Impact of acid secretion, gastritis, and mucus thickness on gastric transfer of antibiotics in rats

P V Sherwood, J I D Wibawa, J C Atherton, N Jordan, D Jenkins, D A Barrett, P N Shaw, R C Spiller

Background and aims: The success of Helicobacter pylori eradication regimens depends on gastric pH, inflammation, and mucus thickness. Our aim was to investigate the effects of acid secretion, inflammation, and mucus on gastric antibiotic transfer.

Subjects and methods: A total of 134 anaesthetised rats were given metronidazole, amoxicillin, or clarithromycin intravenously and gastric contents were aspirated via an indwelling cannula. Acid secretion was controlled by either omeprazole or pentagastrin while gastritis was induced by infection with H pylori or dosing with iodoacetamide. Mucolysis was achieved by instilling pronase into the gastric lumen.

Results: Metronidazole transfer increased with acid secretion and fell with omeprazole, independently of gastric pH. Clarithromycin was also transferred with acid but was then rapidly degraded. Omeprazole prevented this degradation, raising gastric luminal concentrations. Omeprazole did not alter amoxicillin transfer. Gastritis induced by H pylori did not alter gastric transfer of metronidazole and amoxicillin but that of clarithromycin was increased by 23%. However, gastritis induced by iodoacetamide reduced clarithromycin transfer without any effect on metronidazole or amoxicillin transfer. Pronase treatment increased amoxicillin transfer fourfold and metronidazole by 66% but reduced clarithromycin transfer by 35%.

Conclusions: Metronidazole and clarithromycin are predominantly transferred with gastric acid rather than by an acid trapping mechanism. Pronase increases the appearance of amoxicillin and metronidazole in gastric secretions.

MATERIALS AND METHODS

All experiments were performed in accordance with a UK Home Office Project Licence, using a total of 134 male Wistar rats weighing 250–370 g. Rats were fasted on a wire bottomed cage for 24 hours with free access to water. Anaesthesia was induced with a 2.7 ml/kg intraperitoneal dose of a 1:1:2 mixture of Hypnorm (fentanyl/fluanisone), midazolam, and H2O.

Vital signs were monitored using an intracarotid blood pressure transducer connected to a computer recording system. Hydration was maintained with an infusion of 0.9% saline via a tail vein. At laparotomy, a cannula cuffed with two "O" rings was inserted through a duodenal incision into the gastric antrum via the pylorus. The stomach was washed with 0.9% saline until the aspirate was free of debris and then 1.5 ml of saline was instilled. After a 30 minute equilibration period to allow gastric blood flow to stabilise, a bolus intravenous dose of antibiotic was given at the start of the two hour sampling period, followed by a continuous infusion. The following doses were administered: metronidazole 9 mg/kg bolus, 3.6 mg/kg/h infusion; amoxicillin 11 mg/kg bolus, 20 mg/kg/h infusion; and clarithromycin 26 mg/kg bolus, 7.3 mg/kg/h infusion.

Carotid artery blood samples (0.3 ml) were taken at 15 minute intervals for two hours; plasma was separated by centrifugation and snap frozen in liquid N2. At 15 minute intervals the gastric contents were aspirated by syringe and the volume recorded. Net gastric secretion volume was calculated by subtracting the volume instilled (1.5 ml) from the volume aspirated. pH was measured with a glass electrode before the sample was immediately snap frozen in liquid N2 and later transferred to a –80°C freezer. After two hours, the rat was sacrificed by neck dislocation, the stomach excised and portions were preserved in 10% formalin, H pylori culture...
transport medium, and for mucus thickness measurements in liquid N₂. Mucosal sections were categorised as to the degree of inflammation (none, mild, moderate, or severe), as is conventional in assessing human gastritis by a single expert gastrointestinal pathologist. Sections were stained with toluidine blue for identification of H pylori.

