GASTROENTEROLOGY 2006;130:1169–1180

BASIC–ALIMENTARY TRACT

Gastrin Increases Murine Intestinal Crypt Regeneration Following Injury

PENELOPE D. OTTEWELL,* CARRIE A. DUCKWORTH,* ANDREA VARRO,† ROD DIMALINE,† TIMOTHY C. WANG,§ ALASTAIR J.M. WATSON,* GRAHAM J. DOCKRAY,† and D. MARK PRITCHARD*

Divisions of *Gastroenterology and †Physiology, University of Liverpool, Liverpool, UK; and §Columbia University, New York, New York

Background & Aims: A number of growth factors affect the regeneration of intestinal epithelia following injury, but the effects of amidated gastrin have not previously been assessed. We therefore investigated the effects of gastrin on intestinal regeneration following a range of stimuli. Methods: Intestinal crypt regeneration was assessed in transgenic mice overexpressing amidated gastrin (INS-GAS) and mice in which hypergastrinemia was induced using omeprazole, following γ-radiation, 5-fluorouracil, and dextran sulphate sodium (DSS). Abundance of the CCK-2 receptor was assessed in intestinal epithelia and IEC-6 intestinal epithelial cells following γ-radiation. Results: Four days following 14 Gy γ-radiation, or 2 injections of 400 mg/kg 5-fluorouracil, INS-GAS mice exhibited significantly increased small intestinal and colonic crypt survival compared with their wild-type counterparts (FVB/N). INS-GAS mice treated with 3% DSS for 5 days showed less weight loss and increased colonic crypt regeneration at 8 days compared with FVB/N. Increased small intestinal and colonic crypt survival was also demonstrated following γ-radiation in FVB/N mice rendered hypergastrinemic using omeprazole. The increased crypt survival in INS-GAS mice following 14 Gy γ-radiation was inhibited by administration of a CCK-2 receptor antagonist (YF476). Increased abundance of the CCK-2 receptor was demonstrated in intestinal epithelia following 14 Gy γ-radiation by Western blotting and immunohistochemistry. Similarly, increased CCK-2 receptor mRNA abundance and increased ^{125}I-gastrin binding was demonstrated in IEC-6 cells following 4 Gy γ-radiation. Conclusions: Hypergastrinemia increases regeneration of intestinal epithelia following diverse forms of injury. Induction of the CCK-2 receptor in damaged epithelium confers potential for protection against injury by administration of gastrin.

Radiotherapy and cancer chemotherapy often cause the side effect of intestinal mucositis, manifest clinically as diarrhea and weight loss. Over recent years there has been considerable interest in the use of various growth factors to reduce the severity of mucositis induced by cancer therapy and some agents are currently in human clinical trials.[1] Murine models have been particularly useful for studies of the effects of growth factors on radiation and chemotherapeutic drug-induced intestinal mucositis.

The intestinal epithelium is constantly renewed from stem cells located near the bottom of small intestinal and colonic crypts (reviewed in Potten et al[2] and Booth and Potten[3]). Following administration of a damage inducing stimulus such as γ-radiation, some cells near the bottom of intestinal crypts die by apoptosis (reviewed in Watson and Pritchard[4]). If all crypt cells die, the crypt is reproductively sterilized and disappears within 48 hours. However if 1 or more "clonogenic" cell survives the insult, it rapidly proliferates to regenerate the crypt within 72–96 hours and subsequently the tissue heals by clonal expansion. The number of crypts that survive and regenerate following a cytotoxic insult correlates well with severity of symptoms and survival in animal models. A number of growth factors, such as keratinocyte growth factor, transforming growth factor-β, and interleukin-11 have been shown to affect crypt regeneration in murine intestinal epithelium following γ-radiation.[5–8] Growth factors have been postulated to affect the process of crypt regeneration in a number of ways. For example they may alter the number of clonogenic cells, the susceptibility of clonogenic cells to cell death, the time taken to start regeneration, or the rate of proliferation in regenerating crypts.[7]

Gastrin is a hormone secreted from G-cells in the antrum of the stomach. It has important functions in

Abbreviations used in this paper: 5-FU, 5-fluorouracil; DSS, dextran sulfate sodium; CCK, cholecystokinin.

© 2006 by the American Gastroenterological Association Institute
0016-5085/06/$32.00
doi:10.1053/j.gastro.2005.12.033

regulating acid secretion, proliferation, and differentiation in the gastric mucosa (reviewed in Dockray et al[9]). Gastrin has also been shown to enhance the healing of experimentally induced ulcers in rat stomach.[10] Although precursor forms of gastrin, such as progastrin and glycine-extended gastrin exert well described effects in the normal murine colon,[11–13] fully processed amidated gastrin exerts few effects upon normal intestinal epithelia as the CCK-2 receptor is not usually expressed in this tissue.[14,15] We have now assessed whether amidated gastrin acts as a growth factor influencing the healing of intestinal epithelia following induction of injury. We demonstrate that amidated gastrin significantly increases crypt regeneration within intestinal epithelia following a variety of forms of injury and show that this occurs as a result of signaling via CCK-2 receptors in the intestine. This suggests a novel function of gastrin in protecting the distal intestinal epithelium from injury.

