Local Administration of Interleukin-11 Ameliorates Intestinal Radiation Injury in Rats

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Abstract
Intestinal radiation injury is dose limiting during abdominal and pelvic radiotherapy and critical for the outcome after accidental whole-body radiation exposure. The multifunctional cytokine, interleukin-11 (IL-11), ameliorates the intestinal radiation response, but its clinical use is hampered by severe toxicity after systemic administration. This study addressed whether protection against intestinal radiation injury can be achieved by intraluminal administration of IL-11. Male rats underwent surgical transposition of a 4-cm small bowel loop to the scrotum. For repeated intraluminal drug administration, an ileostomy, proximal to the bowel loop in the scrotum, was created. The transposed intestinal loop was exposed to 5 Gy fractions on 9 consecutive days. Recombinant human IL-11 (rhIL-11; 2 mg/kg/d) or vehicle was given through the ileostomy from 2 days before until 2 weeks after irradiation. At 2 weeks, structural, cellular, and molecular aspects of intestinal radiation injury were assessed. rhIL-11 ameliorated structural manifestations of radiation enteropathy, including radiation injury score (6.5 ± 0.6 in the vehicle group versus 4.0 ± 0.3 in the IL-11 group; P = 0.001), mucosal surface area loss (0.2 ± 0.1 versus 0.5 ± 0.03; P < 0.0001), and intestinal wall thickening (842 ± 66 μm versus 643 ± 54 μm; P = 0.02), reduced postradiation transforming growth factor-β overexpression, and reduced numbers of ED2-positive cells. Postirradiation mucosal mast cell numbers were partially restored by rhIL-11. These data show that local administration of rhIL-11 ameliorates early intestinal radiation injury and support further development of rhIL-11 to reduce manifestations of intestinal radiation injury in the clinic. [Cancer Res 2007;67(19):9501–6]

Introduction
Radiation therapy is used in 70% of cancer patients and is a critical factor in 25% of cancer cures. Advances in treatment planning and radiation delivery techniques have improved the ability to focus the radiation beam to the target volume. Nevertheless, radiation therapy remains dose limited by the tolerance of surrounding normal tissues. The intestine not only is a major dose-limiting organ during abdominal, pelvic, and retroperitoneal radiation therapy (1, 2) but is also one of the critical organs for the outcome of accidental whole-body radiation exposure. Therefore, interventions that reduce intestinal radiation injury (radiation enteropathy) are urgently needed.

Radiation enteropathy is not only the result of radiation-induced intestinal crypt cell death but rather results from a complex interplay of pathophysiologic processes. Inflammatory processes contribute substantially to the pathophysiology of radiation enteropathy (3, 4). During radiation therapy of organized tissues, such as the intestine, acute inflammatory responses occur after each radiation fraction. Many of these inflammatory responses are not resolved within 24 h, thus leading to an accumulation of responses with each radiation fraction that follows. Consequently, the inflammatory response that occurs after a course of fractionated radiation is not adequately resolved, as after physical trauma, but leads to chronic overproduction of proinflammatory cytokines (5, 6). Hence, modifiers of inflammation and/or immune responses may be effective radiation response modifiers in organized tissues (7, 8).

Interleukin-11 (IL-11) is a potent anti-inflammatory cytokine that also has hematopoietic growth factor activity and cytoprotective effects on intestinal crypt cells (9, 10). Recombinant human IL-11 (rhIL-11) is used to reduce thrombocytopenia in patients treated with chemotherapy (11) and has been tested in clinical studies for treatment of inflammatory bowel disease (12). Systemic administration of rhIL-11 improves crypt cell survival and reduces intestinal mucosal injury after total body irradiation in mice (13–15). However, although systemic administration of rhIL-11 is well tolerated in rodent models, severe side effects in human subjects, including significant fluid retention and multisystem organ failure, have limited the implementation of systemic IL-11 in the clinic (16, 17). In an attempt to derive therapeutic benefit from the gastrointestinal protection conferred by rhIL-11, while avoiding systemic side effects, entericoated oral formulations have been developed for intraluminal delivery of this cytokine (18). Indeed, studies in human subjects have confirmed that administration of high oral doses of enteric-coated rhIL-11 result in undetectable systemic levels and no clinical signs of systemic toxicity (18).

