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BIOLOGY CONTRIBUTION

A PROTECTIVE ROLE FOR KERATINOCYTE GROWTH FACTOR IN A MURINE MODEL OF CHEMOTHERAPY AND RADIOTHERAPY-INDUCED MUCOSITIS

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Purpose: To evaluate the activity of palifermin (rHuKGF) in a murine model of mucosal damage induced by a radiotherapy/chemotherapy (RT/CT) regimen mimicking treatment protocols used in head-and-neck cancer patients.

Methods and Materials: A model of mucosal damage induced by RT/CT was established by injecting female BDF1 mice with cisplatin (10 mg/kg) on Day 1; 5-fluorouracil (40 mg/kg/day) on Days 1–4, and irradiation (5 Gy/day) to the head and neck on Days 1–5. Palifermin was administered subcutaneously on Days −2 to 0 (5 mg/kg/day) and on Day 5 (5 mg/kg). Evaluations included body weight, organ weight, keratinocyte growth factor receptor expression, epithelial thickness, and cellular proliferation.

Results: Initiation of the radiochemotherapeutic regimen resulted in a reduction in body weight in control animals. Palifermin administration suppressed weight loss and resulted in increased organ weight (salivary glands and small intestine), epithelial thickness (esophagus and tongue), and cellular proliferation (tongue and salivary glands).

Conclusions: Administration of palifermin before RT/CT promotes cell proliferation and increases in epithelial thickness in the oral mucosa, salivary glands, and digestive tract. Palifermin administration before and after RT/CT mitigates weight loss and a trophic effect on the intestinal mucosa and salivary glands, suggesting that palifermin use should be investigated further in the RT/CT settings, in which intestinal mucositis and salivary gland dysfunction are predominant side effects of cytotoxic therapy. © 2006 Elsevier Inc.

Keratinocyte growth factor, Oral mucositis, Epithelial thickness, Ki-67, Salivary gland.

INTRODUCTION

Chemotherapy and radiotherapy eliminate cancer cells, but their nonspecific targeting also destroys normal, healthy cells, particularly in epithelial tissues. Damage to the epithelium of the gastrointestinal tract results in a pathologic condition known as mucositis. This condition, induced by antineoplastic drugs and radiotherapy, is an important, dose-limiting, and costly side effect of anticancer therapy (1, 2). Patients with severe oral mucositis often develop ulcerations that encompass the full thickness of the epithelium and penetrate into the submucosa causing extreme pain. In addition, the clinical sequelae of localized radiotherapy include xerostomia (dry mouth), loss of taste, and increased susceptibility to bacteremia and fungemia from loss of the barrier function of the epithelium, all of which have a negative impact on a patient's quality of life (3). In the United States alone, approximately 400,000 cancer patients per year receiving chemotherapy develop oral complications and 40% of those develop some degree of oral mucositis, a condition in which destruction of the basal epithelium leads to atrophy, thinning, and ulceration of the mucosal epithelium (4). Additionally, cytotoxic regimens often cause myelosuppression, which further increases a patient's risk of developing systemic infections (5–7).

Oral mucositis (OM) is particularly prevalent in patients receiving high-dose myeloablative chemotherapy/radiotherapy regimens that are used in the hematopoietic stem cell transplant setting. The incidence of OM is also high in head-and-neck cancer patients treated with fractionated radiation, with or without chemotherapy. Treatment of patients with head-and-neck cancer presents a particular challenge because radiation therapy frequently damages the normal epithelium of the oral cavity and salivary glands.

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Oropharyngeal mucositis has been characterized as the most debilitating side effect of radiation therapy in patients with head-and-neck cancer and can become a dose-limiting factor in this cancer population (3, 8). The most common interventions for OM include good oral hygiene, topical agents for pain, and parenteral nutrition (9–13). Agents under investigation include amifostine, sucralfate, glutamine, laser therapies, and granulocyte macrophage-colony stimulating factor (GM-CSF) mouthwash (14–20).

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (FGF-7) and binds to the tyrosine kinase KGF receptor (KGFR/FGFR2IIIb), a splice variant of the FGFR2 (21). Activity of KGF is thereby limited to tissues that express KGFR. This receptor is expressed on the epithelial cells of multiple tissues, including the oral cavity, gastrointestinal tract, lung, prostrate, skin epidermis, and hair follicles (22). KGF is secreted by mesenchymal cells and acts as a paracrine mediator for normal epithelial growth and differentiation (21). The well-documented activity of KGF in diverse epithelial tissues has led to extensive evaluation of the growth factor as a potential therapeutic agent for amelioration of cytotoxic therapy-induced mucositis (23–34).

