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Helicobacter pylori and Duodenal Ulcer: Evidence for a Histamine Pathways-Involving Link

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Objectives: **A "gastrin link" has been suggested to explain the statistically relevant association between *Helicobacter pylori* and duodenal ulcer. Given the well known, although not entirely clarified, relationships between gastrin and histamine, the purpose of this study was to assess whether gastric mucosal histamine pathways and, more specifically, histamine-storing cells are involved in the *Helicobacter pylori*–duodenal ulcer route.** ***Methods:*** **Fasting serum gastrin, gastric mucosal histamine content, and mucosal density of both enterochromaffin-like cells and mast cells were compared in 11 *H. pylori*-positive, non-duodenal ulcer subjects, in 16 duodenal ulcer patients (all *H. pylori* positive), and in 11 *H. pylori*-negative control subjects.** ***Results:*** **Fasting serum gastrin concentration and mucosal histamine content were significantly higher in the duodenal ulcer group than in controls, whereas *H. pylori*-positive, non-ulcer subjects had values that were intermediate between those of the other two groups. Enterochromaffin-like cell density was significantly greater in duodenal ulcer patients than in the other groups.** ***Conclusions:*** **These results demonstrate the involvement of histamine pathways in *H. pylori* infection and duodenal ulcer. The most original finding in this study was that enterochromaffin-like cell density is three times greater in duodenal ulcer patients than in *H. pylori*-positive, non-ulcer subjects. This could explain the previous report of an exaggerated acid response to gastrin in duodenal ulcer patients when compared with *H. pylori*-positive, non-ulcer subjects and thus provide further insight into the pathogenesis of ulcers.**

INTRODUCTION

The existence of abnormalities in gastrin release (1) and gastric secretion, consisting mainly of an increase in the average basal and peak acid output when compared to controls (2–4), is well established in duodenal ulcer (DU) and is considered to be a contributing factor to its pathogenesis.

Helicobacter pylori infection has also been shown to be associated with secretion-related abnormalities such as exaggerated meal-stimulated gastrin release (5) and increased gastrin-mediated acid output (6, 7). Moreover, gastrin response to meals and to the gastrin-releasing peptide is increased in *H.pylori*-positive subjects both with and without DU, but acid secretion is three times greater only in those affected by DU (6, 7).

Because a highly significant association exists between *H. pylori* and DU and a pathogenetic role for the infection has been suggested (8), the link between the bacterium and peptic disease may be represented by secretion and/or secretion-related abnormalities (7).

Histamine release is generally recognized as a nodal point in the regulation of gastric secretion, even though some different hypotheses as to its action pathways have been suggested (9–11). Gastric histamine is stored in mast cells, enterochromaffin-like (ECL) cells, and histaminergic neurons (12). The relative importance of these sources in the gastrin- and/or vagus-mediated release of histamine for gastric secretion modulation is only partially known. However, the lack of histaminergic neural fibers in the mucosa makes their direct involvement in acid secretion unlikely (12), whereas mast cells seem much more likely to be involved in gastric mucosal injury-repair mechanisms than in secretion control (13).

In view of the above, the relationships between *H. pylori*, DU, gastric mucosal histamine content, and the density of histamine-storing cells in the mucosa seem interesting and worthy of the investigation undertaken by the present study.

PATIENTS AND METHODS

Patients

Thirty-eight subjects entered the study. These were all part of a series of outpatients who were consecutively referred to our endoscopy unit with symptoms of dyspepsia.

In 16 patients (11 men and five women; mean age 49.4 yr, range 23–69 yr) a DU was endoscopically demonstrated, and all were shown to be *H. pylori* positive by the study

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protocol evaluation. Four of them were smokers (10 or more cigarettes a day).

Twenty-two patients were considered to be affected by functional dyspepsia according to Heading's definition (14) because no macroscopic abnormalities were shown at endoscopy. Eleven of these (five men and six women; mean age 57.0 yr, range 43–70 yr) were *H. pylori* positive as shown by histology and culture. Three of these were smokers. The remaining 11 (eight men and three women, mean age 49.4 yr, range 28–71 yr) were *H. pylori* negative by histology and culture and, because they had neither macroscopic nor histological abnormalities, were considered as controls. Three of these were smokers. None of the subjects in the two latter groups had previously been shown (either radiologically or endoscopically) to be affected by peptic ulcer.

