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BIOLOGY CONTRIBUTION

UP-REGULATION OF ENDOTHELIN TYPE A RECEPTOR IN HUMAN AND RAT RADIATION PROCTITIS: PRECLINICAL THERAPEUTIC APPROACH WITH ENDOTHELIN RECEPTOR BLOCKADE

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Purpose: Rectum radiation damage and fibrosis are often associated with radiation therapy of pelvic tumors. The endothelin (ET) system has been implicated in several fibrotic diseases but never studied in the context of gastrointestinal radiation damage. This study assessed modifications in ET type 1 (ET-1), ET type A receptor (ET_A), and ET type B receptor (ET_B) localization and/or expression in irradiated human rectal tissue and in a rat model of delayed colorectal injury. We also evaluated the therapeutic potential of long-term ET receptor blockade.

Methods and Materials: Routine histological studies of sections of healthy and radiation-injured human rectum tissue were done; the sections were also immunostained for ET_A and ET_B receptors. The rat model involved the delivery of 27 Gy in a single dose to the colons and rectums of the animals. The ET-1/ET_A/ET_B expression and ET_A/ET_B localization were studied at 10 weeks postexposure. The abilities of bosentan and atrasentan to protect against delayed rectal injury were also investigated.

Results: The immunolocalization of ET_A and ET_B in healthy human rectums was similar to that in rat rectums. However, strong ET_A immunostaining was seen in the presence of human radiation proctitis, and increased ET_A mRNA levels were seen in the rat following colorectal irradiation. Immunostaining for ET_A was also strongly positive in rats in areas of radiation-induced mucosal ulceration, atypia, and fibroproliferation. However, neither bosentan nor atrasentan prevented radiation damage to the rectum when given long term. The only effect seen for atrasentan was an increased number of sclerotic vessel sections in injured tissues.

Conclusions: As the result of the overexpression of ET_A, radiation exposure deregulates the endothelin system through an "ET_A profile" in the human and rodent rectum. However, therapeutic interventions involving mixed or specific ET_A receptor blockade do not prevent radiation damage. Further studies are necessary to identify the precise roles of ET in the gastrointestinal response to radiation exposure.

Endothelin, radiation fibrosis, rectum, bosentan, atrasentan.

INTRODUCTION

Because of the substantial morbidity and mortality involved, the chronic and progressive nature of radiation-induced injury to healthy tissue can have a serious effect on patients' quality of life. Added to this is the fact that with the significant progress that has been made in radiation dose delivery and modulation, there is an increasing number of cancer survivors who must cope with these treatment-related side effects. Indeed, the different parts of the gastrointestinal (GI) tract that can be affected by radiation still limit the radiation dose that can be delivered in patients with pelvic tumors. The rectum is particularly at risk in these patients, since it is routinely present in the irradiation field of, for example, patients with prostate tumors.

The delayed manifestation of intestinal radiation toxicity is characterized by an abnormally sustained repair process that can lead to transmural fibrosis, which is associated with

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Conflicts of interest: none.

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inflammation, extracellular matrix deposition, vascular sclerosis, and muscular dystrophy. The cellular and molecular mechanisms of GI radiation injury include oxidative stress, stem cell death, vascular damage with proinflammatory and prothrombotic factors activation, and the release of numerous inflammatory and fibrogenic cytokines and growth factors. One of the major growth factors produced during tissue repair is transforming growth factor-beta 1 (TGF-β1), which has long been associated with radiation injury and fibrosis in healthy tissue (1). From the standpoint of a possible preventive therapy, Zheng *et al*. (2) successfully limited radiation enteropathy in mice, using a recombinant soluble TGF-β type II receptor. Much effort is also being put into identifying more selective fibrogenic agents that might operate downstream in the TGF-β1 network.

One well-recognized key fibrogenic cytokine and downstream mediator of the actions of TGF-β1 is endothelin-1 (ET-1) (3, 4), a 21-amino-acid peptide first identified for its potent vasoconstrictor activity, which is governed by two receptor subtypes: ET receptor type A (ET_A) and type B (ET_B) (5). ET-1 is secreted by many cell types, including endothelial cells, smooth muscle cells, fibroblasts, epithelial cells, leukocytes, and macrophages. ET_A and ET_B are present on the surface of numerous cell types, illustrating the pleiotropic potential of ET-1 outside the cardiovascular system (6). The role for ET-1 in fibrosis was first observed in patients with scleroderma (systemic sclerosis [SSc]). In particular, the fibroblasts of SSc patients were found to produce elevated amounts of ET-1 (7). In addition, an increase in the ET-1 concentration has been observed in the plasma, bronchoalveolar fluid, fibrotic skin, and lung tissues of SSc patients (8–10). On the basis of these findings, bosentan, a dual ET_A/ET_B antagonist, is currently used in SSc patients to manage pulmonary hypertension and digital ulcers (11, 12).

