Specific therapeutic schemes of omeprazole affect the orientation of *Helicobacter pylori*

Marina Azevedo-Vethacke¹, Désirée Garten¹, Claudia Groll¹, and Sören Schreiber¹*

¹Ruhr-Universität Bochum, Institut für Physiologie, Im Lottental 36, 44801 Bochum, Germany

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*Corresponding author:

Soeren Schreiber
Institut fuer Physiologie
Ruhr-Universitaet Bochum
Im Lottental 36
44801 Bochum, Germany

phone: +49 -234 -32 29126
fax: +49 -234 -32 14191
e-mail: soeren.schreiber@rub.de
ABSTRACT

Until now it remains unclear how proton pump inhibitors (PPI) support the *H. pylori* therapy. We tested whether the PPI omeprazole act on the spatial orientation of *H. pylori* in the gastric mucus of infected Mongolian gerbils. Following repetitive PPI administration once daily but not following single doses or administration every 8 hours, the bacterial spatial distribution changed, indicating a loss of orientation. Therefore, the therapeutic scheme of PPI administration may affect efficiency of treatment.
INTRODUCTION

The gastric pathogen *H. pylori* infects about half of all humans (15,22), causes ulcers (14) and gastric adenocarcinomas (4,7). To cure *H. pylori* infection a combined treatment with antibiotics and a proton pump inhibitor for gastric acid suppression is used. Despite frequent administration in conventional triple therapies (12) and the sequential therapies currently being developed (8,9,13,23), the effect of PPIs in therapy for this infection is poorly understood. A finding of major importance was that combined treatment with a PPI causes an increased concentration of the regularly used antibiotic clarithromycin in the gastric mucus (11). Due to the interdependent regulation of acid and mucus secretion, a PPI may reduce mucus secretion (10). In the case of substances with low gastric clearance rates, decreased mucus secretion after administration of a PPI could enhance their concentration in the mucus. However, this observation does not explain the mechanism of action of PPIs, since antibiotic schemes without the macrolide clarithromycin (using, e.g., the well-penetrating fluoroquinolone moxifloxacin (1,16)) are similarly effective.

Consequently, we tested the new hypothesis that the representative proton pump inhibitor omeprazole may act on the spatial orientation of *H. pylori* within the gastric mucus.

BODY

We established a chronic *H. pylori* SS1 infection in the Mongolian gerbil, (Meriones unguiculatus; Hsd:MON) (Harlan & Winkelmann, Indianapolis, USA), administered single and repetitive doses of omeprazole and studied the effect on the bacterial density and distribution within the gastric mucus layer. Single doses of up to 20 µM omeprazole were administered by intraperitoneal perfusion which functioned as a dialysis. Repetitive doses of 10 µM omeprazole were injected intraperitoneally (ip) during a short inhalation anesthesia. Figure 1 shows the omeprazole plasma concentration and the gastric lumen pH after ip administration of 20 µM omeprazole (For details see appendix).
After the last injection, the bacterial distribution in the mucus was measured in nanoliter samples of mucus from the antrum region to determine the number of colonizing bacteria. Using digital microscopic imaging, it was possible to reconstruct the bacterial distribution with respect to the tissue surface as previously described (19,21).

Following different schemes of PPI administration the number of bacteria per nanoliter of mucus observed within the different mucus layers is shown in table 1. Single doses of up to 20 µM (35 µg/ml) omeprazole and two subsequent doses of 10 µM (0.25 mg i.p.) given in time intervals of 8 hours did not affect bacterial orientation. Nearly all bacteria were located within the juxtamucosal mucus layer, with a larger percentage in the first 15 µm above the tissue surface. The bacterial density and distribution within the different mucus layers in these groups were similar to those of the untreated control animals. Thus, a loss of orientation did not occur.

However, administration of 0.25 mg omeprazole twice with a time interval of 24 hours resulted in a decreased density of bacteria colonizing the juxtamucosal mucus layer and an increase in the density of bacteria colonizing the central and luminal mucus layers, indicating that the bacteria had spread into these more luminal mucus layers (Table 1).

H. pylori are normally found to be aligned parallel to one another and the tissue surface in the juxtamucosal layer (Figure 2a). However, after two subsequent doses of omeprazole once daily, H. pylori were distributed throughout the juxtamucosal, central, and luminal mucus layers in an unarranged manner (Figure 2b).

