HOSTED BY

Available online at www.sciencedirect.com
ScienceDirect
Journal of Radiation Research and Applied Sciences
journal homepage: http://www.elsevier.com/locate/jrras

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

Mustafa Deniz [a], Beste M. Atasoy [b], Faysal Dane [c], Güray Can [d], Can Erzik [e], Şule Çetinel [f], Berrak Ç. Yeğen [g,*]

[a] Çanakkale Onsekiz Mart University School of Medicine, Department of Physiology, Çanakkale, Turkey
[b] Marmara University School of Medicine, Department of Radiation Oncology, İstanbul, Turkey
[c] Marmara University School of Medicine, Department of Internal Medicine, Division of Medical Oncology, İstanbul, Turkey
[d] Abant İzzet Baysal University School of Medicine, Department of Internal Medicine, Division of Gastroenterology, Bolu, Turkey
[e] Marmara University School of Medicine, Department of Medical Biology, İstanbul, Turkey
[f] Marmara University School of Medicine, Department of Histology and Embryology, İstanbul, Turkey
[g] Marmara University School of Medicine, Department of Physiology, İstanbul, Turkey

ARTICLE INFO

Article history:
Received 20 December 2014
Accepted 22 January 2015
Available online 9 February 2015

Keywords:
GLP-1
GLP-2
Radiation-enteritis
Myeloperoxidase
Glutathione

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.

* *Corresponding author*. Marmara University School of Medicine, Department of Physiology, Başıbüyük mah., Maltepe Başıbüyük yolu No. 9/1, Maltepe 34854, İstanbul, Turkey. Tel.: +90 216 4212222.
E-mail address: byegen@marmara.edu.tr (B.Ç. Yeğen).

Peer review under responsibility of The Egyptian Society of Radiation Sciences and Applications.
http://dx.doi.org/10.1016/j.jrras.2015.01.010

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

of ice-cold potassium phosphate buffer (20 mM K_2HPO, pH 7.4). The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant was discarded. The pellet was then re-homogenized with an equivalent volume of 50 mM K_2HPO containing 0.5% (wt/vol) hexadecyltrimethylammonium hydroxide (Sigma). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianisidine·2HCl. One unit of enzyme activity is defined as amount of the MPO present that causes a change in absorbance of 1.0 min^{-1} at 460 nm and 37 °C (Bradley, Priebat, Christerser, & Rothstein, 1982).

2.4. *Measurement of tissue malondialdehyde (MDA) and glutathione (GSH) levels*

Tissue samples were homogenized in 10 volumes of ice-cold 10% trichloracetic acid and centrifuged at 3000 rpm for 15 min at 4 °C. Supernatant was removed and re-centrifuged at 10,000 rpm at 4 °C for 8 min. The supernatant was transferred to a test tube containing an equal volume of TBA (0.67% w/v), and this mixture was then heated to 90 °C and maintained at that temperature for 15 min. The MDA concentration for each specimen was determined using a spectrophotometer based on its absorbance at 532 nm, and was expressed as nmol/g tissue (Beuge & Aust, 1978). GSH measurements were performed using a modification of the Ellman procedure (Beutler, 1975). Briefly, after centrifugation at 3000 rpm for 10 min, 0·5 ml supernatant was added to 2 ml 0·3 mol/l $Na_2HPO_4·2H_2O$ solution. A 0·2 ml solution of dithiobisnitrobenzoate (0·4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of $1·36 \times 10^5$ M/cm. Results are expressed in μmol GSH/g tissue.

2.5. *Histopathological analysis*

For light microscopic investigations, both ileum and colon samples were fixed in 10% formaldehyde, dehydrated in alcohol series, cleared in toluen end embedded in paraffin. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined under a photomicroscope (Olympus BH 2, Tokyo, Japan). All tissue sections were examined microscopically for the characterization of histopathological changes by an experienced histologist (S.C.) who was unaware of the treatment conditions. Assessment of the IR-induced injury was performed semi-quantitatively using the criteria given in Table 1.

2.6. *DNA fragmentation assay*

Mucosal samples from both tissues were homogenized in 10 volumes of a lysis buffer (5 mM Tris–HCl, 20 mM ethylene diamine tetra-acetic acid (EDTA) and 0·5% (v/v) *t*-octylphenoxypolyethoxyethanol (Triton-X 100; pH 8.0)). Two separate samples of 1 ml each, were taken from the sample and centrifuged at 25,000 *g* for 30 min to separate the intact chromatin in the pellet from the fragmented DNA in the supernatant (Wyllie, 1980). The supernatant was saved and the pellet was re-suspended in 1 ml tris–ethylenediaminetetraacetate

Table 1 – Assessment of the intestinal and colonic injury was performed semi-quantitatively using the described microscopical criteria.

Microscopical score	Ileum	Colon
0	Regular morphology	Regular morphology
1	Subepithelial congestion, slight cellular desquamation at villus tips	Degeneration in superficial epithelial cells and slight accumulation of inflammatory cells
2	Congestion in mucosa, loss of less than half of the villus	Degeneration in both superficial and glandular epithelium, moderate inflammatory cells
3	Loss of more than a half of the villus	Severe desquamation in superficial epithelium, prominent degeneration of glandular epithelium
4	Degeneration extending to submucosa	Degeneration extending to submucosa

buffer (pH 8·0; 10 mM:1 mM). Both the supernatant and the re-suspended pellet were then assayed for DNA content determination by the diphenylamine reaction described by Burton (Burton, 1956).

