

Relationship between gastrin cell number, serum, antral mucosa and luminal gastrin concentration and gastric acidity in antral atrophic gastritis

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Abstract

The aim of our study was to investigate the relationship between gastrin producing cell density with antral mucosa, luminal and serum gastrin concentration in antral atrophic gastritis. Our study group consisted of 17 patients: six with mild atrophic gastritis, seven with moderate atrophic gastritis and four with severe atrophic gastritis. None of the patients had type-A atrophic gastritis but the body mucosa was affected by superficial gastritis at various extent in some. A group of 15 healthy subjects served as control. All subjects underwent gastroscopic examination with multiple bioptic sampling. Radioimmunoassay was used for gastrin determination and photomicroscopy for gastrin producing cell density assessment. Electron microscopy was used to assess the gastrin producing granule density index. Patients with moderate and severe atrophic gastritis showed a lower gastric acidity and acid output as compared to control. Serum gastrin did not show significant differences among the groups. In moderate and severe atrophic gastritis, gastrin producing cell granule density index, gastrin producing cell density and antral mucosa gastrin concentration were significantly lower when compared with control and decreased with advancing of the severity of atrophic gastritis. In atrophic gastritis, however, the latter two measurements were not correlated. In moderate and severe atrophic gastritis luminal gastrin concentration significantly increased, compared with control, after the severity of atrophic gastritis. Gastrin producing cell granule density index and luminal gastrin concentration showed a significant correlation with gastric pH. These data suggest that in antral atrophic gastritis with reduced gastric acidity, the decrement of gastrin producing cells is followed by gastrin producing cell hyperfunction with increased luminal release of gastrin.

There have been several studies investigating the relationship between the counts of gastrin producing cells with serum gastrin concentration and/or gastric acid secretions.¹⁻³ It is often difficult to apply these data to atrophic gastritis, however, in which the distribution of gastrin producing cell has been reported to vary considerably.^{4,5}

The purpose of our study was to study the interrelationships between antral gastrin producing cell density and gastrin concentration in antral mucosa, gastric juice and serum of patients with antral atrophic gastritis and low gastric acid output.

Methods

PATIENTS

The study group consisted of 17 patients (10 men, seven women; mean age 49 years, range 41-62) with biopsy proven antral atrophic gastritis. In all these patients, type-A gastritis had been previously ruled out, however, superficial gastritis in the body, and in some cases in the fundus too, had been recently documented. According to Whitehead's classification⁶ these cases were classified as six mild atrophic gastritis, seven moderate atrophic gastritis and four severe atrophic gastritis. The presence of gastric or duodenal ulcer constituted exclusion criteria. Fifteen subjects, matched for age and sex, with endoscopically and bioptically normal stomach and without previous history of peptic ulcer disease served as control. On the first day, after an overnight fast, blood samples were withdrawn from the cubital vein. On these samples gastrin concentration was determined by using a radioimmunoassay kit (CIS) measuring physiologically active gastrin, both G-17 and G-34. This showed an intraassay coefficient of variation of 6% and intrassay coefficient of variation of 10%. Soon after, gastroscopic examination was performed and five to six bioptic specimens were taken each from the lesser and from the greater curvature in the antrum from within an estimated distance of at least 2 cm proximally to the pylorus. All specimens had a diameter of 2-3 mm and a weight of 3-8 mg and were taken with standard Olympus biopsy forceps. Two specimens were sent for conventional histology (H&E) to a pathologist who was aware of the precise site where a given biopsy was taken. During endoscopic examination, 15-20 ml gastric juice were aspirated for acidity determination. The other bioptic samples were processed for immunocytochemistry as follows. For extraction, the bioptic specimens were weighted wet in 3 ml Pyrex tubes. One millilitre of hot 0.9% NaCl was added to each tube and tubes were quickly thawed in a boiling water bath for 30 minutes. The saline extract was decanted and stored at -20°C until assay. Gastrin content of the biopsy was calculated as the mean of duplicate determinations of three different dilutions and expressed as ng equivalent of the heptadecapeptide gastrin standard per mg tissue. Tissue samples were fixed in Bouin's fluid for 24 h, dehydrated and embedded in paraffin. Sagittal sections, 5 µ thick, were stained using antihuman synthetic gastrin I rabbit serum (CEA-IRE-Sorin, France) and the peroxidase-antiperoxidase method after Sternberger.⁷ A Nikon photo-microscope was used for evaluating the density of gastrin producing cells in the stained sections. Only sections showing the

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TABLE I Gastrin producing cell density, antral mucosa concentration and gastrin producing cell granule density index

