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Review paper

Gastrin antagonists in the treatment of gastric cancer

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The polypeptide hormone, gastrin, is known to promote both the *in vitro* and *in vivo* growth of human gastric cancer. This proliferative activity has been shown to be mediated by high affinity, membrane-associated receptors. This has led to the development of agents with the ability to antagonise gastrin receptor binding, which have been evaluated for their potential clinical value. Other anti-gastrin therapies have been investigated. As gastrin may act as an autocrine mediator of gastric tumor cell proliferation, anti-secretory agents have been evaluated, as have agents which induce the production of neutralising anti-gastrin antibodies *in situ*.

Key words: Gastric cancer, gastrin, receptor antagonists.

Introduction

Carcinoma of the stomach is the fourth most common cause of cancer mortality in the UK after cancer of the bronchus, breast and colon. It accounts for some 10 600 deaths per year and, overall, it is associated with a 5 year survival of around 10%. Operative removal remains the only hope of cure in this disease, but unless the tumor is diagnosed at a very early stage, the prognosis after even apparently curative surgery is very poor. Advanced disease, either in the form of inoperable local disease or distant metastases, is very resistant to any form of therapy; radiotherapy has very little to offer and chemotherapy provides only modest response rates at the expense of significant morbidity.[1]

A new approach to systemic therapy in gastric cancer is therefore urgently needed, not only for the treatment of established advanced disease, but also as an adjunct to surgery. Hormonal treatment offers an attractive approach, as experience in breast and prostate cancer with various endocrine manipulations has shown that significant prolongation of life can be achieved with minimal morbidity. One of the most exciting areas has been the development of hormone receptor antagonists and, in context with this, the estrogen receptor antagonist, tamoxifen, has been shown to induce remission in about 40% of women with advanced breast cancer[2,3] and improve survival after surgery for apparently localized disease.[4]

As yet, hormonal treatment for gastrointestinal cancer has not made any clinical impact, but there are indications that it may do so. Recent work from our group and from others has demonstrated that at least a proportion of gastric cancers are sensitive to gastrin and that treatment with gastrin receptor antagonists may inhibit growth. In this article we shall examine the evidence that gastric cancer exhibits a trophic response to gastrin, look at the available gastrin receptor antagonists, review the effect of these antagonists on gastric cancer and, finally, touch on some alternative approaches to anti-gastrin therapy.

Evidence for gastrin sensitivity in gastric cancer

Gastrin has been shown by many research groups to have a major mitogenic role in the growth of tumors arising within the gastrointestinal tract. This proliferative effect has been shown to be mediated by high affinity receptors. One of the initial studies which revealed a mitogenic effect of gastrin on stomach cancer was carried out by Kobori *et al.*,[5] who showed that the rat gastric cancer cell line BV9 was stimulated *in vitro* by the C-terminal gastrin fragment, tetragastrin. This work has been expanded to the human gastric tumor cell lines TMK1 and MKN45, both of which exhibit mitogenic responses to gastrin during *in vitro* culture.[6,7]

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In vitro technology has been developed which has allowed the gastrin sensitivity of primary human gastric tumor tissue to be evaluated in short-term culture. Using this methodology, our group in Nottingham has shown that 69% of gastric tumors tested displayed a significant trophic response to human gastrin-17.[8] Similarly, a study by Moyer *et al.*[9] revealed that gastrin enhanced the growth of both normal and malignant human gastric mucosal cells. In this same study, the malignant cells appeared to be more sensitive to the growth enhancing effects of gastrin than were their normal counterparts.

In vivo studies have confirmed the growth-promoting role of gastrin. Human gastric cancer cells implanted subcutaneously into nude mice have an elevated growth rate when exposed to human gastrin-17 administered continuously by osmotic mini-pump.[7] Likewise, Sumiyoshi *et al.*[10] showed that the final weight, size and [^{3}H]thymidine labeling index of a poorly differentiated human gastric carcinoma SC-6-JCK, but not a mucinous gastric carcinoma ST-15, was increased by pentagastrin when grown in nude mice. In this same study, an intraperitoneal injection of pentagastrin increased the levels of cyclic AMP in SC-6JCK, suggesting that receptors were involved in mediating the observed effects.

