CLINICAL STUDY

Plasma chromogranin A in patients with autoimmune chronic atrophic gastritis, enterochromaffin-like cell lesions and gastric carcinoids

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Abstract

Objective: In atrophic body gastritis (ABG) chronic hypergastrinaemia stimulates enterochromaffin-like (ECL) cell proliferation with development of cell hyperplasia, dysplasia and possibly type-1 gastric carcinoids. As circulating chromogranin A (CgA) levels are a marker of neuroendocrine tumours, we evaluated the clinical usefulness of CgA assay in ABG patients to detect those with carcinoids.

Design and methods: Plasma CgA levels were measured using a commercial ELISA in 45 healthy volunteers, nine patients with type-1 gastric carcinoids and 43 consecutive ABG patients (21 without and 22 with ECL cell hyperplasia/dysplasia).

Results: CgA levels were significantly higher in ABG patients with and without gastric carcinoids than in healthy subjects (P < 0.001). The highest values occurred in patients with carcinoids (median (interquartile range): 58.1 (44.5–65.3) U/l) and with ECL cell hyperplasia/dysplasia (35.5 (31.8–48.65) U/l) but there were no significant differences in CgA among the various subgroups of ABG patients classified according to ECL cell status. Nevertheless, in ABG patients without carcinoids CgA values correlated with the presence and severity of ECL cell lesions (r = 0.428, P < 0.01). The sensitivity and specificity of the CgA assay in identifying patients with carcinoids were 100 and 23% respectively.

Conclusions: CgA plasma levels reflect the histological degree of ECL cell lesions in patients with ABG but the assay specificity is too low to detect among these patients those with gastric carcinoids.

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Introduction

The incidence of type 1 gastric carcinoids has risen markedly over the last 50 years (1–3). These endocrine tumours develop in 2–12.5% of patients with autoimmune atrophic body gastritis (ABG), with or without pernicious anaemia, although rare cases have been reported in patients with Helicobacter pylori gastritis (3–6). The pathogenetic mechanism is claimed to be the trophic effect of hypergastrinaemia on enterochromaffin-like (ECL) cells in the gastric fundus and corpus (7, 8). Indeed, in ABG the lack of acid inhibitory feedback induces a sustained release of circulating gastrin that stimulates the growth of ECL cells with development of cell hyperplasia (3, 7–9). The spectrum of ECL cell lesions includes hyperplasia (simple, linear and micronodular), dysplasia, and eventually carcinoids (9).

Chromogranin A (CgA) is a 49 kDa acidic glycoprotein that is widely expressed in neuroendocrine cells where it constitutes one of the most abundant components of secretory granules (10). During exocytosis it is co-released with peptidic hormones, biogenic amines and neuropeptides (11, 12). Circulating CgA levels are a sensitive marker for gastroentero-pancreatic endocrine tumours, including gastric carcinoids (11–18). Recent studies indicate that circulating CgA levels correlate positively with ECL cell mass in patients with autoimmune ABG (14, 19), in patients with gastrinoma and multiple endocrine neoplasia syndrome type 1, who may develop potentially malignant gastric carcinoids (20), and in subjects on long-term acid inhibition with proton pump inhibitors, who almost never develop gastric carcinoids (21). However, few data are available on the clinical usefulness of CgA assays to identify, in hypergastrinaemic patients, those with advanced ECL cell lesions such as dysplasia and carcinoids (14, 20). Therefore, the aim of this study was to evaluate the pattern of plasma CgA levels in normal subjects and in a series of patients with autoimmune ABG, without and with ECL cell lesions at various stages including gastric carcinoids.
Subjects and methods

Three groups of subjects participated in this study, performed between January 1999 and June 2003.

Normal subjects

The control group for gastrin and CgA assays consisted of 45 healthy volunteers (10 men and 35 women, aged 31–78 years, mean 63.8 years) recruited among medical staff and acquaintances of patients. These subjects did not undergo gastrointestinal endoscopy. Subjects with anti-parietal cell, anti-thyroid peroxidase (TPO) antibodies and/or a positive H. pylori IgG titre were excluded from the study.

