Prostaglandin E₂ in gastric mucosa of children with *Helicobacter pylori* gastritis: Relation to thickness of mucus gel layer

G Oderda, M D'Alessandro, P Mariani, P Lionetti, M Bonamico, D Dell'Olio, N Ansaldi

Abstract

Aims—To evaluate the changes in mucus gel layer thickness and prostaglandin E₂ (PGE₂) content caused by *Helicobacter pylori* infection in the antral mucosa of children: to assess whether decreased mucus gel thickness is related to PGE₂ production.

Methods—Antral biopsy specimens were taken at endoscopy from 153 children. *H. pylori* gastritis was evident in 45 and normal mucosa in 59. The other 49 children were studied one month after antibiotic treatment that eradicated the infection in 37 of them had been stopped. One antral specimen was immersed in ice-cold saline, put under an inverse microscope with an eyepiece graticule. Mucus gel thickness was measured and then the processed for histological examination; another specimen was weighed and processed for in vitro prostanoid generation.

Results—Mucus gel layer thickness was significantly decreased in children with *H. pylori* gastritis (90 (SD) 29 μ m v 120 (58) μ m in controls, $p < 0.01$) but returned to control values after *H. pylori* had been eradicated. PGE₂ generation was significantly increased in children with *H. pylori* gastritis (1022 (811) ng/g v 641 (473) ng/g in controls, $p < 0.01$). One month after treatment PGE₂ generation significantly decreased in children without infection (880 (534), $p < 0.01$), but was still high where infection persisted. A significant inverse correlation was found between PGE₂ generation and mucus gel layer thickness ($p < 0.05$).

Conclusions—These data suggest that *H. pylori* damages the mucus gel layer, and that the gastric mucosa increases generation of PGE₂ in response to back diffusion of acid and pepsin.

(J Clin Pathol 1993;46:836-839)

Prostaglandin E₂ (PGE₂) has an important role in gastric cytoprotection by influencing several factors which improve the integrity of the gastric mucosal barrier.¹ The mucus gel layer adherent to the gastric mucosa is one of these factors: it protects underlying epithelial cells from acid, by facilitating neutralisation of the mucosal surface, from luminal pepsin and from shear forces during digestion. Its protective property depends on gel viscosity

and thickness.² Incubation of antral biopsy specimens with *H. pylori* filtrate causes degradation of the mucus glycoprotein polymer to glycopeptides and loss of mucus viscosity.³ In children with *H. pylori* gastritis mucus gel layer thickness is less than that of control children⁴ and gastric PGE₂ production is increased in adult patients with *H. pylori* gastritis.^{5,6}

Methods

Antral biopsy specimens were obtained from 153 children (94 males) undergoing upper gastrointestinal endoscopy. Their median age was 11 years (range 1 to 14). In 104 of them endoscopy was performed for dyspeptic symptoms, and in 49 to monitor the response to antibiotic treatment, one month after treatment with amoxicillin (50 mg/kg) and tinidazole (20 mg/kg) to treat *H. pylori* gastritis had been stopped. Endoscopy was performed using an Olympus GIF XP20 after mild sedation with oral diazepam. The endoscope and biopsy forceps were disinfected in 2% glutaraldehyde after each use.

Three antral specimens were obtained from each patient: one was immediately tested by the rapid urease test (CP-test, Gist Brocades, Holland). A second specimen was immediately soaked in ice-cold saline and placed under an inverse microscope with an eyepiece graticule ($\times 200$ magnification) where the mucus gel was seen as a continuous opalescent layer adhering to the mucosa. Layer thickness was measured in 10 distinct sites, as described before.⁷ Results are expressed as the median values of the 10 measurements of each antral specimen. Once thickness had been recorded the same biopsy specimen was fixed in 10% formalin and processed for histological examination. Sections were stained with Giemsa for *H. pylori* and haematoxylin and eosin to determine the presence of gastritis according to the Sydney system.⁸ When granulocytes were present gastritis was considered to be active.