2.7. Statistics

All values are given as means ± SE. Groups of data were compared with ANOVA followed by Tukey's multiple comparison test and the significance level was set at $p < 0.05$.

3. Results

Histopathological analysis revealed that irradiation resulted in severe degeneration of both ileum and colon. Detachments of epithelium extending to submucosa, severe foamy degeneration in glandular epithelium with loss of cells was prominent in the BSA-treated IR group (Figs. 1b and 2b), while the control group demonstrated a regular morphology of ileum and colon (Figs. 1a and 2a). In the ileal tissues of the GLP-1-treated IR group, there was a prominent reorganization of epithelial cells with aberrant vacuolization (Fig. 1c). In the colonic tissues of the GLP-1-treated IR group, the epithelial alignment and lamina propria showed a prominent recovery besides a significant re-production of mucus from the goblet cells (Fig. 2c). In the IR group treated with GLP-2, the ileal tissues showed a marked regeneration in the epithelium, whereas in the glandular cells the regeneration was less than that of the GLP-1-treated IR group, with still ongoing leukocyte accumulation and congestion in the lamina propria (Fig. 1d). In the colonic epithelium, a realigned epithelium along with an overproduction of mucus was observed in both epithelium and glands (Fig. 2d).

Microscopic scores of ileal and colonic tissues, designating IR-induced mucosal damage, were very high in vehicle-treated IR group (3.33 ± 0.09 and 3.45 ± 0.10, where 4 is the maximum score) showing degeneration as compared to tissues of control rats ($p < 0.001$; Fig. 3a). However, the scores of ileal and colonic tissues in the GLP-1- and GLP-2-treated IR groups were significantly lower ($p < 0.01$) than those of the BSA-treated IR group. Similarly, mucosal DNA fragmentation percentage, an indicator of apoptosis, was significantly elevated in the ileum and colon of BSA-treated IR rats ($p < 0.001$; Fig. 3b). GLP-1 and GLP-2 treatments given before irradiation significantly decreased DNA fragmentation ratio in both the ileum ($p < 0.01$ and $p < 0.01$) and colon ($p < 0.001$ and $p < 0.05$).

MDA levels in the ileal and colonic tissues of the irradiated group that has received vehicle pretreatment were significantly higher than those of the non-irradiated control group (Fig. 4a). Pretreatment with GLP-1 reduced IR-induced elevation in MDA levels of both ileal ($p < 0.05$) and colonic ($p < 0.01$)

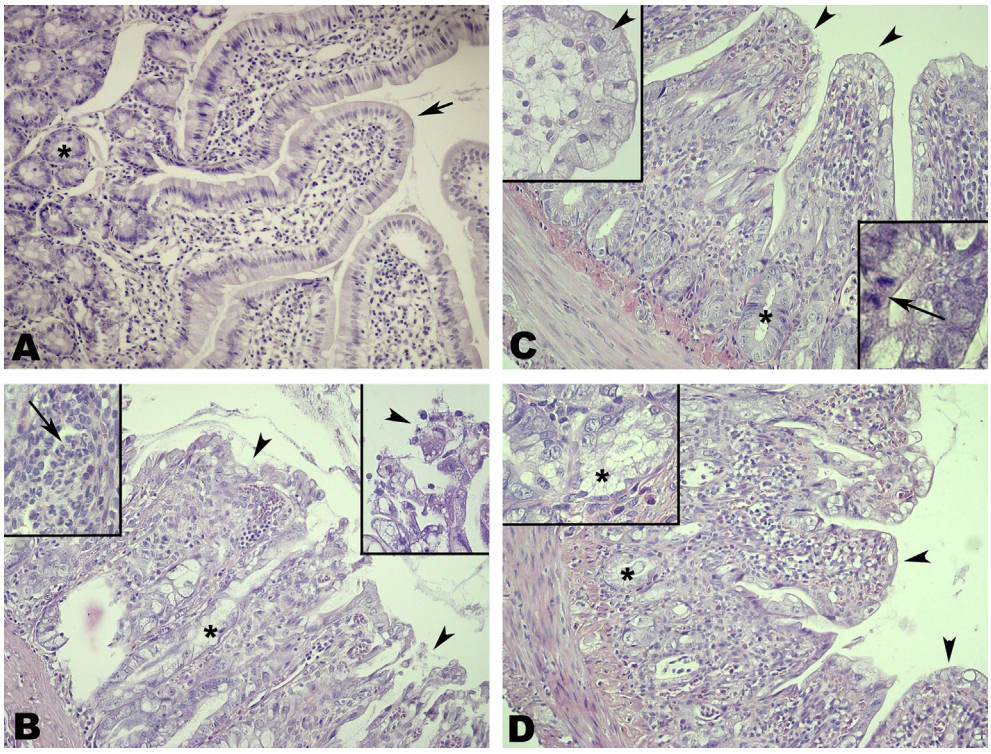

Fig. 1 – Micrographs of the ileal tissues. A. Control group, regular epithelium (arrow) with goblet cells and intestinal glands (*), B. Vehicle-treated irradiated group, the epithelium showed detachments in most of the areas (arrowheads), severe degeneration of the glands (*) accumulation of leukocytes in lamina propria (arrow-insert), C. GLP-1-treated irradiated group, the epithelial cells appear foamy but they demonstrate prominent rearrangement (arrowheads) besides the glands (*), the mitotic cell in the epithelium (arrow-insert), D. GLP-2-treated irradiated group, the epithelium retains its integrity (arrowheads), the glandular structures show re-epithelization in the gland where most of the cells lack nuclei (*-insert); HE, ×200, inserts ×400.

