Plasma levels of progastrin but not amidated gastrin or glycine extended gastrin are elevated in patients with colorectal carcinoma

R K Siddheshwar, J C Gray, S B Kelly

Abstract

Background—The relationship between plasma gastrin levels and colorectal cancer is controversial. When confounding factors which increase plasma gastrin levels are taken into account, it has been shown that gastrin levels are not elevated in patients with colorectal cancer. However, these studies only measured amidated gastrin. Total gastrin (which includes unprocessed, partially processed, and mature forms of gastrin) has been shown to be elevated in patients with colorectal cancer.

Aims—The aim of this study was to determine whether fasting plasma levels of progastrin, amidated gastrin, or glycine extended gastrin are elevated in patients with colorectal cancer or colorectal polyps compared with controls.

Methods—Progastrin, amidated gastrin, and glycine extended gastrin were estimated by radioimmunoassay using the following antibodies: L289, 109–21, and L2. Blood samples were analysed for Helicobacter pylori by an enzyme linked immunosorbent assay.

Results—Median progastrin levels were significantly higher in the cancer group (27.5 pmol/l) than in the polyp (<15 pmol/l) or control (≤15 pmol/l) group (p=0.0001). There was no difference in median levels of amidated gastrin between groups. Median levels of amidated gastrin were significantly higher in H pylori positive patients (19 pmol/l) than in H pylori negative patients (8 pmol/l) (p=0.0022). Median plasma progastrin levels were significantly higher for moderately dysplastic (15 pmol/l) and severely dysplastic (15 pmol/l) polyps (p=0.05).

Conclusions—Plasma levels of progastrin, but not amidated gastrin or glycine extended gastrin, are significantly elevated in patients with colorectal cancer compared with those with colorectal polyps or controls, irrespective of their H pylori status. We conclude that measuring plasma progastrin levels in patients with colorectal cancer is warranted.

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Keywords: progastrin; glycine extended gastrin; colorectal carcinoma

Colorectal cancer is the second most common malignant disease in the UK after lung cancer, and accounts for about 26 000 new cases and 20 000 deaths each year. The relationship between gastrin and the development of colorectal cancer remains controversial. Gastrin stimulates colonic proliferation and growth of colonic cell lines in vitro and colorectal carcinoma in vivo. It has been shown that glycine extended gastrin exerts a mitogenic effect on a human colon cancer cell line and a non-transformed colon cell line. Nemeth and colleagues demonstrated production of progastrin by colorectal tumours. Seva and colleagues observed a proliferative effect of both amidated gastrin and glycine extended gastrin on the exocrine pancreatic cell line AR4-2J. Transgenic mice producing progastrin in the liver have increased plasma progastrin levels and hyperplasia of the colonic mucosa. Koh and colleagues demonstrated decreased epithelial proliferation of the colonic mucosa in gastrin deficient mice. Gastrin receptors have been demonstrated on colorectal carcinomas. Gastrin receptor antagonists and antigastrin antibodies inhibit the growth of colon cancer.

Fasting serum amidated gastrin levels have been shown to be elevated in patients with colorectal cancer compared with controls and to decrease after apparently curative resection of the tumour, suggesting that the elevated serum gastrin seen in these patients may be due, at least in part, to secretion of gastrin by the tumour. This was mainly accounted for by a subgroup of patients who had significantly elevated levels. Kameyama and colleagues found higher serum gastrin levels in patients with colorectal cancer liver metastases. However, none of these studies controlled for the presence of gastric colonisation with Helicobacter pylori, a common infection resulting in chronic gastritis and significantly increased fasting and meal stimulated gastrin levels that decrease after eradication of the organism. It is likely that the subgroups with significantly elevated serum gastrin levels had H pylori infection. Thorburn and colleagues found that serum gastrin levels above the normal range (>90 pg/ml) were associated with a 3.9-fold increased risk of colorectal cancer. Other studies however have found no association between colorectal neoplasia and hypergastrinaemia.

