opinion that in order for omeprazole to exert an optimal antibacterial effect it must be used during the early stage of infection when bacterial numbers are low.

This investigation was carried out with the aid of an award from The Astra Foundation. We are extremely grateful for the helpful comments of Dr P. Matewele.