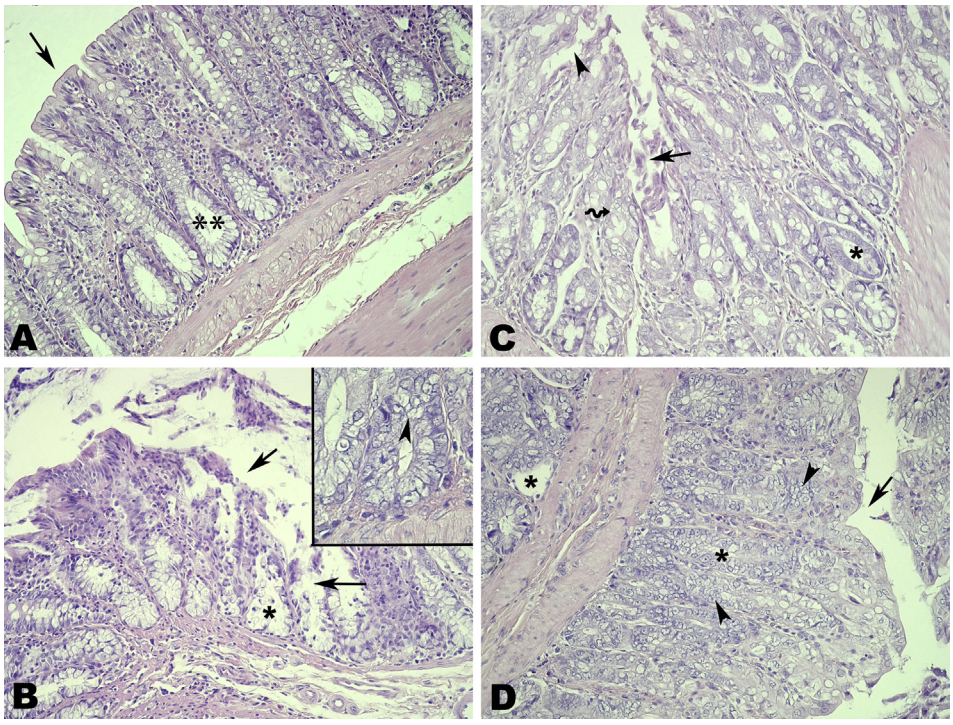

Fig. 2 – Micrographs of the colonic tissues. A. Control group, regular colonic epithelium (arrow) and glands (*), B. Vehicle-treated irradiated group, with severe degeneration of colonic tissue (arrows) extending to muscularis mucosa, loss in the integrity of glandular cells (*), C. GLP-1-treated irradiated group, re-organized epithelium (arrow), crypts (arrowhead) and glands (*) with high density of vacuolization (broken arrow), D. GLP-2-treated irradiated group, epithelial re-organization, high density of vacuoles in glandular cells (*); HE, ×200.

tissues. Similarly, MDA level was significantly decreased in the colonic tissues of GLP-2-pretreated rats that were irradiated ($p < 0.05$), while the reduction in ileal MDA level by GLP-2 treatment was not significant. In accordance with the MDA results, irradiation of BSA-treated rats caused depletion of GSH in both ileum and colon ($p < 0.001$; Fig. 4b). However, GLP-1 pretreatment did not change the reduced GSH levels of either ileal or colonic tissues of irradiated rats, while the GSH levels in both tissues of irradiated and GLP-2-pretreated-rats were not different than those of the control levels.

MPO activity, an indicator of tissue neutrophil infiltration, was significantly elevated in both the ileal and colonic tissues of all the irradiated groups ($p < 0.05–0.01$) as compared to the tissues of non-irradiated control group (Fig. 4c). Neither GLP-1 nor GLP-2 pretreatment did not alter IR-induced ileal injury. In contrary, pretreatment with GLP-2, but not with GLP-1, significantly decreased IR-induced elevation in colonic MPO activity ($p < 0.01$).

4. Discussion

The results of the current study have demonstrated that irradiation-induced oxidative damage of the ileum and colon is ameliorated by both GLP-1 and GLP-2 pretreatments. Both peptides were effective in improving the morphological changes and reducing apoptosis in the ileal and colonic tissues. IR-induced oxidative injury in the colonic tissue, evidenced by elevated MDA levels and MPO activity, as well as depleted GSH levels was reversed by GLP-2, while GLP-1 only reduced the IR-induced elevation in MDA levels. Ileal injury due to IR, as evident with elevated MDA levels, was reduced by GLP-1, but not by GLP-2. On the other hand, ileal GSH replenishment was only reached by GLP-2 treatment. Current findings suggest that these proglucagon gene-derived peptides appear to have varying degrees of 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.