Penman and colleagues found that when confounding factors which increase serum gastrin levels (H2 antagonists, proton pump inhibitors, hypercalcaemia, renal impairment, pernicious anaemia, and H pylori infection) are...
Table 1 Patient demographics in the cancer, polyp, and control groups

<table>
<thead>
<tr>
<th>Diagnosis in controls</th>
<th>Degree of dysplasia</th>
<th>Type of polyp</th>
<th>Dukes’ stage</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diverticular disease</td>
<td>Normal</td>
<td>Severe</td>
<td>Mild</td>
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<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tubulovillous adenoma</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Tubular adenoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Degree of dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>Moderate</td>
<td>Inoperable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>Severe</td>
<td>Inoperable</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Diagnosis in controls</td>
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<td>Number</td>
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<td></td>
<td></td>
<td>Sex (M:F)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Mean age (y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(range)</td>
</tr>
</tbody>
</table>

Patients and methods

Patients

We studied 57 patients with newly diagnosed, histologically proved, sporadic colorectal cancers, 29 patients with colorectal polyps, and 53 controls. Patients undergoing colonoscopy for bowel symptoms and who were found to have normal mucosa or diverticular disease were used as controls. Patients with incomplete colonoscopy, inadequate bowel preparation, metaplastic polyps, or inflammatory bowel disease were excluded. Patients and controls with any of the following conditions were excluded from the study, as these conditions are known to raise plasma gastrin levels: peptic ulcer disease, previous gastric surgery including gastrectomy, vagotomy, pyloroplasty or gastrojejunosotomy, small bowel resection, pernicious anaemia, treatment with acid suppressing drugs (H₂ antagonists or proton pump inhibitors), renal failure, hypercalcemia, recent antibiotic therapy (within one week), patients requiring emergency surgery, or patients with intestinal obstruction. In addition, patients with familial adenomatous polyposis or malignancy at another site were excluded. Informed consent was obtained from all patients and controls and the study was approved by the Newcastle and North Tyneside ethics committee. All blood samples from patients and controls were collected from North Tyneside General Hospital.

Blood samples (10 ml of venous blood) were drawn after an overnight fast from patients with colorectal cancer on the morning of surgery. Fasting blood samples were obtained from 29 patients with colorectal polyps and 53 controls following colonoscopy. Blood samples were collected into lithium-heparin tubes and centrifuged at 3000 rpm for 10 minutes at 4°C and the separated plasma was stored at −20°C until assayed.

Determination of H pylori status

The presence or absence of H pylori infection was determined by an enzyme linked immunosorbent assay (ELISA). This assay detects serum antibodies (IgG) to H pylori in plasma.

Gastrin analysis

Progastrin derived peptides in human plasma were estimated by radioimmunoassay using three different antibodies, as previously described. In all assays, standard curves were prepared with the appropriate volume of plasma stripped of gastrin. Intact progastrin was assayed using antibody L289, which reacts with C terminal fragments of progastrin longer than six residues. C terminal glycine extended gastrins were detected with monoclonal antibody 109–21 (a gift from Dr JH Walsh, UCLA). COOH terminal amidated gastrins were assayed with antibody L2. The “limit of detection”—that is, the sensitivity of the assay—was 4 pmol/l for antibody L2 which detects amidated gastrin. The sensitivity of the 109–21 assay for glycine extended gastrin was 20 pmol/l and the sensitivity of the L289 assay was 15 pmol/l.

Statistical analysis

Statistical analysis was by the Kruskal-Wallis test and Spearman’s rank correlation coefficient.

Results

Patient demographics

Patient demographics are listed in Table 1. In the H pylori positive group with carcinoma, the...
Progastrin levels in colorectal carcinoma

Plasma progastrin levels in control, cancer, and polyp groups. Median values are shown (**p=0.0001, cancers v controls and polyps). (Fig 1)

Table 2 Amidated gastrin levels in control, cancer, and polyp groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Amidated gastrin* (pmol/l)</th>
<th>Percentage of group ≤4 pmol/l</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 (&lt;4–780)</td>
<td>15.1</td>
<td>53</td>
</tr>
<tr>
<td>Cancer*</td>
<td>13 (&lt;4–520)</td>
<td>17.5</td>
<td>57</td>
</tr>
<tr>
<td>Polyp</td>
<td>24 (&lt;4–1600)</td>
<td>10.3</td>
<td>29</td>
</tr>
</tbody>
</table>

*Values are median (range). Differences in median levels of amidated gastrin between groups was not significant.

