This study represents an attempt of showing own author's example of using basic research data as an inspiration for the clinical studies. The project evaluates the role of gastrin in colorectal carcinogenesis as well as the differences of its action in proximal and distal colon. Colonocytes were isolated from Fischer-344 rats and incubated for 2 minutes with gastrin (10^{-8} M). This treatment resulted with 60-70% rise in tyrosine kinase (Tyr-k) and 150-200% in phospholipase C activity as regards to basal levels. In vivo infusion of gastrin for 5 days to Fischer-344 rats resulted with 90-150% increase in distal but not proximal colonic mucosal proliferative activity as well as tyrosine phosphorylation of several colonic mucosal proteins. In clinical study, the mean fasting gastrin level in the control group was significantly lower (p<0.01) than in patients with colorectal cancer before surgery. Mean plasma gastrin level in patients with distal tumor yielded 105.31 ± 12.5 µU/l and was significantly higher than in patients with the proximal tumor site (42.2 ± 3.1 µU/l) (p<0.001). We conclude, that Tyr-k is involved in the mechanism of the trophic action of gastrin, particularly in distal colon. The differences in gastrin concentration in patients with distal and proximal tumors may probably contribute to the distinct pathogenesis and biological properties of those cancers.

Key words: gastrin; experimental study, human study, colorectal cancer

INTRODUCTION

To an outward seeming, the clinical and basic research is very distant from each other and involves different reasoning process. In human studies it takes a serious reflection on how to fit the new hypothesis verification into the narrow space limited by their obvious restrictions. Getting the approval of local ethics
committee is necessary for both human and animal studies, being even sometimes more difficult in the latter. However, the intellectual part of human projects must consider the highest need of safety and compliance in all interventions planned. In addition, the significant problem is related to the increasingly strict law regulations in this area. On the other hand, when you started your research with clinical studies you may be so used to those restrictions, that you do not even realize to what extent they prevent you from developing your ideas.

The clinician introduced into animal or in vitro studies is usually stunned, but also fascinated with the newly discovered boundless possibilities. The areas he can explore extend to the indescribable dimension, which requires broader and changed reasoning but allows going far with more courageous ideas. This is extremely attractive, being free to plan and carry out the experiment exactly verifying all the necessary aspects of our hypotheses.

Basic research is very rewarding, giving a sense of being a part of the world creating the real progress in our understanding of mechanisms regulating all the biological processes. On the other hand, everybody knows that those mechanisms are not identical in all species. In addition, in vitro studies are obviously far from the real living organisms conditions. Therefore, after testing some ideas in experimental or in vitro studies, it is tempting to extend their exploration in humans. This need is due to the fact that - above all - we want to make our data useful for human population: the disease prevention, diagnosis and management.

This study represents an attempt of showing own authors examples of using basic research data as an encouragement for the clinical studies.

The possibility that gastrin may play a significant role in the development of colorectal cancer (CRC) has gained considerable interest over the past decade. Many studies have demonstrated that gastrin stimulates mucosal growth of much of the gastrointestinal tract, including colon (1-4). Gastrin mRNA has been detected by both polymerase chain reaction and Northern blot hybridization in colorectal carcinoma cell lines, normal human colonic mucosa and colorectal cancer (5-7).

The structural and functional properties of the colon are not the same throughout its entire length. Distal segment up to the proximal two thirds of the transverse colon derives from the embryonic midgut, while the distal third of the transverse colon to the upper anal canal origins from the hindgut (8). Furthermore, the midgut segments are supplied with superior mesenteric artery, and hindgut segments - with inferior mesenteric artery (8). Whereas in the proximal colon proliferating cells are located in the midcrypt from where they migrate in both directions, in the distal colon they are found in the crypt base and migrate up toward to the luminal surface (9).

Furthermore, cancer of the left half of the colon is different from cancer of the right one in many clinicopathologic and biologic aspects. Tumor location within the colon may identify distinct genetic categories of the disease. Numerous
genetic abnormalities concerning protooncogenes like K-ras, and tumor suppressor genes: APC, p53 and DCC are observed mostly in distally localized tumors (10,11). A, B, H and Lewis b antigens are expressed in more than 50% of distal colon tumors, and deleted in proximal tumors (12).

Epidemiological studies in the cohort population of more than 120,000 adults showed, that the association of increased gastrin levels and increased risk for colorectal cancer was more pronounced for rectal rather than proximal tumors (13).

The aim of the in vitro study was to determine, if tyrosine kinases (Tyr-k) play a role in mediating the growth promoting action of gastrin signal transduction pathway in distal versus proximal colonic mucosa. The aim of the human study was to estimate fasting serum gastrin levels before and after surgery for colorectal cancer (CRC) and dependently on the clinical stage of the disease as well as to evaluate the possible differences in plasma gastrin, CEA and CA 19-9 in patients with proximal and distal colorectal cancer.

