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Heparin-binding EGF-like growth factor (HB-EGF) protects the intestines from radiation therapy-induced intestinal injury☆

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Abstract

Purpose: Radiation therapy (RT) often induces enteritis by inhibiting proliferation and inducing apoptosis. Heparin-binding EGF-like growth factor (HB-EGF) has been shown to protect the intestine in several animal injury models. The objective of this study was to examine whether HB-EGF affects RT-induced intestinal injury.

Methods: HB-EGF or PBS was administered intraperitoneally to mice daily for 3 days, followed by total body irradiation (TBI). Three days after TBI, intestinal segments were harvested, and BrdU immunohistochemistry was performed to identify proliferating crypts (n = 25). Four days after TBI, intestinal segments were harvested and assessed for histologic injury (n = 34), and FITC-dextran was administered via gavage with serum FITC-dextran levels quantified to determine gut barrier function (n = 18).

Results: Compared to non-HB-EGF-treated irradiated mice, administration of HB-EGF to irradiated mice led to a significantly increased percentage of proliferative crypts (72.6% *vs.* 50.5%, $p = 0.001$), a significantly decreased percent of histologic sections with severe histologic injury (13.7% *vs.* 20.3%, $p = 0.005$), and significantly reduced intestinal permeability (18.8 μg/mL *vs.* 22.6 μg/mL, $p = 0.02$).

Conclusions: These results suggest that administration of HB-EGF protects the intestines from injury after exposure to radiation therapy. Administration of HB-EGF may represent a novel therapy for the prevention of radiation enteritis in the future.

Sixty to ninety percent of cancer patients receiving abdominal or pelvic radiation therapy develop some degree of intestinal injury [1]. Signs and symptoms of mucositis, dysmotility, and obstruction vary in onset, duration, and severity. The potential development of radiation enteritis or

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proctitis has turned the intestine into a dose-limiting organ for the administration of abdominal and pelvic radiation therapy. Without the availability of preventive therapy, radiation enteritis remains a concern for patients intolerant of radiation therapy, often limiting the use of this highly effective treatment modality.

Toxic exposure to radiation damages cellular DNA, often resulting in the loss of cellular proliferation or even immediate cell death. Rapidly dividing cells, such as mucosal crypt cells, are most sensitive to this type of injury. Radiation exposure reduces the ability of crypt cells to proliferate and differentiate into epithelial cells, limiting the ability of the mucosa to recover from the injury. Apoptosis, increased reactive oxygen species production, and severe inflammatory changes ensue throughout the mucosal lining and the wall of the intestine [2]. In acute radiation enteritis, these pathologic changes typically result in ulcer formation, mucositis, and gut barrier dysfunction. Injury leading to chronic radiation enteritis is often characterized by malabsorption, dysmotility, obstruction, or fistula formation [1].

Management of acute radiation enteritis ranges from antibiotics and antiemetics to parenteral nutritional support or surgical intervention. Patients are typically treated with antidiarrheal, antiemetic, and spasmolytic agents. Similarly, treatment options for chronic radiation enteritis are dependent on the underlying pathology and often include prokinetics, antibiotics, and dietary alterations [1]. While therapeutic options for symptomatic relief have been identified, reduced radiation dosages and fractions are often required [2].

Initially identified in 1990 as a secreted product of cultured human macrophages, heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a glycoprotein member of the EGF family [3]. Through interactions with EGF receptor subtypes ErbB1 and ErbB4, as well as the HB-EGF-specific receptor *N*-arginine dibasic convertase (Nardilysin) [4], HB-EGF promotes cell proliferation and migration of many cell types including smooth muscle cells, fibroblasts, and epithelial cells [3,5]. Using several different animal models of intestinal injury, we have shown that administration of HB-EGF down-regulates pro-inflammatory cytokine expression [6], preserves intestinal epithelial cell proliferation [7], and decreases reactive oxygen species production [8]. We further showed that administration of HB-EGF protects the lungs from remote organ injury after intestinal ischemia/reperfusion injury [9]. The therapeutic effects of HB-EGF in the intestine have been explored in our laboratory using animal models of necrotizing enterocolitis [10], ischemia/reperfusion injury [11], and hemorrhagic shock and resuscitation [12]; however, the ability of HB-EGF to protect the intestines from radiation injury has never been investigated. The present study was designed to evaluate the effects of HB-EGF in an animal model of radiation therapy-induced intestinal injury.

1. Methods

1.1. Mouse model of radiation therapy-induced enteritis

The following experimental protocol followed the guidelines for the ethical treatment of experimental animals as approved by our Institutional Animal Care and Use Committee (protocol #01802AR). Eight to twelve week-old female B6D2F1 mice (Jackson Laboratory, Bar Harbor, ME) were randomized to the following three groups: (1) control mice that were non-irradiated and received 200 μL of PBS intraperitoneally (IP) once daily for three consecutive days (no RT group) (n = 19); (2) mice that received 200 μL of PBS IP once daily for three consecutive days followed by exposure to total body irradiation (TBI) at a dose of 10 Gray in a 137 cesium-based gamma irradiator (IBL 437, CIS Bio International) (RT group) (n = 28); or (3) mice that received intraperitoneal HB-EGF [800 μg/kg in 200 μL of phosphate-buffered saline (PBS)] (Trillium Therapeutics Inc., Toronto, Ontario, Canada) once daily for three consecutive days followed by TBI (RT + HB-EGF group) (n = 30).

At the end of the experiment, mice were euthanized by cervical dislocation under inhaled 2% isoflurane, USP (Baxter Healthcare Corporation, Deerfield, IL).

1.2. Mucosal crypt cell proliferation

Mucosal crypt cell proliferation was determined using bromodeoxyuridine (BrdU) immunohistochemistry (IHC). BrdU is a uridine derivative that can be incorporated into DNA during the synthesis-phase of the cell cycle as a thymidine substitute. Mice were randomized to the following three groups: (1) no RT (n = 8); (2) RT (n = 8); and (3) RT + HB-EGF (n = 9). Animals received BrdU (30 mg/kg) (Invitrogen Corporation, Camarillo, CA) IP 69 h after TBI, with non-irradiated animals receiving BrdU simultaneously. Four hours later, animals were sacrificed and the gastrointestinal tract was removed. Portions of duodenum, jejunum, and ileum were collected from every animal and fixed in 10% neutral buffered formalin for 24 h, paraffin-embedded, and sectioned at 4-μm thickness. Sections were then stained using the BrdU Streptavidin-Biotin System (Invitrogen Corporation, Camarillo, CA) following the manufacturer's instructions. Proliferating cells were identified by positive nuclear staining, indicative of BrdU incorporation. Mucosal crypts containing 3 or more BrdU-positive cells were considered to be proliferative crypts. Results were quantified as the percentage of proliferative crypts per intestinal segment.

1.3. Histologic injury score

The degree of radiation-induced injury was assessed in mice 4 days after receiving radiation therapy. Mice were