Indeed, gastrin receptors have been demonstrated on gastric tumors in a number of studies. The gastric cell line TMK-1 is gastrin sensitive and the effects of the hormone appear to be mediated via a high-affinity receptor on the cell membrane.[6] In addition, Singh *et al.*[11] have shown that the human gastric cell line AGS has specific gastrin binding sites with an affinity similar to that seen on the normal fundic cells in the rat. However, Weinstock and Baldwin[12] have performed a very detailed study on five cell lines derived from human gastric carcinomas using radioligand binding and found low affinity receptors but no proliferative response. This would indicate that low affinity receptors may not mediate the mitogenic effect of gastrin. There has been little work done on primary human gastric cancers, but Kumamoto *et al.*[13] found specific gastrin binding sites on four out of five of such tumors and that binding was particularly predominant on poorly differentiated tumors.

Thus it would appear that gastric cancer can be sensitive to the trophic effects of gastrin and that this effect is mediated by gastrin receptors. Therapy with gastrin receptor antagonists, therefore, appears to be a feasible option, and the mode of action and range of such compounds is now considered.

Gastrin receptor antagonists

A specific hormone receptor antagonist is a compound which recognizes and binds to the receptor of the hormone in question, but does not possess the stimulatory properties of the hormone itself. Thus, by occupying the receptor, the antagonist blocks the effect of naturally occurring hormone. To be effective, an antagonist must be similar enough to the hormone to allow high-affinity binding to the receptor, but also be sufficiently different so that its intracellular activating effect is negligible.

The gastrin receptor is closely related to the cholecystokinin (CCK) receptor and the development of gastrin receptor antagonists has therefore paralleled that of CCK receptor antagonists. There are various classes of gastrin/CCK receptor antagonists, including cyclic nucleotides, analogs of the antibiotic Virginiamycin, peptides related to gastrin and CCK, derivatives of amino acids and benzodiazepine derivatives. However, only the last three classes have been investigated as anti-tumor agents and, accordingly, the rest of this section will concentrate on these.

Peptides related to gastrin and CCK

In a recent study by Ishizuka *et al.*,[14] it was shown that JMV320, a cyclic CCK analog which is selective for the central CCK_B/gastrin receptor,[15] inhibited the proliferative response of the human stomach cancer line, AGS-P, to human gastrin-17 in a dose-dependant manner.

Amino acid derivatives

The first gastrin antagonist to be developed from an amino acid was proglumide, a glutaramic acid derivative.[16] This was followed by the N-acyl derivative of tryptophan, benzotript,[17] and it has been shown that these compounds can act as competitive antagonists on both the CCK_A and the gastrin/CCK_B receptors.[18,19] It is also of particular interest that proglumide inhibits the trophic effect of pentagastrin on the oxyntic mucosa of the rat as measured by DNA synthesis.[20] Proglumide and benzotript are, however, of relatively low potency and recent modifications to proglumide have resulted in the development of three further antagonists which all have a higher affinity for the gastrin receptor, CR 1509 (lorglumide),[21] CR 1505

(loxiglumide)[22] and CR 2093 (LC Rovati, Communication to the Society for Drug Research, London, March 1991).

Benzodiazepine derivatives

The ability of the benzodiazepine sedatives to act as antagonists of peptide receptors was discovered when an endogenous peptide of rat brain was shown to be capable of displacing 1,4-benzodiazepines from their specific synaptic binding sites.[23] The first benzodiazepine to be identified as a CCK antagonist was asperlicin[24] and this discovery led to the use of the 1,4-benzodiazepine ring structure as the basic template for the development of two highly potent CCK/gastrin receptor antagonists. The first, named L-364,718, has a high selectivity for the CCK_A receptor[25] and the second, L-365,260, has high affinity for the gastrin/CCK_B receptor two orders of magnitude greater than its affinity for the CCK_A receptor. L-365,260 inhibits gastrin-stimulated acid secretion and it has been shown to be a selective gastrin receptor antagonist.[26,27]

The effect of gastrin receptor antagonists on gastric cancer

As we have seen, there is abundant evidence that gastric cancer can be sensitive to the trophic effects of gastrin and that the tumor cells express receptors for the hormone. Gastrin receptor antagonists, therefore, might have therapeutic potential in gastric cancer and in this section we shall examine the evidence for this hypothesis.