Despite the well-documented safety and feasibility of intraluminal administration of rhIL-11, the therapeutic efficacy of this approach for the purpose of ameliorating intestinal radiation injury has not been investigated. Direct intraluminal injection of radiation response modifiers during surgical exteriorization of the bowel has been used in other animal models of radiation enteropathy (19). We recently developed a rat model that allows daily intraluminal administration of response modifiers during and after a course of fractionated irradiation without the need for additional surgery. In this study, we tested whether delivery of rhIL-11 into the gut lumen during localized, fractionated irradiation ameliorates radiation enteropathy development.
Materials and Methods

Surgery. A total of 32 male Sprague-Dawley rats (Harlan), weighing 170 to 195 g on day of arrival, was housed under standardized conditions with controlled temperature and humidity (30–35%) and a 12:12 h light-dark cycle. The rats had free access to standard rat chow (TD8640, Harlan Teklad) and tap water. All animals were conditioned to this environment for 7 days before surgery. The experimental protocol was approved by the University of Arkansas for Medical Sciences (UAMS) Institutional Animal Care and Use Committee. The UAMS animal care facility is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

A model for localized small bowel irradiation in combination with local delivery of drugs to the bowel lumen was used as described in detail before (Fig. 1; ref. 8). In brief, rats were anesthetized with i.m. injections of 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine and orchiectomized, and a loop of distal ileum, located 10 cm from the ileocecal valve, was sutured to the inside of the scrotum. The resulting "scrotal hernia" contained a 4-cm loop of small intestine that was accessible for localized irradiation without additional manipulation.

Subsequently, an antiperistaltic Bishop-Koo type ileostomy was created by making a circular 4-mm skin incision in the left lower quadrant of the abdominal wall. The intestine was divided 12 to 15 cm proximal to the scrotal loop, and the distal limb was pulled through the skin incision and matured to the skin with six to eight full-thickness interrupted 6-0 polypropylene sutures. Intestinal continuity was reestablished by an end-to-side anastomosis between the proximal limb and the distal limb, 6 to 7 cm from the ileostomy. The abdominal incision was closed in two layers (muscle/aponeurosis and skin). After surgery, the animals were maintained in an incubator with controlled temperature until completely awake. During the first postoperative week, patency of the ileostomy was ensured by daily flushing of the stoma at the skin surface. Subsequently, the ileostomies were perfused with a daily dose of rhIL-11 (Neumega, Wyeth) or vehicle was given from 2 days before the start of (sham) irradiation until 2 weeks after the end of (sham) irradiation (a total of 24 intraluminal injections of rhIL-11). rhIL-11 was dissolved in 0.9% NaCl containing 0.1% human serum albumin (HSA; Sigma) and given directly into the bowel lumen through the ileostomy with a metal olive-tipped gavage needle at a daily bolus of 2 mg/kg body weight (injection volume, 750 μL). Vehicle-treated rats were injected with 0.9% NaCl, containing 1.8% HSA (injection volume, 750 μL), to deliver the same amount of total protein as delivered to the rhIL-11–treated rats. Special care was taken to avoid backflow of the drug or vehicle by holding the rats in an upright position for at least 10 s after injection. The daily drug injection did not require anesthesia.

Radiation enteropathy is classified as early (acute) or delayed (chronic). This study focused on the early effects of radiation. Rats were euthanized 2 weeks after the last day of (sham) irradiation. Three specimens of irradiated and unirradiated intestine (~10 cm proximal of the scrotal loop) were obtained. One specimen was snap frozen in liquid nitrogen and transferred to −80°C for RNA extraction, one specimen was fixed in formalin for histopathology and morphometry, and one specimen was fixed in methanol-Carnoy's solution (methanol/chloroform/glacial acetic acid, 6:3:1) for immunohistochemical analysis.

Radiation injury score. H&E-stained sections were used to assign radiation injury score (RIS), which provides a global measure of the severity of structural radiation injury. It has been extensively used and validated in our laboratory (21). Briefly, seven histopathologic variables of radiation injury (mucosal ulcerations, epithelial atypia, thickening of subserosa, vascular sclerosis, intestinal wall fibrosis, ileitis cystica profunda, and lymph congestion) were assessed and graded from 0 to 3. The sum of the scores for the individual alterations constitutes the RIS. All specimens were evaluated in a blinded fashion by two separate researchers, and discrepancies in scores were resolved by consensus.