Palifermin (ΔN23KGF), a recombinant form of human KGF, differs from the endogenous human KGF through the deletion of the first 23 N-terminal amino acids, which improves protein stability. Palifermin is approved in North America, Australia, and Europe to decrease the incidence and duration of severe oral mucositis in patients with hematologic malignancies receiving myelotoxic therapy and hematopoietic stem cell transplantation. The safety and efficacy of palifermin in the nonhematologic setting have not been established. Thus the current study was designed to evaluate the activity of palifermin in a murine model subjected to a highly myeloablative radiotherapy/chemotherapy (RT/CT) regimen similar to the conditioning regimen used in the treatment of head-and-neck cancer patients.

MATERIALS AND METHODS

Recombinant human KGF production

Recombinant human KGF (palifermin) was produced in *Escherichia coli,* refolded, purified to homogeneity by conventional chromatography techniques, and tested to verify that it was endotoxin free. Palifermin was assayed in the BALB/MK keratinocyte cell line, as described previously (35). Palifermin was freshly prepared on each day of injection by reconstitution in phosphate-buffered saline to a final concentration of 0.5 mg/mL. Palifermin was administered subcutaneously at an injection volume of 0.2 mL per mouse. Dosage and route of administration were based on previous murine studies (29, 30).

Mice maintenance

Female BDF1 mice (Charles River Laboratories, Wilmington, MA) were used in all experiments. The protocols were performed at the Amgen Inc. Thousand Oaks site, which is accredited by the International Association for the Assessment and Accreditation of Laboratory Animal Care.

Radiotherapy and chemotherapy administration

Mice were injected with a single dose of intraperitoneal (IP) cisplatin (10 mg/kg; Bristol Myers Squibb, Princeton, NJ) on Day 1 at approximately 8:00 am. Cisplatin is used in the clinical setting as a radiosensitizer (36). To model multi-dose chemotherapy, mice were injected with 5-fluorouracil (5-FU) (40 mg/kg/day; Roche division Hoffmann-La Roche, Inc, Nutley, NJ) IP at approximately 9:00 AM on Days 1 to 4. This 5-FU regimen typically induces rapid loss of body weight followed by some recovery of body weight in surviving animals (29). To model clinical radiotherapy, mice were irradiated (5 Gy) at approximately 10:00 AM on Days 1 to 5 by exposure to a Gamma Cell 40 cesium source (Atomic Energy of Canada Limited, Kanata, ON, Canada). Mice were anesthetized with ketamine (Vetamine; Mallinckrodt Vet, Mundelein, IL) and xylazine (Rompu; Phoenix Scientific Inc, St. Joseph, MO) and placed in iron lead shields with only their heads exposed to the radiation to minimize whole-body exposure. The final concentration of anesthesia was ketamine 6.1 mg/mL and xylazine 0.4 mg/mL. A single dose of the drugs was administered intraperitoneally in a volume of 0.5 to 0.55 mL per mouse (the mice weighed 20–25 g). The final dose for a mouse weighing 20 g was ketamine 150 mg/kg and xylazine 10 mg/kg. The mice were euthanized by CO_2 asphyxiation on Days 1, 6, and 8. Before initiation of the RT/CT regimen, mice received either palifermin (5 mg/kg/day for 3 days) or vehicle control (saline). Mice euthanized on Day 8 received a final injection of palifermin (5 mg/kg) post-RT/CT on Day 5. This pre-RT/CT and post-RT/CT administration of palifermin is referred to as palifermin therapy.

Analysis of body weight

Mice were weighed daily to determine changes in body weight over time. Mice were monitored closely for signs of morbidity for the duration of the experiments.

Target tissues and evaluated endpoints

The small intestine and salivary glands were weighed (wet weight) to establish the effect of palifermin on organ weight. Tongue, esophagus, and salivary glands were fixed, processed, and blocked in paraffin to facilitate morphometric analysis and evaluation by *in situ* hybridization and immunohistochemistry.

Tissues retrieved included the tongue (bisected longitudinally to capture dorsal and ventral surfaces), esophagus, small intestine, and salivary glands (submandibular and sublingual). Cell proliferation in the tongue, esophagus, small intestine, and salivary glands was examined through Ki67 (Novo Castra Laboratories Ltd., Newcastle-upon-Tyne, UK) staining or bromodeoxyuridine (BrdU) (Aldrich Chem. Co., Milwaukee, WI) incorporation. For the BrdU labeling, the mice were injected with BrdU (50 mg/kg) 1 h before sacrifice for tissue harvesting.

Epithelial thickness

Epithelial thickness (μm) was measured for tongue (dorsal and ventral surfaces) and esophagus. Morphometry of epithelial thickness was performed on hematoxylin and eosin–stained tissue sections using the MetaMorph image analyzer (Universal Imaging Corporation, Downingtown, PA). The full thickness of the nonkeratinized layer from the basement membrane of the germinal layer to the interface with the keratinized nonnucleated layer of the dorsal and ventral surfaces of the tongue was divided into 21 to 24 equally spaced positions along the length of the surface in each of these regions (three to six fields per mouse and four measurements