

Effect of increasing *Helicobacter pylori* ammonia production by urea infusion on plasma gastrin concentrations

R S Chittajallu, W D Neithercut, A M I Macdonald, K E L McColl

Abstract

It has been proposed that the hypergastrinaemia in subjects with *Helicobacter pylori* infection is caused by the action of the ammonia produced by the organism's urease activity on the antral G cells. To investigate this hypothesis we examined the effect on plasma gastrin of increasing the bacterium's ammonia production by infusing urea intragastrically to eight *H pylori* positive duodenal ulcer patients. After a 60 minute control intragastric infusion of dextrose solution at 2 ml/minute, a similar infusion containing urea (50 mmol/l) was continued for four hours. During the urea infusion, the median gastric juice urea concentration rose from 1.1 mmol/l (range 0.3-1.6) to 15.5 mmol/l (range 7.9-21.3) and this resulted in an increase in the ammonium concentration from 2.3 mmol/l (range 1.3-5.9) to 6.1 mmol/l (range 4.2-11.9) ($p < 0.01$). This appreciable rise in ammonia production did not result in any change in the plasma gastrin concentration. The experiment was repeated one month after eradication of *H pylori*, at which time the median basal gastrin was 20 ng/l (range 15-25), significantly less than the value before eradication (30 ng/l range 15-60) ($p < 0.05$). On this occasion, the gastric juice ammonium concentration was considerably reduced at 0.4 mmol/l (range 0.1-0.9) and the urea infusion did not raise the ammonium concentration or change the plasma gastrin concentration. In conclusion, augmenting *H pylori* ammonia production does not cause any early change in plasma gastrin.

More than 95% of patients with duodenal ulcer disease have chronic *Helicobacter pylori* (formerly *Campylobacter pylori*) infection of the gastric antrum,¹ and eradication of this reduces the ulcer relapse rate.² It has recently been shown that patients with the infection have raised plasma gastrin concentrations³ which fall when the infection has been cleared.^{4,5} Because this increase in the plasma gastrin concentration is associated with raised intragastric acidity after meals,⁴ gastrin may be the link between chronic *H pylori* infection and duodenal ulceration.³ The mechanism by which *H pylori* infection increases the plasma gastrin concentration is unknown but could be related to its high urease enzyme activity, which results in high intragastric ammonium ion concentrations in gastric juice.⁶ The bacterium is mainly found under the gastric mucus layer close to the antral epithelial cells and its formation of ammonia may raise the pH of the epithelial surface. Because the release of gastrin

by antral G cells is normally suppressed by intragastric acid,⁷ a rise in mucosal pH due to high concentrations of ammonia could explain the increased gastrin release. There is also evidence from animals that ammonium uptake by G cells is a mechanism by which gastrin release is mediated.⁸

To determine whether the raised plasma gastrin concentration is the result of ammonia production by *H pylori* we have studied the effect of altering the rate of ammonia formation on plasma gastrin concentration by infusing urea intragastrically.

Patients

Eight patients (five men) with a history of endoscopically confirmed duodenal ulceration within the previous year were studied. Their ages ranged from 26 to 62 years. Each of the patients had *H pylori* infection of the gastric antrum confirmed by histology of antral biopsy specimens, rapid urease test (CLO test),⁹ and 14C urea breath test.¹⁰ None had taken any acid inhibitory agents or bismuth preparations in the month before the study.

Methods

The plasma gastrin response to increasing *H pylori* ammonia production was investigated by infusing a urea solution into the gastric antrum. Each patient acted as his or her own control by having the urea infusion repeated one month after a three week course of tripotassium dicitratobismuthate (De-Noltab) 120 mg qid, and metronidazole 400 mg tid designed to eradicate *H pylori*. Confirmation of eradication of *H pylori* was achieved by repeating the endoscopic antral biopsies and 14C urea breath test one month after the completion of treatment.