Age and sex distribution did not significantly differ between the three groups (DU, *H. pylori* positive without DU, and control) (Kruskal-Wallis and Dunn test). None of the 38 subjects had taken proton pump inhibitors, H2-receptor antagonists, nonsteroidal anti-inflammatory drugs, antibiotics, or other relevant drugs in the month preceding the study, and none were suffering from any metabolic or systemic disorder. Alcohol intake was negligible in all subjects. All gave informed consent to undergo the study protocol, which consisted of upper endoscopy with multiple gastric biopsies and basal gastrinemic assessment.

Laboratory methods

Fasting serum gastrin concentration assessment. Fasting serum gastrin concentrations were determined by a specific radioimmunoassay (GASK-PR, CIS Bio International, Gif-Sur-Yvette, France) in 10 ml of blood, which was quickly centrifuged and frozen at -80°C until measurement.

Endoscopy. All the esophagogastroscopies were performed by one of the authors (P.B.) with simple local pharyngeal anesthesia with 2% lidocain (Xylocaine, Astra, Kings Langley, UK). Grasp biopsies (forceps 413 26 180, open diameter 7.7 mm, Endo-Technik, Hilden, Germany) were taken from the upper third of the gastric body on the greater curvature. Four biopsies were put into two vials (two biopsies per vial) containing, respectively, 4 ml of 0.1 N HCl for the determination of histamine and 4% ethyl-dimethylaminopropyl-carbodiimide (Sigma, Milan, Italy) in 0.1 M of phosphate buffer, pH 6.9, as a fixative for the histochemical assessment of histamine-containing cells (15). Five biopsy specimens were also taken for histological evaluation: two from the gastric body as close as possible to those used for the biochemical and histochemical analyses, two from the antrum, and one from the angulus.

Determination of mucosal histamine content. Histamine was measured fluorimetrically in mucosal samples using the method of Shore *et al.* (16) as modified by Lorenz *et al.* (17). The mucosal samples were weighed with a precision balance (H51 AR, Mettler Instrument AG, Zurich, Switzerland) after the aqueous fluid had been carefully wiped off. They were then homogenized in the 4 ml of 0.1 N HCl, where the biopsies had been put immediately after being taken. Two milliters of the mixture was shaken with 1.5 g of NaCl in 10 ml of *n*-butanol in a glass tube and then centrifuged for 5 min at 1800 × *g*. The butanol phase was separated and mixed with 5 ml of 0.1 N NaOH saturated with NaCl in a glass tube. Histamine was redistributed to the aqueous phase by shaking 8 ml of the butanol phase together with 3 ml of 0.1 N HCl and 15 ml of *n*-heptane for at least 6 min.

After centrifugation as above, the organic phase was carefully removed by suction, and 2 ml of the aqueous phase was transferred into a test tube and mixed with 0.4 ml of 1 N NaOH and 0.1 ml of *o'*-phtaldialdehyde solution (1% w/v dissolved in methanol). After exactly 4 min, 0.2 ml of 3 N HCl was added, and the fluorescence was measured in a spectrophotofluorimeter (RF-5000, Shimadzu, Kyoto, Japan) at 21°C. The excitation wavelength was 360 nm, and the fluorescence wavelength was 450 nm. The recovery of added authentic histamine was about 70%. The histamine content was given in nanograms of base per milligram of tissue wet weight. Histamine authenticity was shown by recording the excitation and fluorescence spectra. The chemicals used to prepare the solution for the fluorimetric assay were of Suprapur quality from E. Merck AG (Darmstadt, Germany); *o'*-phtaldialdehyde was from B.D.H. Chemical Ltd. (Poole, UK).

Histology. Gastric biopsy specimens were fixed in formalin, routinely processed, and embedded in paraffin. Sequential 5-μm-thick sections were stained with hematoxylin and eosin and alcian blue/periodic acid-Schiff (pH 2.5) for histopathological evaluation and with a modified Giemsa technique for the detection of *H. pylori*.

Antrum and body biopsy specimens were classified for the gastritis pattern according to the Sydney System (18). Chronic inflammation, neutrophil infiltration (activity), gland atrophy, intestinal metaplasia, and *H. pylori* density were graded on a 0–3 scale for each biopsy site.