MATERIAL AND METHODS

In vitro study

Colonocytes were isolated from Fischer 344 rats according to Roediger and incubated for 2 minutes with gastrin (10^{-8} M) (14). Tyrosine kinase activity according to Dangott (15) and phospholipase C (PLC) activity according to Gupta (16) were determined in cell membranes. The changes in total Tyr-kinase activity were estimated separately in the colonocytes from proximal and distal colon. To further examine the mechanism of gastrin action, the next set of experiments was performed. Tyrphostin, a specific, irreversible inhibitor of Tyr-k and staurosporine (protein kinase C inhibitor) were used in absence and presence of gastrin at 10^{-8} M concentration (17). The results of tyrosine kinase activity were expressed as pmol {^32} P-incorporated per miligram protein. The activity of PLC was estimated on the ground of the radioactivity released in the aqueous layer and expressed as pmole of radioactive phosphatidylinositol hydrolyzed / mg protein.

In vivo study

Fischer-344 rats were implanted with osmotic minipumps that delivered either gastrin G-17 -I (250 ng/kg/h) or equivalent volume of 0,9% NaCl (controls) for 5 days. After 5 days animals were sacrificed, the entire colon was removed, divided into proximal and distal segment and processed separately. Colonic mucosal proliferative activity was determined with 5-bromo-2-deoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) immunoreactivity (Fig. 1). Tyrosine specific phosphorylation of proteins was evaluated in the membrane fraction of colonic mucosa by means of immunoprecipitation with antiphosphotyrosine antibody and sulfate - polyacrylamide gel electrophoresis (Western blot).

Clinical study

The study population comprised 50 patients (22 men, 28 women aged 46-85, mean 64 ± 8,7) with newly diagnosed sporadic colorectal cancer (CRC). In all cases colorectal cancer was diagnosed at colonoscopy and confirmed with pathologic examination of the tumor biopsy specimen. Cancer staging was performed according to the Dukes classification. Patients with tumor
in Dukes’ stage D had metastatic changes located in one or two hepatic segments without lymphatic or peritoneal involvement.

None of the subjects studied received any drugs affecting plasma gastrin level, like gastric antisecretory drugs (H₂ blockers or proton pump inhibitors), non-steroidal antiinflammatory drugs and prostaglandin analogs 28 days before and during the study period (18-20). Patients with other diseases causing endogenous hypergastrinemia, as atrophic gastritis, Zollinger-Ellison syndrome and peptic ulcer surgery were not included into the study (4,21,22). Hazardous drinkers were excluded from the study, since this kind of behavior may also affect gastrin level (23).

In all subjects fasting RIA gastrin levels were measured by radioimmunoassay with GASK-PR Cis Bio International, France with 125I gastrin. In addition, in all subjects studied CA19-9 and CEA with microparticle enzyme immunoassay (MEIA; Abbott Laboratories, Tokyo, Japan) were evaluated. In CRC group gastrin, CEA and CA19-9 have been assessed one day before surgery. In no case was blood sample drawn after bowel lavage since it may result with falsely elevated gastrin level. In all examined patients Helicobacter pylori infection was also assessed with urease test and pathologic examination of biopsies sampled from gastric mucosa at upper gastrointestinal endoscopy.

Informed consent was obtained from all patients, and the study protocol was approved by local Ethical Committee at Medical University, Lodz, Poland.

Statistical analysis

The results were statistically evaluated with Student’s t-test for unpaired values, taking p<0,05 as the level of confidence.

RESULTS

In vitro study

Incubations with gastrin resulted with 60-70% rise in Tyr-k and 150-200% rise in PLC activities as regards to the corresponding basal levels. When processed separately, in proximal colon Tyr-k activation with gastrin was 15-20%, while in distal 70-80% as compared to the buffer control (Fig. 2).

In absence of tyrphostin gastrin caused 61,8% and 150% stimulation of Tyr-k and PLC activities respectively, compared with corresponding controls.
Nevertheless, preincubation of colonocytes with tyrphostin (3.2 µM) completely abolished the gastrin-induced stimulation of Tyr-k activity, whereas staurosporine (20 nM) and tyrphostin themselves did not show any effect on this activity. Similarly, phospholipase C stimulation with gastrin could be totally inhibited with tyrphostin, but not with staurosporine or tyrphostin (Fig. 3).

Fig 2. Total Tyr-kinase activation in colonocytes from proximal and distal colon.