A good deal of work has been done using the gastrin-sensitive rat pancreatic cell line AR42J and, to summarize, it has been shown that *in vivo* gastrin-stimulated growth can be inhibited by L-365,260[28,29] and CR 2093. *In vitro* studies have also demonstrated reversal of gastrin-stimulated cell proliferation using proglumide, lorglumide, loxiglumide and L-365,260.[28,30,31] A number of colon cancer cell lines have also been studied and again evidence exists that the trophic effects of gastrin can be abrogated by receptor antagonists.[29,32,33]

However, the only gastric cancer cell line which has been examined in detail is MKN45. This is a cell line derived from a human gastric adenocarcinoma and our group has studied the effect of the glutaramic acid derivative CR 2093 on the *in vivo* growth of this tumor.[29]

Established xenograft tissue was grafted into 40 nude mice and the experimental animals were given either human gastrin-17 as a subcutaneous infusion delivered by osmotic mini-pump at 10 μg/kg/day from day 0 to 17 or water only, again delivered by pumps. CR 2093 was given by daily intravenous (i.v) injections at 40 mg/kg/day from day 0 to 20, with control mice receiving normal saline. Thus there were four groups of animals initiated:

A pumped water + i.v saline
B pumped gastrin-17 + i.v saline
C pumped water + i.v CR 2093
D pumped gastrin-17 + i.v CR 2093

Tumor growth was assessed by measuring the cross-sectional area of the xenografts and, as can be seen from Figure 1, there was a significantly greater increase in group B when compared with group A, and significantly inhibited growth in group D when compared with group B. There did not, however,

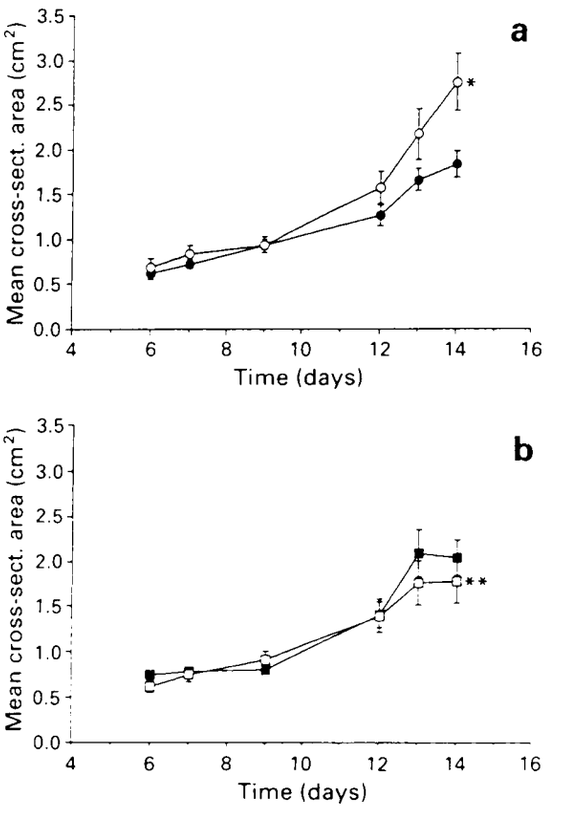

Figure 1. Effect of CR2093 on the basal and gastrin-stimulated growth of MKN45 xenografts. (a) ●, group I, pumped H_2O, saline i.v.: ○, group II, pumped G17 (10 μg mouse/day), saline i.v. (b) ■, group III, pumped H_2O, CR2093 (40 mg kg day) i.v.: □, group IV, pumped G17, CR2093 i.v. $^*p = 0.0216$ from group I. $^{**}p = 0.0454$ from group II ($n = 10$ animals per group). The error bars indicate the SE of the mean. See Watson *et al.*[29]

appear to be any effect of the CR 2093 on the basal growth on MKN45.

