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BIOLOGY CONTRIBUTION

ORAZIPONE, A LOCALLY ACTING IMMUNOMODULATOR, AMELIORATES INTESTINAL RADIATION INJURY: A PRECLINICAL STUDY IN A NOVEL RAT MODEL

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Purpose: Intestinal radiation injury (radiation enteropathy) is relevant to cancer treatment, as well as to radiation accidents and radiation terrorism scenarios. This study assessed the protective efficacy of orazipone, a locally-acting small molecule immunomodulator.

Methods and Materials: Male rats were orchiectomized, a 4-cm segment of small bowel was sutured to the inside of the scrotum, a proximal anteperistaltic ileostomy was created for intraluminal drug administration, and intestinal continuity was re-established by end-to-side anastomosis. After three weeks postoperative recovery, the intestine in the "scrotal hernia" was exposed locally to single-dose or fractionated X-radiation. Orazipone (30 mg/kg/day) or vehicle was administered daily through the ileostomy, either during and after irradiation, or only after irradiation. Structural, cellular, and molecular aspects of intestinal radiation toxicity were assessed two weeks after irradiation.

Results: Orazipone significantly ameliorated histologic injury and transforming growth factor-β immunoreactivity levels, both after single-dose and fractionated irradiation. Intestinal wall thickness was significantly reduced after single-dose and nonsignificantly after fractionated irradiation. Mucosal surface area and numbers of mast cells were partially restored by orazipone after single-dose irradiation.

Conclusions: This work (1) demonstrates the utility of the ileostomy rat model for intraluminal administration of response modifiers in single-dose and fractionated radiation studies; (2) shows that mucosal immunomodulation during and/or after irradiation ameliorates intestinal toxicity; and (3) highlights important differences between single-dose and fractionated radiation regimens. © 2006 Elsevier Inc.

Radiation injuries, Disaster planning, Intestines, Immunomodulation.

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 concentrate the radiation beam to the target volume. However, radiation therapy remains dose limited by the tolerance of surrounding normal tissues. Increased focus on the avoidance of treatment-related side effects in cancer patients, as well as the recently emergent need for medical countermeasures against radiologic accidents or terrorism, have resulted in a resurgence of interest in the development of interventions that may help reduce radiation-induced normal tissue toxicity.

The intestine is a major dose-limiting organ during abdominal, pelvic, and retroperitoneal radiation therapy (1–3). It was previously believed that the severity of intestinal radiation toxicity is determined exclusively by the extent of radiation-induced intestinal crypt cell death. It is now recognized that radiation-induced changes in cellular function, as well as secondary alterations, notably inflammatory changes, contribute substantially to the pathophysiologic manifestations of intestinal radiation toxicity. These considerations apply particularly to clinical (fractionated) radiation therapy regimens, because successive fractions of radiation inflict injury in cells and tissue compartments that exhibit progressive inflammatory changes (4). Consequently, modifiers of inflammatory responses or immune function have attracted significant attention as potential radiation response modifiers.

Orazipone (3-[[4-(methylsulfonyl)phenyl]methylene]-2,4-

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pentanedione), or OR-1384, is a locally-acting immunomodulator (5–8) originally developed for use in inflammatory bowel disease. Orazipone has potent anti-inflammatory effects in the gut mucosa and effectively reduces bowel inflammation in several preclinical animal models of inflammatory bowel disease (6, 7). Because orazipone is rapidly metabolized upon absorption into inactive compounds, the compound is devoid of systemic immunosuppressive properties and its actions are restricted to the bowel mucosa. However, for the same reason, orazipone must be delivered directly into the bowel lumen in the experimental setting, where enterosoluble capsules cannot be used.

This study reports a novel rat model, in which our previously established "scrotal hernia" model for localized small bowel irradiation was combined with a proximal anteperistaltic ileostomy. The ileostomy provided access for daily administration of orazipone directly into the gut lumen during and after localized small bowel irradiation. The study showed that orazipone ameliorated several aspects of intestinal radiation toxicity. Moreover, the study also demonstrated important differences between single-dose and fractionated irradiation in terms of development of injury and optimal scheduling of response modifiers.