**Acid secretion/suppression experiments**

Two dosing regimens of intravenous omeprazole were used to suppress gastric acid secretion. A single bolus dose (10 μmol/kg) was used for the metronidazole experiments. For the amoxicillin and clarithromycin experiments, 20 μmol/kg omeprazole was administered initially and again at 45 minutes into the sampling period, a regimen which gave similar but more consistent acid suppression. In stimulated acid secretion experiments, intravenous bolus doses of pentagastrin (25 μg/kg) were given every 15 minutes. Where intragastric pH was controlled (table 1) the following buffers were instilled instead of saline: pH 2.7: glycine/HCl buffer, 300 mosmol/kg; pH 6.0: 2-[(N-morpholino)ethanesulphonic acid]

**Induction of gastritis**

Four H pylori strains were prepared for dosing into 32 rats weighing 180–220 g; two Hel73 toxigenic strains (rat and mouse passaged variants), the Sydney strain, and a fresh clinical isolate. Using the method of Li and colleagues, rats were gavaged with 2 ml of H pylori in suspension (5×10⁷ cfu/ml of each strain) at midday and at 4 pm for two days. One rat died early during surgery and no data were obtained; seven rats that had neither surgery nor antibiotics were sacrificed at 12 weeks and their stomachs sampled for histology. Vital signs of the rats remained within normal limits throughout the experiments.

**Gastric mucolysis experiments**

Gastric mucus dissolution was achieved using pronase, a non-specific protease active at neutral pH. All pronase treated rats were also given intravenous omeprazole. The stomach was washed with saline initially, and then 1.5 ml of a 20 mg/ml solution of pronase (Sigma) in pH 7.4 phosphate buffered saline (osmolality 312 mosmol/kg) was instilled instead of saline for the 30 minute equilibration period and the two hour experiment. The dose of pronase (440 tyrosine units/kg) was chosen to achieve a concentration similar to that used in human trials. Rat stomachs were prepared for mucus thickness measurements using the method of Jordan and colleagues. Mucus continuity was expressed as a percentage of the mucosal surface covered by mucus.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Gastric metronidazole transfer: effect of acid secretion and luminal pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous drug</td>
<td>Omeprazole</td>
</tr>
<tr>
<td>Gastric luminal solution n</td>
<td>0.9% saline</td>
</tr>
<tr>
<td>Median gastric aspirate pH (range)</td>
<td>5.74 (4.39–6.30)***</td>
</tr>
<tr>
<td>Gastric aspirate Cmax (mg/l)</td>
<td>14.2 (2.7)††</td>
</tr>
<tr>
<td>Gastric clearance (l/min)</td>
<td>87 (14) ††</td>
</tr>
<tr>
<td>Gastric transfer fraction (%)</td>
<td>7.1 (1.3) ††</td>
</tr>
</tbody>
</table>

Unless otherwise stated, values are mean (SD).

***p<0.001 versus pentagastrin+saline and omeprazole+glycine/HCl buffer.††p<0.01 versus all other groups.‡‡p<0.01 versus pentagastrin+saline and pentagastrin+MES. MES, 2-(N-morpholino)ethanesulfonic acid.

**Antibiotic analysis**

Concentrations of metronidazole, amoxicillin, and clarithromycin in plasma and gastric aspirate samples were determined using modified narrow bore high performance liquid chromatography techniques based on methods previously reported. Aliquots of 50 μl plasma or 200–500 μl gastric aspirate were used for analysis. Controls were prepared by spiking plasma and 0.9% saline with stock antibiotic solutions. The presence of pronase in the sample had no effect on the quantification of any of the antibiotics. The limit of quantification was 0.015 mg/l for metronidazole, 0.04 mg/l for amoxicillin, and 0.10 mg/l for clarithromycin. The precision of the assays (defined as the relative standard deviation for repeated measurements of the same concentration) over the range of concentrations studied was ±1.4–13.1%, and deviation from linearity was <1%.

**Calculations and statistics**

Gastric antibiotic clearance (in ml/min) for the 120 minute experiments was calculated as:

\[
\text{Gastric clearance} = \frac{\Sigma (Gastric aspirate volume per minute \times antibiotic concentration)_{0-120}}{\text{plasma steady state concentration}_{0-120}}
\]

Gastric transfer fraction was calculated by dividing gastric clearance by plasma clearance. Both parameters enable a description of the relative transfer of antibiotics across the gastric mucosa under different experimental conditions by correcting for intersubject variation in plasma antibiotic concentrations.

Sample statistics were calculated using SPSS 8.0 software (SPSS Inc., Chicago, USA). The Mann-Whitney U test was used to compare the results of the two groups; the Kruskal-Wallis H-test was used to compare three or more groups. A p value <0.05 was regarded as statistically significant.

