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Hypergastrinemia increases gastric epithelial susceptibility to apoptosis

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Abstract

Plasma concentrations of the hormone gastrin are elevated by *Helicobacter pylori* infection and by gastric atrophy. It has previously been proposed that gastrin acts as a cofactor during gastric carcinogenesis and hypergastrinemic transgenic INS-GAS mice are prone to developing gastric adenocarcinoma, particularly following *H. pylori* infection. We hypothesised that the increased risk of carcinogenesis in these animals may partly result from altered susceptibility of gastric epithelial cells to undergo apoptosis.

Gastric corpus apoptosis was significantly increased 48 h after 12Gy γ-radiation in mice rendered hypergastrinemic by transgenic (INS-GAS) or pharmacological (omeprazole treatment of FVB/N mice) methods and in both cases the effects were inhibited by the CCK-2 receptor antagonist YM022. However, no alteration in susceptibility to γ-radiation-induced gastric epithelial apoptosis was observed in mice overexpressing progastrin or glycine-extended gastrin. Apoptosis was also significantly increased in gastric corpus biopsies obtained from *H. pylori*-infected humans with moderate degrees of hypergastrinemia.

We conclude that hypergastrinemia specifically renders cells within the gastric corpus epithelium more susceptible to induction of apoptosis by radiation or *H. pylori*. Altered susceptibility to apoptosis may therefore be one factor predisposing to gastric carcinogenesis in INS-GAS mice and similar mechanisms may also be involved in humans.

Keywords: Gastrin; Stomach; Apoptosis; Radiation; Gastric carcinoma

1. Introduction

The development of intestinal type distal gastric adenocarcinoma is strongly associated with prolonged infection with the bacterium *Helicobacter pylori* [1]. Carcinogenesis proceeds via the pre-neoplastic stages of atrophic gastritis, intestinal metaplasia and dysplasia [2]. Both *H. pylori* infection per se and the resulting gastric atrophy are associated with moderate increases in the serum concentration of the gastric antral hormone, gastrin. It has therefore been proposed that hypergastrinemia acts as a cofactor during gastric carcinogenesis [3].

Evidence in support of this hypothesis has recently been provided from the analysis of transgenic mice in which expression of members of the gastrin family of peptides has been altered. Transgenic hypergastrinemic INS-GAS mice spontaneously develop gastric atrophy which eventually leads to gastric adenocarcinoma by 20 months of age [4]. This process is greatly accelerated by infection with *H. felis* or *H. pylori* [4–6]. The process of gastric carcinogenesis in INS-GAS mice, particularly the development of gastric atrophy, is delayed in the presence of elevated serum concentrations of glycine-extended gastrin [7].

Several mechanisms may contribute towards the increased susceptibility of INS-GAS to developing gastric neoplasia. Gastrin itself has been shown to modulate a number of key cellular pathways including proliferation, apoptosis, differentiation and

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migration as well as regulating gastric acid secretion (reviewed in [8,9]). We hypothesised that members of the gastrin family of peptides may affect the likelihood of organisms developing gastric adenocarcinoma by modulating the susceptibility of gastric epithelial cells to undergo apoptosis. We have therefore performed a systematic analysis of gastric epithelial apoptosis induced by the standard stimulus of γ-radiation, in mice in which expression of members of the gastrin family of peptides have been manipulated transgenically or pharmacologically, using techniques which we have recently described and validated [10]. Our data suggest that hypergastrinemia renders epithelial cells in the gastric corpus more susceptible to undergoing apoptosis following stimulation by γ-radiation. In addition we demonstrate that gastric corpus apoptosis is increased in *H. pylori*-infected humans with moderate degrees of hypergastrinemia.

2. Materials and methods

2.1. Animals

Inbred FVB/N mice were purchased from Harlan UK Ltd, (Bicester, UK). Transgenic mice were bred in house. INS-GAS mice contain a gastrin transgene consisting of 0.4 kb of the insulin promoter upstream of the human gastrin coding sequence, resulting in the overexpression of gastrin in pancreatic β-cells and elevated (>150 pM) serum levels of human amidated gastrin [4]. h-GAS mice are transgenic for 1.3 kb of the human gastrin promoter and the coding sequence of human preprogastrin, resulting in overexpression of the transgene in hepatocytes and elevated (1–100nM) serum levels of human progastrin [11]. MTI/G-Gly transgenic mice express a mutated gastrin gene downstream of the mouse metallothionein promoter and demonstrate elevated serum and colonic mucosal concentrations of glycine-extended gastrin [12]. INS-GAS/G-Gly mice were generated by crossing INS-GAS and MTI/G-Gly mice and overexpress both amidated gastrin and glycine-extended gastrin [7]. All mice were housed under routine animal house conditions and were fed a commercially prepared pelleted diet and given water *ad libitum*. The animals were maintained on a 12:12 h light–dark cycle and all irradiations were performed between 09.00 and 11.00. Experiments were conducted with UK Home Office approval.

