CHROMOGRANIN A- CONTAINING ENDOCRINE CELLS IN THE GASTRIC MUCOSA OF PATIENTS WITH HELICOBACTER PYLORI ASSOCIATED CHRONIC GASTRITIS TYPE B

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ABSTRACT

The aim of the present study was to examine the chromogranin A containing endocrine cells in gastric mucosa of patients with Helicobacter pylori associated chronic gastritis type B, histological inflammation and interactions among these parameters. In 35 patients (12 males and 23 females, median age 47 years, range 21-86 years) the gastric mucosal tissue was obtained by endoscopic biopsy from the antrum and corpus. Slides were stained with haematoxylin and eosin, alcian blue (pH 2.5), periodic acid Schiff, modified Giemsa for Helicobacter pylori, and chromogranin A to visualize endocrine cells. Biopsy specimens were assessed histologically according to the Sydney system. The mean number of stained cells was calculated by counting the number of cells per visual field when using a x 40 objective and a x10 eye piece. Cell counts were made on two visual fields in one section from each specimen (three specimen per site). We studied 24 patients positive for Helicobacter pylori and 11 negative patients. The median chromogranin A containing endocrine cell number in antrum and corpus mucosa was significantly lower in Helicobacter pylori positive than in Helicobacter pylori negative patients (respectively p< 0.01, p< 0.05). There was an inverse correlation between endocrine cell numbers and Helicobacter pylori of the gastric mucosa. On the other hand, endocrine cell numbers of the antral gastric mucosa significantly decreased with inflammatory change associated with Helicobacter pylori gastritis but did not in corpus. This decrease was correlated with the grade of active inflammation and chronic inflammation, which was in close relation to Helicobacter pylori infection. Besides endocrine cell were irregularly distributed, close to inflammatory areas, or near lymphoid follicles. These results indicate that, suggesting a close relationship between Helicobacter pylori and gastric chromogranin A regulation. A close correlation between an decrease in
gastric chromogranin levels and the histologic activity was also present, suggesting that certain peptide-immune reactions in the gastric mucosa exist in HP infection.

Key words: chromogranin A, Helicobacter pylori, chronic gastritis

INTRODUCTION

Chronic gastritis is a multifactorial condition resulting from an interplay between genetic predisposition, gastric bacterial infection, and environmental factors. The studies about the link between chronic gastritis, Helicobacter pylori (HP), and gastric endocrine cells has risen, especially in the modern era of potent antisecretory treatment. How HP is usually acquired and its route of transmission are unknown. A urea-urease-ammonia system is thought to be one of the pathophysiological mechanisms of gastric mucosal damage in HP infection, but the mechanism of HP-induced gastroduodenal mucosal damage remains obscure. Host factors that influence the severity of gastritis include the secretory state of the stomach.

The gastric mucosa is rich in endocrine cells (EC) than most other regions of the gut. In the antral portion the three major EC populations (G cells, D cells and enterochromaffin cells) contain immunohistochemically identified secretory products (gastrin, somatostatin, and serotonin, respectively), enabling their visualization at light microscopic level. The oxyntic mucosa of the human stomach harbors at least five different endocrine cell types: enterochromaphin-like (ECL) cells, A-like (or X cells), somatostatin cells (D), 5-hydroxy-tryptamine (5-HT)-containing EC cells, and D1 or P cells; the latter two cell types are notably scarce. Chromogranins occur in secretory granules of most neuroendocrine cells, together with amines and/or regulatory peptides. All major EC types were found to express chromogranin A (CgA) or CgA-derived peptides in various peripheral organs. CgA, first demonstrated in the adrenal medulla, belongs to a family of soluble acidic proteins known to occur in many peptide hormone-producing cells, including gut EC. Chromogranin-positive cells included all EC identified by the other staining techniques.

The aim of the present study is to examine the number and distribution of the CgA containing EC in the antral and corpus gastric mucosa of patients with HP associated chronic gastritis type B, to look for a possible correlation among changes in histologic inflammation, HP and EC parameters in cases.