**RESULTS**

Vital signs of the rats remained within normal limits throughout the experiments.

**Histology**

Control rats had histologically normal gastric mucosa following the 2.5 hour experiment. H pylori dosed rats all developed mild to moderate mixed inflammatory cell infiltration of the gastric mucosa, with mainly sparse colonisation by H pylori. None showed any macroscopic mucosal ulceration. Marked vascular engorgement was a striking histological feature in iodoacetamide dosed rats. Iodoacetamide caused a similar degree of inflammatory cell infiltrate as H pylori gastritis. Pronase treated gastric mucosa was macroscopically normal but showed marked histological changes. The superficial epithelial layer was fissured with shedding of some apical cell layers. In
Gastric mucus thickness

Gastric mucus thickness in \(H. pylori\) infected rats and controls was similar, whether or not surgery was performed (fig 1). Mean mucus thickness was 87 (29) \(\mu\)m in iodoacetamide treated rats compared with 162 (21) \(\mu\)m in controls (p<0.05). Coverage of gastric mucus in control rats was 100% whereas in iodoacetamide rats there were areas of denuded gastric mucosa with only 88% coverage overall. Pronase markedly thinned gastric mucus creating large gaps; overall, coverage was reduced to 60%. Mean mucus thickness was reduced to 30 (13) \(\mu\)m (p<0.001 vs controls).

**\(H. pylori\) culture**

\(H. pylori\) was cultured from the gastric mucosa in 86% of infected controls but no positive cultures were obtained from the stomachs of rats that received antibiotics which showed similar degrees of histological gastritis and a similar density of colonising \(H. pylori\).

**Gastric secretion**

This was higher in pentagastrin dosed rats compared with those receiving high dose omeprazole (3.3 (1.6) ml/kg/h vs 2.0 (1.2) ml/kg/h; p=0.019). Neither \(H. pylori\) infection nor iodoacetamide gastritis significantly affected gastric juice secretion volume. Gastric secretion was much greater in pronase treated rats compared with controls (8.3 (1.6) ml/kg/h vs 2.0 (1.2) ml/kg/h; p<0.001).

**Metronidazole transfer**

During pentagastrin dosing, gastric aspirate metronidazole concentrations were markedly higher than those seen at very similar gastric pH levels during omeprazole dosing (fig 2, table 1). Neither \(H. pylori\) nor iodoacetamide gastritis affected the gastric metronidazole transfer rate but mucus digestion with pronase produced a 66% increase in gastric metronidazole transfer rate (p=0.005 vs control) (table 2).

**Amoxicillin transfer**

There were no significant effects of acid secretion/suppression, iodoacetamide, or \(H. pylori\) induced gastritis on gastric amoxicillin transfer. Pronase caused a fourfold increase in the gastric clearance of amoxicillin (p<0.001; table 3) with rising gastric aspirate amoxicillin concentrations as the experiment progressed (fig 3).

**Clarithromycin transfer**

Gastritis induced by \(H. pylori\) caused a 23% increase in gastric clarithromycin clearance compared with controls (table 4). Gastritis induced by iodoacetamide caused a small but significant reduction in the gastric transfer fraction of clarithromycin compared with controls; gastric clearance was also reduced but just failed to reach statistical significance (p=0.06). Gastric clarithromycin transfer was reduced by 35% in pronase treated rats (fig 4). Pentagastrin dosing resulted in a significantly lower gastric clearance and gastric transfer fraction of clarithromycin compared with omeprazole dosed rats. However, subsequent analysis of an unknown compound in the gastric aspirate samples revealed that clarithromycin had been broken down into the inactive acid degradation product, decladinose-clarithromycin. Taking this degradation into account, gastric clearance and gastric transfer fraction of clarithromycin during pentagastrin dosing were 222 \(\mu\)l/min and 3.92%, respectively, significantly greater (p<0.001) than that seen during omeprazole dosing.