Further administration of 0.25 mg omeprazole every 24 hours for up to 5 days resulted in a cumulative reduction of the bacterial load to below 5% of the colonization density in the juxtamucosal mucus observed in controls.
Thus, omeprazole only affected the orientation of *H. pylori* when two or more doses were administered at time intervals of 24 hours, as the bacterial density and distribution in the juxtamucosal, central, and luminal mucus layers were significantly different in these animals when compared to the animals treated with the other schemes (significance data in Appendix Table A2). These observations may result from a modification of the bicarbonate-dependent mucus pH gradient, which guides *H. pylori* orientation within the gastric mucus layer (19).

Due to the continuous mucus flow caused by mucus secretion in the glands and degradation at the luminal surface (20), a precise bacterial orientation is required to prevent *H. pylori* from being vertically swept away into the lumen. The pH gradient in the mucus layer from an acidic lumen pH to a more neutral pH at the epithelial surface is generated by the simultaneous secretion of mucus, bicarbonate and acid. *H. pylori* senses the local acidity of this pH gradient using the chemotaxis receptor TlpB (6) and relays the information to the flagellar motor (3). Acid suppression by the PPI may alter the bicarbonate and pH regulation in the gastric antrum, which would in turn disturb the *Helicobacter* orientation. Interestingly, neither a single dose of omeprazole nor two subsequent doses administered at short time intervals contributed to a loss of bacterial orientation. However, when the second (and further) omeprazole dose(s) was/were given at a regular time interval of 24 hours, a loss of bacterial orientation was observed. Hence, the long-lasting neutralized gastric lumen pH following a single or two doses administered in a shorter interval does not act on *Helicobacter* orientation, whereas the changes in the course of gastric pH values following the second dose given after a time interval of 24 hours are fatal for *H. pylori*. Figure 3 shows the omeprazole plasma concentration and the gastric luminal pH following a single dose of PPI in comparison to the effect of two subsequent administrations either every eight or every 24 hours.

![Figure 3 next to here-](image-url)
PPI administration once daily disturbed the vertical orientation of *H. pylori*, most likely through a complex time course of acid suppression, thereby destroying the guiding
bicarbonate-dependent mucus pH gradient. *Helicobacter pylori* which lose their guiding
gradient in the gastric mucus, spread over the entire mucus layer and into the lumen (19).
Dependent on the actual pH, remaining activity of pepsin C in the lumen causes a loss of
*H. pylori* motility (17,18), the bacteria are irretrievably lost and the bacterial load is reduced.
In contrast, two subsequent PPI administrations in shorter time intervals, in which the gastric
pH remained neutralized, had no effect on *H. pylori* orientation. Therefore, the time course of
the gastric lumen pH that was particularly fatal for bacterial orientation was characterized by
a recovery of acid secretion after the last PPI dose and a rapid neutralization following the
next dose. These experimental data match the remarkable clinical observations that lower
doses (2) or lower frequencies (5) of PPI administration result in improved or unchanged
eradication rates.
We conclude that repetitive omeprazole administration every 24 hours causes complex
changes in gastric acid secretion which impair the bacterial orientation. This finding indicates
that the therapeutic scheme of proton pump inhibitor administration may be of importance for
an efficient cure of *H. pylori* infection which requires further clinical examination.
Funding

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Competing Interests

The authors declare that no competing interests exist.
REFERENCES


OMEPRAZOLE AFFECTS HELICBACTER ORIENTATION


**FIGURE LEGENDS**

**FIG. 1**
Course of omeprazole plasma concentration and gastric lumen pH following PPI-injection. The omeprazole plasma concentration (green line, measured by HPLC) and the changes in the gastric lumen pH (red and blue line) are shown following an intraperitoneal injection of omeprazole in the Mongolian gerbil. The omeprazole was eliminated within less than one hour after administration. The neutralization of the gastric lumen pH, initially detectable in the corpus region, began 15 - 20 min following the intraperitoneal injection, extended to the antrum region, and reached a gastric pH of nearly 7. The luminal pH started to decrease three hours after omeprazole administration, and within 12 hours, acid secretion recovered.