After a 16 hour overnight fast a dual lumen size 16 F gastric tube, which has multiple ports over its distal 6 cm (Andersen Inc, New York), was passed nasogastrically and positioned radiographically so that its tip lay in the distal part of the stomach. An intravenous cannula was inserted into the antecubital vein. At the end of a 30 minute basal period the stomach was emptied, and over the following hour dextrose solution (328 mmol/l) that did not contain urea was infused into the stomach at a rate of 2 ml per minute. After this control period, a dextrose solution containing urea (50 mmol/l) was infused at a rate of 2 ml per minute for four hours. The concentration of dextrose in the urea solution was reduced by 50 mmol/l so that the osmolalities of the two infusions were the same. In addition, the pH of the solutions was reduced to 1.8 by

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adding 1 ml concentrated HCL per 500 ml dextrose to prevent them raising intragastric pH.

Venous blood samples were obtained at 30 minute intervals throughout the experiment for plasma gastrin determination and were collected in lithium heparin tubes containing 4000 KIU aprotinin (Trasylol). The blood was centrifuged at 3000 g for 10 minutes at 4°C and the plasma was stored at -20°C. During the intragastric infusions, 10 ml samples of gastric juice were collected every 15 minutes for measurement of the ammonium and urea concentration and determination of pH. At the end of each hour all the gastric contents were aspirated to prevent the accumulation of infusate.

The plasma gastrin concentration was measured by radioimmunoassay using antibody R98 which has a lower limit of detection of 5-10 ng/l.¹¹ Gastric aspirate urea concentrations were measured by a urease method (SMAC I Technicon, Basingstoke, UK) and ammonium concentrations by an enzymatic method (SIGMA, Dorset, UK). The pH of the gastric aspirate was measured with a glass electrode (Radiometer ETS 822).

Statistical significance was assessed by the Wilcoxon signed rank sum test. The study was approved by the Western Infirmary Ethical Committee and each patient gave informed written consent.

Results

In seven patients the *H pylori* infection was successfully eradicated as confirmed by the absence of organisms and resolution of gastritis seen on antral biopsy specimens, negative CLO test, and negative 14 C urea breath test four weeks after completing the antibacterial treatment. Their median 20 minute breath test value was 135% of administered dose per mmol CO₂ × kg body weight × 100 (range 104-251) before treatment and fell to 4.5 (range 0.6-7) one month afterwards. The one patient in whom the organism was not cleared was excluded from further analysis.

BASAL VALUES

The median concentration of ammonium ions in the basal gastric aspirate before eradication of *H pylori* was 4.4 mmol/l (range 1.8-14.7) and this fell after eradication to 0.7 mmol/l (range 0.3-1.4) ($p < 0.02$) (Fig 1). The median concentration of urea in the basal gastric aspirate was 1.1 mmol/l (range 0.3-1.6) and rose to 2.5 mmol/l (range 1.0-3.4) after eradication ($p < 0.02$). The median plasma concentration of gastrin was 30 ng/l (range 15-60) and this fell to 20 ng/l (range 15-25) ($p < 0.05$) after eradication. The median pH of the basal gastric aspirates did not change significantly - it was pH 1.7 (range 1.2-1.9) before and 1.6 (range 1.2-1.7) after eradication of *H pylori*.

EFFECT OF INTRAGASTRIC INFUSIONS

During the 60 minute control infusion of dextrose solution, both before and after eradication, there was a progressive fall in urea and