Materials and Methods

Animals

Mice used were INS-GAS and their wild-type counterparts FVB/N. INS-GAS mice contain a transgene consisting of 0.4 kb of the insulin promoter upstream of the human gastrin coding sequence. This results in the overexpression of gastrin in pancreatic β-cells and elevated serum concentrations of human amidated gastrin.[16] FVB/N mice were obtained from B+K (Hull, UK). We also assessed hGAS ($hg^{+/+}$) mice, which express increased serum concentrations of human progastrin,[11] $G^{-/-}hg^{+/+}$ mice (which express human progastrin but no forms of murine gastrin) and $G^{-/-}hg^{-/-}$ mice (which express no gastrin).[13] To investigate the importance of basal concentrations of gastrin we also compared gastrin knockout mice[17] and their wild-type counterparts (C57BL/6). Male mice, 10–12 weeks old, were used in most experiments, with a minimum of 6 animals in each experimental group. Female mice were used for the DSS experiments because of fighting between male mice when caged together for the 11-day time course of this experiment. Mice were fed a commercially prepared pelleted diet and allowed water ad libitum. All animals were maintained in a conventional, nonspecific pathogen free, mouse facility on a 12:12-hour light–dark cycle, and all experiments were conducted during the day time. Experiments were performed with home office approval.

Irradiation

Mice were exposed to whole-body γ-irradiation using a ^{137}Cs source at a dose rate of 2.6 Gy/min. All irradiation treatments were begun between 09.00 and 10.00. Animals were sacrificed 96 hours after irradiation. Three hours prior to sacrifice, animals were injected with 0.02 mg/kg vincristine sulphate (Sigma, Poole, UK) IP to facilitate detection of regenerating crypts.

To assess whether the effects observed were specific to hypergastrinemia, FVB/N mice were rendered hypergastrinemic by gavage with 75 mg/kg omeprazole (AstraZeneca, Luton, UK) suspended in 0.25% methylcellulose (Sigma). Effects of gastrin acting via the CCK-2 receptor were blocked by IP injection of 10 μmol/kg YF476 (a gift from Yamanouchi [Osaka, Japan]) dissolved in polyethylene glycol 300 (Sigma). Omeprazole or YF476 was given 24 hours prior to exposure to 14 Gy γ-radiation and daily up until sacrifice.

5-Fluorouracil–Induced Enteritis

5-Fluorouracil (5-FU; 400 mg/kg; Sigma), dissolved in 20% DMSO/80% saline was administered by IP injection at 10.00 and again at 16.00. Mice were sacrificed 3 or 4 days posttreatment. Three hours prior to sacrifice, animals were injected with 0.02 mg/kg vincristine sulphate (Sigma) IP.

Dextran Sulfate Sodium–Induced Colitis

Female FVB/N and INS-GAS mice were given 3% DSS in the drinking water for 5 days and then water ad libitum. Mice were weighed daily and were inspected for diarrhea. Groups of mice were humanely killed at days 8 and 11 and the intestines were removed, processed, and scored as described below.

Tissue Preparation and Scoring

Following sacrifice, intestines were removed and fixed in Carnoy's solution, then embedded in paraffin wax. Transverse sections (3–5 μm) of small intestine and colon were prepared and stained with H&E as previously described.[12] Ten transverse sections per small intestine or colon were scored for numbers of surviving crypts.[18] A surviving crypt was defined as containing ≥10 adjacent healthy looking epithelial cells and a lumen. The widths (at the widest point) of 15 surviving crypts were also measured to allow size correction.[18] Such a correction factor adjusts for the probability of overscoring larger regenerating crypts or underscoring smaller ones. Data are presented as percentage of surviving crypts (± standard deviation [SD]) compared with control following correction for crypt width.

For 5-FU–treated samples, the number of cells per hemicrypt was also scored in 10 separate small intestinal crypts and 10 midcolonic crypts per mouse. This is an alternative technique for assessing the toxic effects of 5-FU and reflects the previous observation that 5-FU in the current dosing regime causes decreases in the cell number and height of both small intestinal and colonic crypts.[19] Data are presented as percentage of number of cells per hemicrypt (± SD) following treatment compared with control as previously described.[19]

Western Blotting

Intestinal epithelial cells were prepared by using a modified Weiser technique.[20] Intestines were excised and incised along their length to expose the epithelial surface. After washing in PBS the intestines were immersed in Weiser solution for 45 minutes.[20] The contents were shaken vigor-

Download English Version:

https://daneshyari.com/en/article/3297776

Download Persian Version:

https://daneshyari.com/article/3297776

Daneshyari.com