

Effects of *Helicobacter pylori* on histamine and carbachol stimulated acid secretion by human parietal cells

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Abstract

Helicobacter pylori (*H pylori*) infection is associated with hypo, normal, and hypersecretory disorders of the gastric mucosa. Pathophysiological pathways by which *H pylori* interacts with acid secretion are still unclear. The effects of *H pylori* on (¹⁴C) aminopyrine uptake by human parietal cells were examined as an indirect assay for acid secretion. Isolated oxyntic glands were stimulated with submaximal concentrations of histamine or carbachol and incubated with sonicates of different *H pylori* strains. Omeprazole and sonicates of *Campylobacter jejuni* served as positive and negative controls, respectively. Two of four *H pylori* strains reduced hydrochloric acid sequestration within the parietal cells significantly and in a dose dependent manner in up to 80%. Interaction with acid secretion may therefore constitute a factor contributing to a distinct pathogenicity of *H pylori* strains.

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Helicobacter pylori (*H pylori*) causes or is at least associated with different clinical and histological entities such as chronic gastritis, peptic ulcer disease, and even gastric carcinoma. Under these conditions acid secretion varies from hypo to hyperchlorhydria. Recently it has been shown that sonicates of *H pylori* strongly inhibit acid secretion by rabbit parietal cells.¹ Rabbits, however, are not natural hosts of *H pylori*. In humans *H pylori* infection may cause acute epidemic gastritis associated with hypochlorhydria.^{2,4} Whether hypochlorhydria occurs in every short term *H pylori* infection is as unknown as the influence of longterm *H pylori* infection on parietal cell function. We have examined the effects of sonicates of different *H pylori* strains on acid secretion from isolated human oxyntic glands, using ¹⁴C-aminopyrine (¹⁴C-AP) uptake by the parietal cells. This well established assay indirectly reflects acid production.

Methods

ISOLATED OXYNTIC GLAND PREPARATION

Isolated gastric glands were prepared from patients who had had an endoscopic biopsy and who gave informed consent before the investigation. Six to 10 biopsy specimens were taken 10 cm aboral from the cardia using a GIF XQ 10 or XQ 20 endoscope and an FB 19K biopsy forceps (Olympus). All patients had a negative HLO-test (Delta West). Only data from patients

(n=12) with histologically normal oxyntic mucosa who had not taken any drugs inhibiting or buffering acid secretion during the last week were evaluated. The specimens were immediately taken to the laboratory in cold (4°C) phosphate buffered saline. According to the method of Fellenius⁵ the specimens were minced, washed twice in phosphate buffered saline, and treated with collagenase (Collagenase type I, Sigma) for 120 minutes. Then the solution was filtered to separate the glands from the connective tissue. The glands were washed in respiration medium containing human albumin (1 mg/ml) and glucose (2 mg/ml). After removing the supernatant, 0.5 ml concentrated glands were resuspended in 3 ml respiration medium. One hundred µl of this suspension containing 100-300 µg glands (dry weight) were incubated with 3×10⁻⁴ M histamine (Sigma, Lot 49F5002) or with 10⁻⁴ M carbamylcholine chloride (Sigma, Lot 39T0478) and with sonicates of 2, 4, or 6 mio colony forming units (CFU) *H pylori* in addition to ¹⁴C-aminopyrine. Incubations with omeprazole (3×10⁻⁸ M) and sonicates of *Campylobacter jejuni* (4 mio CFU) served as positive and negative controls. The isolated gland preparation of each patient (n=12) was aliquoted for the following ¹⁴C-aminopyrine uptake tests: native (2 tests), stimulation (4 tests), *H pylori* strain A-D (8 tests), omeprazole (2 tests), and *Campylobacter jejuni* (2 tests). Accordingly, each gland preparation was tested for adequate stimulation with histamine and carbachol as well as for the effect of *H pylori*/*Campylobacter jejuni*, and omeprazole on histamine and carbachol stimulated parietal cell secretion.

HELICOBACTER PYLORI PREPARATIONS

Four fresh isolates conforming to standard phenotype criteria for *H pylori* were grown for 72 hours at 37°C in BHI medium under microaerophilic conditions. Bacteria were suspended in phosphate buffered saline (10⁻⁸ CFU/ml). Suspensions of *H pylori* and of *Campylobacter jejuni* were subjected to a 4×30 seconds sonification before they were tested in the isolated gland preparations. In addition sonicates of *H pylori* strain A were incubated with 50 µg/ml pronase E Typ XXV (Sigma) for 30 minutes at 37°C or with 50 µg/ml trypsin (Merck) for 12 hours at 20°C or were heat treated for 30 minutes at 100°C.

PARIETAL CELL FUNCTION

After 120 minutes incubation at 37°C with the respective stimulant/inhibitor the glands were centrifuged and the supernatant was withdrawn.