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### Table 2 Gastric metronidazole transfer: effect of Helicobacter pylori, iodoacetamide, and pronase

<table>
<thead>
<tr>
<th>Intravenous drug</th>
<th>Omeprazole</th>
<th>Omeprazole</th>
<th>Omeprazole</th>
<th>Omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric luminal solution</td>
<td>0.9% saline</td>
<td>0.9% saline</td>
<td>(H. pylori) infected</td>
<td>Iodoacetamide</td>
</tr>
<tr>
<td>Preparation</td>
<td>Normal</td>
<td>Normal</td>
<td>(H. pylori) infected</td>
<td>Normal</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Gastric aspirate (C_{\text{max}}) (mg/l)</td>
<td>14.2 (2.7)</td>
<td>13.9 (3.4)</td>
<td>12.2 (3.7)</td>
<td>20.5 (6.6)*</td>
</tr>
<tr>
<td>Gastric clearance (l/min)</td>
<td>87 (14)</td>
<td>84 (19)</td>
<td>89 (28)</td>
<td>145 (41)*</td>
</tr>
<tr>
<td>Gastric transfer fraction (%)</td>
<td>7.1 (1.3)</td>
<td>5.4 (1.4)*</td>
<td>7.2 (2.1)</td>
<td>9.9 (2.5)*</td>
</tr>
</tbody>
</table>

Values are mean (SD). *p<0.05 versus control.
such as metronidazole might passively diffuse from gastric contamination of gastric juice occurs. In contrast with human studies, no pyloric loss or bile minimising intersubject variability (age, sex, weight, strain).

The anaesthetised rat model provides an opportunity to investigate the mechanisms of gastric antibiotic transfer by

DISCUSSION

The anaesthetised rat model provides an opportunity to investigate the mechanisms of gastric antibiotic transfer by minimising intersubject variability (age, sex, weight, strain). In contrast with human studies, no pyloric loss or bile contamination of gastric juice occurs and the gastric transport of novel compounds can readily be evaluated. Pentagastrin or omeprazole dosing gave gastric pH values similar to those found in humans during basal acid output and omeprazole pre-dosing. Rat plasma antibiotic concentrations were comparable with those found in human pharmacokinetic studies.

Two other animal models of gastric antibiotic secretion have been reported. The Ussing chamber model uses rat gastric mucosa placed in a Lucite chamber bathed by solutions on the mucosal and serosal sides. In this model high serosal antibiotic concentrations were necessary, the drug diffusion path was different from in vivo as there was no intact gastric blood supply, and mucosal acid secretion was not proven to occur. The recently described explanted embryonic human stomach model of gastric antibiotic secretion used human gastric tissue and could also be infected with *H pylori* but its great complexity limits its usefulness for studies on gastric pharmacokinetics.

During normal gastric acid secretion conditions, weak bases such as metronidazole might passively diffuse from gastric capillaries across the gastric mucosa, become ionised by the low pH of gastric juice, and then become “trapped” in the gastric lumen in accordance with the pH partition hypothesis. An alternative possibility is that, in addition to passive diffusion, weak bases might accumulate in the acid secretory apparatus of parietal cells and subsequently be secreted into the gastric lumen with gastric acid. According to the latter hypothesis, transport of weak bases into the gastric lumen should be independent of luminal pH. “Acid trapping” may still occur, but will take place within the secretory apparatus, rather than within the lumen. By controlling gastric acid secretion with either omeprazole or pentagastrin, and gastric luminal pH with iso-osmotic pH buffers, this model was able to distinguish between these possibilities. Gastric metronidazole transfer rates observed during pentagastrin treatment, with a median gastric luminal pH held at either 2.54 or 6.00 with buffers, were very similar. Moreover, omeprazole markedly reduced the gastric metronidazole secretion rate both at gastric pH 2.77 and at pH 5.74. Therefore, it is the rate of acid

### Table 3

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Intravenous drug</th>
<th>Omeprazole</th>
<th>Pentagastrin</th>
<th>Omeprazole</th>
<th>Omeprazole</th>
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<tr>
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<td>9</td>
</tr>
<tr>
<td>Median gastric aspirate pH (range)</td>
<td>6.09 (5.53–6.44)</td>
<td>2.27 (2.11–3.41)***</td>
<td>6.23 (6.01–6.39)</td>
<td>6.21 (5.99–6.38)</td>
<td>6.45 (6.33–6.57)***</td>
<td></td>
</tr>
<tr>
<td>Gastric clearance (l/min)</td>
<td>2.9 (1.9)</td>
<td>3.3 (2.5)</td>
<td>2.3 (0.6)</td>
<td>1.3 (0.4)</td>
<td>12.0 (3.8)***</td>
<td></td>
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<tr>
<td>Gastric transfer fraction (%)</td>
<td>0.15 (0.11)</td>
<td>0.12 (0.09)</td>
<td>0.15 (0.05)</td>
<td>0.10 (0.04)</td>
<td>0.86 (0.14)***</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD), except for pH.

