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ALIMENTARY TRACT

Histamine concentration of gastric mucosa in *Helicobacter pylori* positive and negative children

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Abstract

The histamine concentration was determined by enzymatic isotopic method in biopsy specimens of oxyntic mucosa from 37 children. Nineteen of the 37 had *Helicobacter pylori* associated gastritis (9 with duodenal ulcer). The histamine concentration in the *H pylori* negative group was mean (SD) 54·1 (23·1) μg/g fresh weight, and that in the *H pylori* positive group was 26·3 (14·2) μg/g ($p<0·01$). There was also a significant difference between *H pylori* positive patients with duodenal ulcer (19·8 (6·3) μg/g) and those without ulcer (31·4 (17·9) μg/g) ($p<0·05$). These results suggest that *H pylori* positive patients, especially those with duodenal ulcer, have reduced 'stored' histamine, perhaps because of increased histamine liberation.

The aetiology and pathogenesis of duodenal ulceration are not fully understood. It is currently accepted that peptic ulceration arises from a disturbance in the balance between acid production and mucosal resistance.[1] A number of features are thought to be important in the pathogenesis of duodenal ulcer disease but clinical and epidemiological data now suggest that *Helicobacter pylori* plays a major role in most duodenal ulcer patients.[2-5] In fact, the micro-organism has been observed in up to 100% of subjects with this disorder and it can be eliminated by antibiotic treatment, leading to complete healing in some cases.[4] Nevertheless, the mechanism by which the micro-organism promotes ulcer formation is uncertain.

Levi *et al* recently proposed that urease produced by *H pylori* breaks urea up into ammonia, raising the antral surface pH, which increases gastrin release and therefore gastric acid secretion.[6] In addition, it was shown that the serum gastrin concentration of children with upper gastrointestinal disorders[7] and of adults with duodenal ulcer fell after eradication of *H pylori* with antibiotics.

If increased acid secretion is linked to *H pylori* in the pathogenesis of duodenal ulcer disease the amount of histamine in oxyntic mucosa of *H pylori* positive and negative patients with and without duodenal ulcer should be measured, because histamine has been proposed as the final common mediator for all parietal cell secretagogues.[9-11] We therefore determined the histamine concentration in oxyntic mucosa of *H pylori* positive and negative children. We believe children are a suitable population for duodenal ulcer study because factors such as smoking, alcohol, and drugs, which are important in modifying expression of the disease in adults, are not present among them. Furthermore, we are not aware of any other study evaluating the histamine concentration of gastric mucosa in children.

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Patients and methods

This project was approved by the Ethics Committee of Hospital das Clínicas, Universidade Federal de Minas Gerais, Brazil, and informed consent to the study was obtained from the parents of all the patients.

STUDY SUBJECTS

Thirty seven patients (27 boys) undergoing endoscopy for investigation of upper abdominal pain and who did not have a history of smoking or underlying disorders were included in the study. None had received non-steroidal anti-inflammatory drugs, histamine H_2 receptor antagonists, or any other medication for at least 30 days before the study. Their ages ranged from 1 to 16 years (mean age 9·2 years). Nineteen of the patients had *H pylori* associated gastritis. In this group nine children had endoscopically proved duodenal ulcer (eight boys, mean age 12·4 years) and 10 did not (8 boys, mean age 9·0 years).

Endoscopy was performed either under general anaesthesia or sedation (diazepam and pethidine) and local anaesthesia (lignocaine). The biopsy forceps were disinfected with 70% ethanol.

Biopsy specimens were collected from the mid region of the greater curvature of the gastric body for histamine determination, culture, and histology, and from the lesser curvature of the antrum for culture,[5] preformed urease test,[12] carbolfuchsin staining,[13] and histological examination.[14]

HISTAMINE ANALYSIS

Three fragments taken from oxyntic mucosa were used for histamine determination. They were immediately frozen and were stored in liquid nitrogen until they were processed.