Carcinoid patients

This group included nine patients (one man and eight women, aged 45–87 years, mean 63.8 years) with newly diagnosed, untreated, type 1 gastric carcinoids. Eight of them had multiple carcinoids, ranging in size from 0.4 to 1.1 cm, in the corpus and/or fundus, and the ninth had a solitary carcinoid measuring 0.9 cm in size in the fundus. They all underwent upper gastrointestinal endoscopy. Any abnormality was biopsied and six biopsy specimens (two from fundus, corpus and antrum) were taken. In all cases histology confirmed the diagnosis of well differentiated tumours, restricted to the mucosa–submucosa, with concomitant ABG. No patient had histological/serological evidence of H. pylori infection. All nine patients had anti-parietal cell (n = 8) and/or anti-intrinsic factor (n = 5) antibodies. Seven had pernicious anaemia with low serum vitamin B₁₂ levels (<148 pmol/l), normal serum folate levels (>7 nmol/l) and high total homocysteine (tHCY) plasma levels (>20 μmol/l). The other two patients had serum vitamin B₁₂ values in the lower normal range (165 and 176 pmol/l), with iron deficiency anaemia in one. Associated diseases were primary hypothyroidism in one patient and primary hypothyroidism and diabetes mellitus type 1 in another. Anti-TPO antibodies were found in six patients.

Non-carcinoid ABG patients

In this group we included 43 consecutive patients (eight men and 35 women, aged 31–88 years, mean 54.7 years) with pernicious anaemia, 21 referred to us with this diagnosis who were on long-term parenteral vitamin B₁₂ treatment and in haematological remission, and 22 with a new diagnosis. The main haematological findings (mean and range) in untreated patients were: haemoglobin 98 (71–132) g/l, erythrocyte count 2.9 (1.7–4.3) × 10¹² cells/l, mean corpuscular volume 115 (90–140) fl, serum vitamin B₁₂ 84 (35–140) pmol/l, serum folate 18 (7.5–26.7) nmol/l and plasma tHCY 42 (28.4–83.0) μmol/l. Anti-parietal cell antibodies were present in 38 patients and anti-intrinsic factor antibodies in 24. All the patients underwent upper gastrointestinal endoscopy with at least six gastric biopsies as described above. Pathological examination of the specimens demonstrated the presence of ABG in all patients: mild in three, moderate in 10 and severe in 30 (22). ECL cell status was independent of vitamin B₁₂ treatment and was classified according to Solcia et al. (9), with the lesions graded as follows: 0 = absent, 1 = simple hyperplasia, 2 = linear hyperplasia, 3 = micronodular hyperplasia, and 4 = dysplasia. Eleven patients had simple hyperplasia, four linear hyperplasia, six micronodular hyperplasia, and one dysplasia with micronodular hyperplasia. Another patient had gastric carcinoids and was excluded from this group and included in the previous one; thus in this series of consecutive pernicious anaemia patients only 1 of 44 (2.3%) had gastric carcinoids. Associated autoimmune diseases were primary hypothyroidism in eight patients, diabetes mellitus type 1 in two, hypoparathyroidism in one, Addison’s disease in one, and Sjogren’s syndrome in one. Anti-TPO antibodies were detected in 21 patients. Three women patients, aged 60–72 years, had primary hyperparathyroidism at the time of the study; their serum calcium levels were 2.87, 3.07 and 2.94 mmol/l (normal range 2.02–2.59 mmol/l) and plasma parathyroid hormone levels 98, 175 and 128 ng/l (normal range 10–65 ng/l). Two of them had parathyroid adenoma at subsequent surgery and the third parathyroid hyperplasia.

Exclusion criteria for all patients were H. pylori infection (according to histology and/or positive H. pylori IgG titre), treatment with antisecretory drugs, renal failure, severe hepatic failure, and concomitant malignancy.

All the subjects gave their informed consent to the study, which was approved by the local ethics committee.

Methods

Venous blood samples were drawn into tubes containing EDTA between 0800 and 1000 h after an overnight fast, 1 h before gastrointestinal endoscopy in all patients. The samples were centrifuged at 4 °C and plasma separated and stored in aliquots at −30 °C until assayed. Haemolysed samples were discarded to avoid spuriously elevated CgA levels (23).

