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BIOLOGY CONTRIBUTION

GLUCAGON-LIKE PEPTIDE-2 IMPROVES BOTH ACUTE AND LATE EXPERIMENTAL RADIATION ENTERITIS IN THE RAT

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Purpose: **Acute and/or chronic radiation enteritis can develop after radiotherapy for pelvic cancers. Experimental and clinical observations have provided evidence of a role played by acute mucosal disruption in the appearance of late effects. The therapeutic potential of acute administration of glucagon-like peptide-2 (GLP-2) against acute and chronic intestinal injury was investigated in this study.**

Methods and Materials: **Intestinal segments were surgically exteriorized and exposed to 16.7 or 19 Gy X-rays. The rats were treated once daily with vehicle or a protease-resistant GLP-2 derivative for 14 days before irradiation, with or without 7 days of GLP-2 after treatment. Macroscopic and microscopic observations were made 2 and 15 weeks after radiation exposure.**

Results: **In the control animals, GLP-2 induced an increase in intestinal mucosal mass, along with an increase in villus height and crypt depth. GLP-2 administration before and after irradiation completely prevented the acute radiation-induced mucosal ulcerations observed after exposure to 16.7 Gy. GLP-2 treatment strikingly reduced the late radiation damage observed after 19 Gy irradiation. Microscopic observations revealed an improved organization of the intestinal wall and an efficient wound healing process, especially in the smooth muscle layers.**

Conclusion: **GLP-2 has a clear therapeutic potential against both acute and chronic radiation enteritis. This therapeutic effect is mediated through an increased mucosal mass before tissue injury and the stimulation of still unknown mechanisms of tissue response to radiation damage. Although these preliminary results still need to be confirmed, GLP-2 might be a way to limit patient discomfort during radiotherapy and reduce the risk of consequential late effects.** © 2007 Elsevier Inc.

Glucagon-like peptide-2, Radiation enteritis, Consequential late effect, Mucosal ulceration, Fibrosis.

INTRODUCTION

The exposure of healthy intestinal tissues to radiation, such as during radiotherapy to the abdomen or pelvis, can be associated with the development of acute and/or chronic pathologic features generally referred to as radiation enteritis (1, 2). Severe acute diarrhea is a common side effect of pelvic radiation, occurring in 5–10% of treated patients, followed by intestinal fibrosis and occlusion (3, 4). Acute radiation enteritis is characterized by epithelial ulceration and mucosal and submucosal inflammation, and the transmural effect of chronic radiation enteritis is characterized by excessive extracellular matrix deposition, vascular sclerosis, and muscular dystrophy (5).

Epithelial renewal is contingent on a fragile balance between the continuous production of cells in the proliferative compartment and cell extrusion at the top of the villi. Microvascular endothelial cell apoptosis, local ischemia, and death of stem cells in the progenitor compartment can contribute to induce epithelial disruption, which leads to impaired intestinal barrier function, loss of surface epithelium, and severe mucosal inflammation (6–10). An increasing number of experimental and clinical data have supported evidence of a ''consequential'' effect (*i.e.,* a role played by acute epithelial disruption and subsequent additional trauma to underlying tissues) in the development of late sequelae of radiotherapy (11). One potential therapeutic strategy to limit

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the consequential late adverse effects might, therefore, be the reduction of the severity of acute mucosal injury during radiotherapy. Attempts have been made to limit radiation- or chemotherapy-induced oral or intestinal epithelial injury using growth factors such as keratinocyte growth factor (KGF), epidermal growth factor, and transforming growth factor-β3 (12–15). In this study, we assessed the therapeutic relevance of glucagon-like peptide-2 (GLP-2), an intestinotrophic peptide specific to the gastrointestinal mucosa, in a model of experimental radiation enteritis (16).

Glucagon-like peptide-2 is a pro-glucagon–derived peptide synthesized in, and released from, enteroendocrine L cells in the small and large intestine. GLP-2 displays important intestinotrophic properties, which were first identified in case reports of patients with glucagon-secreting tumors who developed giant villi (17). GLP-2 inhibits enterocyte apoptosis and stimulates intestinal crypt cell proliferation in the small intestinal epithelium, contributing to increasing mucosal mass and, thereby, the exchange surface area (18–20). Increased plasma GLP-2 levels have also been associated with mucosal damage in human subjects with inflammatory bowel disease, suggesting a potential role for this peptide in epithelial restitution and/or regeneration (21). Several studies conducted in rodent models have demonstrated that GLP-2 promotes intestinal adaptation after massive small bowel resection and prevents total parenteral nutrition-induced villus atrophy (22, 23). GLP-2 also reduces the severity of ischemia/reperfusion-associated mucosal damage and is efficient in the treatment of intestinal inflammation (24, 25) and chemotherapy-induced enteritis in rodents (26). One study showed that GLP-2 injection 14 days before whole body irradiation in mice increased crypt stem cell survival (27).