Subjects (n)	G-cells/mm ²	AGC (ng/mg)	G-cell GDI
Controls (15)	87 (39)	13.8 (1.6)	2.29 (0.09)
Mild atrophic gastritis (6)	74 (31)	12.4 (2.9)	2.11 (0.12)
Moderate atrophic gastritis (7)	45 (17)*	9.7 (1.8)*	1.72 (0.05)*
Severe atrophic gastritis (4)	26 (22)†	8.8 (1.5)*	1.28 (0.08)†

AGC: antral mucosa gastrin concentration; Gastrin producing cell GDI: gastrin cell granule density index; AG: atrophic gastritis; p-values indicate significance of difference from normals: *p<0.05; †p<0.01

whole area between surface and muscularis mucosae and with intact mucosa were counted. The mean density of antral gastrin producing cells/mm² determined in five or more specimens was used. On a different day, a standard 6 µg/kg pentagastrin stimulated gastric acid test was done. Further, on four samples of gastric juice obtained from the 30 min basal collection, gastrin concentration was determined. This was done within two months on neutralised samples stored at -20°C as described by Knight *et al.*⁸ For electron microscopy the tissues were immediately fixed in 3% glutaraldehyde at low pH, to better preserve G-17, washed in buffered solution, dehydrated in acetone and embedded in Vestopal. The morphometric method described by Creutzfeldt *et al.*,⁹ was used to quantitatively assess the gastrin producing cell granule population in ultrathin sections stained with uranyl acetate. Therefore, 'full', 'intermediate full', 'intermediate empty' and 'empty' granules scored 4, 3, 2 and 1 respectively. Gastrin producing cell granule density index was defined as the product of the number of each granule type multiplied by its assigned scoring factor and divided by the total number of granules.

STATISTICAL ANALYSIS

All data were expressed as mean (SD). Results were evaluated statistically by the Mann-Whitney test and by the rank correlation coefficient of Spearman.

Results

Table I shows the result of gastrin producing cell density, antral mucosa gastrin concentration and gastrin producing cell granule density index.

All these three measurements were shown to decrease in relation to the progression of the severity of gastritis. This trend achieved a statistical significance in patients with moderate atrophic gastritis and, particularly, in patients with severe atrophic gastritis. In this group, compared with controls, the decrease of gastrin producing cell density and of antral mucosa

gastrin concentration was 69% and 37%, respectively. A weak correlation ($\rho=0.40$, $p<0.05$) was found between gastrin producing cell density and antral mucosa gastrin concentration in the overall study group. In the atrophic gastritis group however, the two measurements did not correlate at all. Table II shows the results of the determination gastrin concentration in the serum and in the gastric juice (as a mean of four different determinations) together with gastric acid pH value, BAO and MAO.

Serum gastrin concentration showed no significant difference among the groups. Luminal gastrin concentration was significantly higher in patients with moderate atrophic gastritis ($p<0.05$) and in those with severe atrophic gastritis ($p<0.01$). As expected, with the progression of the severity of atrophic changes a significant decrease of gastric acidity (moderate atrophic gastritis and severe atrophic gastritis: $p<0.05$) and basal and stimulated gastric acid output (severe atrophic gastritis: $p<0.05$) was noted when compared with controls. There was no significant difference in the volume of the basal collection among the groups and no patients showed pentagastrin fast achlorhydria. No correlation was found between fasting serum gastrin and any of the other measurements. Taking into account all 32 samples, gastric pH significantly correlated with luminal gastrin concentration ($\rho=0.88$, $p<0.01$) and with gastrin producing cell granule density index ($\rho=0.83$, $p<0.01$).

Discussion

In the present study, the concentration of serum gastrin was found to be comparable in all different groups considered and did not correlate with any of the other measurements examined. This result is in accordance with the reported lack of correlation between fasting serum gastrin and gastrin producing cell density¹⁰ and with acid secretion.¹¹ Petersen *et al.*¹² reported a positive correlation between fasting serum gastrin and BAO but this did not appear from our study.