Fig 3. Phospholipase C (pmol $^{32}$P $^3$H hydrolized/mg protein) activation in colonocytes. (G - gastrin, TP- tyrphostin, ST - staurosporine)
In vivo study

Infusion (osmotic minipump) of gastrin G-17-I for 5 days to Fischer-344 rats resulted in a significant (90-150%) increase in proliferative activity in the distal colonic mucosa (BrdU-positive cells/crypt from 5,1 ± 1,7 in saline-infused controls to 12,9 ± 2 in the hormone-infused rats, PCNA: 4,6 ± 0,8 to 8,8 ± 0,8) (p<0,001). In contrast gastrin caused no apparent change in BrdU or PCNA immunoreactivity in proximal colon (PCNA/BrdU-positive cells/crypt: 8,2 ± 1,5 in controls versus 7,2 ± 1,4 in gastrin treated rats). Most of the BrdU-positive cells in both control and gastrin-treated rats were found to be located at or near the base of the crypt. As has been observed with BrdU, most of the PCNA immunoreactive cells in the distal colon were located at the bottom of the crypt, whereas in the proximal colon they were found in the middle of the crypt.

Gastrin-induced stimulation of colonic mucosal proliferative activity was associated with a marked increase in phosphorylation of proteins with molecular weight of 55, 60, 70 and 170 kDa, which was demonstrated by densitometric analysis of the autoradiograph.

Clinical study

Mean fasting plasma gastrin in CRC patients before surgery was 83, 75 ± 15,8 µU/ml which was significantly higher (p<0,01) than in controls, in whom the respective value was: 40,3 ± 3,9 µU/ml. However, 59 days after surgery mean gastrin levels yielded 37,8 ± 4,8 µU/ml and was no more significantly different from this of the control group. Similarly, mean CEA and CA 19-9 levels were significantly higher (p<0,01) in patients with CRC before surgery (15,59 ± 1,0 ng/ml and 15,8 ± 0,6 U/ml respectively) than after tumor resection (3,2 ± 0,6 ng/ml and 2,9 ± 0,2 U/ml, respectively) (Table 1).

Statistical analysis revealed the significant positive correlation between the plasma gastrin concentration and CRC stage according to Dukes’ classification: r= 0.63 (p<0.01). In addition, the significant positive correlation between gastrin and both, CEA and CA 19-9 levels in the group of the patients has been noted: r = 0,63 and r = 0,51, respectively (p<0,05).

Mean plasma gastrin level in patients with distal tumor yielded 105,31 ± 12,5µ U/l and was significantly higher than in patients with the proximal tumor site (42, 2 ± 3,1 µU/l) as well as in controls (p<0,001). No significant difference

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>After surgery</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin (mU/ml)</td>
<td>83,75 ± 15,8</td>
<td>37,8 ± 4,8</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>CEA (ng/ml)</td>
<td>15,59 ± 1,0</td>
<td>3,2 ± 0,6</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>CA 19-9 (U/ml)</td>
<td>15,8 ± 0,6</td>
<td>2,9 ± 0,2</td>
<td>&lt;0,01</td>
</tr>
</tbody>
</table>
between mean plasma gastrin in patients with proximal tumors and the control group was seen. Mean CEA and CA 19-9 plasma level in patients with distal tumors was respectively 9,1 ± 1,1ng/ml and 19,9 ± 2,1 U/ml, while in proximal tumors - 1,48 ± 0,1 U/ml and 1,8 ± 0,2 U/ml (Table 2; p<0,01).

**Table 2.** Mean CEA and CA 19-9 concentration in proximal and distal colorectal cancer

<table>
<thead>
<tr>
<th>Tumor marker</th>
<th>Proximal colorectal cancer</th>
<th>Distal colorectal cancer</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA (ng/mL)</td>
<td>1,48 ± 0,1</td>
<td>9,1 ± 1,1</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>CA 19-9 (U/mL)</td>
<td>1,8 ± 0,2</td>
<td>19,9 ± 2,1</td>
<td>&lt;0,01</td>
</tr>
</tbody>
</table>

The prevalence of Helicobacter pylori infection in patients with CRC was 40%, which was no significantly different from the value obtained in the control group (50%).

**DISCUSSION**

The possibility that gastrin may play a significant, but not fully elucidated, role in colon carcinogenesis has been confirmed in our studies: in vitro, in animal model and in clinical study. In presented experiments we have observed in our in vitro preparations an increase in both Tyr-kinase and phospholipase C activities in response to gastrin. It also appeared that gastrin significantly stimulated tyrosine phosphorylation of a number of proteins. The molecular mass of some of them corresponds to the molecular mass of PCL. The significance of increased Tyr-kinase activity with a resultant rise in tyrosine phosphorylation of some membrane proteins is not fully understood. However, recent evidence suggests that tyrosine phosphorylation, catalyzed by Tyr-kinase, plays an important regulatory role in cell proliferation, differentiation and transformation (24-27). The observation that several oncogene products (28,29), as well as receptors of many growth factors (30,31) possess Tyr-k activity suggests that induction of cellular growth and transformation are also associated with alteration in phosphotyrosine content.