Ionizing radiation is currently one of the main modalities in cancer therapy. The incidence of radiation enteritis is increasing because of the current trend of combined adjuvant chemotherapy and radiation strategies (Ooi, Tjandra, & Green, 1999). Radiation enterocolitis is one of the most feared and potentially life-threatening complications of abdominal and pelvic radiation affecting the quality of patients' life (Mann, 1991). In high doses, cell death may occur as a direct consequence of GI epithelial damage (Hauer-Jensen, 1990), which is progressive in nature and its treatment is non-rewarding. Therefore, the morbidity and mortality due to complications of radiation enterocolitis remain high (Earnest & Trier, 1989). Radiobiologists have recognized that the most critical target of ionizing radiation passing through living tissues is the DNA in the nucleus and mitochondria of most cells. Exposure of cells

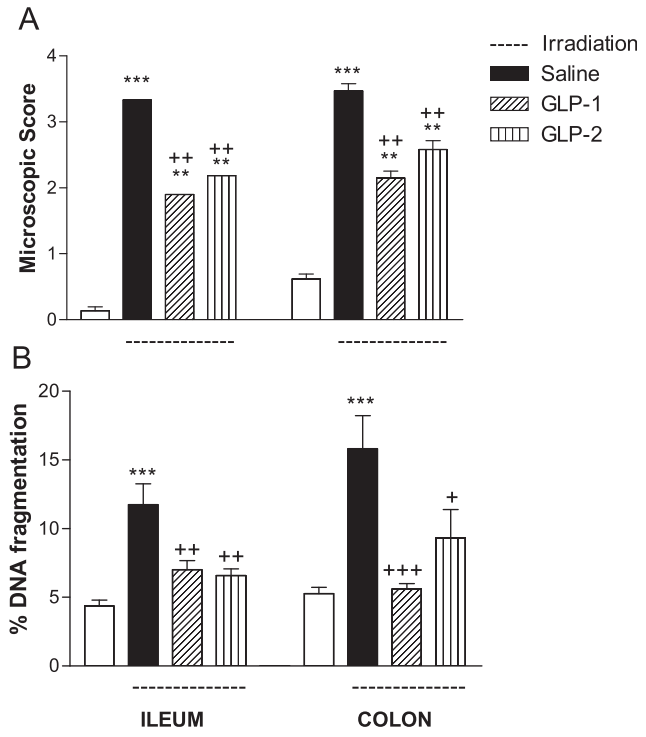

Fig. 3 – Microscopic damage scores (a) and DNA fragmentation ratios (%) (b) in the ileum and colon of rats treated with, GLP-1 or GLP-2 before they were irradiated (------). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to non-irradiated control group; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.01$ compared to saline-treated irradiated group.

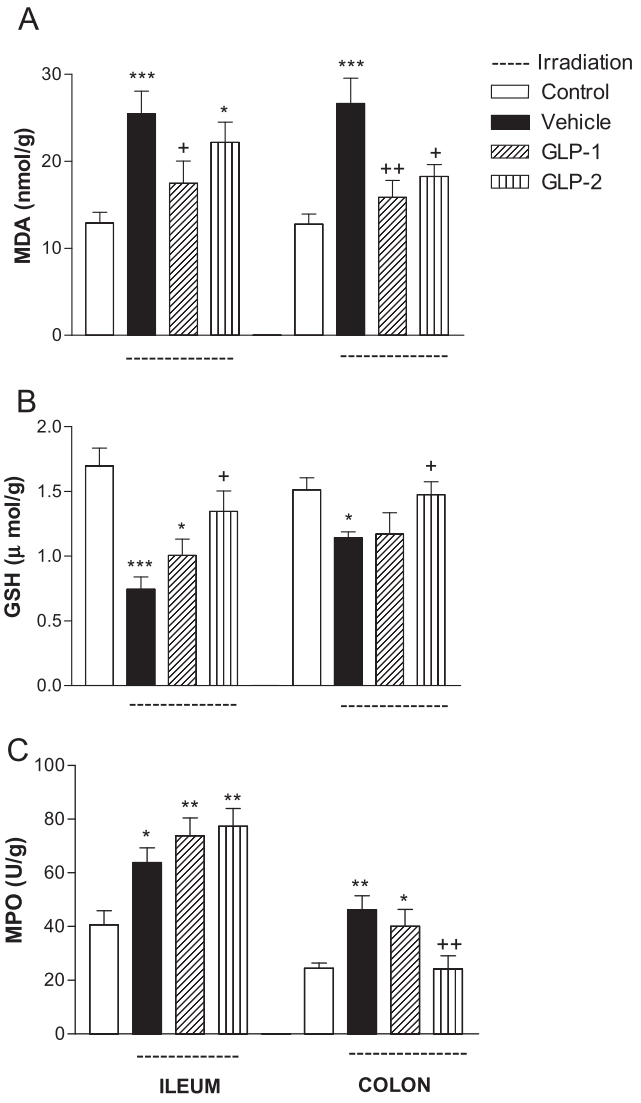

Fig. 4 – Malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity in the ileum and colon of rats treated with vehicle (saline), GLP-1 or GLP-2 before they were irradiated (------). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared to non-irradiated control group; $^{+}p < 0.05$, $^{++}p < 0.01$, compared to vehicle-treated irradiated group.