Intestinal mucosal surface area. Radiation-induced decrease in mucosal surface area (MSA) is a sensitive variable of small bowel radiation injury. MSA was measured in vertical H&E-stained sections using a stereologic projection/cycloid method as described by Baddeley et al. (22) and adapted by us to our model system (21). This technique does not require assumptions about the shape or orientation distribution of the specimens and thus circumvents problems associated with most other procedures for surface area measurement.

Intestinal wall thickness. Intestinal wall thickening is a measure of both reactive intestinal wall fibrosis and intestinal smooth muscle cell hyperplasia. Intestinal wall thickness (encompassing submucosa, muscularis externa, and subserosa) as well as the thickness of the subserosa alone were measured with computerized image analysis (Image-Pro Plus, Media Cybernetics) in 10 fields per section (40x objective), three measurements per field.

Mast cell numbers. Sections were stained with 0.5% toluidine blue in 0.5 N HCl for 7 days followed by a 10 min-incubation in 0.7 N HCl. The number of mast cells in 10 fields per section (40x objective) was considered a single value for statistical analysis.

Immunohistochemistry and computed image analysis. Immunohistochemical staining for ED2, myeloperoxidase, transforming growth factor-β (TGF-β), and collagen types I and III was done with methods established and optimized in our laboratory. Tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was blocked with 1% H2O2 in methanol for 30 min. Nonspecific binding was reduced with 10% normal goat or 10% normal rabbit serum (Vector Laboratories) in 3% dry powdered milk in TBS for 1 h. Sections were incubated with mouse anti-ED2 (1:200; Serotec), rabbit anti-myeloperoxidase (1:200; Dako), pan-specific rabbit anti-TGF-β (1:300; R&D Systems), goat anti-collagen I (1:100; Southern Biotechnology Associates), or goat anti-collagen III (1:100; Southern Biotechnology Associates).
Associates) for 2 h. This was followed by a 30-min incubation with the following biotinylated antibodies: goat anti-mouse IgG (for ED2, 1:400; Sigma), rabbit anti-goat IgG (for collagen I and III, 1:400; Vector Laboratories), or goat anti-rabbit IgG (for TGF-β and myeloperoxidase, 1:400; Vector Laboratories). Sections were incubated with avidin-biotin-peroxidase complex for 30 min (Vector Laboratories) and developed in TBS containing 0.5 mg/mL 3,3-diaminobenzidine tetrahydrochloride and 0.003% H2O2.

Quantitative assessment of immunoreactivity was done using computerized image analysis (Image-Pro Plus) as described and validated before (23). Areas positive for immunoreactivity of extracellular matrix-associated TGF-β, collagen I, or collagen III were measured in 20 fields per section (40× objective). Cells positive for ED2 or myeloperoxidase were identified by color thresholding. The number of positive cells per 10 fields (40× objective) was considered a single value for statistical analysis.

Statistics. Statistical analysis was done with software packages Number Cruncher Statistical Systems 2000 (NCSS) and Statistical Package for the Social Sciences 14.0 (SPSS). Differences in end points were tested with ANOVA, with radiation and rhIL-11 as fixed factors, and post hoc multiple-range testing as appropriate. Univariate comparisons were done with the Mann-Whitney test. A P value of <0.05 was considered significant.

Results

Similar to what has been shown in humans, administration of rhIL-11 by the local route was well tolerated and not associated with clinical signs of toxicity.

As reported in previous studies, irradiation induced significant structural alterations in the intestinal wall, resulting in a significant increase in RIS (P < 0.001), intestinal wall thickness (P < 0.001), and thickness of the subserosa (P = 0.01) and a significant reduction in MSA (P < 0.001; Fig. 2).

Local administration of rhIL-11 had no effects on intestinal structure in sham-irradiated animals. In contrast, in irradiated animals, rhIL-11 administration was associated with a significant reduction in RIS (P = 0.001; Fig. 2A). Of all RIS variables, mucosal ulceration, subserosal thickening, and vascular sclerosis were reduced most markedly by rhIL-11. Likewise, rhIL-11 significantly reduced total intestinal wall thickness (P = 0.02; Fig. 2B) and subserosal thickness (P = 0.02; Fig. 2C) in irradiated intestine. rhIL-11 also ameliorated radiation-induced loss of MSA (P < 0.0001; Fig. 2D).

TGF-β is mechanistically involved in both early and delayed intestinal radiation responses. Radiation induced a small increase in extracellular matrix-associated TGF-β immunoreactive area (radiation*IL-11 interaction component: P = 0.01). rhIL-11 significantly reduced TGF-β after irradiation (P = 0.004), bringing it back to levels similar to unirradiated control intestine (Fig. 3).