The present study was aimed at extending these previous observations to both acute and chronic radiation enteritis and investigating the therapeutic benefits of a novel protease-resistant GLP-2 derivative in a model of localized small intestinal radiation injury in the rat.

METHODS AND MATERIALS

Chemicals

A dipeptidylpeptidase (DDP)-IV–resistant GLP-2-analogue (NNC-103-0066), provided by Novo-Nordisk A/S (Copenhagen, Denmark), was used. DPP-IV can be found in many epithelial and several mesodermal cells, especially at the site of physiologic barriers, as well as in a soluble form in the blood plasma (28, 29). Protection from DPP-IV degradation enhances the plasma concentration of intact GLP-2 and its intestinotrophic effects in both mice and rats (30), suggesting that DPP-IV degradation is strongly implicated in overall GLP-2 metabolism. The intestinotrophic effect obtained on unirradiated parts of the bowel was similar to that of controls, suggesting that the DPP-IV–resistant analogue was preserved in the context of intestinal inflammation.

Animals and experimental procedure

The experiments were conducted in compliance with French regulations for animal experimentation (Ministry of Agriculture, Act 87-848, October, 19 1987) and were approved by the Institut de Radioprotection et de Sûreté Nucléaire's ethics committee. Male Wistar rats (weight, 275–300 g; CERJ, Le Genest, France) were maintained on a 12-hour light/dark cycle. They were given standard rat chow diet and had free access to water.

We included 108 rats, with 6 rats in each group. The nonfasted rats were anesthetized by inhalation (TEM anesthésie, Limoges, France) of 5% isoflurane (Forène, Abbott France, Rungis, France) and maintained under anesthesia with 2.5% isoflurane during irradiation. The rats first underwent laparotomy and were placed under a 5-mm-thick lead shield. A 6–8-cm-long ileal segment (15 cm from the ileocecal valve) was exteriorized, placed over the lead shield through a 2 $\times$ 3-cm opening and exposed to X-rays (225 kV, 17 mA, 0.5-mm Cu-filter). A single dose of either 16.7 or 19 Gy was delivered at 0.98 Gy/min). Sham irradiation was performed by maintaining the exteriorized ileal segment over the lead shield for 19 minutes without radiation exposure. During irradiation, the animals were wrapped in a heating pad (40°C), and the abdominal organs were covered with gauze moistened with sterile saline solution.

A single dose of GLP-2 derivative (1,000 μg/rat daily) or vehicle (NaCl 0.9%) was administered daily by subcutaneous injection. The rats were treated with GLP-2 or vehicle according to three protocols: from Day -16 to Day -2 (before irradiation only); from Day 0 to Day +14 (before and during irradiation); and from Day -16 to Day -2 followed by Day 0 to Day +7 (both before and after irradiation; henceforth referred to as the GLP-2 group). Acute observations were made 2 weeks after exposure to 16.7 Gy. Given the results obtained during the acute phase, the GLP-2 therapeutic benefit during the chronic phase was investigated 15 weeks after 19 Gy exposure in GLP-2 group only.

The selected dose of GLP-2 was shown to induce a 40% increase in villus height (data not shown).

Tissue harvesting and macroscopic observations

The whole small intestine was removed, rinsed with warm saline to avoid muscular contractions, and weighed. The length of the small intestine was measured using a 4.2-g weight to provide constant tension.

Four segments were removed for histologic examination: the duodenum, jejunum (equal distance between duodenum and irradiated segment), irradiated segment, and terminal ileum. All segments were weighed individually.

Histologic analysis

The tissues were fixed in 10% formaldehyde and embedded in paraffin. The 5-μm-thick sections were stained with hematoxylin-eosin-saffron. The percentage of ulcerated mucosa was measured on 1-cm-longitudinal tissue sections using the VisioScan 2000 image analysis software (Biocom SA, Les Ulis, France).

Morphometric analyses were performed using the Visiol@b 2000 image analysis software (Biocom SA). Villus height, crypt depth, intestinal wall thickness, and muscularis propria were measured (10–20 measurements/slide). The thickness of the submucosa was obtained by the difference between the intestinal wall thickness and the muscularis propria. The intraindividual variation was about 10%. The data are presented as the mean values derived from six tissue sections from different rats $\pm$ the standard error of the mean.

Sirius red collagen staining. The tissue sections were incubated with saturated picric acid containing 0.1% Sirius red for 1 h in the dark and were counterstained with Mayer's hematoxylin.

α-Smooth muscle actin immunostaining. The tissue sections were incubated with an antibody against α-smooth muscle actin (clone 1A4, Sigma-Aldrich, France) diluted at 1:250 for 1 h. The sections