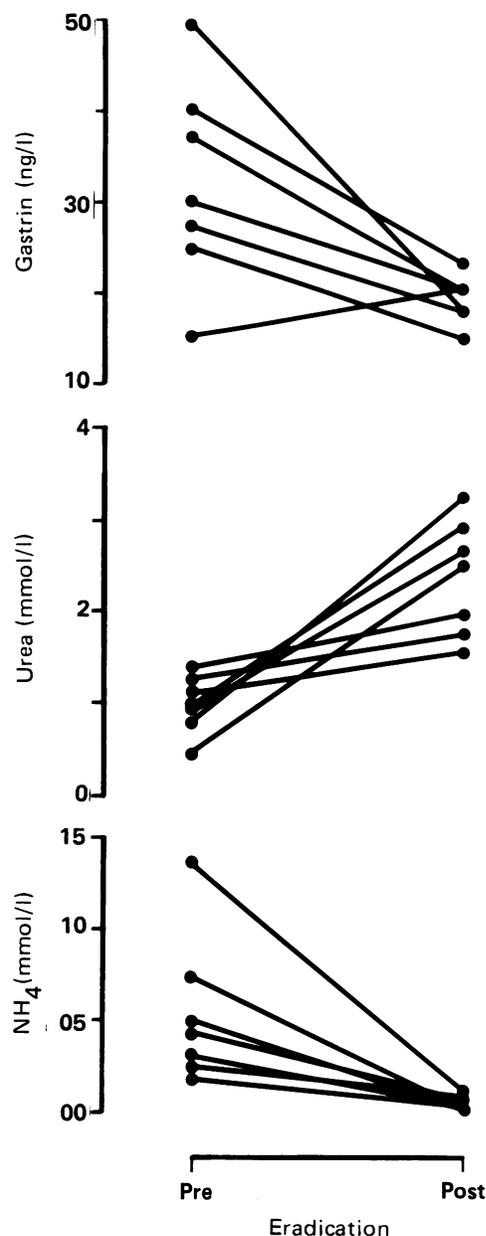


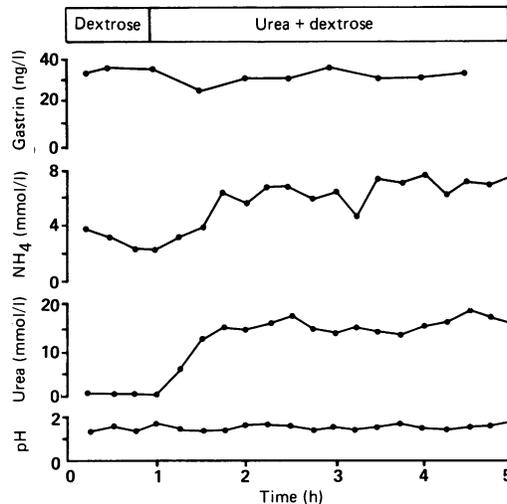
Figure 1: Gastric juice concentrations of urea and ammonia and plasma gastrin concentrations before and after eradication of *Helicobacter pylori* infection in 7 duodenal ulcer subjects. After eradication plasma gastrin concentration and gastric juice ammonium concentration were lower ($p < 0.05$, $p < 0.02$ respectively) and the gastric juice urea concentration increased ($p < 0.02$).

ammonium concentrations in the gastric aspirate due to dilution by the infusate (Figs 2 and 3). Plasma gastrin concentration did not change over this period.

Before eradication of *H pylori*, the urea infusion resulted in a rise in the gastric aspirate urea concentrations which reached a plateau after 60 minutes at a median value of 15.5 mmol/l (range 7.9-21.3) (Fig 2). The median gastric juice ammonium concentration immediately before beginning the urea infusions was 2.3 mmol/l (range 1.3-5.9) and rose over 90 minutes to reach a median plateau value of 6.1 mmol/l (range 4.2-11.9). This rise in ammonium production was not accompanied by any change in plasma gastrin concentration (Fig 2).

After eradication of *H pylori*, the rise in gastric juice urea concentration during urea infusion

Figure 2: Effect of intragastric infusion of urea on gastric juice concentrations of urea and ammonium, intragastric pH and plasma gastrin concentration in 8 patients with *Helicobacter pylori* infection of the gastric antrum. (Values are medians.)



was similar to that before eradication (Fig 3). On this occasion, however, there was no rise in the ammonium concentration. The median value immediately before beginning the infusion was 0.4 mmol/l (range 0.1–0.9) and it was 0.4 mmol/l (range 0.3–1.2) at the end of the infusion. The median gastrin concentration, which was lower after eradication of *H pylori*, was unaffected by the urea infusion.

