Drugs of the Future 2001, 26(9): 865-872

CCC: 0377-8282/2001

Review Article

Antigastrin hormone therapy in the treatment of colorectal cancer

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Overview

During the 1980s, it became apparent that cancer of the gastrointestinal (GI) tract may be under hormonal control, as shown for breast, prostate and ovarian malignancies. A number of candidate hormones were tested, including bombesin and cholecystokinin (CCK), but only gastrin has stayed the course. This has led to the development of antigastrin agents, which have entered phase III clinical trials in patients with GI malignancies.

Initially, gastrin was evaluated as an endocrine mediator of tumor growth. The proliferative effects of exogenously administered gastrin peptides were found to be exerted on established cell lines and human tumor specimens both *in vitr o*and *in viv o*(1). This was consolidated by a number of studies showing CCK_2/gastrin receptors on GI tumor cells (1, 2).

In the early 1990s, researchers began to show activation of the gastrin gene within epithelial cells of colorectal adenocarcinomas, resulting in the production of gastrin peptides with the potential to mediate proliferation in an autocrine/paracrine manner. As the species produced were largely composed of immature gastrin peptides, their biological significance was unknown until it was confirmed that they were proliferative in their own right (1-3). In the last five years, research efforts have been focused on the receptor(s) interacting with precursor gastrin forms. A number of splice variants have been shown and the potential for both identifying tumor-specific CCK_2 receptor expression and delineating their role in promoting potential autocrine/paracrine pathways has attracted much attention.

This review will focus on the findings which have confirmed the role of gastrin as a central oncogene in GI adenocarcinoma progression and the therapeutic rationales for blocking its action.

Gastrin as a central growth factor

The gastrin gene – a target of β-catenin-mediated transcription in the colonic adenoma-carcinoma sequence

The importance of gastrin gene expression in GI tumorigenesis has recently been consolidated by confirming the gene as a downstream target of β-catenin-mediated transcription (4). The gastrin gene has a β-catenin/Tcf4 response element within its promoter, highlighting it as a transcriptional target (4). Thus, the gastrin autocrine pathway may be operational early in the adenoma-carcinoma sequence within the colon and may play a role in CCK_2 receptor upregulation and tumor progression due to deregulated proliferation.

Gastrin – an antiapoptotic factor

In line with its early expression, evidence supports the role of gastrin as a survival factor, as it has been shown to circumvent apoptosis in a number of experimental systems. Todisco *et al*. showed that gastrin stimulation of the CCK_2 receptor-expressing rat pancreatic cell line AR42J reduced apoptosis following serum withdrawal (5). Gastrin stimulated phosphorylation and subsequent activation of the survival-inducing protein kinase B (PKB)/Akt. The PKB/Akt gene includes a multifunctional protein kinase that has recently been discovered to be activated in many cancers, promoting survival and resistance to chemotherapy and radiation (6), and is at the crossroads of multiple intracellular pathways, stimulating antiapoptotic, proproliferative/cell cycle mitogenic and increased metabolic signals (7).

Furthermore, autocrine gastrin produced by the colorectal cancer cell line HCT 116 reduced activation of caspase 3 and upregulated cytochrome c Vb. This

resulted in decreased sensitivity of the cells to proapoptotic stimuli by retaining cytochrome c within the mitochondria (8). Camptothecin-induced activation of caspases 3 and 9 was also reduced by progastrin in the intestinal cell lines IEC18 and IEC6 (9).

Gastrin – a transcriptional activator

Gastrin has been shown to activate the transcription of a number of genes via interaction with the CCK_2 receptor. When a rat gastric epithelial cell line was transfected with the CCK_2 receptor, gastrin stimulation resulted in an upregulation of heparin-binding epidermal growth factor (HB-EGF) and amphiregulin gene expression (10). Furthermore, processing and release of HB-EGF and tyrosine phosphorylation of the EGF receptor were increased (10). The relevance of the relationship between gastrin and EGF receptor ligands in the malignant scenario has been strengthened by the finding that gastrin and HB-EGF are coexpressed at the gene and protein level in gastric cancer and they are significantly correlated (11). There is also concomitant activation of the gastrin and c-erbB2 genes on the same amplicon in intestinal gastric cancer, thus further strengthening the relationship (12). A correlation between HB-EGF and gastrin has recently been shown for colorectal tumors (13). This relationship is significant in that HB-EGF has been shown to be a potent mitogen and migration factor for vascular and endothelial cells, acting in concert with vascular endothelial growth factor (VEGF) and other angiogenic factors (14, 15).