**p<0.001 versus control.

### Table 4

<table>
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<tr>
<th>Preparation</th>
<th>Intravenous drug</th>
<th>Omeprazole</th>
<th>Pentagastrin</th>
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<tr>
<td>Median gastric aspirate pH (range)</td>
<td>6.13 (5.89–6.35)</td>
<td>2.58 (2.30–2.85)****</td>
<td>6.19 (6.15–6.46)</td>
<td>5.83 (5.58–6.34)</td>
<td>6.60 (6.56–6.72)****</td>
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<tr>
<td>Gastric aspirate Cmax (mg/l)</td>
<td>8.7 (1.8)</td>
<td>4.4 (4.5)*</td>
<td>10.3 (1.6)</td>
<td>6.7 (2.2)</td>
<td>7.3 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Gastric clearance (l/min)</td>
<td>128 (31)</td>
<td>30 (33)***</td>
<td>158 (22)*</td>
<td>95 (29)**</td>
<td>82 (18)***</td>
<td></td>
</tr>
<tr>
<td>Gastric transfer fraction (%)</td>
<td>2.28 (0.25)</td>
<td>0.53 (0.63)****</td>
<td>2.09 (0.22)</td>
<td>1.72 (0.41)***</td>
<td>1.33 (0.35)*****</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD), except for pH.

* p<0.05, **p=0.06, ***p<0.01, ****p<0.001 versus control.
H pylori infection, iodoacetamide gastritis was associated with more severe histological changes. There was vascular engorgement and mucus thinning, not seen with iodoacetamide. The rats reduced food and fluid intake and lost weight. These complex possibly conflicting effects suggest that iodoacetamide was not a useful model of H pylori gastritis. Iodoacetamide did not cause any significant changes in gastric antibiotic clearance. Gastric clarithromycin transfer fraction was reduced, perhaps as an artefact of low body weight in iodoacetamide dosed rats.

The apparent benefit of pronase in clinical studies could be caused by either disruption of the normal habitat of H pylori, increasing its susceptibility to antibiotics, or by affecting antibiotic transfer. Our studies show that the latter mechanism is important. Pronase caused pronounced degradation of the gastric mucus layer and also considerable signs of epithelial cell disruption. There was a marked increase in gastric secretion volume, possibly by inducing copious protective mucus secretion. Although we did not measure blood contamination from the damaged mucosal surface, this could have contributed to the rise in gastric amoxicillin level, as plasma has a high concentration of amoxicillin relative to gastric aspirate. It may partially explain the reduction in aspirate clarithromycin level, as clarithromycin is usually concentrated in the lipid outer membrane of H pylori. The apparent benefit of pronase in clinical studies could be caused by either disruption of the normal habitat of H pylori, increasing its susceptibility to antibiotics, or by affecting antibiotic transfer. Our studies show that the latter mechanism is important. Pronase caused pronounced degradation of the gastric mucus layer and also considerable signs of epithelial cell disruption. There was a marked increase in gastric secretion volume, possibly by inducing copious protective mucus secretion. Although we did not measure blood contamination from the damaged mucosal surface, this could have contributed to the rise in gastric amoxicillin level, as plasma has a high concentration of amoxicillin relative to gastric aspirate. It may partially explain the reduction in aspirate clarithromycin level, as clarithromycin is usually concentrated in gastric juice, which may have been diluted with plasma containing a lower concentration of clarithromycin. The progressive rise in amoxicillin transfer rate during the experiment suggests amoxicillin traverses the mucosa via the paracellular route due to tight junction disruption, or that mucin is a considerable barrier to amoxicillin transfer that is gradually digested away during the experiment. The progressive reduction in gastric clarithromycin transfer rate during the pronase experiments strongly suggests that mucin is not a significant barrier to gastric clarithromycin secretion but that an active process requiring a healthy epithelium has been gradually degraded by pronase. The safety and efficacy of mucolytics with antibiotics is worthy of future research in human clinical trials.

ACKNOWLEDGEMENTS

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