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Radiation-induced oxidative injury of the ileum and colon is alleviated by glucagon-like peptide-1 and -2

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ABSTRACT

Purpose: The present study was conducted to characterize the possible therapeutic effects of glucagon-like peptide (GLP)-1 and GLP-2 against oxidative damage in the ileum and colon of irradiated rats.

Methods and materials: Sprague-Dawley rats of both sexes received either a single dose of GLP-1 (0.1 nmol/kg, intraperitoneally, ip; $n = 6$) 10 min before abdominal irradiation (IR) or two consecutive doses of GLP-2 (7 nmol/kg, ip; $n = 6$) at 30 and 10 min before IR, while another group was administered vehicle ($n = 6$) 10 min before IR. Control rats ($n = 6$) received vehicle treatment without IR. On the fourth day of IR, samples from ileum and colon were removed for histological analysis, for the determination of myeloperoxidase (MPO) activity, malondialdehyde (MDA) and glutathione (GSH) levels, as well as DNA fragmentation ratio, an index of apoptosis.

Results: IR-induced oxidative injury in the colonic tissue of vehicle-treated rats, evidenced by elevated MDA levels and MPO activity, as well as depleted colonic GSH levels, was reversed by GLP-2, while GLP-1 reduced IR-induced elevations in colonic MDA levels. IR-induced injury with elevated ileal MDA levels was reduced by GLP-1, while replenishment in GSH was observed in GLP-2-treated rats.

Conclusion: Current findings suggest that GLP-1 and GLP-2 appear to have protective roles in the irradiation-induced oxidative damage of the gut by inhibiting neutrophil infiltration and subsequent activation of inflammatory mediators that induce lipid peroxidation.

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1. Introduction

During radical pelvic or abdominal radiotherapy of the genitourinary or lower gastrointestinal (GI) tumors, exposure of healthy intestinal tissues to radiation often results in acute and long-term GI symptoms due to the physical disruption of the sensitive regenerative epithelium of the intestinal mucosa (Andreyev, 2007; Molla & Panes, 2007; Rubio & Jalnas, 1996). Although the pathogenesis of radiation enteritis is not clear, it is presumed to be an inflammatory process in which various mediators such as eicosanoids, cytokines, and reactive oxygen species (ROS) take place (Linard, Ropenga, Vozenin-Brotons, Chapel, & Mathe, 2003; MacNaughton, 2000). It has long been recognized that the most critical target of irradiation passing through living tissues is the DNA (Daly, Bertagnoli, De Cosse, & Morton, 1999). Additionally, lipids and proteins present in the structure of the cells are also attacked by ROS generated following irradiation (Agrawal, Chandra, & Kale, 2001). Consequently, in order to control the acute gastrointestinal injury and to reduce the impact of long-term effects of radiotherapy on gastrointestinal function, dietary, pharmacological and physical interventions as well as optimization of radiotherapy techniques are considered (Yeoh, 2008). Current biological modifiers targeting oxidative damage for radioprotection have a limited success, because oxidative tissue injury due to ionizing radiation ultimately begins by radiolytic hydrolysis and formation of ROS (Vijayalaxmi, Tan, Herman, & Thomas, 2004). Thus, to boost defense mechanisms against oxidative injury, there is a need for new potent and non-toxic antioxidant compounds.

Glucagon like peptide-1 (GLP-1) and GLP-2 represent the major secretory peptides derived from the posttranslational processing of the proglucagon gene expressed in the enteroendocrine L cells localized in the intestine with multiple local and systemic actions (Drucker, 2005). Numerous animal studies have suggested that GLP-2 is a potent intestinal mitogen in rodents (Dubé & Brubaker, 2007; Estall & Drucker, 2006) and it was shown to increase absorptive surface area and intestinal weight by stimulating epithelial cell proliferation, inhibiting apoptosis and leading to enlarged crypts and villi. GLP-2 also increases the capacity for nutrient absorption and increases the activity of nutrient transporters and epithelial brush-border digestive enzymes (Martin, Wallace, & Sigalet, 2004). Independent of its proliferative actions, GLP-2 has anti-inflammatory effects (Sigalet et al., 2007). Similarly, GLP-1 has attenuated inflammation-induced microvascular permeability and protected mesenteric endothelium (Dozier et al., 2009). GLP-1 was shown to ameliorate myocardial ischemia/reperfusion injury in rabbits (Matsubara et al., 2009), with a possible protective effect directly on myocytes (Ban et al., 2008). Similarly, GLP-1 has exerted an anti-apoptotic effect in isolated pancreatic beta-cells (Drucker, 2003).

Based on these reports, this study was designed to characterize the possible therapeutic effects of GLP-1 and GLP-2 against irradiation-induced oxidative damage of the ileum and colon in rats by evaluating the extent of tissue injury through biochemical and histological analyses.

2. Materials and methods

2.1. Animals

Sprague Dawley rats of both sexes (240–280 g) were obtained from Marmara University Animal Center (DEHAMER). They were kept at a constant temperature of 22 ± 2 °C with light–dark cycles of 12 h and fed a standard diet and water ad libitum. The experimental protocol was approved by the Marmara University Animal Care and Use Committee.

2.2. Experimental design

After an overnight fasting, anesthetized rats (ketamine, 100 mg/kg and chlorpromazine, 12.5 mg/kg, intraperitoneally; ip) were irradiated with a linear accelerator (LINAC, Saturne 42, 800 series, General Electric, Buc, France) producing 6 MV photons at a focus 100 cm distant from skin, where each animal received an 11-Gy ionizing radiation (IR) to whole abdominal area. GLP-1 and GLP-2 (Sigma Chemical, St. Louis, MO) were prepared in 0.1% bovine serum albumin (BSA, Sigma Chemical). The rats received ip either a single dose of GLP-1 (0.1 nmol/kg; $n = 6$) 10 min before the IR or two consecutive doses of GLP-2 (7 nmol/kg; $n = 6$) at 30 and 10 min before IR, while another group of animals were given vehicle (BSA; $n = 6$) 10 min before they were irradiated. Rats were returned to their home cages following the irradiation procedure. Control rats ($n = 6$) received BSA without IR.

Rats were decapitated on the 4th day of IR, and samples from ileum and colon were fixed in formaldehyde for histological analysis, while additional ileal and colonic samples were stored at −80 °C for the determination of myeloperoxidase (MPO) activity, malondialdehyde (MDA), glutathione (GSH) levels and DNA fragmentation.

2.3. Measurement of tissue myeloperoxidase activity

MPO is an essential enzyme for normal neutrophil function, which is released from the stimulated neutrophils along with other tissue-damaging substances (Kettle & Winterbourn, 1997). MPO activity was measured in tissues in a procedure similar to that documented by Hillegas, Griswold, Brickson, and Allbrightson-Winslow (1990). Tissue-associated myeloperoxidase (MPO) activity was determined in 0.2–0.3-g samples. Tissue samples were homogenized in 10 volumes