The *Reg* family of genes is believed to be activated during regeneration of intestinal mucosa (16). Gastrin has been shown to stimulate the expression of the *Reg* gene and protein in cultured enterochromaffin-like (ECL) cells (17). Long-term administration of the proton pump inhibitor (PPI) lansoprazole strongly increased *Reg* gene expression and moderately elevated HB-EGF gene expression, which was reversed by inclusion of a CCK_2 receptor antagonist (17). The relevance to colon cancer was highlighted by a recent study showing that regenerative processes involving *Reg* may assist progression in colorectal malignancy, *Reg* gene expression positively correlating with recurrence of colorectal cancer (18). Its correlation to gastrin gene expression remains to be elucidated.

Gastrin – role in growth factor shedding and angiogenesis

The role of gastrin in expression and shedding of HB-EGF, as initially shown by Miyazaki et al. (10), is gaining much interest in the malignant setting. It was recently shown that gastrin peptides increase activated MMP-2 secretion in the human colon cancer LoVo cell line, resulting in enhanced invasion (19). As MMPs are implicated in HB-EGF shedding (20), gastrin may play a central/complementary role, culminating in increased invasion and enhanced endothelial interactions in the malignant scenario.

Gastrin and p53

Hypergastrinemia in the *Mastomys* carcinoid model revealed that the incidence of mutated *p53* in mice with serum hypergastrinemia was elevated (21). *In vitro* gastrin stimulation of the AGS gastric cancer cell line increased expression of *p53,* reinforcing the findings that gastrin may interact with *p53,* influencing derivation of the malignant phenotype (22).

Gastrin and COX-2

Recent evidence suggests that both autocrine- and endocrine-acting gastrin may impact upon the expression of cyclooxygenase type 2 (COX-2) expression. Our research group has shown, by transfecting a gastrin sense and antisense plasmid into pancreatic tumor cells, that COX-2 is positively modulated by endogenous gastrin expression (23). Furthermore, COX-2 and gastrin gene expression were significantly correlated in a series of human pancreatic tumor specimens (23). In the colorectal setting, exogenously administered gastrin increases COX-2 gene expression in the human colon cancer cell line HT-29 (24) and the mouse colon cell line C26 (25).

Overview

Gastrin is now gaining recognition as an oncogene, with the potential to induce amplification of multiple pathways, all aimed at maximizing the malignant potential of GI mucosa, including the colon, as summarized in Figure 1.

Role of gastrin in the development of colon cancer

Elevation of serum gastrin levels is a common occurrence through a number of pathological and physiological mechanisms (2). Firstly, it occurs as a result of pernicious anemia, the pathology of which involves the production of autoimmune antibodies directed against parietal cells, resulting in reduced acid secretion and increased gastrin output (2). Secondly, it can occur in patients with Zollinger-Ellison syndrome due to the hypersecretion of the peptide by the malignant tissue (2). Finally, it is a documented side effect of *Helicobacter pylori* (HP) infection and administration of proton pump inhibitors (2). This leads to an increase in mainly amidated, mature forms of gastrin which have the potential to interact with CCK_2 receptors and mediate proliferation (2). Colonic proliferation has been shown to be altered in conditions of

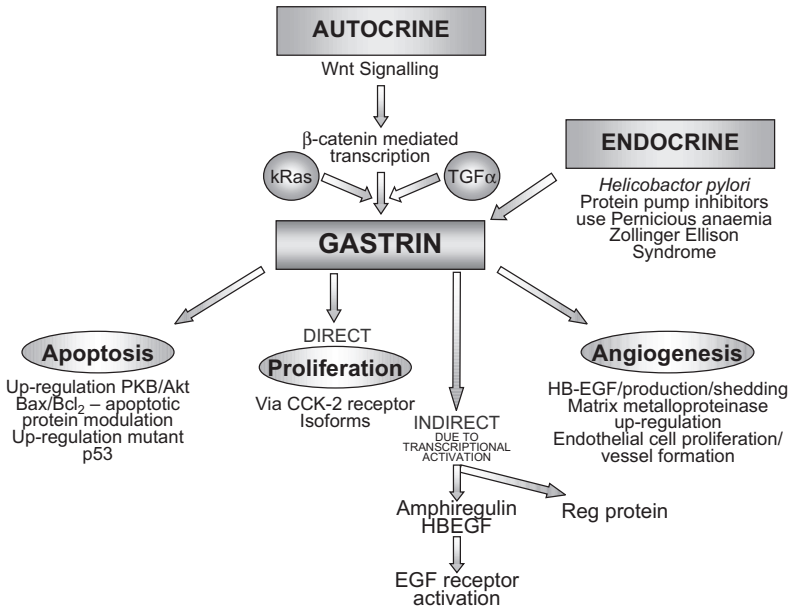

Fig. 1. Central oncogenic role of gastrin peptides.

hypergastrinemia attributable to anemia and the Zollinger-Ellison syndrome, with normal crypt cell proliferation increased, as determined using the DNA analog bromodeoxyuridine (26, 27), with movement of the proliferative compartment towards the top of the crypt, as seen in patients with an increased risk of developing colon cancer (26, 28, 29).