to ionizing radiation results in immediate and widespread oxidative damages to DNA by both direct and indirect mechanisms (Zhou et al., 2006). About 60–70% of cellular DNA damage produced by ionizing radiation is caused by $^{\cdot}OH$, formed from the radiolysis of water (Ward, 1988). These occur primarily by interaction of free radicals with DNA bases and, to a lesser extent, with DNA sugars (Karbownik & Reiter, 2000). Besides DNA, lipids and proteins are also attacked by free radicals induced by ionizing radiation (Vijayalaxmi, Herman, & Meltz, 1998). The radio-protectors can elicit their action by various mechanisms, such as suppression of the formation or detoxification of the radiation-induced species, target stabilization and enhancing the repair and recovery processes (Nair, Parida, & Nomura, 2001). Since rapidly proliferating intestinal crypt cells are particularly sensitive to radiation, various chemicals and nutrients have been used in the past for prevention and treatment of adverse effects of radiation on intestinal mucosa. However, there is still need for new potent anti-inflammatory agents in attenuating the deleterious effects of irradiation on gut epithelium.

Although the number of identifiable cells undergoing spontaneous apoptosis in the normal intestinal crypt compartment is low, intestinal injury after exposure to ionizing radiation results in marked induction of apoptosis in the crypt compartment (Coopersmith & Gordon, 1997; Ijiri & Potten, 1983; Watson & Pritchard, 2000). When the animals were exposed to low-dose radiation (1–5 cGy), a rapid increase in the rate of apoptosis of the intestinal crypts has occurred mainly in the stem cells of the crypts (Potten & Grant, 1998). Previously, Drucker and colleagues (Drucker, Erlich, Asa, & Brubaker, 1996; Tsai, Hill, Asa, Brubaker, & Drucker, 1997) have reported the growth-promoting properties of GLP-2 and have shown that it regulates both cell proliferation and apoptosis and promotes intestinal growth in intact animals. Similarly, our findings verified that GLP-2 and GLP-1 reduced the percentage of apoptotic cells after irradiation, demonstrating a marked reduction in the apoptosis of ileal and colonic mucosa.

In rats that have underwent massive small bowel resection, GLP-2 has exerted trophic effects on jejunal growth and improved mucosal glutathione redox status throughout the bowel (Washizawa et al., 2004). The observations have shown that GLP-2 is an intestinotrophic peptide stimulating crypt cell proliferation, nutrient transporter expression, and intestinal blood flow (Martin et al., 2004) via specific receptors on the enteric neurons and enteroendocrine cells (Bjerknes & Cheng, 2001; Guan et al., 2006). Furthermore, a physiological role was attributed to GLP-2 in the restoration of epithelial growth through modulation of crypt-cell proliferation and villus apoptosis following re-feeding of fasted mice (Shin, Estall, Izzo, Drucker, & Brubaker, 2005). Independent of its proliferative actions, GLP-2 has also been shown to produce anti-inflammatory effects in different models of intestinal inflammation by improving mucosal healing (Boushey, Yusta, & Drucker, 1999; Drucker, Yusta, Boushey, DeForest, & Brubaker, 1999; L'Heureux & Brubaker, 2003), which may involve its stimulatory effect on mesenteric arterial blood flow (Deniz, Bozkurt, & Kurtel, 2007). In accordance with the aforementioned studies, the current study demonstrated that pretreatment with GLP-2 alleviated irradiation-induced oxidative damage of the colon and ileum. Similarly, Booth, Booth, Williamson, Demchyshyn, and Potten (2004) have reported the trophic effects of GLP-2 on the murine small intestinal epithelium following whole body irradiation using the crypt microcolony assay as a measure of stem cell survival. Furthermore, in rats with chemotherapy-induced enteritis GLP-2 has also resulted in an increase in jejunal wet weight, crypt depth, and villus height (Tavakkolizadeh et al., 2000; Torres et al., 2007) have demonstrated that GLP-2, administrated both before and after localized radiation exposure, reduced the severity of acute and chronic experimental radiation-induced intestinal injury in the rat, but their observations were only based on macroscopic and microscopic evaluation of the radiation enteritis. However, in none of the studies that have shown the intestinotrophic effects of GLP-2, the antioxidant effect of the peptide was not studied before.

Although no studies associated with its trophic effects on the GI cells are present, GLP-1 has also been shown to produce cytoprotective and antiapoptotic effects in both pancreatic beta cells and myocardial cells (Bose, Mocanu, Carr, Brand, & Yellon, 2005; Buteau, Roduit, Susini, & Prentki, 1999). GLP-1 was reported to activate the epidermal growth factor receptor (Buteau, Foisy, Joly, & Prentki, 2003) and stimulate skin and hair growth (List, He, & Habener, 2006). Similar to GLP-2, GLP-1 was also shown to exert anti-inflammatory effects. Ethanol- or stress-induced gastric mucosal damage was prevented by GLP-1 (Isbil-Buyukcoskun, Gulec, Cam-Etoz, & Ozluk, 2009). GLP-1 has increased cardiomyocyte viability after ischemia-reperfusion injury of isolated hearts and has reduced myocardial injury in mice along with increased coronary blood flow (Ban et al., 2008). In the present study, GLP-1 was efficient to alleviate irradiation-induced lipid peroxidation and apoptosis in both ileum and colon.