Histochemistry. After fixation with carbodiimide for 8 h, part of each biopsy was embedded in paraplast, and part was cryoprotected with 20% sucrose, cryosectioned, and fixed in acetone (15). Some sections were exposed to ultraviolet radiation to abolish autofluorescence before staining, and other sections were stained without ultraviolet exposure. Histamine was labeled with rabbit polyclonal antibodies (lot HA19C, a generous gift of Dr. Pertti Panula), diluted 1:500, followed by fluorescein isothiocyanate-conjugated goat anti-rabbit antiserum (Sigma). Mast cells were stained with tetramethylrhodamine isothiocyanate-conjugated avidin (Immunotec, Marseille, France) (19). Endocrine cells were stained with a prediluted monoclonal antibody against human chromogranin A (clone PHE5, Ortho Diagnostic Systems, Milan, Italy) (20). The sections were examined with an Axioskop microscope (Zeiss, Oberkochen, Germany) equipped for epifluorescence. The specificity of the immunostainings was tested by omitting the first antibodies or by

TABLE 1
*Fasting Serum Gastrin Concentration and Mucosal Histamine Content in DU, H. pylori-Positive, Non-DU, and Control Subjects**

	Gastrin (ng/ml)	Histamine (ng/mg wet weight)
DU (I) (n = 16)	59.14 ± 4.44	33.18 ± 2.51
H. pylori-positive, non-DU (II) (n = 11)	44.05 ± 3.54	25.71 ± 3.04
Control (III) (n = 11)	38.02 ± 1.77	19.68 ± 2.19

* Values are mean ± SEM. Statistical analyses were performed with the Kruskall-Wallis and Dunn tests. Gastrin: I *vs* II, NS; I *vs* III, $p < 0.001$; II *vs* III, NS. Histamine: I *vs* II, NS; I *vs* III, $p < 0.005$; II *vs* III, NS. NS = not significant.

substituting them with unrelevant ones; in some experiments, anti-histamine antiserum was preadsorbed with a histamine-succilylated ovalbumine conjugate (1–10 μg/ml) for 24 h before applying to sections. No reactivity was observed in any control sections.

For morphometry, the numbers of all ECL cells and all mast cells were counted, and the surface area was measured in one frozen section per biopsy, double immunostained for histamine and chromogranin A. All the cells stained by both antibodies were counted as ECL cells, and those that were stained by anti-histamine antibodies only were counted as mast cells. The results are expressed as number of cells per square millimeter.

Statistical analysis. Statistical evaluation of the data was performed by means of Kruskal-Wallis and Dunn tests for nonparametric multiple comparisons and the Spearman rank correlation test and the Mann-Whitney test for independent samples. The significance limit was fixed at $p = 0.05$, two-tailed probability test.

RESULTS

Fasting serum gastrin concentrations

Mean serum gastrin concentrations were higher in the DU group than in the others. However, differences were significant only between the DU and control groups. *H. pylori*-positive, non-DU subjects had intermediate gastrin concentration values, but differences between them and both the control and DU patients were not significant (Table 1).

Mucosal histamine content

Results of the assessment of mucosal histamine content are given in Table 1. Their correspondence to gastrin findings is quite close, with significantly higher values in DU subjects than in control subjects and intermediate values in *H. pylori*-positive, non-DU subjects.

Histological findings

Scores for each of the main morphological changes in the two groups showing histological abnormalities are given in Table 2.

ECL cell and mast cell density

ECL cell density was more than three times greater in the DU group than in the other groups. On the other hand, no differences at all were shown between *H. pylori*-positive, non-DU subjects and control subjects (Table 3). ECL cells were interspersed among the epithelial cells with the greatest frequency in the deepest third of the gastric glands.

Mast cell density was almost identical in the three groups (Table 3). They were located mostly in the superficial third of the mucosa, and some were close to the glands and even appeared to be in contact with epithelial cells.

Relationships between fasting serum gastrin concentration, histamine mucosal content, and histamine-storing cell density

Considering the whole series of 38 subjects, fasting serum gastrin concentration was positively correlated with both histamine mucosal content ($r_s = 0.4368$, $p < 0.001$) and ECL cell density ($r_s = 0.5125$; $p < 0.005$). Histamine mucosal content was also correlated with ECL cell density ($r_s = 0.3719$; $p < 0.05$).

DISCUSSION

This study shows relevant differences in the gastrin–histamine pathways pattern between subjects affected with DU and control subjects and lesser differences between these two groups and *H. pylori*-positive, non-DU subjects. This places the latter group in an intermediate position between the others. The study also, and more importantly, shows a three times greater ECL density in DU subjects (all *H. pylori* positive), not only when compared to controls but also when compared to *H. pylori*-positive, non-DU subjects, whereas mast cell density is similar in the three groups.

