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Review Article

Animal Models to Study the Role of Long-Term Hypergastrinemia in Gastric Carcinogenesis

Reidar Fossmark,[1,2] Gunnar Qvigstad,[1,2] Tom Chr. Martinsen,[1,2] Øyvind Hauso,[1,2] and Helge L. Waldum[1,2]

[1] *Department of Gastroenterology and Hepatology, St. Olavs Hospital HF, Trondheim University Hospital, 7006 Trondheim, Norway*
[2] *Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway*

Correspondence should be addressed to Reidar Fossmark, reidar.fossmark@ntnu.no

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Patients with chronic hypergastrinemia due to chronic atrophic gastritis or gastrinomas have an increased risk of developing gastric malignancy, and it has been questioned whether also patients with hypergastrinemia caused by long-term use of acid inhibiting drugs are at risk. Gastric carcinogenesis in humans is affected by numerous factors and progresses slowly over years. When using animal models with the possibility of intervention, a complex process can be dissected by studying the role of hypergastrinemia in carcinogenesis within a relatively short period of time. We have reviewed findings from relevant models where gastric changes in animal models of long-term hypergastrinemia have been investigated. In all species where long-term hypergastrinemia has been induced, there is an increased risk of gastric malignancy. There is evidence that hypergastrinemia is a common causative factor in carcinogenesis in the oxyntic mucosa, while other cofactors may vary in the different models.

1. Introduction

Many patients have gastric hypoacidity and secondary hypergastrinemia due to atrophic gastritis or the use of proton pump inhibitors, whereas patients with gastrinomas have hypergastrinemia and increased gastric acidity. There is evidence that patients with atrophic gastritis have an increased risk of both enterochromaffin-like (ECL) cell carcinoids as well as gastric adenocarcinomas [1–4]. Patients with gastrinomas also have an increased risk of ECL cell carcinoids [5–7] and may develop gastric signet ring cell carcinomas [8]. However, there is no direct evidence that Proton Pump Inhibitors (PPI) increases the risk of developing gastric malignancy, but micronodular ECL cell hyperplasia is seen after 5 years of PPI use [9]. Carcinogenesis in humans is considered a multistep process progressing over years where various factors may influence. To study the contribution of single factors in carcinogenesis, various animal models can be useful. The major advantage of using animal models is that carcinogenesis is relatively reliable and often progresses in months allowing stepwise tumour development to be studied in detail.

Much of the knowledge we have of regulation of acid secretion is derived from animal studies and also applies to growth regulation of the oxyntic mucosa. Gastrin released from antral G-cells is the main regulator of acid secretion and binds to the CCK-2/gastrin receptor located on the ECL cell that secretes histamine which in turn stimulates parietal cells to secretion of hydrochloric acid [10, 11].

Although the evidence of the gastrin-ECL-parietal cell axis came from studies of the effects of various acid secretagogues in isolated rat stomachs in the 1980s, more recent studies confirm these findings. Fluorescein-labelled CCK-8 binds to ECL cells but not parietal cells [12], and gastrin does not stimulate acid secretion in either histidine-decarboxylase (HDC) deficient [13] or H2 receptor deficient [14] mice. These findings are relevant to understand the trophic and carcinogenic effects of long-term hypergastrinemia, where the target cell of gastrin, the ECL cell, is pivotal.

In this paper we review findings from animal studies on the role of long-term hypergastrinemia in gastric carcinogenesis.

2. Animal Models

2.1. Rats. In 1985 it was published that rats with life-long acid inhibition by dosing the insurmountable histamine 2-blocker loxtidine developed ECL cell carcinoids [15]. Initially it was speculated whether the carcinogenic effect was specific for this compound, but shortly after it became known that the proton pump inhibitor omeprazole caused a 15-fold increase in plasma gastrin [16], tripled the ECL cell density [17] and resulted in a 20% increase in oxyntic mucosal thickness after only 10 weeks administration. Life-long administration of omeprazole moreover resulted in ECL cell carcinoids in rats [18]. As both omeprazole and loxtidine cause profound gastric hypoacidity and subsequent hypergastrinemia is was hypothesized that hypergastrinemia caused ECL cell carcinoid development. Several following studies were in support of this hypothesis. Infusion of gastrin was found to stimulate self-replication of ECL cells [19], and partial corpectomy (also causing hypergastrinemia) resulted in ECL cell hyperplasia [20] and ECL cell carcinoids [21] in the remaining oxyntic mucosa. Long-term administration of the competitive H2-blocker ranitidine also has the ability to induce ECL cell carcinoids when given in large enough doses [22]. Finally, the administration of ciprofibrate induces ECL cell carcinoids [23] in rats without gastric hypoacidity [24], but causes hypergastrinemia through a direct effect on the antral G-cell [25]. The induction of ECL cell carcinoids by ciprofibrate clearly demonstrates that it is hypergastrinemia and not hypoacidity that drives ECL cell carcinogenesis.