Dukes’ classification was not available in two patients as they were inoperable but preoperative biopsies were positive for carcinoma in both patients. In one patient, the carcinoma was found to be arising in a polyp. All patients had one carcinoma and four patients also had a polyp present. This was a tubulovillous adenoma in three patients and a tubular adenoma in one patient. In the H pylori negative group with carcinoma, two patients had two rectal cancers, both of which were Dukes’ stage B. One patient had one cancer in the ascending colon and one at the hepatic flexure, both of which were Dukes’ stage B. In three patients, the carcinoma was found to be arising in a polyp. Two more patients had one polyp each, both of which were tubulovillous adenomas.

In the H pylori positive group with polyps, 14 patients had one polyp and four patients had two polyps. Three of the patients with two polyps had two tubulovillous adenomas and one had two tubular adenomas. In the H pylori negative group with polyps, 10 patients had one polyp and one patient had three polyps.

PLASMA GASTRIN LEVELS

Median progastrin levels in the cancer group (27.5 pmol/l (range 15–132)) were significantly higher than in either the control group (≤15 pmol/l (15–54)) (p=0.0001) or the polyp group (≤15 pmol/l (15–240)) (p=0.0001, Kruskal-Wallis test) (Fig 1). In the control group, 49 of 53 (92.5%) patients had progastrin levels <15 pmol/l compared with 17 of 29 (58.6%) in the polyp group and 15 of 56 (26.8%) in the cancer group. Progastrin levels were not measured in one patient with cancer due to an insufficient sample. Median amidated gastrin levels did not differ significantly between groups (Table 2). The cancer group were significantly older than the control and polyp groups and a preliminary analysis showed progastrin to be significantly related to age. However, as Table 3 demonstrates, comparing levels of progastrin within age groups shows that progastrin levels were higher in cancer patients, irrespective of age. Thus the apparent association between age and progastrin is likely to be due to the age imbalance of the groups and therefore spurious. There was no apparent relationship between age and amidated gastrin (Spearman rank correlation coefficient 0.05; p=0.5). There was no significant difference in progastrin levels between the sexes (Kruskal-Wallis test, p=0.3). There was borderline significance in median amidated gastrin levels between the sexes (males 9.5 pmol/l, females 16 pmol/l) (p=0.08).

The groups were fairly well matched in terms of proportion of H pylori positive patients; 55% of the control group, 60% of the cancer group, and 62% of the polyp group. There was no statistically significant effect of H pylori status on progastrin levels (Table 4). Amidated gastrin levels were significantly higher in H pylori positive patients (19 pmol/l) than in H pylori negative patients (8 pmol/l) (Kruskal-Wallis test, p=0.0022) (Table 5). There was no significant difference in plasma levels of progastrin or amidated gastrin depending on the site (Kruskal Wallis, p=0.9 and p=0.4, respectively), Dukes’ stage (Kruskal Wallis, p=0.2 and p=1) (Table 6) or grade (Kruskal Wallis, p=0.4 and p=0.2) (Table 7) of the cancer. There was no significant difference in plasma levels of progastrin or amidated gastrin between tubulovillous adenomas and tubular adenomas. Median plasma progastrin levels were significantly higher for moderately dysplastic polyps (38 pmol/l) (n=18) compared with mildly dysplastic polyps (15 pmol/l) (n=8) and severely dysplastic polyps (15 pmol/l) (n=3) (Kruskal-Wallis test, p=0.05). There was no significant difference in amidated gastrin levels between groups (p=0.1).
Levels of amidated gastrin and progastrin greater than 100 pmol/l were detected in the following numbers of subjects respectively: controls (4, 0), cancers (4, 3), and polyps (2, 2). The mean values of amidated gastrin and progastrin >100 pmol/l, respectively, were as follows: controls (300 pmol/l, 0), cancers (313 pmol/l, 119 pmol/l), and polyps (960 pmol/l, 192 pmol/l). The highest recorded level of amidated gastrin was 1600 pmol/l whereas the highest level of progastrin was 2400 pmol/l. All but two controls had levels of glycine extended gastrin less than the limit of detection (<20 pmol/l). Two controls had levels of 58 pmol/l (H pylori positive) and 100 pmol/l (H pylori negative), respectively.

Discussion

To our knowledge, this is the first study to demonstrate that plasma levels of progastrin, but not amidated gastrin or glycine extended gastrin, are significantly elevated in patients with colorectal cancer but not in patients with colorectal polyps or controls.