MATERIALS AND METHODS

Patients

We examined 35 patients (23 females and 12 males) of a median age 47 years (ranging from 21 to 86) with clinical and morphological signs of chronic gastritis type B. Adult patients undergoing upper endoscopy for routinely accepted clinical indications were studied. None had received antibiotics, nonsteroidal anti-inflammatory drug or proton pump inhibitors prior to the eradication therapy. All patients gave detailed informed consent in accordance with the Helsinki Declaration.
Endoscopy and Histology

Endoscopy was performed without premedication. Topical anesthesia (Xylocaine) of the pharynx facilitated introduction of the endoscope. Endoscopic examinations were performed by a single investigator. For the study, six separate biopsy specimens were obtained from the antrum and from the corpus. All biopsy specimens for histological examination were fixed in 10% formalin, embedded in paraffin wax on the oriented edge, and cut into 5 µm thick sequential sections. For optimal histological evaluation, all gastric biopsy specimens included surface epithelium and muscularis mucosa. Of the 35 subjects evaluated, 5 corporal mucosal biopsy were excluded from histological assessment because their biopsy specimens were too small for adequate evaluation. All tissue sections were stained with haematoxylin and eosin (H&E) for general histological analysis, with alcian blue (pH 2.5), and periodic acid-Schiff. The colonization of HP in the gastric mucosa was estimated semi-quantitatively in modified Giemsa stained specimens. The specimens were examined in a blinded manner by a pathologist and scored in accordance with the Sydney system. The severity and activity of gastritis, atrophy, and intestinal metaplasia, in addition to HP density, were evaluated according to the updated Sydney system. The following histological parameters were semiquantitatively scored, as described previously: (1) bacterial peak density grade (0, absent; 1 to 3, from few and isolated bacteria to colonies); (2) polymorphonuclear cell infiltrate (activity) (0, absent; 1 to 3, from mild to severe); (3) mononuclear cell infiltrate (chronic inflammation) (0, absent; 1 to 3, from mild superficial to severe glandular); (4) intestinal metaplasia (0, absent; 1 to 3, type I to type II). There was the nonatrophic antral-predominant, body sparing HP gastritis.

Immunohistochemistry

Specimens of corpus and antral mucosa were immunostained for EC, using a monoclonal antibody against human CgA. For immunohistochemical detection of CgA, 4µm sections were cut from formalin-fixed paraffin-embedded mucosa samples. The sections were deparaffinized and dehydrated through a series of xylene and graded alcohol. The standard ABC technique was performed using the primary monoclonal antibodies against the CgA antigen (prediluted Biogen, USA). Endogenous peroxidase activity was blocked by incubation of the sections in 3% hydrogen peroxide in methanol at room temperature for 10 minutes and washed in phosphate-buffered saline (PBS). Antigen retrieval was undertaken with the sections placed in a plastic slide rack in a plastic beaker containing 0.01 M sodium citrate buffer (pH 6.0) and heated in a microwave oven for three periods of 5 min. After each 5 min period the level of the buffer was restored to a predetermined level with distilled water. After heating, the container was removed from the oven and allowed to cool for 30 min. Non-specific antibody binding was blocked by incubation of the sections with normal horse serum for 20 min at room temperature in a humidified chamber. Subsequently, they were incubated for 60 min with 1:200 diluted mouse-anti-CGA. Following primary antibody incubation, the sections were rinsed in PBS for ten min and incubated in biotinylated anti-mouse antibody for 30 min at room temperature. After washing in PBS they were incubated in avidin-biotinylated horseradish peroxidase complex for 30 min at room temperature. The antibody-biotin complex was demonstrated
by incubating in PBS containing 0.1% hydrogen peroxide for 5 min, and the sections were counterstained with haematoxylin for 2 min and dehydrated through graded alcohols, cleared and mounted. Cell counts were made on two fields in one section from each specimen (three specimens per site). We examined only sections showing whole area between surface and muscularis mucosa and with an intact mucosa. The mean number of stained cells was calculated by counting the number of cells per visual when using a x 40 objective and x 10 eye piece. Some of the cells were rounded; others were elongated. Chromogranin-positive cells were arranged around the entire glands but mainly in the upper half of antral glands.

Statistical Analysis

Data were expressed as the means ± SD for each group. A statistical analysis was performed by using the SPSS software package. The Mann-Whitney U-test was used for two-group comparisons of continuous variables. The correlation between two items were analyzed by the Pearson test. In all statistical tests, P values of <0.05 or less were considered to represent significant differences.