2.2. Mice. The consequences of long-term hypergastrinemia have also been studied in mice by the administration of antisecretagogues and by the use of various genetically modified mice.

Administration of loxtidine for two years to mice induced carcinoids in the oxyntic mucosa [26], whereas a similar study with the proton pump inhibitor omeprazole did not show development of such tumours [18]. However, the mice were given the same dose omeprazole according to weight that had previously been given to rats (400 μmg/kg/day) without measuring the effect on gastric acidity and serum gastrin in mice. Later we have shown that mice are remarkably resistant to proton pump inhibitors with respect to inhibition of gastric acid secretion and require an extremely high dose (1750 μmg/kg/day subcutaneously) to induce profound hypoacidity and hypergastrinemia [27]. Consequently, the omeprazole study [18] was inconclusive and the potential tumorigenic effect of long-term administration of PPIs in mice is not settled.

In transgenic INS-GAS mice, it is possible to study the effect of hypergastrinemia without gastric hypoacidity [28]. Overexpression of gastrin leads to 4-fold increase in plasma gastrin and gastric hypersecretion mimicking human gastrinomas. Young INS-GAS mice have an increased ECL cell number, but with time, the INS-GAS mice lose both parietal cells and cells that can be identified as ECL cells and some develop adenocarcinomas in the oxyntic mucosa at the end of their lifespan. Inoculation with *Helicobacter felis* further increases plasma gastrin and accelerates carcinogenesis considerably [28]. Moreover, the carcinogenesis is synergistically inhibited by administration of histamine 2 receptor (loxitidine) and gastrin receptor antagonists (YF476) [29] indicating a role for both mediators in carcinogenesis. The reason why hypergastrinemic INS-GAS mice develop tumours with an adenocarcinoma phenotype, whereas mice and rats develop ECL cell carcinoids after long-term acid inhibition is not known.

A study comparing different mice models suggests that the carcinogenic effect of Helicobacter infection is mediated by gastrin. INS-GAS, C57BL/6 and gastrin deficient mice were inoculated with *Helicobacter felis* and whereas hypergastrinemic mice developed dysplasia in the oxyntic mucosa, dysplasia was not found in gastrin-deficient mice [30].

Other mice models as well can be used to study the consequences of hypergastrinemia secondary to gastric hypoacidity. H^+K^+ATPase beta subunit-deficient mice are anacidic and have a 4- to 7-fold increase in serum gastrin and show hyperplasia of the oxyntic mucosa [31, 32], whereas hyperplasia is not seen in H^+K^+ATPase beta subunit and gastrin double knockout mice. H^+K^+ATPase beta subunit-deficient mice have an increase in ECL cell number compared to controls, but carcinoma development in the oxyntic mucosa is rare and expression of neuroendocrine markers within the carcinoma could not be detected [33]. Similar changes have been described in H^+K^+ATPase alpha subunit-deficient mice [34].

The role of histamine has been studied using HDC-deficient mice that show gastric hypoacidity and a threefold increase in plasma gastrin levels [35]. In animals aged 8 to 12 weeks there were no differences in mucosal thickness suggesting that histamine mediates the trophic effect of gastrin, but not via the histamine-2 receptor, since histamine-2 receptor deficient mice are hypergastrinemic and have a hypertrophic oxyntic mucosa [14]. Long-term studies addressing the carcinogenic effects of hypergastrinemia in the absence of histamine have not been published.

Gastrin-deficient mice do not have basal acid secretion [36] thus providing a model for studying the effects of gastric hypoacidity without hypergastrinemia. These mice develop antral tumours classified as adenocarcinomas [37] which are attributed to bacterial overgrowth and subsequent formation of carcinogenic substances [38, 39].

H^+K^+ATPase and gastrin double knockedout mice are anacidic without gastrin [31], hence they do not develop a hypertrophic oxyntic mucosa, demonstrating that gastrin is responsible for these changes.