The potential effect of gastrin on progression through the adenoma-carcinoma sequence was recently reinforced when it was shown that CCK_2 receptors are expressed on adenomas of all grades and stages, thus allowing such premalignant lesions to be acted upon by gastrin, the extent determined by the level of gastrin elevation and the density of receptor expression (30). Limited studies relevant to the human situation have been performed in this field, although the incidence of colonic polyps has been shown to be elevated in patients with HP (31). In preclinical models, this area has generally been clouded with controversy, with the majority of studies evaluating the effect of hypergastrinemia induced by proton pump inhibitors on the development of carcinogen-induced colon cancer showing no deleterious effect (32-34). However, in a more physiologically relevant model involving the APC^{Min} mouse model of familial adenomatous polyposis (FAP) and in a human colonic adenoma xenograft grown in nude mice, PPI-induced hypergastrinemia resulted in increased proliferation and reduced survival (35, 36). Finally, a carefully controlled clinical study has consolidated the preclinical findings by showing that elevated gastrin levels (> 90 pg/ml) were associated with a 3-4-fold increased risk of colorectal cancer development (37).

CCK_2/gastrin receptor isoforms

Gastrin mediates its physiological effects through a G-protein-coupled seven transmembrane domain receptor known as the CCK_B, CCK_2 or gastrin receptor. A number of splice variants of the receptor have now been identified. These include a long form, which, due to differential splicing in the fourth exon, results in formation of a receptor with an additional five amino acids in the third intracellular loop, a domain associated with signal transduction (38, 39). No significant differences in ligand affinity have been shown, although ligand binding to the long isoform results in sustained intracellular Ca^{2+} release, which may have implications with regard to functional effects mediated by the receptor (38).

The use of an alternative exon 1, named exon 1b, leads to an amino terminal-truncated receptor (40). The resultant receptor lacks the first extracellular domain of the receptor, which contributes towards the ligand binding pocket. The truncated receptor has been shown to be coexpressed with the gastrin gene in a series of GI cell lines, and may therefore contribute towards mediating the autocrine pathway (41). Finally, there are CCK_2 receptors with retained introns, such as intron 2 and 4 (42, 43). Retention of intron 4 leads to a 69-amino-acid insert into the third intracellular G-protein-coupled loop of the CCK_2 receptor (43). Expression of the receptor in 3T3 fibroblasts led to sustained ligand-independent Ca^{2+} mobilization and a 2.5-fold increase in growth rate (43). The receptor variant was selectively expressed on colon adenocarcinoma when compared to normal colonic mucosa (43).

There are many divergent reports of CCK_2 receptor gene expression in GI adenocarcinomas, as shown in

Table I: Gene expression of CCK_2 isoforms in colon tumors: Summary of literature.

CCK_2 isoform Authors	% CCK_2 receptor isoform gene expression of colon tumors				
	Short	Long	Δ	Intron 4	CCKC
Biagini (44)	76.5	23	NE	NE	100
Clerc (45)	16.6	NE	NE	NE	NE
Matsushima (46)	20	NE	NE	NE	NE
Hellmich (43)	NE	NE	NE	100	NE
Hoon Kang (47)	68	0	NE	NE	EN

NE: not evaluated

Table I. Using a CCK_2-specific antiserum, the 74-kDa classical CCK_2 receptor has been shown on a panel of colorectal cell lines (48) and a series of human colorectal tumors (49). However, radioligand binding and *in vitro* receptor autoradiography on human colorectal tumor specimens (50) have not demonstrated receptor expression.

Such studies highlight the complexity surrounding the accurate measurement of CCK_2 receptor expression of human colon cancers, which is further compounded by the diversity of the ligands, both serum- and tumor-associated, which may bind to such receptors. Studies so far have provided evidence for (51, 52) and against (53, 54) precursor gastrin binding to isoforms of the CCK_2 receptor, and until the role of such receptors in the autocrine pathway has been fully delineated, functional studies with the most appropriate ligands cannot be optimally performed.

