HELICOBACTER PYLORI

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

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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 mucolysis 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.

The development of *Helicobacter pylori* treatment regimens has been largely empirical. There are however several marked discrepancies between in vitro and in vivo efficacy, indicating the importance of gastric distribution of antibiotics. The efficacy of delivery of the antibiotic to the gastric mucus layer where *H pylori* resides has been surprisingly little studied. As *H pylori* is becoming more resistant to standard antibiotics,[1] there is a need to discover the fundamental mechanisms of gastric antibiotic delivery in order to help the design of novel agents.[2] Clinical trials using amoxicillin, metronidazole, and clarithromycin have indicated the key importance of raising intragastric pH by means of proton pump inhibitors.[3] Studies using less potent regimens have also indicated that eradication rates in patients with an inflamed stomach are greater than in those with minimal inflammation.[4-6] This suggests that penetration of some antibiotics might be facilitated by inflammation just as transport of amoxicillin into cerebrospinal fluid is enhanced in meningitis.[7] Gastric mucus, whose function it is to protect the gastric epithelium from injurious substances, may also provide a barrier to antibiotic penetration, and mucolytic agents have been used with good effect to enhance eradication rates.[8 9] The aim of this study was to examine the role of acid secretion and gastric luminal pH on the gastric transfer rate of the three most commonly used anti-*H pylori* antibiotics, metronidazole, amoxicillin, and clarithromycin using a new anaesthetised rat model of gastric antibiotic transport. This model allows independent control of both acid secretion and intragastric pH. The effect of gastritis on gastric antibiotic transfer rate was also investigated after inducing gastritis by either infecting rats with *H pylori* or addition of iodoacetamide to the drinking water. Finally, gastric mucus was disrupted with pronase and the effect on gastric antibiotic secretion rate determined.

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 H_2O. 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 N_2. 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 N_2 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

Table 1 Gastric metronidazole transfer: effect of acid secretion and luminal pH

Intravenous drug	Omeprazole	Pentagastrin	Omeprazole	Pentagastrin
Gastric luminal solution	0.9% saline	0.9% saline	Glycine/HCl buffer	MES buffer
n	8	7	8	8
Median gastric aspirate pH (range)	5.74 (4.39–6.30)***	2.54 (2.32–2.79)††	2.77 (2.69–2.82)	6.00 (5.81–6.03)
Gastric aspirate C_{max} (mg/l)	14.2 (2.7)‡‡	50.8 (8.5)	14.8 (1.8)‡‡	66.0 (28.0)
Gastric clearance (l/min)	87 (14) ‡‡	352 (68)	97 (11) ‡‡	429 (172)
Gastric transfer fraction (%)	7.1 (1.3) ‡‡	24.5 (5.0)	7.2 (0.8) ‡‡	27.8 (10.2)

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) ethanesulphonic acid.

transport medium, and for mucus thickness measurements in liquid N_2. Mucosal sections were categorised as to the degree of inflammation (none, mild, moderate, or severe), as is conventional in assessing human gastritis[10] 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 buffer at pH 6.0, 300 mosmol/kg.

Induction of gastritis

Four *H pylori* strains were prepared for dosing into 32 rats weighing 180–220 g: two Hel73 toxigenic strains[11] (rat and mouse passaged variants), the Sydney strain, and a fresh clinical isolate. Using the method of Li and colleagues,[11] rats were gavaged with 2 ml of *H pylori* in suspension (5×10^8 cfu/ml of each strain) at midday and at 4pm 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 *H pylori* culture and histology.

Chemical gastritis was induced by adding 0.1% w/v iodoacetamide (Sigma, Poole, UK) to the water supply of a further group of rats for one week prior to surgery. This reduced food and fluid intake but all animals survived the study period.

Gastric mucolysis experiments

Gastric mucus dissolution was achieved using pronase, a nonspecific protease active at neutral pH.[12] 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.[8 9] Rat stomachs were prepared for mucus thickness measurements using the method of Jordan and colleagues.[13] Mucus continuity was expressed as a percentage of the mucosal surface covered by mucus.

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.[14–16] 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:

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

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 concentration.[17]