For grading the severity of antral inflammation mononuclear cells and granulocytes were quantified as described before.⁹ A third antral specimen was weighed and processed for ex vivo prostanoid generation according to the method of Whittle *et al.*¹⁰ PGE₂ content was measured by radioimmunoassay (NEN Du Pont, Boston, Massachusetts). PGE₂ assay sensitivity was 0.13 pg/added and the antibody to PGE₂ showed a cross-reactivity of 3.7% with PGE₁ and less than 0.5 with other prostanoids. Results were expressed as ng/g

Department of
Paediatric
Gastroenterology,
University of Turin
G Oderda
D Dell'Olio
N Ansaldi

Second Surgical
Clinic, First
Paediatric Clinic La
Sapienza University,
Rome, Italy
P Lionetti
M Bonamico
M D'Alessandro
P Mariani

Correspondence to:
G Oderda, Paediatric
Gastroenterology, University
of Turin, Piazza Polonia 94,
10126 Torino, Italy.

Accepted for publication
7 April 1993

Table 1 Endoscopic and histological findings in children undergoing endoscopy for dyspeptic symptoms or after antibiotics for *H. pylori* gastritis

	With dyspeptic symptoms		After treatment	
	<i>H. pylori</i> + <i>n</i> = 45	<i>H. pylori</i> - <i>n</i> = 59	<i>H. pylori</i> + <i>n</i> = 12	<i>H. pylori</i> - <i>n</i> = 37
Endoscopy:				
Normal	13	55	9	21
Nodular antrum	28	1	3	16
Duodenal ulcer	3	2		
Gastric ulcer	1	1		
Antral mucosa:				
Normal		48		15
SCG: active	20		2	
non-active	25	11	10	22

SCG: Superficial chronic gastritis

wet weight of antral biopsy specimens.

Statistical evaluation of parametric data was based on Student's *t* test for unpaired data for non-parametric data for the Wilcoxon rank sum test, and correlations on Pearson's correlation coefficient.

Results

The urease test and Giemsa stained antral sections showed the presence of *H. pylori* in 45 children with dyspeptic symptoms, all of whom had superficial chronic gastritis; 59 children were negative for *H. pylori*—most of them with normal antral mucosa. Twelve of the children studied one month after antibiotic treatment were still infected; in 37 *H. pylori* had been eradicated. The endoscopic and histological findings are shown in table 1.

The thickness of the mucus gel layer was significantly less in children with *H. pylori* gastritis when compared with *H. pylori* negative children ($p < 0.01$). As well as being thinner the gel layer varied considerably in thickness from site to site; in control children it was more evenly distributed (fig 1). There was no correlation between mucus gel layer thickness and severity of antral inflammation, as defined by the inflammation score, and no difference between children with active or non-active superficial chronic gastritis. In 11 *H. pylori* negative children in whom histological examination showed mild superficial chronic gastritis thickening of the mucus gel layer, this was similar to that in control children. In children studied after antibiotic treatment the mucus gel layer thickness was significantly increased when compared with patients who were still infected ($p < 0.001$) and was similar to controls when *H. pylori* had been eradicated; when *H. pylori* was still present mucus thickness was not significantly different (table 2).

PGE₂ generation in antral mucosa was significantly greater in children with *H. pylori* gastritis compared with controls ($p < 0.01$, table 2). No correlation was found between PGE₂ and severity of antral inflammation. In *H. pylori* positive children PGE₂ production tended to be higher in patients with nodular antritis compared with children with a normal looking antral mucosa (1136 (928) ng/g, *v* 744 (633) ng/g, but the difference failed to reach significance ($p = 0.05$). Similarly, PGE₂ generation was mildly but not significantly greater in *H. pylori* positive children with active superficial chronic gastritis (1161 (896) ng/g *v* 648 (433) ng/g in non-active superficial chronic gastritis. Seven children had duodenal or gastric ulcers; PGE₂ generation in their antral mucosa was lower (357 (97) ng/g in four who were *H. pylori* positive and 622 (128) ng/g in three who were *H. pylori*

Figure 1 Appearance of mucus gel layer (between arrows), adhering to a normal antral mucosa viewed under an inverse microscope (A). Gel layer was thicker and more regularly distributed than in infected mucosa (B), where it showed considerable variation in thickness between different sites of the biopsy specimen.