The pH of gastric aspirates remained between 1.5 and 2.0 throughout the studies.

Discussion

In agreement with previous work,⁴ this study has shown that there is a considerable lowering of plasma gastrin concentrations after eradication of *H pylori* infection in duodenal ulcer subjects. The finding that increasing *H pylori* ammonia production failed to change the plasma gastrin concentration does not lend support to the hypothesis that the hypergastrinaemia is caused by the ammonia. It seems unlikely that the degree of increase in ammonia production was inadequate as the rise in the ammonium ion concentration at the antral epithelial surface, where the bacteria are found close to the gastrin secreting G cells, would have been even greater than the three fold rise noted in the gastric aspirate. It should be stressed, however, that the failure to cause a further increase in gastrin by augmenting ammonia production does not

exclude the possibility that the raised gastrin value was caused by the organism's ammonia production. The amount of ammonia produced by the bacterium under normal conditions may be sufficient to produce the maximum gastrin response via this mechanism.

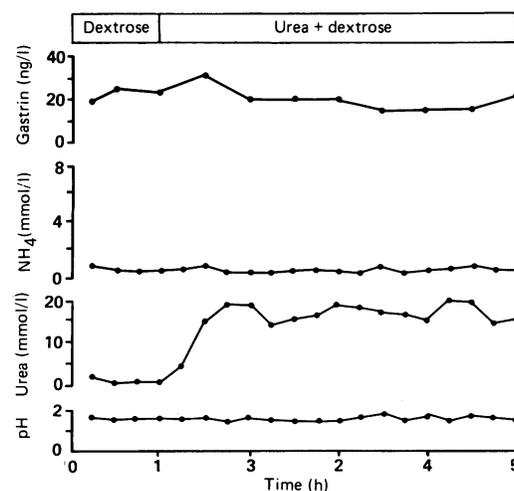
It has been proposed by Levi *et al* that the increased plasma gastrin concentrations are caused by the organism's ammonia production raising antral surface pH.³ This would interfere with the physiological suppression of gastrin release by luminal acid. Our finding that augmenting *H pylori* ammonia production fails to stimulate gastrin release does not refute this hypothesis. The local alkalinising effect of *H pylori* under basal conditions may already be sufficient to prevent suppression of gastrin release by gastric acid. In addition, the duration of the urea infusion in our study was four hours and earlier studies have shown that gastric alkalinisation was required for five hours before there was any rise in serum gastrin.¹² The effect of antral alkalinisation on serum gastrin in humans is controversial, however, as a more recent study could not detect any increase after raising gastric pH for 10 hours.¹³ Unfortunately, there are no studies of the effect of gastric alkalinisation on serum gastrin in patients of known *H pylori* status.

Other mechanisms by which *H pylori* infection of the gastric antrum may be stimulating excessive gastrin release need to be considered also. Eradication of the infection results in resolution of the antral gastritis and it is possible that it is the inflammatory cell infiltrate in the region of the G cells that is responsible for the hypergastrinaemia. A recent study has indicated that the hypergastrinaemia correlates better with antral gastritis than with *H pylori* infection.¹⁴ As the gastritis associated with the bacterial infection may be at least partly caused by the damaging effects of the ammonia,¹⁵ it may be very difficult in practice to differentiate the effects of the two.

This study also provides information on the regulation of *H pylori* ammonia production in vivo. The almost undetectable intragastric concentrations of urea found before eradication of the organism and the rapid rise in gastric ammonia concentration after the infusion of urea indicate that the gastric juice concentration of urea and its rate of diffusion across the gastric mucosa are factors that limit the generation of ammonia by *H pylori*. It has been proposed that the organism's ammonia production is a means by which it protects itself from intragastric acid by producing a local alkaline microenvironment.¹⁶ As gastric pH varies constantly and rapidly, such a protective mechanism should be under pH dependant control rather than under the control of substrate availability, which cannot be altered. The main function of the enzyme is more likely to be to scavenge nitrogen from urea for use in the synthesis of amino acids and nucleic acids.