RESULTS

We examined 35 patients (12 males and 23 females) of a median age 47 years (ranging from 21 to 86 years) with clinical and morphological signs of chronic gastritis type B. Of the 35 subjects evaluated, 5 corporal mucosal biopsy were excluded from histological assessment because their biopsy specimens were too small for adequate evaluation. There was the nonatrophic antral-predominant gastritis, body sparing HP gastritis. The grade of inflammation was significantly higher in the antrum than in the corpus (p<0.01). HP infection was positive in 24 of 35 patients (69%) in antrum and 9 of 30 patients (30%) in corpus by histology. HP density was significantly higher in the antrum than in the corpus in type B gastric mucosal inflammation patients (p<0.01) (Figure 1). The corporal mucosa in 10 of the patients showed no inflammatory changes. Neither age nor sex (male/female ratio) was different between the HP positive and negative patients. Prevalence of HP infection in the antrum was increased in the grade of inflammation, but not in the corpus mucosa (p<0.01, p>0.05, respectively). Gastric biopsy sections (antrum and corpus) from 35 patients with Type-B gastritis were also examined for density of EC. EC were irregularly distributed, close to inflammatory areas, or near lymphoid follicles (Figure 2).
Figure 1. H. Pylori in the gastric mucosa (GiemsaX40).

Figure 2. Chromogranin A positive cells in the antral gastric mucosa of a patient with HP associated chronic gastritis (ABCX20).
Decreased CgA containing EC number in antrum and corpus mucosa were found significantly in HP positive than in HP negative patients (respectively p< 0.01, p< 0.05). There was an inverse correlation between EC numbers and HP of the gastric mucosa. On the other hand, EC numbers of the antral gastric mucosa significantly decreased with chronic and active inflammatory change associated with HP gastritis but did not in corpus (p<0.05, p>0.05 respectively). This decrease was correlated with the grade of active and chronic inflammation, which was in close relation to HP infection. The number of CgA containing EC of the gastric mucosa decreased with age, but not significantly, and there was no sex difference. The prevalence and density of lymphoid follicles and aggregates in gastric antral mucosal biopsies correlated closely with HP infection (p<0.001). There was a significant correlation between lymphoid follicles and aggregates with severity and activity of inflammation in the gastric mucosa. Only one patient with HP gastritis had mild atrophic changes and intestinal metaplasia in the antrum. The presence and numbers of CgA positive cells did not correlate with intestinal metaplasia and atrophy of the gastric mucosa.

DISCUSSION

HP infection induces a spectrum of changes in gastric morphology and gastric function. At the one end of the spectrum, there is the nonatrophic antral-predominant, body sparing HP gastritis, that is characteristic of duodenal ulcer patients and that produces marked acid hypersecretion. At the other end of the spectrum is the atrophic pangastritis or body predominant gastritis that is characteristic of gastric cancer patients and induces marked acid hyposecretion. These different patterns and severity of gastritis might be explained by factors related to the bacterium and related to the host. HP associated gastritis is seen most commonly in the antrum, but it can also cause pangastritis. In patients with chronic gastritis, HP is found in at least 90 % of cases. In our study, there was seen nonatrophic antral-predominant, body sparing HP gastritis, and HP was found in 69% of cases.