It has been shown that the number of gastrin producing cells decreases in relation with the progression of the severity of gastric mucosal atrophic changes.¹³⁻¹⁵ Keuppens *et al.*,¹⁶ using gastrectomy specimens from patients with peptic ulcer, found a significant inverse correlation between the mean gastrin producing cell concentration and the degree of extension of antral gastritis. Significant variations of the gastric cell count occur from one area to another in the gastric antrum and between the lesser and the greater curvature. Further, in case of antral gastritis a gastrin producing cell population

TABLE II Serum and luminal gastrin concentration, gastric pH and gastric acid output

Subjects (n)	Serum gastrin (pg/ml)	Luminal GC (pg/ml)	Gastric pH	BAO	MAO (mmol/h)
Control (15)	54.2 (11.4)	144.3 (115.8)	2.14 (1.10)	3.0 (1.2)	16.4 (3.6)
Mild AG (6)	60.1 (14.2)	138.4 (157.4)	2.30 (2.02)	2.4 (1.4)	14.2 (4.4)
Moderate AG (7)	56.8 (18.6)	238.7 (103.9)*	3.83 (0.89)*	1.7 (0.8)	12.0 (2.1)
Severe AG (4)	63.2 (21.4)	281.8 (132.0)†	4.13 (0.94)*	0.8 (1.2)*	8.2 (1.3)*

Luminal GC: mean of four determinations of gastrin concentration in gastric juice; AG: atrophic gastritis; p-values express significant difference v controls: *p<0.05; †p<0.01

assessment on gastrectomy specimens proves to be even more cumbersome.¹⁶ Understandably, in our study we had to rely on multiple biopsy sampling from which any inference about the total gastrin producing cell population is an impossible task. As pointed out by Royston *et al*,¹⁷ however, gastrin producing cell numbers can be roughly assessed by measuring the gastrin producing cell density from representative antral biopsies. Taking into account such limitations, it would appear that our results are in keeping with Keuppens's observation. Patients with severe atrophic gastritis showed the most marked decrease of gastrin producing cell density when compared with control [26 (22) v 87 (39) $p < 0.01$]. It must be said that in this group of patients, however, gastrin producing cell degranulation, showing poor immunolabelling, might have contributed to further lower the gastrin producing cell density. Also antral mucosa gastrin concentration decreased and generally followed the severity of mucosal atrophy. This decrement was of a lesser degree compared with the drop of gastrin producing cell density. Patients with moderate and severe atrophic gastritis, although had a significantly lower antral mucosa gastrin concentration when compared with control ($p < 0.05$), showed a wide overlap of values. This suggests that the decrease of gastrin producing cell density is not strictly followed by a proportional fall of the mucosal content of gastrin as it was also shown by a lack of correlation between these two measurements in the atrophic gastritis group. This finding may be explained by our further observation that, with advancing degree of atrophy, gastrin producing cells showed a proportional hyperfunction. A low gastrin producing cell granule density index, that is, higher percentage of 'empty' granules, stands for higher functional activity⁹ and it was significantly present in patients with moderate and with severe atrophic gastritis ($p < 0.05$ and $p < 0.01$, respectively) when compared with control.

Gastrin release in gastric juice was first shown by Jordan and Yip,¹⁸ however, methodological difficulties using radioimmunoassay have not made this measurement widespread in man. Knight *et al*⁸ has provided consistent evidence that luminal gastrin derives by secretion from antral gastrin producing cells into the gastric lumen. We found that patients with moderate and severe atrophic gastritis had significantly higher luminal gastrin concentration when compared with control ($p < 0.05$ and $p < 0.01$ respectively). From the study of Solcia *et al*¹⁹ it has been hypothesised that the routes by which gastrin reaches the lumen after release from gastrin producing cells start from the intra-epithelial basal and lateral parts of the gastrin producing cell. No gastrin granules have been described in the apical part of the cells, as also noted by us (data not shown), and therefore, such indirect pathway implies tight junctions permeable to gastrin. Further ultrastructural studies are needed to elucidate why these patients seem to release gastrin preferably into the lumen, being their serum gastrin not significantly elevated. Luminal gastrin concentration showed a significant correlation ($\rho = 0.88$, $p < 0.01$)

with gastric pH. This finding is in keeping with the data of Fiddian-Green *et al*²⁰ who showed that an increased rate of acid secretion was accompanied by the disappearance of endogenous and exogenous immunoreactive gastrin from gastric juice. Recently, Gutierrez *et al*²¹ in a group of duodenal ulcer and vagotomised patients showed that luminal gastrin release varied in function of gastric acidity. In the present study, gastrin producing cell granule density index highly correlated with gastric pH ($\rho = 0.83$, $p < 0.01$) so that a lower acidity was associated with a higher gastrin producing cell activity. This finding is in accordance with the concept of a feedback mechanism between gastric acidity and gastrin producing secretion.²²

These data suggest the conclusive hypothesis that in patients with antral atrophic gastritis and low gastric acidity, a decreased gastrin producing cell density is followed by a compensatory gastrin producing cell functional hyperactivity with a higher luminal release of gastrin.

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