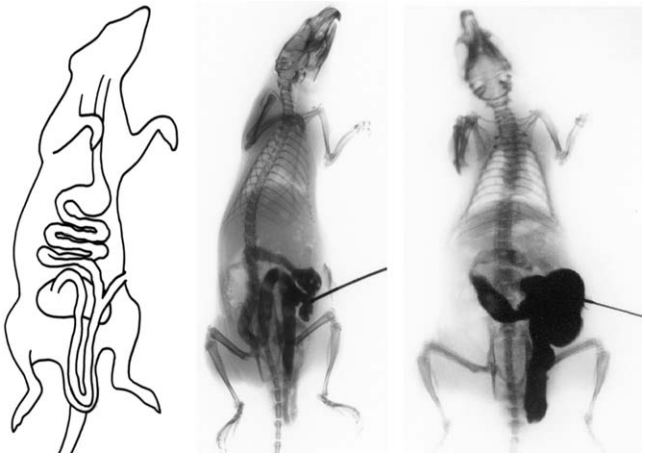

Fig. 1. The anteperistaltic ileostomy model for intraluminal delivery of response modifiers. A loop of distal small bowel is sutured to the inside of the scrotum in an orchiectomized male rat. The intestine proximal to the transposed loop is divided and the distal end is brought out as an end ileostomy. Intestinal continuity is re-established by an end-to-side intestinal anastomosis. Left panel: Cartoon showing the ileostomy relative to the transposed loop of small intestine. Middle panel: x-ray after injection of 1.5 mL contrast showing how the contrast passes easily through the intestine, including into the transposed loop in the scrotum. Right panel: x-ray after injection of 2.5 mL contrast to demonstrate complete filling of the intestinal loop in the scrotum.

METHODS AND MATERIALS

Surgery

A total of 77 male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 170–195 g on the day of arrival, were housed under standardized conditions with controlled temperature and humidity (30–35%) and a 12–12-h light–dark cycle. Thirteen animals were used for model development, and the remaining 64 rats were used for immunomodulation experiments as described in detail later. The rats had free access to standard rat chow (TD8640, Harlan Teklad, Madison, WI) and tap water. All animals were conditioned to this environment for seven days before surgery. The experimental protocol was reviewed and approved by the University of Arkansas for Medical Sciences (UAMS) Institutional Animal Care and Use Committee (IACUC). The UAMS animal care facility is fully accredited by the American Association for Accreditation of Laboratory Care (AAALAC).

A previously described model for localized small bowel irradiation (9, 10) was modified to allow local delivery of orazipone directly into the intestinal lumen just proximal to the irradiated segment by creating an anteperistaltic Bishop-Koop type ileostomy (Fig. 1).

After overnight fasting, rats were anesthetized with 60 mg/kg ketamine hydrochloride (Ketaset, Aveco, Fort Dodge, IA) and 10 mg/kg xylazine (Gemini, Rugby, Rockville Centre, NY) intramuscularly. The animals were orchiectomized and a loop of distal ileum, about 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 would be accessible for subsequent localized irradiation without additional manipulation.

To create the ileostomy, a circular 4-mm skin incision was made in the left lower quadrant of the abdominal wall. The bowel was divided approximately 12–15 cm proximal to the transposed (scrotal) loop, and the distal limb was pulled through the skin incision after bluntly splitting the abdominal wall muscles. The ileostomy was matured to the skin with 6–8 full-thickness interrupted 6–0 polypropylene sutures. Intestinal continuity was re-established by end-to-side anastomosis between the proximal limb and the distal limb, 4–6 cm from the ileostomy. The end-to-side anastomosis was created by making an approximately 5-mm longitudinal antimesenteric incision in the distal limb, placing 6–0 polypropylene stay sutures, and then running 6–0 polypropylene sutures between stay sutures. The patency and appropriate location of the anastomosis was tested by injecting 1.5 mL saline before closing the abdominal incision in two layers (muscle/aponeurosis and skin) with 4–0 running monofilament nylon sutures. After surgery the animals were maintained in an incubator with controlled temperature until completely awake. During the postoperative period (between surgery and start of drug administration), the stomas were cannulated every 1–3 days with a lubricated olive-tipped gavage needle to ensure patency at the level of the skin opening.

Irradiation

Irradiation was performed after three weeks postoperative recovery using a Seifert Isovolt 320 x-ray machine (Seifert X-Ray Corporation, Fairview Village, PA). Irradiation characteristics were: 250 kV, 15 mA, with 3 mm Al added filtration. The resulting half-value layer was 0.85 mm Cu and the dose rate was 4.49 Gy/min. Calibration and dosimetry have been described in detail elsewhere (10).

After anesthesia intramuscularly with 60 mg/kg ketamine hydrochloride (Ketaset, Aveco) and 10 mg/kg xylazine (Gemini, Rugby), the rats were placed in a supine position on a PVC board and exposed to irradiation of the small bowel segment in the "scrotal hernia." Rats were randomly assigned to receive sham irradiation, irradiation with a single dose of 21 Gy, or fractionated irradiation with 5.6 Gy daily for eight consecutive days.