Plasma gastrin and CgA levels were measured as previously described (15, 24) using commercially available kits (GammaDab Gastrin [¹²⁵I] RIA Kit (DiaSorin, Inc., Stillwater, MN, USA) and DAKO Chromogranin A ELISA Kit (Dako A/S, Glostrup, Denmark)). For the gastrin assay the intra- and interassay coefficient of variation (CV) values were 3.6 and 5.9% respectively. The
CgA enzyme immunoassay uses polyclonal antibodies raised in rabbits against a 23 kDa C-terminal fragment of human CgA and the same fragment as standard. Results are given as U/l. In our laboratory the 95% confidence detection limit was 2.1 U/l and the intra- and interassay CV values were 4.1 and 6.7% respectively. The normal range established in 135 healthy subjects was 2.6–35.4 U/l.

Statistics

Results are given as median and interquartile range. Differences between groups were evaluated by the Kruskal–Wallis test followed by Dunn’s multiple comparison test. Relationships between variables were assessed by linear regression analysis, and the Spearman coefficient (\( r_s \)) was employed to evaluate the correlation between ECL cell status and CgA and gastrin plasma levels in the patients. A \( P \) value < 0.05 was considered statistically significant. The sensitivity, specificity, positive and negative predictive values of the CgA assay were calculated as previously described (25) using the upper reference limit of normal subjects as cut-off and considering the ABG patients as a population in which it is clinically sensible to suspect gastric carcinoids.

Results

The results are summarised in Table 1 and Fig. 1. The upper reference limits for gastrin and CgA defined as 2 s.d. above the mean levels of our normal subjects were 95 ng/l and 20.5 U/l respectively. ABG patients without and with gastric carcinoids had plasma gastrin and CgA levels significantly higher than those of healthy subjects (Table 1). The presence of hypothyroidism, diabetes mellitus type 1 and primary hyperparathyroidism did not affect the results significantly (data not shown).

As shown in Fig. 1, all ABG patients, with and without carcinoids, had abnormally high gastrin levels, and CgA levels were above the normal range in all nine with carcinoids and in 33 of the non-carcinoid group, 13 of the 21 without and 20 of the 22 with ECL cell hyperplasia/dysplasia. Patients in the non-carcinoid group with ECL cell hyperplasia/dysplasia had median gastrin and CgA values (964 (833.5–1337) ng/l and 35.5 (31.8–48.65) U/l) intermediate between those of the same group without ECL cell hyperplasia (665 (419–886) ng/l and 23.5 (15.6–31.4) U/l) and those of the gastric carcinoid patients (1381 (1081–1400) ng/l and 58.1 (44.5–65.3) U/l). However, no significant differences in gastrin and CgA plasma levels were found among these three groups, or among the five subgroups of patients without carcinoids subdi-vided according to ECL cell lesion stage. Nevertheless, in the non-carcinoid group, there was a significant correlation between ECL cell status with lesions graded from 0 to 4 and plasma gastrin \( (r_s = 0.474, P < 0.01) \) and plasma CgA \( (r_s = 0.428, P < 0.01) \).

Log-transformed CgA and gastrin plasma levels correlated significantly in the ABG patients with gastric carcinoids \( (r^2 = 0.547, P = 0.023) \) and without gastric carcinoids \( (r^2 = 0.251, P = 0.0006) \) whereas a similar correlation was not found in healthy subjects \( (r^2 = 0.001, P = NS) \).

The sensitivity and specificity of CgA assay for gastric carcinoids were 100% (95% confidence interval (CI), 66–100%) and 23% (95% CI, 12–39%) respectively, with a positive predictive value of 21% (95% CI, 10–37%) and a negative predictive value of 100% (95% CI, 69–100%).