2.2. Assessment of apoptosis

Groups of 10–12 week old mice were subjected to 12Gy γ-radiation using a ^{137}Cs source and were sacrificed 6 h or 48 h afterwards. The radiation dose and timepoints have previously been established as optimal for assessing γ-radiation-induced gastric epithelial apoptosis [10]. Ligatures were tied around the distal oesophagus and proximal duodenum and the stomach lumen was infiltrated with 4% formal saline. Following fixation, the tissue was paraffin embedded and 3–5 μm sections were cut and stained with haematoxylin and eosin. 20 antral and 40 corpus half glands per mouse were scored on a cell positional basis by a single observer (DMP) for morphologically apoptotic cells as previously described for this tissue [10]. We have recently demonstrated that in murine gastric epithelium, assessment of apoptosis by morphological criteria in H and E sections is

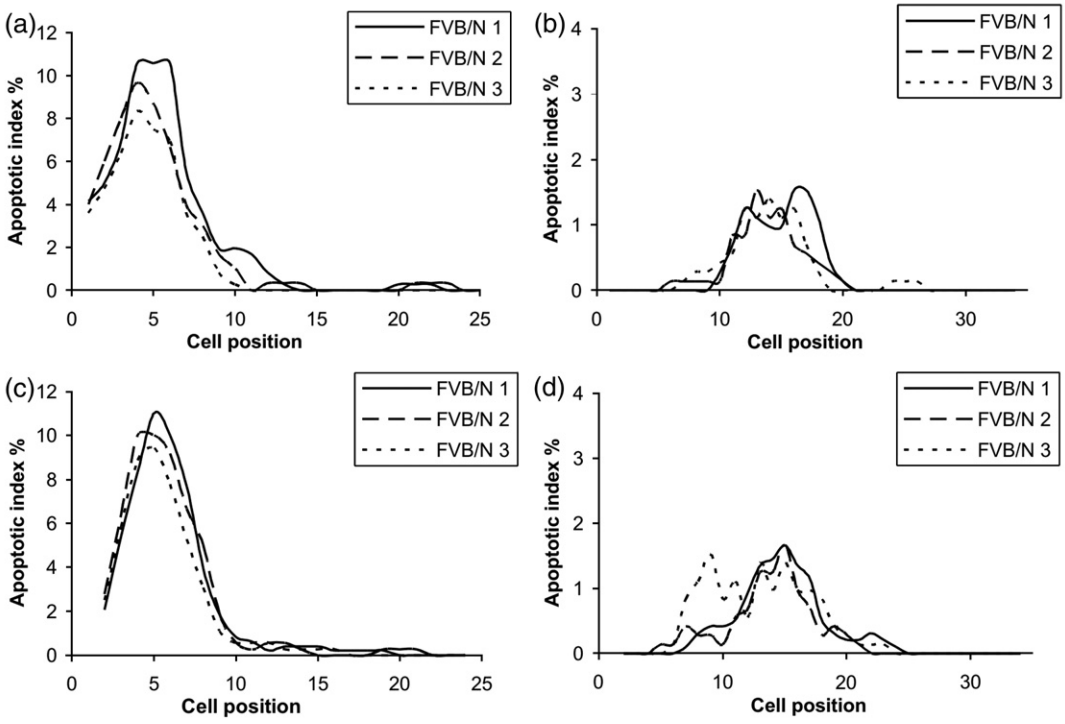

Fig. 1. Cell positional distribution of apoptosis in antrum (a and c) and corpus (b and d) of 3 groups of FVB/N wild-type mice 6 h (a and b) and 48 h (c and d) following 12Gy γ-radiation (3 independent experiments each of 6–8 male mice per experimental group).