Figure 2 PGE₂ production (ng/g wet weight of antral biopsy specimen) in antral mucosa of 104 children with and without *H. pylori* gastritis in relation to mucus gel layer thickness (μm) measured in fresh antral biopsy specimens ($r = 0.289$, $p < 0.05$).

negative), but the difference was not significant, probably because the numbers were small.

A significant inverse correlation was found between levels of PGE₂ generation and mucus gel layer thickness ($r = 0.289$, $p < 0.05$) (fig 2). After treatment and eradication of *H. pylori* PGE₂ generation was significantly decreased compared with patients still infected ($p < 0.01$) and was similar to that of controls (table 2).

Discussion

Our results showed that the mucus gel layer covering the epithelial surface of antral mucosa was significantly thinner and more irregularly distributed in children with *H. pylori* gastritis. Its thickness was inversely correlated with increased PGE₂ generation.

The mechanisms of interaction between *H. pylori* and gastric mucosa are still not well understood. Several pathogenic mechanisms for the mucosal damage have been proposed.¹¹ Disruption of the mucus coat is known to occur in gastric disease, and the process has been attributed to the enhanced activity of intraluminal pepsin.¹² More recent data, however, have provided some evidence that *H. pylori* proteases and lipases may be responsible for mucus protein and lipid degradation, thus undermining gel layer

integrity.^{13,14} On the other hand, *H. pylori* may reduce mucosal peptic activity by accelerating removal of pepsin into the gastric lumen.¹⁵ After disintegration of the glycoprotein polymeric structure and formation of glycopeptides that no longer possess viscous and gel-forming properties, mucus gel exhibits only a limited hydrogen ion retardation capacity.³ Hydrochloric acid secreted by gastric glands can penetrate the mucus gel layer by producing a viscous fingering pattern dependent on pH, mucin concentration, and acid flow rate: hydrochloric acid in the lumen is prevented from diffusing back to the epithelium by the high viscosity of mucus gel on the luminal side.¹⁶ By reducing the dimension and physicochemical qualities of the mucus gel layer *H. pylori* may render the stomach epithelium more vulnerable to damage by luminal acid and pepsin. As a response to mucosal damage PGE₂ generation was increased in the children studied. This has already been shown in adults with duodenal¹⁷ or gastric ulcers¹⁸ regardless of *H. pylori* status and in patients with *H. pylori* gastritis.^{5,6}

We did not find a significant difference in PGE₂ generation in children with more severe gastritis or in those with polymorphonuclear cell infiltration of the antral mucosa as has been found in adults.⁵ This finding was surprising because PGE₂ in the inflamed gastric mucosa is mostly synthesised by the lamina propria macrophages.^{19,20}

The most interesting of our findings was the inverse correlation between mucus gel layer thickness, as a marker of mucus damage, and PGE₂ generation. The lack of correlation between severity of inflammation and PGE₂ generation, and the inverse correlation with mucus gel layer thickness, seem to suggest that the micro-organism causes the mucus gel damage first. This deficient mucosal protection mechanism facilitates back diffusion of acid and pepsin and the gastric mucosa responds by increasing PGE₂ generation in order to protect itself from the noxious gastric juice.

Some preliminary data were presented to the AGA Meeting in Washington in May 1992 and have been published as an abstract in *Gastroenterology* 1992;101:A137.

- 1 Robert A. Cytoprotection by prostaglandins. *Gastroenterology* 1979;77:761-7.
- 2 Allen A, Hutton DA, Leonard AJ, Pearson JP, Sellers LA. The role of mucus in the protection of the gastroduodenal mucosa. *Scand J Gastroenterol* 1986;21(suppl 125):71-7.
- 3 Sarosiek J, Slomiany A, Slomiany BL. Evidence of weakening of gastric mucus integrity by *Campylobacter pylori*. *Scand J Gastroenterol* 1988;23:585-90.