Two mechanisms have been proposed to explain the origin of elevated plasma gastrin levels in patients with colorectal carcinoma. The first mechanism suggests that the tumour is the origin of gastrin production—that is, “autocrine secretion”. Ciccotosto and colleagues’ suggested by Ciccotosto and colleagues. Ciccotosto and colleagues’ also reported that H pylori positive patients with colorectal cancer had significantly higher levels of circulating non-amidated gastrin than H pylori negative patients (5.2-fold and 2.3-fold, respectively). In contrast, we were unable to find any significant effect of H pylori on progastrin levels.

Table 5 Effect of H pylori status on amidated gastrin levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Amidated gastrin (pmol/l)</th>
<th>Number</th>
<th>Amidated gastrin (pmol/l)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>H pylori −ve</td>
<td>6.5</td>
<td>24</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>Cancer</td>
<td>8</td>
<td>23</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>Polyp</td>
<td>9</td>
<td>11</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>Overall</td>
<td>8*</td>
<td>58</td>
<td>19*</td>
<td>80</td>
</tr>
<tr>
<td>Overall % (≤4)</td>
<td>17</td>
<td></td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

*Median amidated gastrin levels were significantly higher in H pylori positive than in H pylori negative patients, p=0.0002 (Kruskal-Wallis test).

Table 6 Effect of Dukes’ stage on progastrin and amidated gastrin levels

<table>
<thead>
<tr>
<th>Dukes’ stage</th>
<th>Number</th>
<th>Median progastrin* (pmol/l)</th>
<th>Number (%) progastrin ≥15 pmol/l</th>
<th>Median amidated gastrin* (pmol/l)</th>
<th>Number (%) amidated gastrin ≥4 pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>22</td>
<td>3 (42.9)</td>
<td>20</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>B</td>
<td>21</td>
<td>24 (n=20)</td>
<td>5 (21.5)</td>
<td>11</td>
<td>4 (19.1)</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>42</td>
<td>6 (22.2)</td>
<td>12</td>
<td>3 (11.1)</td>
</tr>
</tbody>
</table>

*There was no significant effect of Dukes’ stage on progastrin levels (Kruskal-Wallis, p=0.2). †There was no significant effect of Dukes’ stage on amidated gastrin levels (Kruskal-Wallis, p=1).

Table 7 Effect of grade of carcinoma on progastrin and amidated gastrin levels

<table>
<thead>
<tr>
<th>Grade</th>
<th>Number</th>
<th>Median progastrin* (pmol/l)</th>
<th>Median amidated gastrin* (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>6</td>
<td>33</td>
<td>49.5</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>42</td>
<td>30 (n=41)</td>
<td>11</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>6</td>
<td>17.5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

*There was no significant effect of grade of the carcinoma on progastrin levels (Kruskal-Wallis, p=0.4). †There was no significant effect of grade of the carcinoma on amidated gastrin levels (Kruskal-Wallis, p=0.2).
would have required upper gastrointestinal endoscopy with biopsies. The cancer group (mean age 70 years) was older than the polyp group (mean age 65 years) or the control group (mean age 56 years). There were more men in the cancer group (32 men and 25 women) and polyp group (20 men and 10 women) and more women in the control group (26 men and 40 women). In this study, we found no association between age or sex and progastrin levels. A number of previous studies have shown elevated levels of amidated gastrin in patients with colorectal neoplasia but none of these studies controlled for the presence of *H. pylori*. In addition, these studies only measured levels of circulating amidated gastrin and not progastrin or glycine extended gastrin. Apart from this study, there has only been one other study which controlled for the presence of *H. pylori* and which measured unprocessed and partially processed gastrins in patients with colorectal cancer. We found that amidated gastrin levels were significantly higher in *H. pylori* positive patients compared with *H. pylori* negative patients. These findings are similar to Penman and colleagues but at odds with Ciccolosto and colleagues. Ciccolosto and colleagues have also shown that fasting amidated gastrin levels for the *H. pylori* positive group with colorectal cancer were significantly higher than those for the *H. pylori* positive control group. By contrast, we found no significant difference between these two groups (table 5).

Smith and colleagues and Seitz and colleagues found significantly elevated gastrin levels in patients with colorectal polyps and colorectal cancer compared with controls. Gastrin levels were modestly elevated in patients with polyps but patients with colorectal cancer had markedly elevated gastrin levels. All but two controls in our study had levels of glycine extended gastrin which were below 20 pmol/l. This was the "limit of detection" of our assay which was higher than for the other forms of gastrin and higher than the assay used by Ciccolosto and colleagues. Ciccolosto and colleagues found that glycine extended gastrin was detected in 44% of tumours. They found that fasting levels of glycine extended gastrin in *H. pylori* positive (16±4 pmol/l) and *H. pylori* negative (12±2 pmol/l) patients with colorectal cancer were significantly lower than in *H. pylori* positive (22±6 pmol/l) and *H. pylori* negative (21±1 pmol/l) controls, respectively.