One of the characteristic features of HP induced mucosal damage is an inflammatory response in the host mucosa. Prevalence of HP infection in the antrum was increased in the grade of chronic inflammation and of active inflammation, but not in the corpus mucosa (p<0.01 ve p<0.001; p>0.05 respectively). HP infection stimulates the release of inflammatory cytokines such as interleukin-8 (IL-8) and the expression of IL-8 mRNA in the gastric mucosa. It is well known that inflammatory cytokines including IL-1 beta, IL-8 and TNF-alpha modulate gastric acid secretion. Therefore, the host immune response by the cytokines may cause these disparate pathways in gastric acid secretion. The gastric mucosa is richer in EC than most other regions of the gut. Light microscopically, EC are visualized immunohistochemically on the basis of their content of peptided and/or biogenic amines that are secreted or on the basis of a number of markers that are common for all of them. For the quantitative assessment of EC we preferred CgA as a selective marker, since in humans it gives an opportunity for visualizing all the EC in the gastric mucosa. In our study, there was an inverse correlation between EC numbers and HP of the gastric mucosa. Decreased CgA containing EC number in antrum and corpus mucosa were found significantly in HP positive than in HP negative patients (p< 0.01, p< 0.05 respectively). We have also demonstrated that, EC
numbers of the antral gastric mucosa significantly decreased with chronic and active inflammatory change associated with HP gastritis but did not in corpus. This decrease was correlated with the grade of active inflammation and chronic inflammation, which was in close relation to HP infection. Recently, a close relationship between inflammatory cytokines and gastric neuropeptides has been demonstrated in animal cultured cells in vitro. Clinic investigations showed that HP infection induced an increase in the plasma level of gastrin. HP exerts diverse effects on gastric acid secretion. It can result in increased acid secretion, decreased acid secretion, or no overall change in gastric acid secretion. The effect of HP infection on acid secretion depends on the pattern of gastritis induced by the infection. In particular, the effect depends on the distribution of the gastritis between the antral and body region of the stomach and on the degree of atrophy of the mucosa produced by gastritis. In the literature several mechanisms that may be related to hypergastrinemia accompanying HP infection are discussed. Thus Levi et al. suggested that hypergastrinemia is due to the elevated gastric pH resulting from the HP ammonia production. Later it had been found that the gastrin plasma concentration is not influenced by the ureas activity and ammonia production by the microorganism. It is well known that gastrin shows a trophic effect on the EC and especially on the ECL cell in the corporal gastric mucosa. The ECL cell in the oxyntic mucosa, which together with gastrin (G) cell in the antral mucosa, regulate the secretion of gastric acid, is the abundant neuroendocrine cell of the stomach. According to certain authors, in patients with duodenal ulcer and hypergastrinemia, the number of EC in the corporal gastric mucosa is enlarged while others accept that it remains unchanged. EC proliferation in the corporal gastric mucosa is chiefly observed in Zollinger-ellison syndrome and chronic atrophic gastritis type A. Our results showed that the number of CgA containing EC in the corporal gastric mucosa of patients with HP associated chronic gastritis type B is nor enlarged. We found no atrophic changes in the corporal gastric mucosa in our patients which explains the increase of proliferation in the EC population. Although the underlying mechanisms by which HP infection suppresses CgA activity remain unclear, a growing body of evidence suggests that several cytokines related to HP infection might play a key role in CgA regulation. However, few studies on the relationship among inflammation, cytokine and CgA in HP related pathogenesis have been reported so far. The mechanism by which the infection or accompanying inflammation results in this depletion of CgA is unclear. Possible explanation of how HP infection reduces the number of EC include: alkalisation of the microenvironment as a result of ammonia production from urea by HP urease, bioactive substance produced by the bacterium itself, exaggerated antral gastrin relase caused by HP, inflammation of the gastric mucosa induced by the infection and cytokines overproduced by the inflammatory infiltrate, increase in degradation of EC that may have an effect on neuroendocrine cell regulation. Taken together, these data suggest that inflammation, have relationships with antral CgA regulation of HP infected mucosa in chronic gastritis type B. Besides EC were irregularly distributed, close to inflammatory areas, or near lymphoid follicles. The proximity of EC to prominent inflammatory zones may indicate modulate the immune response. This studies, however, may not be representative of the situation in vivo because HP is not in direct contact with EC at the level of the gastric glands. Further studies should be done to clarify these relationships.
In most patients with HP infection, there is relatively little atrophy, and the inflammation is more marked in the antrum but also involves to some extent the corpus mucosa. In our study, there was the nonatrophic antral-predominant, body sparing HP gastritis. Metaplastic intestinal epithelium also harbors EC that may present hyperplastic and neoplastic changes. In the present study we could not find any correlation between HP, CgA with intestinal metaplasia and atroph, which is in apparent contrast to previous study showing such a correlation.

From this points of view, much investigation must be done to verify the mechanisms involved in the depletion of the gastric CgA concentration accompanying HP infection. In our preliminary study, an inverse correlation was present between antral CgA concentrations with inflammation and HP. Further studies are required to determine HP to affect gastric endocrine cells.

COMPETING INTERESTS

The authors declare no competing interest.

REFERENCES