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
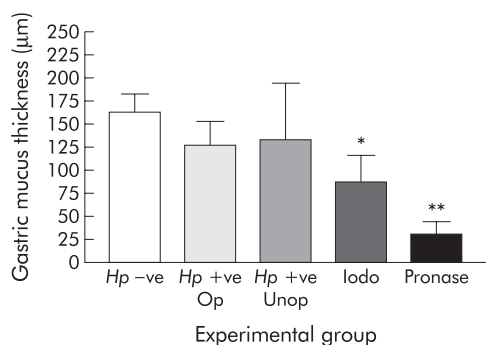
Figure 1 Effect of *Helicobacter pylori*, iodoacetamide (iodo), and pronase on gastric mucus thickness. *Hp*–ve, *H pylori* negative control (no surgery); *Hp*+ve Op, *H pylori* infected (had surgery); *Hp*+ve Unop, *H pylori* infected control (no surgery).
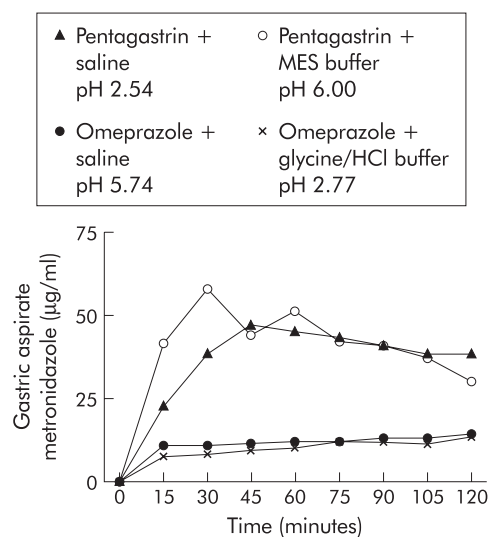

Figure 2 Effect of acid secretion and luminal pH on gastric metronidazole transfer. MES, 2-(*N*-morpholino) ethanesulphonic acid.

some sections vascular engorgement, as seen in iodoacetamide treated rats, was observed. There was however no abnormal inflammatory cell infiltration of the pronase treated rat stomachs.

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) µm in iodoacetamide treated rats compared with 162 (21) µm 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) µm (p<0.001 *v* 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 *v* 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 *v* 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 *v* 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 µl/min and 3.92%, respectively, significantly greater (p<0.001) than that seen during omeprazole dosing.

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

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

Values are mean (SD).
*p<0.05 versus control.

Table 3 Gastric amoxicillin transfer: effect of acid secretion, *Helicobacter pylori*, iodoacetamide, and pronase

Intravenous drug	Omeprazole	Pentagastrin	Omeprazole	Omeprazole	Omeprazole
Gastric luminal solution	0.9% saline	0.9% saline	0.9% saline	0.9% saline	Pronase
Preparation	Normal	Normal	*H pylori* infected	Iodoacetamide	Normal
n	8	8	8	8	9
Median gastric aspirate pH (range)	6.09 (5.53–6.44)	2.27 (2.11–3.41)***	6.23 (6.01–6.39)	6.21 (5.99–6.38)	6.45 (6.33–6.57)***
Gastric aspirate C_{max} (mg/l)	1.9 (1.3)	1.84 (1.13)	2.79 (0.84)	1.21 (0.40)	11.4 (1.9) ***
Gastric clearance (l/min)	2.9 (1.9)	3.3 (2.5)	2.3 (0.6)	1.3 (0.4)	12.0 (3.8) ***
Gastric transfer fraction (%)	0.15 (0.11)	0.12 (0.09)	0.15 (0.05)	0.10 (0.04)	0.86 (0.14) ***

Values are mean (SD), except for pH.
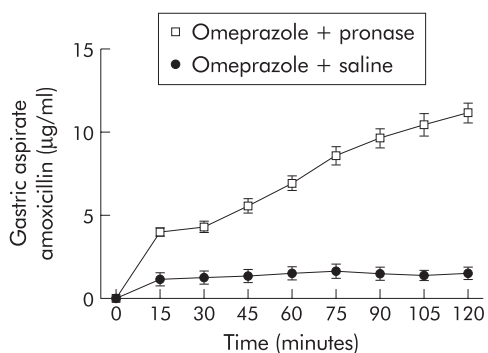*** $p < 0.001$ versus control.

Figure 3 Effect of pronase on gastric amoxicillin transfer.

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[17] 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.[17] 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.[18] 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.[19]

During normal gastric acid secretion conditions, weak bases such as metronidazole might passively diffuse from gastric
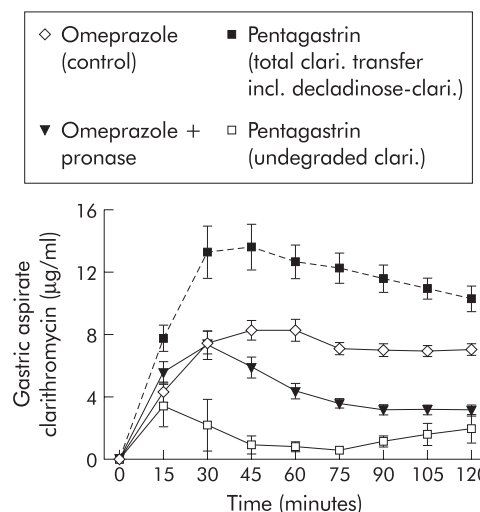

Figure 4 Effect of acid secretion and pronase on gastric clarithromycin (clari.) transfer.