The early intestinal radiation response is associated with accumulation of macrophages and granulocytes. Hence, significant increases in the number of ED2-positive cells (macrophages) and myeloperoxidase-positive cells (mainly neutrophils) were observed after irradiation. Large numbers of ED2-positive cells were especially found in areas with mucosal ulceration. Treatment with rhIL-11 significantly reduced the number of ED2-positive cells after irradiation (P = 0.02; Fig. 4A), whereas the reduction in the number of myeloperoxidase-positive cells did not
reach statistical significance \((P = 0.1; \text{Fig. 4B})\). The number of mast cells was significantly reduced at 2 weeks after irradiation \((P < 0.001)\). Small numbers of mast cells were especially found in areas with mucosal ulceration. rhIL-11 partially restored mast cell numbers after irradiation \((P = 0.006)\). Interestingly, rhIL-11 administration also increased mast cell numbers in sham-irradiated intestine (Fig. 4C).

At the early time point used in this study, no changes were found in immunoreactive area for collagen I or collagen III and rhIL-11 did not affect collagen I or collagen III immunoreactivity (data not shown).

**Discussion**

This study shows unequivocally that intraluminal delivery of rhIL-11 significantly ameliorates structural, cellular, and molecular manifestations of early radiation injury after local fractionated irradiation of rat small bowel.

rhIL-11 may exert its protective effects in radiation enteropathy by a variety of mechanisms. First, IL-11 is a potent anti-inflammatory cytokine. Reduced numbers of neutrophils and macrophages after rhIL-11 treatment of irradiated intestine suggest that IL-11 may indeed interfere with the postradiation intestinal inflammatory response. rhIL-11 reduces production of many inflammatory mediators by macrophages, including IL-6, IL-12, tumor necrosis factor-\(\alpha\), IL-1\(\beta\), and nitric oxide (24). Analogous to the findings in our study, rhIL-11 reduced myeloperoxidase activity and transcript levels of profibrotic and proinflammatory cytokines, including TGF-\(\beta\), in a rat model of inflammatory bowel disease (25). The role of TGF-\(\beta\) in radiation injury and radiation-induced adverse tissue remodeling is well established (26–28). The reduced postradiation increase in TGF-\(\beta\) in the current study may therefore contribute to the observed reduction of structural manifestations of radiation enteropathy.

*In vitro* crypt cell apoptosis is significantly reduced by rhIL-11 as early as 1 day after combined radiation and chemotherapy in mice (29). Moreover, *in vitro* studies show that rhIL-11 has cytoprotective effects on intestinal epithelial cells, coinciding with an up-regulation of the IL-11 receptor complex and heat shock protein 25 (10, 30). These studies are consistent with a direct cytoprotective effect of rhIL-11 on intestinal crypt cells, which could contribute to reduced mucosal alterations after irradiation. It is possible that at least part of the protective effect on crypt cells is simply the result of induction of transient cell cycle arrest (31), somewhat similar to TGF-\(\beta\), which is assumed to protect intestinal crypt cells by inhibiting their progression through G\(_1\) (32).

Mucosal mast cell numbers are greatly reduced 2 weeks after small bowel irradiation in rats (8). rhIL-11 treatment was associated with increased mast cell numbers both in irradiated and unirradiated intestine. Members of the IL-6 family of cytokines, including IL-11, can induce mast cell proliferation (33), which might explain the increased mast cell numbers in the present study. Because of the protective role of mast cells in the early intestinal radiation response (34), it is conceivable that the beneficial effect of rhIL-11 may be, at least partly, because of the increased number of mast cells.

**Figure 3.** Effects of rhIL-11 on TGF-\(\beta\) immunoreactivity at 2 wk after small bowel irradiation in rats \((9 \times 5\text{ Gy})\). Local administration of rhIL-11 significantly reduced extracellular matrix–associated TGF-\(\beta\) immunoreactive area (per 100 \(\mu\text{m}^2\)). Columns, average; bars, SE.