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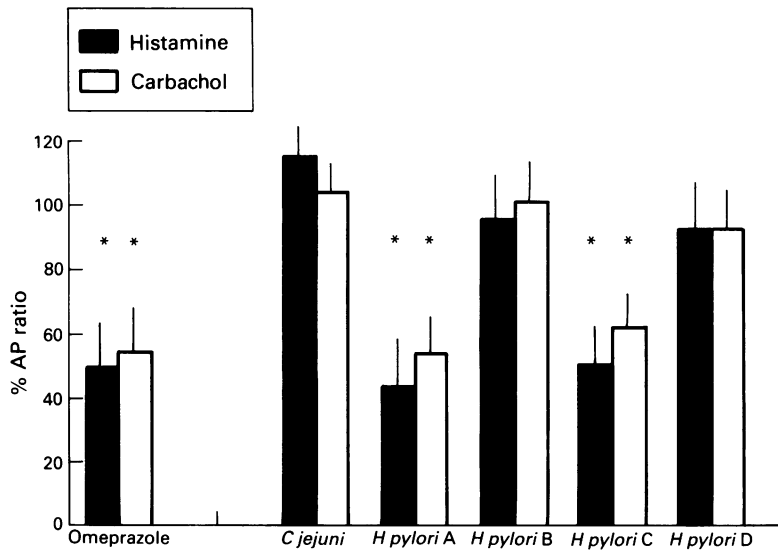


Figure 1: Percentage ^{14}C aminopyrine (AP) ratio (mean (SD)) of histamine ($300\ \mu\text{M}$) or carbachol ($100\ \mu\text{M}$) stimulated gastric glands. Comparison of four different *H. pylori* strains, *Campylobacter jejuni* (4 mio colony forming units), and omeprazole ($30\ \mu\text{M}$). * $p < 0.05$.

Glands were dried, weighed, and solubilised in sodium dodecyl sulphate (SDS, Bio Rad). Intracellular acidification was determined by ^{14}C aminopyrine ($10^{-6}\ \text{M}$; 94,7 mCi/mmol Dupont) uptake into the parietal cells. The ratio between ^{14}C -AP in the intraglandular and the extraglandular compartment (AP ratio = dpm pellet \times 100/2 \times dry weight pellet \times dpm supernatant) was used as an estimate of acid production by the parietal cells.⁵ Radioactivity of the samples was counted in a United Technologies Packard 2000CA scintillation counter.

CELL VIABILITY

To rule out cytotoxic effects, trypan blue exclusion tests and tritiated leucine incorporation tests as indirect parameter of protein synthesis were performed in each experiment.

STATISTICS

Because the data had a Gaussian distribution and the standard deviations were roughly equal, the results were analysed by Student's *t* test for paired data. A *p* value < 0.05 was considered to be significant.

Results

As determined by dye exclusion tests and leucine

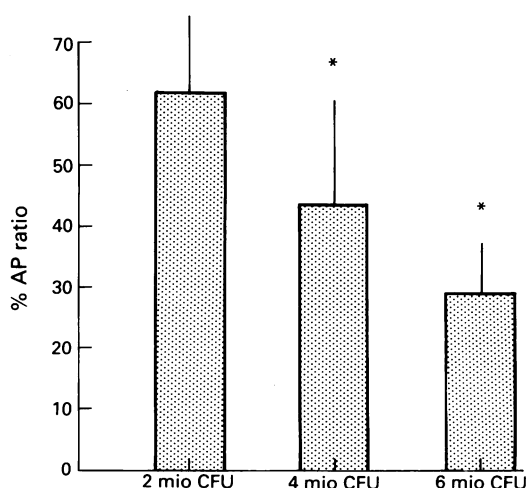


Figure 2: Percentage ^{14}C aminopyrine (AP) ratio (mean (SD)) of histamine ($300\ \mu\text{M}$) stimulated gastric glands. Comparison of 2, 4, and 6 mio colony forming units (CFU) *H. pylori* of strain A. * $p < 0.05$.