Tissue MPO activity is frequently utilized to estimate tissue neutrophil accumulation in inflamed tissues (Bradley et al., 1982). In the present study, the presence of increased neutrophil accumulation, as assessed by elevated MPO activity in the ileum and colon indicates that radiation-induced oxidative injury involves the contribution of neutrophil accumulation. However, the observed reduction in MPO activity strongly suggests that ongoing neutrophil recruitment is significantly reduced by GLP-2 therapy, but not by GLP-1. Thus, the protective effect of GLP-2 against irradiation involves an inhibitory effect on inflammation-enhanced MPO activity. On the other hand, GSH, a well-known antioxidant, provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage (Ross, 1988), while depletion of tissue GSH is one of the primary factors that permits lipid peroxidation to occur (Szabo, Nagy, & Plebani, 1992). In the present study, the decrease in tissue GSH levels may be due to its consumption during the oxidative stress induced by irradiation. On the other hand, treatment with GLP-2, but not GLP-1, prevented GSH depletion or preserved the GSH stores, suggesting the antioxidant and free-radical scavenging effect of GLP-2. The present histopathologic results revealed that radiation-induced intestinal and colonic damage and inflammation were ameliorated when the animals were treated with either of the peptides, verifying their protective effect against radiation injury.

The cellular localization of intestinal GLP-2 receptor expression has not yet been identified, but it was suggested that intestinal cells expressing the GLP-2 receptors are likely to be protected from cell death associated with exposure to genotoxic stress in vivo (Boushey, Yutsa, & Drucker, 2001). Given the proliferative effects of GLP-1 and GLP-2, prolonged therapies with these peptides may be an increased risk for tumorigenesis (Durai, Yang, Gupta, Seifalian, & Winslet, 2005). A study in mice has shown that GLP-2 administration can accelerate the growth of chemically induced intestinal tumors (Thulesen et al., 2004). On the other hand, GLP-2 significantly improved survival, reduced bacterial infection, and decreased intestinal damage in mice treated with chemotherapeutic agents, but did not impair chemotherapy effectiveness in tumor-bearing mice, demonstrating that the protective effects of GLP-2 are not diminished in the setting of active tumor (Boushey et al., 2001). Thus, in the current study the short-term pretreatment with either GLP-1 or GLP-2 before irradiation is not expected to impair radiotherapy effectiveness.

In conclusion, the most important and innovative conclusion of the present work is that GLP-1 and GLP-2, by their antioxidant properties, protected the vulnerable intestines and colon against oxidative damage. These results suggest that supplementing cancer patients with adjuvant therapy of these peptides may afford significant protection against radiation-induced oxidative organ injury.

Authors' contributions

MD was involved in the conception and design of the experiment; acquisition, analysis and interpretation of data; drafting the manuscript. BMA was involved in the conception and design of the irradiation procedure; interpretation of data; drafting the manuscript. FD was involved in the design of the

experiment; acquisition and analysis of data; revising the manuscript. GC was involved in the design of the experiment; analysis and interpretation of data; drafting the manuscript. CE was involved in the acquisition, analysis and interpretation of DNA fragmentation data. SC was involved in the acquisition, analysis and interpretation of histological data. BCY was involved in the conception and design of the experiment; acquisition, analysis and interpretation of data; drafting the manuscript and revising it critically for important intellectual content; and giving final approval of the version to be published.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Agrawal, A., Chandra, D., & Kale, R. K. (2001). Radiation induced oxidative stress: II studies in liver as a distant organ of tumor bearing mice. *Molecular and Cellular Biochemistry, 224*, 9–17.

Andreyev, J. (2007). Gastrointestinal symptoms after pelvic radiotherapy: a new understanding to improve management of symptomatic patients. *Lancet Oncology, 8*, 1007–1017.

Ban, K., Noyan-Ashraf, M. H., Hoefer, J., Bolz, S. S., Drucker, D. J., & Husain, M. (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation, 117*, 2340–2350.

Beuge, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*, 302–310.

Beutler, E. (1975). Glutathione in red blood cell metabolism. In E. Beutler (Ed.), *A manual of biochemical methods* (pp. 112–114). New York, NY, USA: Grune & Stratton.

Bjerknes, M., & Cheng, H. (2001). Modulation of specific intestinal epithelial progenitors by enteric neurons. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 12497–12502.

Booth, C., Booth, D., Williamson, S., Demchyshyn, L. L., & Potten, C. S. (2004). Teduglutide ([Gly2]GLP-2) protects small intestinal stem cells from radiation damage. *Cell Proliferation, 37*, 385–400.

Bose, A. K., Mocanu, M. M., Carr, R. D., Brand, C. L., & Yellon, D. M. (2005). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes, 54*, 146–151.