Several other genetically modified mice with gastric hypoacidity have been made, but studies on long-term gastric changes have so far not been published. That is, mice where the gene encoding KCNE2 (a potassium channel ancillary subunit) is knockedout are hypoacidic and hypergastrinemic, and these mice have marked hyperplastic changes in the oxyntic mucosa at age 3 months [40].

2.3. Japanese Cotton Rats. Animals from a strain of Japanese cotton rats develop spontaneous gastric carcinomas restricted to the oxyntic mucosa with a marked female preponderance [41]. The animals developing carcinomas were later found to have gastric hypoacidity, secondary hypergastrinemia, and pronounced ECL cell hyperplasia [42]. The oxyntic mucosa in hypergastrinemic cotton rats has marked hyperplasia of chromogranin A, synaptophysin, and HDC immunoreactive cells and a proportion of the tumour cells are chromogranin A, pancreastatin, HDC, and Sevier-Munger positive [42–45]with similar changes found in mRNA expression [46, 47]. Between the age of two and six months, a proportion of female cotton rats develop gastric hypoacidity by an unknown mechanism, and develop carcinomas after approximately four months of hypergastrinemia. Several studies have demonstrated the importance of gastrin in tumour development as carcinomas are prevented by injections of a gastrin receptor antagonist (YF476) [43], by removal of antral gastrin by antrectomy [48] or by administration of the somatostatin analogue octreotide [47]. Male cotton rats that are made hypergastrinemic due to either administration of loxtidine [49] or by partial corpectomy [50] also develop carcinomas in the oxyntic mucosa. The ECL cell ultrastructure and neuroendocrine immunoreactivity in hypergastrinemic animals have been observed over time and ECL cells gradually lose characteristics suggesting that ECL cells undergo dedifferentiation during transformation stimulated by hypergastrinemia [45].

The cotton rat model demonstrates that tumours with an adenocarcinoma phenotype and neuroendocrine characteristics are induced by gastric hypoacidity and hypergastrinemia and probably develop through dedifferentiation of ECL cells.

2.4. Mongolian Gerbils. Studies in both rodents and man have associated infection with *Helicobacter spp.* with development of gastric carcinomas. In Mongolian Gerbils ("desert rats") infection with *H. pylori* leads to development of chronic gastritis, peptic ulcers, atrophy of the gastric mucosa, intestinal metaplasia, and finally gastric carcinomas [3, 51], thus a disease that parallels what is found in humans.

There seems to be a close relationship between *Helicobacter* infection, elevated gastrin, and development of gastric carcinomas. Hypergastrinemia is a risk factor for gastric carcinomas irrespective of *Helicobacter* infection in both rodents and man. Infection with *H. pylori* induces a 5- to 10-fold rise in serum gastrin in Mongolian Gerbils [52] and increases with time [53]. Two phenotypic different gastric tumours can be found in Mongolian Gerbils after long-term infection with H. pylori; ECL cell carcinoids [3, 53] and presumably adenocarcinomas [3, 52, 53], suggesting that these two malignant tumours develop through similar mechanism. Interestingly, there is an increase in CgA positive cells up to week 50, which decreases from week 50 to 100 [54], resembling the dedifferentiation seen in other models for studying effects of long-term hypergastrinemia. It has also been demonstrated that regenerating (reg) gene expression correlates with hypergastrinemia in H pylori infected animals [55].

2.5. Mastomys. One of the animal models contributing to our understanding of gastric carcinoid tumours (neuroendocrine tumours, NETs) is the African rodent *Mastomys Natalensis* of the family *Muridae.* Already in the 1950s, it was discovered that strains of Mastomys had the propensity to develop multicentric gastric tumours that were first misclassified as adenocarcinomas [56, 57]. These tumours were later reclassified as gastric neuroendocrine tumours [58, 59], originating from the ECL-cell [60, 61], similar to the human type I (associated to atrophic gastritis) and type II (associated with gastrinoma) gastric carcinoids. The carcinoids in Mastomys are found in about 50 percent of aged animals (1-2 years) and are located to the oxyntic mucosa. The pathological changes seen preceding the development of tumours are summarized in three stages: stage I with linear hyperplasia of ECL cells, stage II with the development of ECL-cell nodules restricted to gastric glands, and stage III with ECL- cell growth below the lamina propria [62]. The direct cause of the ECL cell neoplasia in Mastomys is uncertain, however closely linked to gastrin and the CCK-2 receptor activity. The Mastomys CCK2/gastrin receptor has been transfected to COS-7 cells, and this receptor has an enhanced basal level of activity compared to the human receptor [63]. Gastrin is, however, also necessary in the pathogenesis of the carcinoids as the CCK2/gastrin receptor antagonist YF476 inhibits both ECL hyperplasia and gastric tumour development [64]. Loxtidine-induced hypergastrinemia moreover promotes the development of carcinoids in Mastomys [61]. The density of ECL cells was three times that of controls after 24 weeks of loxtidine treatment and 1/4 of the animals had gross tumours [61]. ECL cell hyperplasia and dysplasia, but not tumours, have been shown to be reversible after cessation of loxtidine treatment, suggesting that the tumour cells become independent of hypergastrinemia at some point in the neoplastic process [65]. The somatostatin-analogue octreotide has inhibitory effects on both gastrin cells and ECL cells and is found to prevent loxtidine-induced ECL hyperplasia [66].