Discussion

In agreement with previous studies (14, 19, 21), our findings demonstrated that high plasma gastrin and CgA levels, which correlated one with the other, are a characteristic feature of patients with ABG. The highest values for both gastrin and CgA occurred in patients with type 1 gastric carcinoids and in those with ECL cell hyperplasia/dysplasia. Moreover, in patients without gastric carcinoids plasma gastrin and CgA levels correlated positively with the histological degree of ECL cell lesions scored according to the Solcia et al. classification from 0 to 4 (9). These data are in line with those of Borch et al. (14) who found a significant correlation between plasma CgA levels and the densities of endocrine cells in the atrophic fundic mucosa of patients with autoimmune ABG. Also in gastrinoma patients (13, 20) and in patients on long-term acid suppression therapy for gastro-oesophageal reflux disease (21) serum CgA levels have been shown to reflect the degree of gastric ECL cell proliferative changes. Overall, these observations suggest that ECL cell mass is a major determinant for CgA elevations in hypergastrinaemic states. Moreover, in animal models of sustained hypergastrinaemia there is evidence that gastric ECL cells

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Gastrin levels (ng/l)</th>
<th>CgA levels (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>45</td>
<td>56 (50.1–67)</td>
<td>12.3 (9.1–14.6)</td>
</tr>
<tr>
<td>ABG patients without gastric carcinoids</td>
<td>43</td>
<td>886 (564–1100)*</td>
<td>32 (22.2–41.5)*</td>
</tr>
<tr>
<td>ABG patients with gastric carcinoids</td>
<td>9</td>
<td>1381 (1081–1400)*</td>
<td>58.1 (44.5–65.3)*</td>
</tr>
</tbody>
</table>

* \( P < 0.001 \) vs healthy subjects (Kruskal–Wallis test followed by Dunn’s multiple comparison test).
are the main source of serum CgA breakdown products (26). However, as CgA is widely expressed in neuroendocrine cells (10), it is likely that in ABG patients the circulating CgA pool is also affected by CgA release from gastrin-secreting cells of the gastric antrum. This interpretation is consistent with some previously reported data in patients with gastrinoma (18, 27) and could account for the finding in our study that

**Figure 1** Plasma gastrin and chromogranin A (CgA) levels in normal subjects (controls) and in patients with autoimmune atrophic body gastritis (ABG) with and without gastric carcinoids. Patients without gastric carcinoids are subdivided according to the presence of enterochromaffin-like (ECL) cell hyperplasia/dysplasia (ECL 0 and ECL +). In the inserts the histological degree of ECL cell lesions is scored as follows: 0 = absent, 1 = simple hyperplasia, 2 = linear hyperplasia, 3 = micronodular hyperplasia, and 4 = dysplasia. Gastrin levels are plotted logarithmically to accommodate extreme values. The broken lines indicate the upper references limits defined as 2 s.d. above the mean gastrin and CgA values of our normal subjects.
62% of patients without ECL cell hyperplasia had CgA plasma levels above the normal range. Nevertheless, due to the well known difficulties of distinguishing a normal ECL cell pattern from simple hyperplasia (4, 9), we cannot exclude the possibility that some of our patients in whom the ECL cell pattern was classified as normal actually had simple hyperplasia. Moreover, the specificity of the DAKO CgA assay is not very high, ranging from 83 to 86% in different studies (17, 18, 23), which could also account for some positive results in our patients without ECL cell hyperplasia.

As regards the clinical usefulness of the CgA assay in ABG, we found no significant differences in median plasma CgA values among the various subgroups of ABG patients classified according to ECL cell status, and although the sensitivity of the assay in identifying patients with gastric carcinoids was excellent (100%), the specificity was too low (23%). These fogs are similar to those previously reported by Borch et al. (14) and Bashir et al. (20) and confirm that the value of CgA plasma levels in the diagnosis of type I gastric carcinoids is very limited, although, due to the negative predictive value of 100%, the finding of normal CgA levels should actually exclude the condition.

In the present study the coexistence of autoimmune endocrinopathies and primary hyperparathyroidism did not influence CgA levels. The association of autoimmune ABG with immunologically mediated endocrine gland failure is well known (19, 28) and occurred in about 25% of our patients. Also, sporadic reports have described an association between pernicious anaemia and primary hyperparathyroidism (29–31), which was observed in 3 of 52 of our patients (6%). However, the relevance of the latter association is unclear and indeed it could be a chance one as both diseases are relatively common in women over 60, although other still unknown pathogenetic mechanisms cannot be excluded.

In conclusion, CgA plasma levels reflect the histological degree of ECL cell lesions in patients with ABG but the assay cannot replace upper gastrointestinal endoscopy to detect and monitor gastric carcinoid tumours in patients with hypergastrinaemic states in whom also CgA levels are abnormally high.

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