In Nottingham we have derived a cell line from MKN45 by selection and regrafting of gastrin-sensitive xenografts. This line, which we have called MKN45G, is not sensitive to gastrin itself, but has elevated *in vitro* growth in serum-free medium compared with the parent cell line. Its growth can be inhibited by anti-gastrin antiserum and immunohistochemical staining of the cell has revealed gastrin immunoreactivity not seen with MKN45.[34] There is thus good reason to assume that MKN45G enhances its own growth by means of endogenous gastrin and we have therefore studied the effect of a gastrin antagonist on the *in vitro* proliferation of this cell line.[31]

Lorglumide at a concentration of 2×10^{-5} M induced a significant inhibition of growth in serum-free medium to 88% of control and a concentration of 3×10^{-5} M produced inhibition to 30% (Figure 2). In order to control for a direct toxic effect, Trypan blue exclusion was carried out and, as can be seen from Figure 2, the cells remained viable. In addition, the inhibition achieved with the higher concentration of antagonist was reversed to 60% of basal growth by the addition of gastrin-17 at 10^{-5} M. Proglumide, on the other hand, had no effect on MKN45G at concentrations of up to

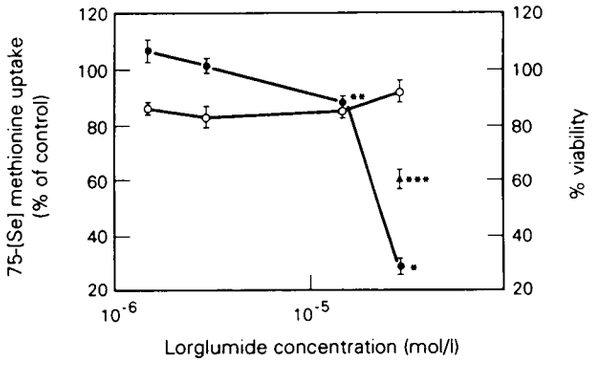

Figure 2. Effect of lorglumide on the [^{75}Se]selenomethionine uptake (●) and the viability (○) of MKN45G grown in serum-free culture medium and the effect of reversal of the inhibitory effect of lorglumide at 3×10^{-5} mol/l with 10^{-5} mol/l G17 (▲, as measured by label uptake). The mean and SE are expressed ($n = 5$ replicates). $^{*}p < 0.001$, $^{**}p < 0.02$ when compared with the untreated control and $^{***}p < 0.001$ when compared with the inhibition induced with 3×10^{-5} mol/l lorglumide, as analyzed by Student's *t*-test. Lorglumide at a concentration of 3×10^{-5} mol/l inhibited the basal growth of MKN45G, although the viability of the remaining cells was unaffected. Partial reversal of the inhibition was achieved by co-incubation with 10^{-5} mol/l G17. See Watson *et al.*[31]

3×10^{-4} M, probably because of its lower affinity for the gastrin receptor.

Thus, the studies relating to the effect of gastrin receptor antagonists on the growth of gastric cancer cell lines are fairly limited; however, there is certainly good evidence that the trophic effects of gastrin on gastrointestinal tumors can be abrogated by such compounds. Of course, it is impossible to extrapolate from these studies to the clinical situation with any degree of certainty and it is only clinical trials which can provide the necessary information regarding therapeutic efficacy.

In gastric cancer, there has been one phase III trial of proglumide carried out by Harrison *et al.*[35] In this study, 110 consecutive patients were recruited and, before randomization, were stratified first by sex and then by stage of disease. Within the groups, patients were randomized to receive 800 mg proglumide four times daily until death or to have no treatment other than surgery where appropriate. Sixteen of the patients stopped taking proglumide for a variety of reasons including nausea, vomiting and non-specific malaise.

At the last analysis of this trial, 67 (61%) of the patients had died over a 2 year period with a mean follow up of 48 weeks and a median survival of 31 weeks. Life-table analysis of survival revealed no difference between the two groups (Figure 3), with a 95% confidence interval for survival in the proglumide group of 260–474 days compared with 230–272 days for the control group. Subgroup analysis likewise revealed no differences.

It is disappointing that this study should be negative, but it must be emphasized that proglumide is of very low potency when compared with other antagonists of the gastrin receptor and thus may not be an ideal agent. In addition, it is not known how many of the patients had gastrin receptor-positive tumors and this may also have a bearing on the interpretation of the results.