The present demonstration of *H. pylori* in all subjects of the DU group is not surprising and is consistent with the very high prevalence of infection previously reported in patients with DU (21).

The possibility of explaining different outcomes of *H. pylori* infection on the basis of differences in bacterial virulence factors (22) was not explored in the present study. On the contrary, the main purpose of this study was to further investigate the so-called "gastrin link" (23) between *H. pylori* and DU and, in particular, to determine whether this link involves histamine pathways, as suggested by the previously reported central role of histamine in regulating gastric secretion (9–11). In our opinion, the results obtained in this respect are interesting.

The starting point was the previous demonstration of 1) increased serum gastrin concentration after intravenous infusion of gastrin-releasing peptide or after a meal in *H. pylori*-positive subjects compared to control subjects (6, 7, 24) and 2) exaggerated acid response to gastrin in DU subjects when compared to *H. pylori*-positive, non-DU subjects (6, 7). These findings, in addition to other gastric secretion abnormalities, were fully described by El-Omar *et*

TABLE 2
*Scores for the Histological Features in DU and H. pylori-Positive, Non-DU Subjects**

	Antrum		Body	
	DU (n = 16)	*H. pylori*-positive, non-DU (n = 11)	DU (n = 16)	*H. pylori*-positive, non-DU (n = 11)
Inflammation	2.13 ± 0.09	2.18 ± 0.12	1.07 ± 0.12	1.45 ± 0.25
Activity	2.50 ± 0.13	2.27 ± 0.20	0.93 ± 0.21	1.54 ± 0.25
Atrophy	0.25 ± 0.11	0.46 ± 0.21	0 ± 0	0.27 ± 0.20
Intestinal metaplasia	0.25 ± 0.11	0.36 ± 0.20	0 ± 0	0 ± 0
H. pylori density	2.94 ± 0.06	2.82 ± 0.12	1.87 ± 0.13	2.54 ± 0.21

Values are mean ± SEM. Statistical analyses were performed with the Mann-Whitney test. Only inflammation and *H. pylori* density, exclusively in the body, differed significantly between the DU and *H. pylori*-positive, non-DU groups ($p < 0.05$ and $p < 0.01$, respectively).

TABLE 3
*Histamine-Storing Cell Density in the Mucosa of DU, H. pylori-Positive, Non-DU, and Control Subjects**

	ECL cell density (n/mm^2)	Mast cell density (n/mm^2)
DU (I) (n = 16)	2.40 ± 0.24	7.46 ± 0.55
H. pylori-positive, non-DU (II) (n = 11)	0.78 ± 0.15	8.73 ± 0.58
Controls (III) (n = 11)	0.70 ± 0.10	8.34 ± 0.66

* Values are mean ± SEM. Statistical analyses were performed with the Kruskall-Wallis and Dunn tests. ECL cells: I *vs* II, $p < 0.001$; I *vs* III, $p < 0.001$; II *vs* III, NS. Mast cells: I *vs* II, I *vs* III, II *vs* III, NS. NS = not significant.

al. (6, 7) and were not investigated further in our study. However, in our series the increasing concentrations of serum gastrin and mucosal histamine from control subjects to *H. pylori*-positive, non-DU subjects and finally to DU subjects seem to indirectly support the hypothesis of the "gastrin link" between *H. pylori* and DU. The finding concerning gastrin is consistent with most previous reports (4, 6, 7, 24, 25). Our findings concerning histamine, although in agreement with findings by Domschke *et al.* (26), are sharply at variance with some previous reports of lower histamine mucosal concentration in DU subjects compared to control subjects (27–29). However, one of these studies (28) investigated DU subjects after only 1 wk of suspension of the H2-receptor antagonist therapy, which is known to affect histamine mucosal concentration (30). Moreover, the length of the history of DU was not specified, and this is an important aspect because the progressive development of *H. pylori*-associated chronic gastritis could certainly affect acid secretion and mucosal histamine concentration in the course of the natural history.

Another study (29) dealt exclusively with children, who were likely to have had a recent onset of DU/*H. pylori* infection. With regard to the third study (27), in addition to the lack of history information, histological abnormalities were not looked for either in the DU group or in the so-called control group, which therefore seems questionable as such. On the other hand, our DU patients were compared with subjects who had macroscopically and histologically normal gastric mucosa, were affected with long-lasting chronic ulcers (mean 13.2 yr of symptoms, range 3 months-35 yr), and had not been previously treated (four patients) or had interrupted treatment at least 1 month previously. In addition to these methodological reasons, the dynamic equilibrium between the new synthesis and release within the paracrine mechanism of histamine action (31), together with the possible different stages in the progression of the natural history of *H. pylori* gastritis (32), could account for the discrepancies between the different reports on histamine findings.