Gastrin species implicated in the autocrine pathway in colorectal tumor cells

Progastrin

Progastrin has been shown to be a secreted end product in the colorectal cancer setting by a number of groups (55-57). Such tumor secretion results in elevated levels of non-amidated gastrin levels in colorectal cancer patients, which can be attributable to the presence of tumors (58, 68). The biological effects of high levels of serum progastrin have been investigated in the hGAS transgenic mouse (59). In this model, elevated levels of serum progastrin resulted in an increase in the proliferation index of the normal colonic mucosa. The mice were also predisposed to generating aberrant crypt foci, colonic adenoma and carcinomas in response to a chemical carcinogen (60, 61). Thus, increased tumor-associated serum gastrin levels in colorectal cancer patients may exacerbate proliferation of the tumor and also increase the incidence of synchronous or metastatic tumor development.

Gly-gastrin

Colon cancer cell lines also secrete glycine-extended gastrin (Gly-gastrin) (56). Gly-gastrin promotes the growth of colonic mucosa in a transgenic mouse model (62) and in colon cancer cell lines when exogenously applied (63), and acts as an autocrine factor in the non-transformed colon cell line YAMC.

Options for blockade of gastrin-mediated growth pathways

Gastrin receptor blockade

A multitude of drugs which block the CCK_2 receptor have been described over the last decade (2). One of the first series were glutamic acid derivatives which include proglumide. This CCK_2 receptor antagonist was used in a number of preclinical assessments and, despite poor potency for the CCK_2 receptor, inhibited basal and gastrin-stimulated growth of colonic cell lines, and also of the mouse colon cancer tumor MC26 when grown in syngeneic mice (1). This led to a small clinical study in patients with advanced colon cancer. Twenty-five patients received proglumide and 16 placebo. Of the patients on proglumide, seven had adverse effects associated with the treatment and were taken off the trial. No survival benefit was shown (64).

A number of more highly potent CCK_2 receptor antagonists were then described, including the benzodiazepam derivative L-365260, which was shown to have high affinity for the CCK_2 receptor (in the nM range) and reversed gastrin-stimulated GI tumor growth in a number of preclinical studies (1).

A more recently described CCK_2 receptor antagonist, Gastrazole (JB-5008), was shown to be highly potent in preclinical studies and has been used in an open-label pilot clinical trial in patients with pancreatic cancer. When compared to historical controls, a significant survival advantage was suggested (65).

Although the evidence supporting Gly-gastrin as a ligand for the CCK_2 receptor is variable, a number of CCK_2 receptor antagonists block the effect of Gly-gastrin peptides, thus circumventing potential autocrine gastrin pathways. The potent, selective CCK_2 receptor antagonist YM-022 was shown to reverse both G17- and GlyG17-stimulated proliferation of the human colon cancer LoVo cell line, in addition to inhibiting basal growth (66). As LoVo is known to have an activated gastrin gene, it is likely that the antagonist is able to circumvent a potential

autocrine growth pathway. Additionally, the CCK_2 receptor antagonist JMV-1155 reduced the GlyG17-stimulated growth of the human colon tumor DLD1 *in vivo* (66).

Due to the number of CCK_2 receptor isoforms that have been described, the divergence in colon tumor expression reported by research groups and the uncertain role of the CCK_2 receptor in mediating the proliferative effects of GlyG17, receptor blockade may not be at present a fruitful therapeutic avenue. However, if tumor-specific receptors are identified with distinct ligand-binding properties, selective agents may be designed to block such receptors.

Antisense gastrin gene therapies

An antisense DNA construct of the gastrin gene should circumvent the production of all tumor-associated gastrin peptides. This was shown to be a highly therapeutic approach when a retroviral construct containing antisense gastrin was transfected into human colon cancer cell lines *in vitro* (67). When the transfected colon tumor cells were grown *in vivo,* significant growth suppression was observed (67). However, appropriate delivery and targeting systems for systemic administration of gene therapies remain to be discovered. Intratumoral injection of such an agent could be appropriate for certain tumors, but would not be amenable for the treatment of diffuse tumors such as colorectal micrometastases. Furthermore, if a systemic approach is developed, it may be associated with toxicity, as it would target G cells and block all physiologically relevant gastrin species.

Gastrin immunoneutralization

One main area which at present is likely to generate fruitful clinical products involves the neutralization of specific gastrin peptides maximally implicated in the promotion of colonic tumor growth. These would include amidated G17, GlyG17 and progastrin. G34 has not been shown to result in tumor proliferation to date, although glycine-extended G34 has been found to be secreted by pancreatic tumors (42).