2.6. The Norwegian Lundehund. The Norwegian Lundehund, a small Norwegian spitz breed, is a working dog developed for hunting puffins (*Fratercula arctica*), especially in the northern part of Norway. The breed was nearly eradicated during the Second World War because of the spread of canine distemper virus, and the present population originates from only five dogs.

Lundehunds often suffer from the "Lundehund syndrome": intermittent diarrhoea, hypoproteinemia, ascites, subcutaneous oedema, weight loss, and lethargy. Affected dogs are also known to develop chronic atrophic gastritis and are predisposed to the development of gastric tumours, two conditions that are rare in other breeds. The chronic atrophic gastritis is associated with loss of parietal cells and linear hyperplasia of ECL cells [67]. These findings are consistent with decreased acid secretion and long-term hypergastrinemia in these dogs.

Gastric carcinomas in dogs usually arise in the pyloric area. However, in Lundehunds the tumours most often arise

in the fundic/corpus area, that is, in the oxyntic mucosa [67]. When examining the tumours by means of histochemistry and immunohistochemistry, the neoplastic cells show neuroendocrine and more specifically ECL cell differentiation in half of the tumours [67]. Thus, it is likely that the carcinomas originate from the ECL cell secondary to the long-term trophic effect of hypergastrinemia. The neoplastic process thus parallels the development of tumours associated with pernicious anemia in man [68–70]. About half of the gastric carcinomas in Lundehund show neuroendocrine differentiation. However, during neoplastic progression an increasing number of mutations lead to dedifferentiation of tumour cells with reduced concentrations or complete loss of normal cell markers, as shown in Japanese cotton rats [71] and man [72]. This may explain why it is difficult to detect neuroendocrine and ECL cell markers in some tumours [70] and makes it possible that tumours which fail to express such markers may still be of neuroendocrine origin.

The effects of long-term administration of acid inhibitors has not been studied in the Norwegian Lundehund, but beagle dogs have been given omeprazole daily for 7 years [73]. There were no changes in the gastric mucosa at termination including ECL cell numbers, but the dogs receiving omeprazole had similar fasting and meal-stimulated plasma gastrin levels compared to controls which means the dogs had not received an adequate dose of PPI.

3. Discussion

Although the incidence of gastric cancer in western countries is decreasing, the incidence of adenocarcinomas of the diffuse type is increasing [74], being the subtype of adenocarcinomas that often develop in patients with hypergastrinemia and have neuroendocrine differentiation [70, 75, 76]. Recently it was also reported that in USA there is a significant increase of noncardia gastric adenocarcinomas in whites among younger cohorts [77], while the cause of these new trends is difficult to determine from epidemiological data alone. The relevance of animal models where hypotheses can be tested and new are generated is obvious, as animal models allow intervention by introducing or eliminating factors thought to affect carcinogenesis. Hypergastrinemia is seen in many models of gastric carcinogenesis and seems to be a common causative factor in otherwise different circumstances. In all species where long-term hypergastrinemia has been induced, an increased risk of gastric malignancy is observed, whether hypergastrinemia is accompanied by either gastric hypoacidity or hyperacidity.

In some animal models hypergastrinemia induces malignancy with either carcinoid or adenocarcinoma phenotype. However, findings from Mongolian gerbils and Japanese cotton rats suggest that these tumours develop by similar mechanisms and derive from ECL cells, thus resembling patients with atrophic gastritis who have an increased risk of developing both types of tumours. More experiments are needed to identify the mechanisms that determine the tumour phenotype.

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