A role for ET-1 in several other fibrotic disorders in different organs has also been observed. This role served as the basis for the development of therapies for these disorders that can oppose the effects of ET-1, and, indeed, the therapeutic efficacy of mixed ET_A/ET_B or selective ET_A receptor blockade has now been demonstrated in several preclinical rodent models of fibrosis, such as carbon tetrachloride- or bile duct ligation-induced hepatic fibrosis (13, 14), N(G)-nitro-L-arginine methyl ester–induced renal vascular fibrosis (15), the myocardial fibrosis in deoxycorticosterone acetate-salt hypertensive rats (16), and bleomycin-induced lung fibrosis (17).

However, as far as we are aware, the effects of colorectal irradiation on the ET system have never been studied. The aim of this study was (1) to characterize the modifications of ET_A/ET_B localization in irradiated human rectum; (2) to monitor ET-1/ET_A/ET_B expression and ET_A/ET_B localization in a preclinical model of radiation-induced colorectal delayed injury in rats; and (3) to evaluate the therapeutic potential effect of chronic ET receptor blockade with bosentan, a dual ET_A/ET_B antagonist, or atrasentan, a selective ET_A antagonist, on colorectal radiation fibrosis.

METHODS AND MATERIALS

Human tissues

Human tissues were obtained, following institutional ethical guidelines (Gustave Roussy Institute) and French Medical Research Council guidelines. Tissue samples from 10 patients treated for rectal adenocarcinoma with preoperative radiotherapy (a total of 45 Gy, delivered in a fraction of 2 or 1.8 Gy) were included in this study. Tumors were surgically resected at 5 to 7 weeks posttreatment. For each patient, specimens of healthy tissue were taken from the irradiated field adjacent to the tumor and from microscopically healthy mucosa distant from the tumor. Slides were stained with hematoxylin-eosin-saffron.

Chemicals

Bosentan was kindly provided by Actelion Pharmaceuticals, Ltd., Switzerland, and atrasentan by Abbott Laboratories.

Animals and experimental procedures

Experiments were conducted in compliance with French regulations for animal experimentation (Ministry of Agriculture, Act 87-848, 19 Oct 1987) and approved by the ethics committee of the Institut de Radioprotection et de Sûreté Nucléaire. Male Sprague-Dawley rats (275–300 g; Charles River, L'Abresle, France) were maintained on a 12-h light-dark cycle. Animals were given standard rat chow and had free access to water.

Nonfasted animals were anesthetized by inhalation (TEM anesthesia; Limoges, France) of isoflurane (AErrane; Baxter SA, Lessines, Belgium). A single dose of 27 Gy (1.4 Gy/min) of gamma irradiation was delivered by a cobalt-60 source through a 2-x-3-cm window centered on the colorectal region.

Tissue sampling and preservation

All animals were euthanized at 10 weeks postexposure, except for animals in a preliminary study performed on day 7. Table 1 shows the treatment groups and numbers of animals per group. After animals were euthanized, the distal colon and rectum were removed and a small ring of tissue obtained from the middle of the irradiated area was kept for molecular analyses. The muscularis propria (MP) was separated from the mucosa/submucosa by gentle dissection and frozen in RNAlater RNA stabilization reagent (Qiagen SA, Courtaboeuf, France). The mucosa and submucosa were also separated but only for studies of acute radiation effects. The remaining tissue was fixed in 10% formaldehyde, and these samples were used for histological studies. Each tissue specimen was cut into three or four pieces that were 0.5 cm long. Paraffin embedding was done to allow for colorectal cross-sectioning.

Drug administration

Bosentan, which was suspended in raspberry-flavored gelatin (Jello; Kraft Foods), was administered once daily in a bolus at 10:00 A.M. Bosentan treatment (100 mg/kg/24 h) was started 2 days before irradiation and was maintained until the end of the experiment. The dose of bosentan and the pretreatment period were in agreement with conditions recommended by Actelion (3). Control rats were given the gelatin without bosentan. Because colorectal irradiation is associated with a severe reduction in food intake during the first 2 weeks postexposure, oral gavage was used during this period in animals that did not eat the gelatin.

Atrasentan, a selective and soluble ET_A receptor antagonist, was administered in the drinking water, and treatment (10 mg/kg/24 h) was started 3 days before irradiation and continued until the end