To our knowledge, ECL cell density has not been thoroughly investigated before in similar groups of subjects. Thus, the most original and interesting finding in our study is represented by the three times greater ECL cell density in DU subjects compared not only to control subjects but also to *H. pylori*-positive, non-DU subjects. Even though increased gastrin release (both basal and after stimulus) has been shown in all *H. pylori*-positive subjects (5–7, 24, 33), an exaggerated acid response to gastrin stimulation has been demonstrated only in those affected by DU (6, 7). This exaggerated response has been considered pathogenetically relevant for DU (6, 7). Thus, our findings of significantly greater ECL cell density may explain why only a few of the *H. pylori*-positive subjects develop DU. Because gastrin-stimulated histamine release depends on ECL cell density (34), an increased pool of ECL cells seems capable of an exaggerated histamine release. This could affect acid secretion more considerably by amplifying the effect of similarly increased gastrinemic concentrations because of the role of histamine as a major limiting factor in maximal gastrin- and hence meal-stimulated acid secretion (35, 36). This hypothesis, to explain the disproportionate acid response to gastrin in infected patients with DU, may be additional or alternative to that of an impairment within the gastric mucosa of the inhibitory control of gastric secretion played by peptides such as cholecystokinin, secretin, etc. (7).

Because it is well known that hypergastrinemia is capable of promoting ECL cell hyperplasia (13, 37–40), DU could be hypothesized as the result of a two-step process. In the first phase *H. pylori* infection could determine, possibly through abolition of the inhibitory effect of cholecystokinin

(41) and/or suppression of antral somatostatin (33, 42), increased serum gastrin concentrations. Long-term hypergastrinemia would then promote hyperplasia of histamine-releasing ECL cells in some *H. pylori*-positive subjects (second phase). Only the interaction of the persisting increased gastrin levels with this modified pool of histamine-releasing cells would result, because of the role of histamine as a limiting factor in gastrin- and meal-stimulated gastric secretion (36), in a critical enhancement of gastric secretion abnormalities and, possibly, DU. None of the other pathogenetic factors that have classically been considered as relevant for DU (increased gastric cell mass, gastric metaplasia of the duodenum, cag A-positive *H. pylori* strains, and blood-groups-related differences) contrast with this hypothesis but could, indeed, help to explain the possibility of different outcomes of *H. pylori* infection.

The finding of an almost identical mast cell density in the three groups of our series does not support a major role for mast cells and mast cell histamine, either in regulating gastric secretion or in sustaining gastric mucosal inflammatory changes, associated with *H. pylori* infection. A similar message seems to be expressed by the lack of correlations between serum gastrin concentration, histamine mucosal content, and mast cell density. On the contrary, the existence of positive correlations between serum gastrin concentration, histamine mucosal content, and ECL cell density indirectly supports the relevance of the ECL cell-released histamine in gastric secretion modulation (13). This finding confirms the previously shown dose-dependent trophic effect of gastrin on ECL cells in the rat stomach (40). Therefore, these findings concerning mast cell and ECL density suggest different control mechanisms and different functions for the two cell types; the former seems to play a marginal (if any) role in the regulation of acid secretion (13, 43, 44), whereas that of the latter seems important.

In conclusion, this study has provided indirect evidence of ECL cells as a major source of the histamine involved in the regulation of gastric secretion. Furthermore, it has demonstrated involvement of a histamine pathway in the pathogenesis of gastric secretion changes occurring in the *H. pylori*–DU route. The latter finding, on the basis of the well known relationships between gastrin, histamine, and acid secretion, indirectly supports the "gastrin link" between *H. pylori* and DU. Nonetheless, the most important finding of the study is the three times greater ECL cell density in DU patients compared to *H. pylori*-positive, non-DU subjects. This finding could explain why among the *H. pylori*-positive subjects, an exaggerated acid response to gastrin is shown only by those affected with DU. Further investigations are needed to understand whether this is a pathogenetically relevant event or simply an epiphenomenon. If the first case holds true, clarifying hypotheses concerning certain aspects of the natural history of *H. pylori* infection-DU could be proposed.

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