In conclusion, though lowering intragastric ammonia concentration by eradicating *H pylori* is accompanied by a lowering of the plasma gastrin concentration, augmenting the bacterium's ammonia production is not accompanied by a further increase in plasma gastrin. The

Figure 3: Effect of intragastric infusion of urea on gastric juice concentrations of urea and ammonium, intragastric pH, and plasma gastrin concentration in 7 patients after eradication of *Helicobacter pylori* infection. (Values are medians.)



mechanism by which *H pylori* raises plasma gastrin concentrations, therefore, remains unclear.

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- 1 Marshall BJ, McGeachie DB, Rogers PA, Glancy RG. Pyloric campylobacter infection and gastroduodenal disease. *Med J Aust* 1985; **142**: 439–44.
- 2 Marshall BJ, Goodwin CS, Warren JR, *et al*. Prospective double blind trial of duodenal ulcer relapse after eradication of *campylobacter pylori*. *Lancet* 1988; **ii**: 1437–42.
- 3 Levi S, Haddad G, Ghosh P, Beardshall K, Playford R, Calam J. *Campylobacter pylori* and duodenal ulcers; the gastrin link. *Lancet* 1989; **i**: 1167–8.
- 4 McColl KEL, Fullarton GM, Nujumi AM El, Macdonald AM, Brown IL, Hilditch TE. Lowered gastrin and gastric acidity after eradication of *Campylobacter pylori* in duodenal ulcer. *Lancet* 1989; **i**: 499–500.
- 5 Oderda G, Holton J, Altare F, Vaira D, Ainley C, Ansaldi N. Amoxicillin plus tinidazole for *Campylobacter pylori* gastritis in children: assessment by serum IgG antibody, Pepsinogen I and gastrin levels. *Lancet* 1989; **i**: 690–2.
- 6 Marshall BJ, Langton SR. Urea hydrolysis in patients with *Campylobacter pyloridis* infection. *Lancet* 1986; **i**: 965–6.
- 7 Oberhelman HA, Woodward ER, Zubiran JM, Dragstedt LR. Physiology of the gastric antrum. *Am J Physiol* 1952; **169**: 738–48.
- 8 Lichtenberger LM, Graziani LA. Possible importance of dietary ammonia in the postprandial release of gastrin (G). *Gastroenterology* 1981; **80**: 1212.
- 9 Marshall BJ, Warren JR, Francis GJ, Langton S, Goodwin CS, Blincolm ED. Rapid urease test in the management of *Campylobacter pyloridis* associated gastritis. *Am J Gastroenterol* 1987; **82**: 200–10.
- 10 Bell GD, Weil J, Harrison G, *et al*. 14 C urea breath test analysis, a non invasive test for *Campylobacter pylori* in the stomach. *Lancet* 1987 **i**: 1367–8.
- 11 Ardill JES. The measurement of gastrin by radio-immunoassay. [PhD Thesis.] Belfast: Queens University, 1973.
- 12 Peters MN, Feldman M, Walsh JH, Richardson CT. Effect of gastric alkalinisation on serum gastrin concentration in humans. *Gastroenterology* 1983; **85**: 35–9.
- 13 Peterson WL, Walsh JH, Richardson CT. Cimetidine blocks antacid-induced hypergastrinaemia. *Gastroenterology* 1986; **90**: 48–52.
- 14 Wyatt JI, Rathbone BJ, Green DM, Primrose J. Raised fasting serum gastrin in chronic gastritis is independent of *Campylobacter pylori* status and duodenal ulceration. [Abstract]. *Gut* 1989; **30**: A1483.
- 15 Yoshida H, Imoto I, Shida Y, Suzuki S. The significance of *Campylobacter pylori* infection on the development of gastric mucosal injury – urease – protease theory. [Abstract]. *Gastroenterology* 1989; **96**: A562.
- 16 Goodwin CS, Armstrong JA, Marshall BJ. *Campylobacter pyloridis*, gastritis and peptic ulceration. *J Clin Pathol* 1986; **39**: 353–65.