**FIG. 2**
Spatial alignment of *H. pylori* within the mucus under normal conditions and following omeprazole administration. Shown is the juxtamucosal mucus of the *H. pylori* infected Mongolian gerbil at 1000-fold magnification. Figure B is a digital addition of three focus planes lying upon another. Epithelial cells of the mucosal surface are visible at the bottom of the micrographs. A: In untreated animals, the bacteria moved parallel to the cellular surface, within a distance of 0 - 25 µm. B: Following two days of PPI administration, once daily, the bacteria lost their sorted alignment and spread over the entire mucus layer.

**FIG. 3**
Omeprazole plasma concentration, gastric lumen pH, and effect on bacterial orientation following different therapeutic schemes. The course of omeprazole plasma concentration (green line) and gastric lumen pH (blue line) are shown following a peak plasma concentration of 10 µM omeprazole. A: The administration of omeprazole in time intervals of
eight hours caused a long-lasting neutralization and had no effect on bacterial orientation. B: Following an interval of 24 hours after administration, acid secretion recovered, and the second dose of omeprazole led to a rapid re-neutralization of the gastric lumen, inducing a loss of bacterial orientation. C: When the second dose of PPI was substituted by a solvent, the orientation of *H. pylori* was not affected.
### TABLE 1

<table>
<thead>
<tr>
<th>Untreated controls</th>
<th>Juxtamucosal</th>
<th>Central</th>
<th>Luminal</th>
<th>Analyzed samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>Bacteria / nl mucus Mean ± SD</td>
<td>4560 ± 400</td>
<td>2280 ± 430</td>
<td>90 ± 60</td>
<td>60 ± 50</td>
</tr>
<tr>
<td>Two ip injections of solvent, time interval 24 hours</td>
<td>Juxtamucosal</td>
<td>Central</td>
<td>Luminal</td>
<td>Analyzed samples</td>
</tr>
<tr>
<td>Bacteria / nl mucus Mean ± SD</td>
<td>5050 ± 980</td>
<td>2010 ± 310</td>
<td>70 ± 30</td>
<td>50 ± 30</td>
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<tr>
<td>Two ip injections of omeprazole, time interval 8 hours</td>
<td>Juxtamucosal</td>
<td>Central</td>
<td>Luminal</td>
<td>Analyzed samples</td>
</tr>
<tr>
<td>Bacteria / nl mucus Mean ± SD</td>
<td>5740 ± 1310</td>
<td>2260 ± 400</td>
<td>40 ± 20</td>
<td>70 ± 60</td>
</tr>
<tr>
<td>First dose omeprazole, second dose solvent, time interval 24 hours</td>
<td>Juxtamucosal</td>
<td>Central</td>
<td>Luminal</td>
<td>Analyzed samples</td>
</tr>
<tr>
<td>Bacteria / nl mucus Mean ± SD</td>
<td>4790 ± 840</td>
<td>1800 ± 440</td>
<td>30 ± 20</td>
<td>30 ± 10</td>
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<tr>
<td>Two ip injections of omeprazole, time interval 24 hours</td>
<td>Juxtamucosal</td>
<td>Central</td>
<td>Luminal</td>
<td>Analyzed samples</td>
</tr>
<tr>
<td>Bacteria / nl mucus Mean ± SD</td>
<td>3310 ± 560*</td>
<td>2230 ± 840</td>
<td>300 ± 130*</td>
<td>120 ± 30*</td>
</tr>
</tbody>
</table>

* Significant difference in students t-test, $P \leq 0.05$ (more information in Appendix table A2)
Figure 1

OMEPRAZOLE AFFECTS HELICBACTER ORIENTATION

Corpus pH
Antrum pH
Omeprazole concentration

Omeprazole concentration (µM)

Time (min)  Time (h)

Corpus pH
Antrum pH
Omeprazole concentration

Omeprazole concentration (µM)

Time (min)  Time (h)
Figure 2

A

B

Mucosa

Mucus

Mucosa

Mucus

10 µm

10 µm
OMEPRAZOLE AFFECTS *HELCBACTER* ORIENTATION

**Figure 3**

A. Gastric lumen pH, following repetitive doses of PPI  
Time interval of 7 - 8 hours

B. Gastric lumen pH, following repetitive doses of PPI  
Time interval of 24 hours

C. Gastric lumen pH, following PPI and solvent  
Time interval of 24 hours