Boushey, R. P., Yusta, B., & Drucker, D. J. (1999). Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis. *American Journal of Physiology – Endocrinology and Metabolism, 277*, E937–E947.

Boushey, R. P., Yutsa, B., & Drucker, D. J. (2001). Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. *Cancer Research, 61*, 687–693.

Bradley, P. P., Priebat, D. A., Christerser, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation. Estimation of neutrophil content with an enzyme marker. *Journal Investigative Dermatology, 78*, 206–209.

Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal, 62*, 315–323.

Buteau, J., Foisy, S., Joly, E., & Prentki, M. (2003). Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes, 52*, 124–132.

Buteau, J., Roduit, R., Susini, S., & Prentki, M. (1999). Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia, 42*, 856–864.

Coopersmith, C. M., & Gordon, J. I. (1997). Gamma-ray-induced apoptosis in transgenic mice with proliferative abnormalities in their intestinal epithelium: re-entry of villus enterocytes into the cell cycle does not affect their radioresistance but enhances the radiosensitivity of the crypt by inducing p53. *Oncogene, 15*, 131–141.

Daly, J. M., Bertagnoli, M., De Cosse, J. J., & Morton, D. L. (1999). Oncology. In S. I. Schwartz (Ed.), *Principles of surgery* (pp. 335–345). New York: McGraw-Hill.

Deniz, M., Bozkurt, A., & Kurtel, H. (2007). Mediators of glucagon like peptide 2 induced blood flow: responses in different vascular sites. *Regulatory Peptides, 142*, 7–15.

Dozier, K. C., Cureton, E. L., Kwan, R. O., Curran, B., Sadjadi, J., & Victorino, G. P. (2009). Glucagon-like peptide-1 protects mesenteric endothelium from injury during inflammation. *Peptides, 30*, 1735–1741.

Drucker, D. J. (2003). Glucagon-like peptide-1 and the islet beta-cell: augmentation of cell proliferation and inhibition of apoptosis. *Endocrinology, 144*, 5145–5148.

Drucker, D. J. (2005). Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nature Clinical Practice Endocrinology & Metabolism, 1*, 22–31.

Drucker, D. J., Erlich, P., Asa, S. L., & Brubaker, P. L. (1996). Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 7911–7916.

Drucker, D. J., Yusta, B., Boushey, R. P., DeForest, L., & Brubaker, P. L. (1999). Human [Gly2]GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 276*, G79–G91.

Dubé, P. E., & Brubaker, P. L. (2007). Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. *American Journal of Physiology – Endocrinology and Metabolism, 293*, E460–E465.

Durai, R., Yang, W., Gupta, S., Seifalian, A. M., & Winslet, M. C. (2005). The role of the insulin-like growth factor system in colorectal cancer: review of current knowledge. *International Journal of Colorectal Diseases, 20*, 203–220.

Earnest, D. L., & Trier, J. S. (1989). Radiation enteritis and colitis. In M. H. Sleisinger, & J. S. Fordtran (Eds.), *Gastrointestinal disease* (pp. 1369–1382). Philadelphia: WB Saunders.

Estall, J. L., & Drucker, D. J. (2006). Glucagon-like peptide-2. *Annual Review of Nutrition, 26*, 391–411.

Guan, X., Karpen, H. E., Stephens, J., Bukowski, J. T., Niu, S., Zhang, G., et al. (2006). GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. *Gastroenterology, 130*, 150–164.

Hauer-Jensen, M. (1990). Late radiation injury of the small intestine. Clinical, pathophysiologic and radiobiologic aspects. *Acta Oncologica, 29*, 401–415.

Hillegas, L. M., Griswold, D. E., Brickson, B., & Allbrightson-Winslow, C. (1990). Assessment of myeloperoxidase activity in whole rat kidney. *Journal of Pharmacological Methods, 24*, 285–295.

Ijiri, K., & Potten, C. S. (1983). Response of intestinal cells of differing topographical and hierarchical status to ten