Table 2 Mucus gel layer thickness and PGE₂ generation in children undergoing endoscopy for dyspeptic symptoms or after antibiotics therapy for *H. pylori* gastritis

	With dyspeptic symptoms		After treatment	
	<i>H. pylori</i> + $n = 45$	<i>H. pylori</i> - $n = 59$	<i>H. pylori</i> + $n = 12$	<i>H. pylori</i> - $n = 37$
Median (μm)	90*	120	90†	150
(range)	(40-180)	(90-360)	(40-240)	(90-240)
PGE ₂ ng/g				
Mean (1SD)	1022 (811)*	641 (473)	1585 (1215)*	880 (534)

* $p < 0.01$. † $p < 0.001$.

- 4 Oderda G, Dell'Olio D, Forni M, Morra I, Ansaldi N. Mucus gel layer adherent to gastric mucosa in children with and without *Helicobacter pylori* gastritis. *Eur J Gastroenterol* 1991;3(suppl 1):S8.
- 5 Taha AS, Boothman P, Holland P, et al. Gastric mucosal prostaglandin synthesis in the presence of *Campylobacter pylori* in patients with gastric ulcers and non-ulcer dyspepsia. *Am J Gastroenterol* 1990;85:47-50.
- 6 Avunduk C, Suliman M, Gang D, et al. Gastroduodenal mucosal prostaglandin generation in patients with *Helicobacter pylori* before and after treatment with bismuth subsalicylate. *Dig Dis Sci* 1991;36:431-4.
- 7 Kerss S, Allen A, Garner A. A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. *Clin Sci* 1982;63:187-95.
- 8 Misiewicz JJ, Tytgat GNJ, Goodwin CS, et al. The Sydney System: a new classification of gastritis. In: *Working Party Reports of the World Congress of Gastroenterology*. Oxford: Blackwell Scientific Publications, 1990:1-10.
- 9 Oderda G, Vaira D, Dell'Olio D, et al. Serum pepsinogen I and gastrin concentration in children positive for *Helicobacter pylori*. *J Clin Pathol* 1990;43:151-5.
- 10 Whittle BJR, Higgs GA, Eakins KE, Moncada A, Vane JR. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature* 1980;284:271-3.
- 11 Blaser MJ. Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. *Gastroenterology* 1992;102:720-7.
- 12 Pearson JP, Ward R, Allen A, Roberts NB, Taylor WH. Mucus degradation by pepsin: comparison of mucolytic activity of human pepsin 1 and pepsin 3: implications in peptic ulceration. *Gut* 1986;27:243-8.
- 13 Slomiany BL, Bilski J, Sarosiek J, et al. *Campylobacter pylori* degrades mucin and undermines gastric mucosal integrity. *Biochem Biophys Res Comm* 1987;144:307-14.
- 14 Slomiany BL, Kasinathan C, Slomiany A. Lipolytic activity of *Campylobacter pylori*: effect of bismuth subcitrate (De Nol). *Am J Gastroenterol* 1989;84:506-10.
- 15 Yahav J, Oderda G, Diver-Haber A, Keller N, Jonas A. Mucosal peptic activity during *Helicobacter pylori* infection in pediatric patients. *Gut* 1992;33:1162-5.
- 16 Bhaskar KR, Garik P, Turner BS, et al. Viscous fingering of HCl through gastric mucin. *Nature* 1992;360:458-61.
- 17 Cunliffe WJ, Allen A, Hutton DA, Pearson JP, Venables CW. Adherent gastric mucus thickness in patients with gastroduodenal disorders. *Gut* 1982;25:A1182-3.
- 18 Hawkey CJ. Synthesis of prostaglandin E₂, thromboxane B₂ and prostaglandin catabolism in gastritis and gastric ulcer. *Gut* 1986;27:1484-92.
- 19 Morley J, Bray MA, Jones RW, et al. Prostaglandins and thromboxane production by human and guinea pig macrophages and leucocytes. *Prostaglandins* 1979; 17:730-6.
- 20 Smith PD, May UE, Perez-Perez GI, Blaser MJ, Whal IM. *Helicobacter pylori* (HP) induces blood monocytes and mucosal macrophages, but not enterocytes, to produce eicosanoids. *Gastroenterology* 1992;101:A330.