**Figure 4.** Effects of rhIL-11 on intestinal inflammation 2 wk after small bowel irradiation in rats \((9 \times 5\text{ Gy})\). A, local administration of rhIL-11 significantly reduced numbers of ED2-positive cells (macrophages, 10 fields per section with a 4\(\times\) objective). B, local administration of rhIL-11 nonsignificantly reduced numbers of myeloperoxidase (MPO)-positive cells (mainly neutrophils). C, numbers of mast cells were increased by rhIL-11 both in irradiated and unirradiated intestine. Columns, average; bars, SE.
Interactions between the enteric nervous system and intestinal mast cells are required for maintaining mucosal homeostasis and for an appropriate response to injury (35). Interestingly, studies in an inflammatory bowel disease model in rats showed that the intestinal neural response was improved after rhIL-11 treatment (36). This observation has interesting parallels to radiation enteropathy, where sensory nerve ablation has been shown to greatly exacerbate the early intestinal radiation response (37). It is tempting to speculate that improved neural responses and interaction between the enteric nervous system and intestinal mucosal mast cells may contribute to the protective effects of rhIL-11 in radiation enteropathy.

S.c. injections of rhIL-11 reduce intestinal radiation injury in animal models (13–15) and ameliorate inflammatory bowel disease in humans (12). However, although systemic administration of IL-11 does not cause overt toxicity in rodents, it is associated with substantial toxicity in humans (16, 17), thus limiting its use in the clinic. To derive therapeutic benefit from gastrointestinal effects of rhIL-11, and at the same time prevent unwanted systemic side effects, enteric-coated oral formulations that allow intraluminal delivery of this cytokine without systemic absorption have been developed (18). In human subjects, clinical studies have verified that administration of high oral doses of enteric-coated rhIL-11 does not result in detectable levels of this cytokine in the systemic circulation and does not cause clinical or biochemical signs of systemic toxicity (18). Because of the documented clinical safety of intraluminal rhIL-11, the current preclinical study was designed to investigate the potential benefits of intraluminal delivery of rhIL-11 on intestinal radiation injury. Previously, intraluminal administration of radiation response modifiers has been successful in amelioration of radiation enteropathy in animal models (19). An advantage of the model used in the present study is that the administration through a continent ileostomy allows repeated drug administration for extended periods. This is particularly useful with clinically relevant fractionated radiation schedules and also allows injection during the postradiation period.

Although previous studies have clearly documented the safety of the intraluminal delivery route of rhIL-11 in humans (18), it is prudential to consider the possibility that use of this cytokine in the setting of intestinal radiation mucositis (where the epithelial barrier is defective) could lead to absorption and some degree of systemic toxicity. Therefore, the use of rhIL-11 in patients who undergo radiation therapy should preferably be investigated according to a carefully planned dose escalation protocol. However because of the size of the mature protein (177 amino acid residues), systemic absorption, even when mucosal barrier integrity is lost, is considered unlikely.

Although local delivery of rhIL-11 to the bowel lumen during radiation therapy is unlikely to result in increased rhIL-11 levels at the site of nongastrointestinal tumors, it is prudent to ascertain that this method of rhIL-11 delivery does not stimulate cancer growth or reduce the sensitivity of cancer cells to the cancer treatment in question. Studies of the expression of IL-11 and the IL-11 receptor in colon cancer have been contradictory (38, 39), but IL-11 has no direct effect on the growth of colon carcinoma cell lines in vitro (40). Moreover, whereas s.c. injections with recombinant IL-11 protects mice from thoracic radiation injury, the radiosensitivity of pulmonary breast cancer metastases is not affected (41). Consistent with the presumed absence of a growth-promoting effect on malignant tumors, s.c. injection of rhIL-11 has been approved by the Food and Drug Administration as a supportive treatment in adults with solid tumors and lymphomas with severe chemotherapy-induced thrombocytopenia (11).

Taken together, the results reported here suggest that local delivery of rhIL-11 to the bowel lumen may be a promising interventional strategy by which to reduce the risk of radiation enteropathy in cancer patients. The effects of local rhIL-11 treatment on late intestinal radiation injury remain to be determined. However, because rhIL-11 likely interferes with the ongoing inflammatory responses that are induced by irradiation, and early mucosal injury is greatly reduced as shown here, a beneficiary effect of local rhIL-11 treatment on late radiation enteropathy may be inferred.

In conclusion, rhIL-11 ameliorates early intestinal radiation injury after localized fractionated small bowel irradiation in rats. Our data may provide a preclinical basis for future clinical studies to assess local delivery of rhIL-11 as a method to prevent or ameliorate intestinal injury in patients who undergo radiation therapy of tumors in the pelvis or abdomen. These results may also be relevant to the development of medical countermeasures against intestinal injury after accidental radiation exposure or in the radiological terrorism setting.

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References


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