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.[17 20] 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 4 Gastric clarithromycin transfer: effect of acid secretion, *Helicobacter pylori*, iodoacetamide, and pronase

Intravenous drug	Omeprazole	Pentagastrin	Omeprazole	Omeprazole	Omeprazole
Gastric luminal solution	0.9% saline	0.9% saline	0.9% saline	0.9% saline	Pronase
Preparation	Normal	Normal	*H pylori* infected	Iodoacetamide	Normal
n	8	8	8	7	7
Median gastric aspirate pH (range)	6.13 (5.89–6.35)	2.58 (2.30–2.85)****	6.19 (6.15–6.46)	5.83 (5.58–6.34)	6.60 (6.56–6.72)****
Gastric aspirate C_{max} (mg/l)	8.7 (1.8)	4.4 (4.5)*	10.3 (1.6)	6.7 (2.2)	7.3 (2.4)
Gastric clearance (l/min)	128 (31)	30 (33)***	158 (22)*	95 (29)**	82 (18)***
Gastric transfer fraction (%)	2.28 (0.25)	0.55 (0.63)****	2.09 (0.22)	1.72 (0.41)***	1.33 (0.35)****

Values are mean (SD), except for pH.
*$p < 0.05$, **$p = 0.06$, ***$p < 0.01$, ****$p < 0.001$ versus control.

secretion and not luminal pH that determines gastric metronidazole secretion, implying that metronidazole is predominantly transferred with gastric acid. These findings might at first appear paradoxical as in clinical studies coadministration of omeprazole substantially enhanced the effectiveness of regimens containing metronidazole with amoxicillin or clarithromycin. This may be due to enhancement of the stability of clarithromycin and amoxicillin. However, it should also be noted that even during acid suppression in humans, gastric aspirate metronidazole C_{max} is still approximately twice the MIC_{90}. Furthermore, the raised luminal pH ensures that metronidazole (pK_a=2.52) is totally unionised, lipophilic, and therefore available for passive diffusion across the lipid outer membrane of *H pylori*.

At the gastric pH (approximately pH 6) achieved following administration of omeprazole, amoxicillin exists as a hydrophilic zwitterion with a logD (a measure of lipophilicity as a function of pH [17]) of −1.6, and therefore would be predicted to transfer poorly across lipid membranes. Our studies confirm this prediction; we were able to detect only very low concentrations of amoxicillin (less than 2 mg/l in the normal stomach) in human gastric aspirates. Omeprazole potentiates the antibacterial effect of amoxicillin by reducing the volume of gastric secretion and hence effectively increasing the drug concentration.[17] Omeprazole also improves the stability of amoxicillin and the susceptibility of *H pylori* to this antibiotic.[21] Studies of gastric transport of amoxicillin in humans are problematic due to contamination of gastric aspirate samples with amoxicillin-rich bile and the difficulty in detecting low concentrations of amoxicillin. We were able to demonstrate a very low gastric amoxicillin transfer rate in the rat consistent with that seen in humans. As bile was excluded in the rat model, we can conclude that in spite of its hydrophilic nature, amoxicillin can penetrate the gastric epithelium.

There was an apparent fourfold reduction in gastric clarithromycin clearance and transfer fraction during pentagastrin stimulated acid secretion. The net result of omeprazole dosing was that clarithromycin became concentrated in the gastric aspirate compared with plasma. In human phagocytes, macrolides are concentrated in a protonated form in acidic lysosomes and to a lesser extent in the cell cytosol.[22] It would therefore be expected that clarithromycin should be concentrated in the gastric mucosal acid secretory apparatus, as observed with metronidazole. An explanation for this discrepancy was suggested by the discovery of biologically inactive decladinose-clarithromycin, an acid breakdown product of clarithromycin, only in the gastric aspirate of pentagastrin dosed rats. The reduction in gastric transfer rate during omeprazole therapy is outweighed by the increase in clarithromycin stability seen at a higher pH (fig 4, manuscript in preparation).

H pylori colonisation of rat gastric mucosa was sparse and caused relatively mild acute inflammation. *H pylori* resides in humans for many years whereas infection in rats was only two months old so that gastric atrophy and effects on acid secretion may not have had time to develop. Perhaps not surprisingly, no difference in gastric mucus thickness or luminal pH achieved during omeprazole dosing was demonstrated; even in chronically infected humans, mucus thickness is not significantly affected except in older subjects.[23]

There was a significant increase in gastric clarithromycin clearance in *H pylori* infected rats compared with the control group. One explanation for this increase might be that clarithromycin is actively taken up by polymorphonuclear leucocytes which act as potential delivery agents for the drug to inflamed tissues as clarithromycin is rapidly lost from leucocytes in culture when extracellular clarithromycin is removed.[22] Clarithromycin is the most highly protein bound of the three antibiotics, and it is interesting that *H pylori* extracts cause albumin leakage from the rat gastric mucosal microcirculation.[24]

Compared with *H pylori* infection, iodoacetamide gastritis was associated with more severe histological changes. There was vascular engorgement and mucus thinning, not seen with *H pylori*. 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,[25] 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 mucus 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 mucus 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.

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