There were a large number of patients with advanced carcinomas in our series. Our study contained seven Dukes' A patients (12.7%), 21 Dukes' B patients (38.2%), and 27 Dukes' C patients (49.1%). This compares with 31% with advanced disease (Dukes' B, C, or D) reported by Kikendall and colleagues, 55% (Dukes' B or C) reported by Smith and colleagues, and 61% (Dukes' B, C, or D) reported by Seitz and colleagues. We found no relationship between progastrin levels and the malignant potential of cancers, as measured by the Dukes' stage or grade of the tumour (tables 6, 7). However, in the analysis of tumour grade (table 7) the numbers are skewed between the groups such that the results are not likely to be meaningful (that is, 42 moderately differentiated carcinomas compared with six in each of the well and poorly differentiated groups). When analysing the degree of dysplasia of the polyps, median progastrin level was significantly higher for moderately dysplastic polyps (p=0.05). However, the numbers are again skewed between the groups such that the results are not likely to be meaningful (that is, 18 moderately dysplastic polyps, eight mildly dysplastic polyps, and three severely dysplastic polyps).

Differences in results between studies may reflect different statistical analyses of samples lacking a normal distribution and containing outliers. Recognition of these outliers is crucial to interpretation of the results. Several studies contained outliers which occurred mainly in the cancer group and which were significantly higher than controls. In our study, there were 10 outliers in the amidated gastrin group (controls, polyps, and cancers) and only five outliers in the progastrin group (controls, polyps, and cancers). The mean level of amidated gastrin in the outliers was 437 pmol/l and the mean level of progastrin was 148 pmol/l. These outliers could not therefore have accounted for the observed differences in progastrin levels in our study.

Progastrin, the primary translation product of gastrin, requires extensive post-translational processing to amidated gastrin for full biological activity. This occurs through the so-called "regulated route". Ciccolosto and colleagues found that progastrin, amidated gastrin, total gastrin, and glycine extended gastrin were present in 100%, 69%, 56%, and 44% of colorectal cancers, respectively. They suggested that progastrins may not be stored in tumours but rather secreted from tumour cells into the extracellular milieu by an alternative secretory pathway, the so-called "constitutive route". This route takes material direct from the Golgi to the cell surface. They found that plasma levels of total but not processed gastrins were increased in patients with colorectal carcinoma. Nemeth and colleagues found that most colorectal cancers contain peptides derived from the gastrin precursor, progastrin, but these tumours do not usually convert progastrin to amidated gastrin. Kochman and colleagues have shown that progastrin is 700-fold more abundant in colorectal cancer than the adjacent normal mucosa and that the ratio of gastrin precursors to gastrin is significantly increased in neoplastic colonic mucosa compared with normal colonic tissue. Progastrin or progastrin derived peptides have been detected in 80–100% of colorectal carcinomas. Van Solinge and colleagues found that human colon carcinomas and normal colonic tissues express the gastrin gene at both mRNA and peptide levels but post-translational maturation is incomplete. Progastrins were detected in all tumours, cell lines, and normal colonic tissue but amidated gastrin occurred rarely. They found that the concentration of progastrin was 10-fold higher in colon carcinomas than in normal colonic tissue.
The results of this study are clearly important and need to be investigated further. We are currently measuring plasma progastrin levels in patients before and after resection of colorectal cancer to see if progastrin levels fall following curative resection of the tumour. We are also measuring progastrin levels in patients with recurrent disease to determine whether plasma progastrin levels may act as a marker for tumour recurrence. This may have important implications for screening strategies for colorectal cancer. If circulating progastrin is an important growth factor for colorectal cancer, then substances which decrease the levels of circulating progastrin or block its effect, for example, gastrin receptor antagonists and anti-gastrin antibodies, may be effective in regulating tumour growth and may have important implications for therapy.

We are grateful for the financial support received from Northumbria Health Care NHS Trust and the Astrea Foundation. We would also like to acknowledge the help of Andrea Varro, Department of Physiology, University of Liverpool, England, for performing the gastrin assays.