A number of investigators have also demonstrated that gastrin stimulates proliferative properties of the colon in rats as well as in humans (32,33). Our results with experimental models have shown differences in distribution of proliferating stem cells between the proximal and distal colonic mucosa. We have also observed that infusion of gastrin for 5 days stimulates distal colonic mucosal proliferative activity with no change in proximal colon, accompanied by parallel alterations in Tyr-k colon activity.

The mechanisms underlying the possible role of gastrin in colorectal carcinogenesis are currently a matter of intensive studies (27,34). Sereti et al.
found gastrin immunoreactivity in cell membranes of poorly differentiated primary CRC tissues and in liver metastases. The presence of gastrin was shown in tumors of poor grading and therefore poor prognosis (35).

In our clinical study we have found that fasting serum gastrin levels in patients with colorectal cancer were nearly twofold higher than in the control group. Ciccosto et al., Smith et al., Wong et al. and others have also observed elevated fasting serum gastrin concentrations in patients with adenomatous polyps and colon cancer (36-38). In the prospective study including more than 120,000 healthy subjects it was demonstrated that elevated plasma gastrin level positively correlated with the increased risk of colorectal cancer occurrence (13). Thornburn et al. suggested that increased plasma gastrin concentration may reflect the autocrine production of this hormone within adenoma and cancer cells (13). Conversely, a number of other clinical studies in patients with CRC have failed to reveal any elevation of plasma gastrin (39-42).

In our study 2 months after curative resection for CRC, plasma gastrin levels returned to the values not different from the values obtained in the control group. Similar results have been reported by Wong et al. and Charnley et al. (38,43). Lamberts et al. have shown, that in 3 out of 12 patients with CRC and hypergastrinemia, gastrin level returned to normal values after tumor resection (44). Still, other authors demonstrated that gastrin levels in patients with CRC were normal and unchanged after surgery (43,45). We have also found the positive correlation between plasma gastrin levels and the tumor stage according to Dukes’ classification. Similar results have been also reported by Smith et al., Upp et al. and others (37,46). Positive correlation of postprandial plasma gastrin level with the tumor stage has been has also been reported by Wong et al (38).

Kameyama et al. suggested that serum gastrin level may serve as a predictor of liver metastasis from CRC (47). Nevertheless Fontanesi et al. and Vanderstraeten et al. did not find any differences between gastrin level in patients with CRC of different Dukes’ stage (40,42). There might be various reasons for these conflicting results. One of the possible explanations is unidentified Helicobacter pylori infection, widespread in human population, which leads to chronic gastric mucosal inflammation with significant fasting and postprandial hypergastrinemia (48). In addition antibiotic use needs to be considered since the decrease of serum gastrin level in the course of CRC may be due to the their frequent use in those patients, resulting with effective H. pylori eradication (7).

The second reason may be the failure to control for factors affecting plasma gastrin level, as: hazardous drinking, performed bowel lavage, taking gastric antisecretory drugs, prostaglandin analogs, non-steroidal antiinflammatory drugs and coexisting diseases, like pernicious anemia, Zollinger Ellison syndrome (8, 48-50). All those factors have been considered and excluded in the present study.

Furthermore, we have compared the changes of plasma gastrin concentration in CRC patients with classical tumor markers levels. CEA and CA 19-9 as well as gastrin were increased in examined group and decreased after tumor resection.
Moreover, the significant correlation between gastrin and both, CEA and CA 19-9 levels in CRC group has been noted.

Elevation of CEA level in patients with CRC and its decrease after curative tumor resection has been frequently reported (51-53). Therefore CEA is commonly used to follow up patients for relapse after CRC surgery (54,55). Nevertheless, CEA and CA 19-9 monitoring of this disease is limited by relatively poor sensitivity and specificity. The need of simultaneous measurement of several tumor markers in order to improve the their accuracy in the tumor aggressiveness prediction has been emphasized (52,53). Corresponding changes of gastrin and CEA as well as CA 19-9 noted for the first time in our study may indicate their parallel production within the tumor tissue.

Concluding, we have shown that gastrin might play an important role in colon carcinogenesis, particularly in distal colorectal cancer. The responsiveness of the colonic mucosa to the growth promoting action of gastrin varies considerably between different regions of the colon. Gastrin stimulates Tyr-kinase and phospholipase C activity in colonocytes from the distal but not from the proximal colon. Our results lend further support to the hypothesis that the colorectal cancers produce gastrin and thus give an increase in systemic gastrin levels (1,2,6). The significance of gastrin as a marker for diagnosis or prognostic purposes in colorectal cancer needs to be further examined.

REFERENCES:


Received: 27 March 2004
Accepted: 28 May 2004

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