EXTRACTION PROCEDURE

Histamine was extracted by slight modification

of the method of Snyder *et al.*[15] The fragments were weighed while thawing, immediately immersed in 0·1 M phosphate buffer pH 7·9 (1 g/20 ml) in a plastic tube, homogenised with a Thomas teflon pestle tissue homogeniser (size 0, Thomas Scientific, Swedesboro, NJ, USA), and heated for 10 minutes in boiling water. After centrifugation at 5000 rpm at 4°C for 10 minutes, the supernatant was lyophilised.

ASSAY

Histamine was assayed in dry residue suspended in 100 µl of 0·1 M phosphate buffer, pH 7·9. Samples (10 µl) were incubated at 37°C for 90 minutes in 1·5 ml Eppendorf tubes with 35 µl of reagent that contained (per ml) 0·05 M sodium phosphate buffer, 12 µl of a rat kidney histamine methyltransferase enzyme preparation,[16] and 10 µl of 20 µCi/ml solution of S-(methyl-^{14}C) adenosyl-L-methionine (specific activity 55 mCi/mmol). The reaction was stopped by the addition of 20 µl of 2% N-methylhistamine dihydrochloride (1:4 methylhistamine dihydrochloride, Sigma Chemical Co, St Louis, USA) in 1·5 M perchloric acid. A solution containing 20 µl of 10 N NaOH and 500 µl of a mixture of isoamyl alcohol and toluene (20:80) was then added to each tube. The tubes were capped, shaken for 10 minutes, and centrifuged for 60 seconds on a Beckman Microfuge (Beckman Instruments). A 300 µl sample of the upper organic layer was transferred to 5 ml of scintillation fluid for assay of radioactivity. The results were expressed as cpm and the histamine concentration per sample was calculated, based on a standard curve constructed with known amounts of histamine.

STATISTICAL METHODS

Statistical analysis was performed by Student's two tailed *t* test or the Mann-Whitney U test when the frequency distribution of the mucosal histamine contents in one group diverged considerably from the other. Differences were taken as significant when $p<0·05$.

Results

H pylori NEGATIVE GROUP

In 18 children who were not shown to have duodenal ulcer at endoscopy, *H pylori* was not isolated and was not observed on histological sections of antral and oxyntic mucosa. Moreover, the urease test was negative and spiral micro-organisms were not seen on carbofuchsin stained smears of antral mucosa. Histopathological analysis showed normal antral mucosa in 14 patients. Three children showed antral and oxyntic chronic gastritis and one with antral gastritis only (Table). In this *H pylori* negative group, the oxyntic mucosa histamine content was mean (SD) 54·09 (23·09) µg/g fresh weight. With regard to sex, there was no significant difference between the mucosal histamine content of male ((n=11) 54·19 (26·22) µg/g) and female ((n=7) 49·22 (17·87) µg/g) patients (p=0·49).

Histological findings in the gastric mucosa of Helicobacter pylori (HP) positive and negative children with and without duodenal ulcer (DU)

Histology	*HP positive*		*HP negative**
	With DU (n=9)	*Without DU (n=10)*	*(n=18)*
Antral mucosa:			
ASCG	5	6	2
SCG	4	4	2
N	0	0	14
Oxyntic mucosa:			
ASCG	2	2	2
SCG	2	2	1
N	5	6	15

ASCG= active superficial chronic gastritis; SCG=superficial chronic gastritis; N=normal.
*None presented with duodenal ulceration.

H pylori POSITIVE GROUP

This group included nine children with and 10 without duodenal ulcer. In the latter group, *H pylori* was isolated from antral and oxyntic mucosa and spiral micro-organisms were seen on histological sections of antral mucosa. The urease test was positive in all the patients.

The histopathological findings in antral and oxyntic mucosa from these patients are given in the Table. All children showed antral superficial chronic gastritis but oxyntic gastritis was observed in only 42·1% of them.

The mucosal histamine concentration of the *H pylori* positive patients was mean (SD) 26·28 (14·19) µg/g. There was a significant difference between this group and *H pylori* negative patients ($p<0·01$). In duodenal ulcer patients, the mucosal histamine content was mean (SD) 19·75 (6·30) µg/g and in *H pylori* positive patients without duodenal ulcer disease it was 31·44 (17·90) µg/g. Duodenal ulcer patients had significantly lower concentrations of mucosal histamine than *H pylori* positive patients without ulcers ($p<0·05$). A significant difference was also observed between *H pylori* negative patients and the following two groups: (a) *H pylori* positive children without duodenal ulcer ($p=0·01$) and (b) duodenal ulcer patients ($p<0·01$).