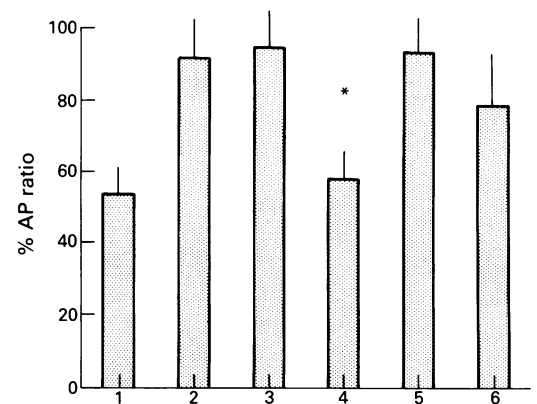


Figure 3: Percentage ^{14}C aminopyrine (AP) ratio (mean (SD)) of histamine ($300\ \mu\text{M}$) stimulated gastric glands. 1 = not histamine stimulated; 2 = histamine + pronase ($5\ \mu\text{g/ml}$); 3 = histamine + trypsin ($5\ \mu\text{g/ml}$); 4 = histamine + trypsin treated *H. pylori* sonicate (4 mio colony forming units (CFU) *H. pylori* A); 5 = histamine + pronase treated *H. pylori* sonicate (4 mio CFU *H. pylori* A); 6 = histamine + heat treated *H. pylori* sonicate (4 mio CFU *H. pylori* A). * $p < 0.05$.

incorporation assays neither *H. pylori* or *Campylobacter jejuni* sonicates had any effect on cell membrane integrity and protein synthesis of the oxyntic glands. Histamine and also carbachol (mean (SD)) stimulated ^{14}C -AP uptake by 92 (18)% and 41 (14)%, respectively. Omeprazole inhibited both histamine and carbachol stimulated ^{14}C aminopyrine uptake by 49 (13)% and 54 (12)% whereas sonicates of *Campylobacter jejuni* had no effect. Two of the four tested *H. pylori* sonicates from different strains inhibited cellular acidification of the parietal cells significantly (Fig 1). This inhibitory effect on the parietal cells was in a dose dependent manner (Fig 2) and was seen in both the histamine as well as in the carbachol stimulated system. The maximal reduction of the histamine and carbachol stimulated parietal cell acidification was 80% and 64% for *H. pylori* A (6 mio CFU) and 62% and 66% for omeprazole ($3 \times 10^{-8}\ \text{M}$), respectively. Heat treatment of strain A for 30 minutes at 100°C reduced the acid inhibitory effect by about 50%. Digestion with pronase eliminated the inhibition totally, whereas trypsin pretreatment of the sonicates resulted in unchanged inhibitions (Fig 3).

Discussion

Support for the assumption that *H. pylori* can affect gastric acid secretion in humans is derived from the clinical finding that under certain circumstances the bacterium may cause acute epidemic gastritis with transient hypochlorhydria²⁻⁴ and from in vitro experiments showing an inhibiting effect of *H. pylori* on acid production by isolated rabbit parietal cells.¹

Our study shows for the first time that *H. pylori* isolated and cultured from infected patients contains a factor that strongly suppresses acid secretion from oxyntic glands of *H. pylori* negative people with normal gastric histology and that this capability differs between *H. pylori* strains. Because only fresh strains of *H. pylori* were used the lack of inhibitory activity seen in two of the strains cannot be attributed to repeated subculture. The nature of the inhibitor and its cellular localisation remain to be defined. Pre-

liminary data suggest that the factor inhibiting acid secretion from rabbit parietal cells is a protein larger than 12–14 000 kDa.¹ Our data support the assumption that the inhibitor is a rather heat stable protein. A precise characterisation and comparison of the factor(s) inhibiting rabbit and human parietal cells, however, is mandatory to prove or refute identity and to gain some insights into their mode of action. As preparations of isolated glands parietal cells have lost their polarity, the site and mode of action of inhibition remain to be determined. Under our experimental conditions the two *H pylori* strains that contained the inhibitor suppressed histamine as well as carbamylcholine stimulated acid secretion. Therefore, it does not seem probable that the acid inhibitor interferes with either the histamine or the acetylcholine receptor of the parietal cell but rather blocks acid secretion by a different mechanism. To further elucidate the mode of action it would be interesting to find out if the inhibitor is membrane bound or cytoplasmic and thereby possibly released by the germ.

Pathogenetic or even clinical implications of our findings must be entirely speculative at this point. Further studies are needed to find out if acid inhibition is a factor facilitating gastric colonisation and contributing to a distinct pathogenicity of different *H pylori* strains. *H pylori* infection has been shown to be associated with hypo, normal, and hyperchlorhydria and with inappropriate hypergastrinaemia.⁶ Hypergastrinaemia seems to be caused by *H pylori*⁷ or by interference of *H pylori* with the somatostatin mediated inhibition of gastrin release from the antral G-cells.⁸ After *H pylori* eradication serum gastrin concentrations decrease⁹ but acid

secretion tends to remain unchanged.¹⁰ One of the possible explanations for this finding is the blockade of the surplus gastrin effect on the parietal cell by the *H pylori* derived acid inhibitor. On the other hand an infection of a subject with an *H pylori* strain devoid of the acid inhibitor could lead to hypergastrinaemia driven acid hypersecretion. The differential expression of an acid inhibiting factor by the different strains could prove to be a key factor of *H pylori* pathogenicity but we are just at the beginning of testing this hypothesis.

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