cytotoxic drugs and five sources of radiation. *British Journal of Cancer, 47*, 175–185.
Isbil-Buyukcoskun, N., Gulec, G., Cam-Etoz, B., & Ozluk, K. (2009). Peripheral GLP-1 gastroprotection against ethanol: the role of exendin, NO, CGRP, prostaglandins and blood flow. *Regulatory Peptides, 152*, 22–27.
Karbownik, M., & Reiter, R. J. (2000). Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. *Proceedings of the Society for Experimental Biology and Medicine, 225*, 9–22.
Kettle, A. J., & Winterbourn, C. C. (1997). Myeloperoxidase: a key regulator of neutrophil oxidant production. *Redox Report, 3*, 3–15.
Linard, C., Ropenga, A., Vozenin-Brotons, M. C., Chapel, A., & Mathe, D. (2003). Abdominal irradiation increases inflammatory cytokine expression and activates NF-kappa B in rat ileal muscularis layer. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 285*, G556–G565.
L'Heureux, M. C., & Brubaker, P. L. (2003). Glucagon-like peptide-2 and common therapeutics in a murine model of ulcerative colitis. *Journal of Pharmacology and Experimental Therapeutics, 306*, 347–354.
List, J. F., He, H., & Habener, J. F. (2006). Glucagon-like peptide-1 receptor and proglucagon expression in mouse skin. *Regulatory Peptides, 134*, 149–157.
MacNaughton, W. K. (2000). Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction. *Alimentary Pharmacology & Therapeutics, 14*, 523–528.
Mann, W. J. (1991). Surgical management of radiation enteropathy. *Surgery Clinics of North America, 71*, 977–990.
Martin, G. R., Wallace, L. E., & Sigalet, D. L. (2004). Glucagon like peptide 2 induces intestinal adaptation in parenterally fed rats with short bowel syndrome. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 286*, G964–G972.
Matsubara, M., Kanemoto, S., Leshnower, B. G., Albone, E. F., Hinmon, R., Plappert, T., et al. (2009). Single dose GLP-1-Tf ameliorates myocardial ischemia/reperfusion injury. *Journal of Surgery Research, 16*, 1–8.
Molla, M., & Panes, J. (2007). Radiation-induced intestinal inflammation. *World Journal of Gastroenterology, 13*, 3043–3046.
Nair, C. K., Parida, D. K., & Nomura, T. (2001). Radioprotectors in radiotherapy. *Journal of Radiation Research, 42*, 21–37.
Ooi, B. S., Tjandra, J. J., & Green, M. D. (1999). Morbidities of adjuvant chemotherapy and radiotherapy for resectable rectal cancer. An overview. *Diseases of the Colon and Rectum, 42*, 403–418.
Potten, C. S., & Grant, H. K. (1998). The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *British Journal of Cancer, 78*, 993–1003.
Ross, D. (1988). Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. *Pharmacology and Therapeutics, 37*, 231–249.
Rubio, C. A., & Jalnas, M. (1996). Dose–time-dependent histological changes following irradiation of the small intestine of rats. *Digestive Disease and Science, 41*, 392–401.
Shin, E. D., Estall, J. L., Izzo, A., Drucker, D. J., & Brubaker, P. L. (2005). Mucosal adaptation to enteral nutrients is dependent on the physiologic actions of glucagon-like peptide-2 in mice. *Gastroenterology, 128*, 1340–1353.
Sigalet, D. L., Wallace, L. E., Holst, J. J., Martin, G. R., Kaji, T., Tanaka, H., et al. (2007). Enteric neural pathways mediate the anti-inflammatory actions of glucagon-like peptide 2. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 293*, G211–G221.
Szabo, S., Nagy, L., & Plebani, M. (1992). Glutathione, protein sulfhydryls and cysteine proteases in gastric mucosal injury and protection. *Clinica Chimica Acta, 206*, 95–105.
Tavakkolizadeh, A., Shen, R., Abraham, P., Kormi, N., Seifert, P., Edelman, E. R., et al. (2000). Glucagon-like peptide 2: a new treatment for chemotherapy-induced enteritis. *Journal of Surgical Research, 91*, 77–82.
Thulesen, J., Hartmann, B., Hare, K. J., Kissow, H., Orskov, C., Holst, J. J., et al. (2004). Glucagon-like peptide 2 (GLP-2) accelerates the growth of colonic neoplasms in mice. *Gut, 53*, 1145–1150.
Torres, S., Thim, L., Milliat, F., Vozenin-Brotons, M. C., Olsen, U. B., Ahnfelt-Rønne, I., et al. (2007). Glucagon-like peptide-2 improves both acute and late experimental radiation enteritis in the rat. *International Journal of Radiation Oncology Biology Physics, 69*, 1563–1571.
Tsai, C. H., Hill, M., Asa, S. L., Brubaker, P. L., & Drucker, D. J. (1997). Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. *American Journal of Physiology – Endocrinology and Metabolism, 273*, E77–E84.
Vijayalaxmi, R. R. J., Tan, D. X., Herman, T. S., & Thomas, C. R., Jr. (2004). Melatonin as a radioprotective agent: a review. *International Journal of Radiation Oncology Biology Physics, 59*, 639–653.
Vijayalaxmi, R. R. J., Herman, T. S., & Meltz, M. L. (1998). Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. *Mutation Research, 297*, 203–208.
Ward, J. F. (1988). DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. *Progress in Nucleic Acid Research & Molecular Biology, 35*, 95–125.
Washizawa, N., Gu, L. H., Gu, L., Openo, K. P., Jones, D. P., & Ziegler, T. R. (2004). Comparative effects of glucagon-like peptide-2 (GLP-2), growth hormone (GH), and keratinocyte growth factor (KGF) on markers of gut adaptation after massive small bowel resection in rats. *Journal of Parenteral and Enteral Nutrition, 28*, 399–409.
Watson, A. J., & Pritchard, D. M. (2000). VII. Apoptosis in intestinal epithelium: lessons from transgenic and knockout mice. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 278*, G1–G5.
Wyllie, H. (1980). Glucocorticoid induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature, 284*, 555–556.
Yeoh, E. (2008). Radiotherapy: long-term effects on gastrointestinal function. *Current Opinion in Supportive and Palliative Care, 2*, 40–44.
Zhou, G., Kawata, T., Furusawa, Y., Aoki, M., Hirayama, R., Ando, K., et al. (2006). Protective effects of melatonin against low- and high-LET irradiation. *Journal of Radiation Research, 47*, 175–181.