Discussion

It has been reported that relatively high histamine values are found in the glandular region of the stomach in all animal species.[17] In man, gastric mucosa histamine is stored in the mast cells, which are most abundant in the body and located close to or adjacent to parietal cells.[11 17]

Histamine has long been recognised as a gastric secretagogue, but only since the introduction of H_2 blockers has its physiological role in regulating gastric acid secretion been unquestionable.[18] Moreover, the H_2 blockers also inhibit acid secretion stimulated by gastrin[19] and by vagal nerves.[20] Because of this it has been proposed that histamine is the final mediator of gastric acid secretion.[10 11] In fact, infusion of pentagastrin, a synthetic analogue of gastrin, produces gastric acid secretion and reduces the mucosal histamine concentration.[17 18] In addition, during pentagastrin infusion, both gastric juice and plasma histamine values increase.[9 10 17] Gastrin evokes a histamine release of such a magnitude that it is

sufficient to explain the whole gastric acid stimulatory effects of this peptide[9 17] Furthermore, it was also shown that electrical stimulation of the vagal nerves elicits an immediate histamine release.[21] It seems, however, that vagal stimulation can also induce acid secretion by another more direct mechanism.[21]

It is well established that duodenal ulcer disease is generally associated with gastric hypersecretion and hyperchlorhydria.[22] Hyperacidity is, without doubt, an important physiological abnormality in most patients. Moreover, patients with chronic duodenal ulceration have significantly lower concentrations of gastric mucosal histamine than control subjects.[23-25] It has been suggested that this finding may be related to a state of high mobilisation of endogenous mucosal histamine with depletion of its stores.[16 24]

In this study we found that *H pylori* positive children, both with or without duodenal ulcer disease, had significantly lower oxyntic mucosal histamine concentrations than *H pylori* negative patients. We also showed that there is a significant difference between *H pylori* positive children with and without duodenal ulcer. This difference could be explained by the fact that duodenal ulcer patients show greater density of *H pylori* than *H pylori* positive patients without duodenal ulcer disease,[26] which could be associated with a greater stimulus to gastric acid secretion. As the low endogenous histamine concentration may be an indirect reflex of an increased acid secretion, *H pylori* seems to be associated with high acid secretion. In fact, it was recently shown that *H pylori* positive patients have increased serum gastrin values that could result in increased acid secretion. Inappropriate hypergastrinaemia cannot be incriminated as the only cause of the increased acid secretion, however, as this can also be stimulated by the vagal nerves as well as by locally released histamine.[10 11 17 18 21] Thus, besides bacterial urease, other bacterial products such as toxins may promote gastric secretion not only by gastrin but also by direct action on histamine release or by direct stimulation of the vagal nerves. In duodenal ulcer patients the constant and strong stimulus to gastric secretion could lead to an exhaustion of mucosal histamine content that could culminate in reduced gastric acid secretion and therefore duodenal healing. The periods of spontaneous exacerbation and remission, characteristically observed in duodenal ulcer disease, could be explained by this mechanism.

We think that although there are still many unanswered questions, there is evidence suggesting that *H pylori* is involved in the pathogenesis of duodenal ulceration by two different mechanisms – firstly by colonising areas of gastric metaplasia in the duodenum, causing active inflammation that may render the mucosa more liable to ulceration[27] and secondly by producing increased acid secretion that leads to a low intraduodenal pH, via gastrin or other gastric secretagogues. The mucosal reparative processes do not operate normally in the presence of hyperacidity.

In conclusion, the present study shows that *H pylori* positive children with and without duodenal ulceration have depleted oxyntic mucosal histamine stores. This finding needs to be confirmed in adults as well as in a larger group of children. Furthermore, we are evaluating gastric mucosal histamine values before and after eradication of *H pylori* to test our hypothesis that this micro-organism is involved in gastric histamine metabolism.

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