Anti-gastrin antibodies were initially shown to be effective at reducing the basal growth of a gastrin-secreting colon cancer cell line, thereby circumventing an autocrine growth pathway (68). Infusion of anti-gastrin antibodies into mice bearing a human colon tumor xenograft also resulted in growth inhibition (69). However, continuous infusion of antigastrin antibodies as a potential therapeutic approach may raise problems due to stability, immunogenicity and achievement of excess/consistent titers to neutralize both tumor-associated and serum gastrin levels, the latter being subject to postprandial surges.

Such limitations can be overcome by the use of an active immunization approach. G17DT is a gastrin immunogen composed of a nonapeptide derived from the amino terminal of human gastrin-17 (70). The G17 fragment is linked to diphtheria toxoid, which acts as an immunogenic carrier (70). The immunogen has been shown to be successful in the generation of G17-specific neutralizing antibodies in both rodent models and cancer patients (71). The G17 epitope is monovalent and cannot complex with more than one antibody molecule, and so complement fixation does not occur. Due to the selectivity of the G17 fragment, antibodies raised do not cross-react with other gastrin hormones such as G34 and smaller *C*-terminal fragments, nor with the related hormone cholecystokinin (CCK). It does, however, react with the glycine-extended form of G17 and therefore is specific to the main forms of gastrin that have been implicated in GI tumor proliferation (72).

G17DT antibodies raised in rabbits have high affinity for G17 and GlyG17 (K_d = 0.15 and 0.47nM, respectively), as measured by a competitive radioimmunoassay (72). The functionality of the antibodies was further confirmed by assessing their ability to competitively displace gastrin ligands from the CCK_2 receptor expressed by a tumor cell line. [^{125}I]-G17 binding to the CCK_2 receptor was successfully inhibited *in vitro* even when rabbit serum was diluted out to 1:100 of the original titer (72).

Active immunization in both a pig model and a rat gastric fistula model confirmed the suppressive effect of the antibodies on G17-mediated acid secretion (71, 72). Furthermore, in the pig model, the proliferation of the normal GI mucosa was maintained through the preservation of alternative gastrin species such as G34 (73).

Preclinical efficacy of G17DT antibodies

In a colorectal cancer setting, the preclinical efficacy of G17DT antibodies has been evaluated in a series of models. A species-specific form of G17DT was used to immunize rats bearing a syngeneic colon tumor (72). Rats were immunized such that antibody titers began to rise during exponential growth of the implanted tumor. Control rats were immunized with an immunogen which lacked the G17 epitope. Active immunization with G17DT resulted in a 70% reduction in tumor area and a 57% reduction in final tumor size when compared to the control rats (72).

Histological analysis revealed that the tumors in the G17DT-treated rats had enhanced necrosis and reduced viable tumor. There was also evidence of an inflammatory infiltrate within the G17DT-immunized but not the control rat tumors (72). Thus, active immunization showed a strong therapeutic effect on established colon tumors. Furthermore, combination studies in which G17DT was administered with 5-FU/leucovorin, given at a dose reflective of that given to humans, generated an augmented therapeutic effect in the same rat colon tumor model (73).

The next step was to evaluate the effect of passive immunization with G17DT antibodies in nude mouse models of human colorectal cancer metastasis. In an initial study, the lung-metastasizing human colon tumor

AP5LV was implanted into the muscle layer of the abdominal wall (74), the primary tumor generated was well vascularized and spontaneously metastasized to the lungs. Antibodies raised by G17DT reduced growth of the primary tumor by 30% and inhibited secondary spread, with the number of lung nodules decreasing by 70%, indicating an effect on take rate. The size of established lesions was reduced by 60%, confirming an antiproliferative effect (74).

Clinical studies

G17DT has been assessed in phase I/II trials in advanced colorectal cancer patients. In a series of 70 patients recruited, it was shown that the G17DT immunogen was well tolerated, with no systemic side effects, and measurable antibody titers developed in over 95% of the individuals immunized. Tumor burden, as assessed by serial CEA chest radiographs and CT scans, did not significantly regress. However, the survival of G17DT patients was compared to a well-matched placebo group, and the median survival time increased from 184 to 338 days ($p = 0.0026$). G17DT is currently being assessed in phase III trials in colorectal cancer and a series of other GI malignancies.

Summary

The rationale for gastrin inhibition is strong. Current agents focus on amidated gastrin species, but only the G17DT immunogen is able to neutralize this and the other potential tumor-secreted forms. Future therapies should be targeted at reducing the action of tumor-specific gastrin peptides to maximize efficacy and tumor selectivity.

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