Other approaches to anti-gastrin therapy

It is believed that gastrin may have an autocrine role in the growth of human gastric cancers as shown by the derivation of the human gastric cancer cell line MKN45G.[34] In addition, gastric immunoreactivity has been demonstrated by flow cytometry in disaggregated primary human gastric tumor samples.[34] Furthermore, Weinstock and Baldwin[12] have postulated that the presence of low affinity gastrin receptors on their panel of gastric

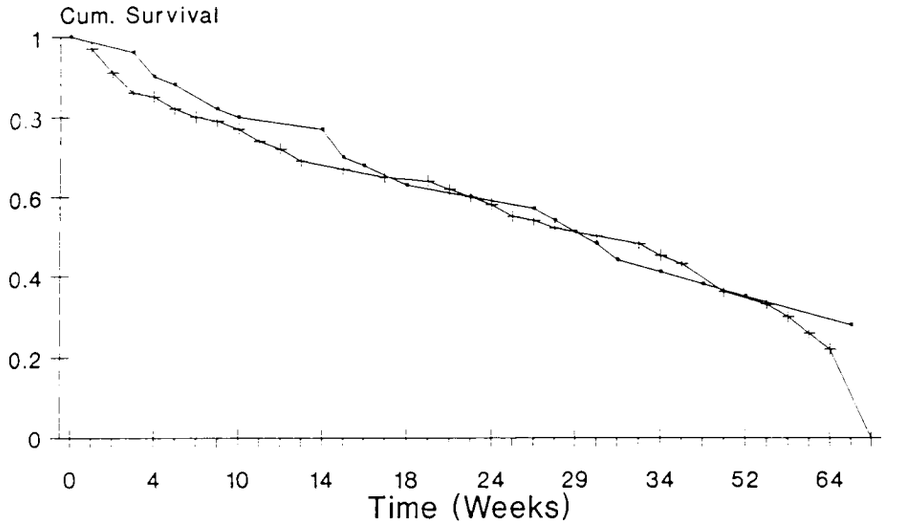

Figure 3. The effect of proglumide on survival in gastric carcinoma: ●, proglumide; +; control. See Harrison *et al.*[35]

tumor lines may be due to autocrine stimulation by gastrin. This opens up the possibility of utilizing anti-secretory agents as a potential therapeutic modality. Two anti-secretory agents have been examined in this respect: Enprostil, an E2 prostaglandin, and SMS 201-995, a long acting somatostatin analog. Both of these agents have been shown to inhibit the growth of MKN45G *in vivo* during administration, but growth recovered to a rate similar to that of controls when treatment was stopped.[36]. This indicates that such agents induce a quiescent cellular state which is reversed when they are withdrawn, allowing the cells to reinstigate the manufacture of gastrin and continue to proliferate.

Another potentially important concept is neutralization of gastrin before it encounters the receptor, as this obviates the need for a high degree of affinity for the receptor and is likely to have a long-lasting effect. It also provides a means of neutralizing cell-associated gastrin, if the hormone is actually released from the cell. Using this concept, an anti-gastrin immunogen composed of the N-terminal of the gastrin-17 molecule linked to diphtheria toxoid has been developed and is undergoing evaluation in Nottingham. Administration of this immunogen results in the production of high titers of neutralizing anti-gastrin antibodies which can be tailored to the particular molecular form or species type required. Initial *in vivo* results in human colorectal tumor models have shown good therapeutic results,[37] although it remains to be seen whether gastric tumors will respond.

This review has attempted to provide an overview of the use of gastrin receptor antagonists in gastric cancer. Accumulating evidence indicates that such tumors proliferate in response to gastrin both *in vitro* and *in vivo*, and that these effects are mediated via hormone receptors. The experimental data on gastrin receptor antagonists reveal that they can have powerful inhibitory effects on gastrin sensitive gastric tumors, but the clinical data are disappointing, possibly reflecting the low potency of the agent used. More potent antagonists exist and these may well have an important clinical role in the future. Alternative approaches to inhibiting gastrin-stimulated growth must not be forgotten, however, and the potential clinical implications of all types of anti-gastrin therapy must be considered together.

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