Establishment of an X-ray Irradiation-Induced Glossitis Model in Rats: Biphasic Elevation of Proinflammatory Cytokines and Chemokines

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ABSTRACT

Oral mucositis is a frequent and serious side effect in patients who receive radiotherapy for head and neck cancer. The purpose of this study was to develop a noninvasive and quantitative model of oral mucositis in rats, investigate the pathophysiology, and evaluate the efficacy of pharmacological interventions. Rats received a single dose of 15 Gy of X-rays to the snout after shielding of the remainder of the rat body with lead plates to protect the body from irradiation (day 0). After irradiation, the macroscopic area of tongue injury gradually increased. The total area of injury and the ulcer-like area reached a maximum on day 7 and then gradually decreased until disappearance on day 28. Expression of proinflammatory cytokines and chemokines occurred transiently within 1–4 hours after irradiation and returned to a normal level at 24 hours. This expression was again observed from days 3 to 5 and increased significantly on day 7, which approximately coincided with the histologic severity of tissue damage. Subcutaneous administration of palifermin at 3 mg/kg per day for 3 consecutive days before irradiation completely prevented ulcer formation in this model. In conclusion, we established a novel model of glossitis in rats, induced by X-ray irradiation, in which biphasic elevations of expression of proinflammatory cytokines and chemokines could be monitored. This model is considered useful to investigate the pathophysiology of oral mucositis and evaluate the preventive effect of pharmacological interventions on oral mucositis induced by X-ray irradiation.

Introduction

Oral mucositis is a common and serious side effect in patients who undergo radiotherapy for head and neck cancer. Oral mucositis has a significant impact on quality of life due to severe pain, as well as difficulties in eating and swallowing (Keefe et al., 2007). Furthermore, oral mucositis may also affect anticancer therapy by limiting the ability of patients to tolerate radiotherapy or chemotherapy. However, neither effective prevention nor therapeutic medications have yet been established (Lalla et al., 2008). To develop a new, effective medication, it is necessary to establish a reproducible and quantitative animal model that enables precise evaluation of the efficacy of test drugs. A number of animal models have often been used in research into oral mucositis, including the mouse radiation model and hamster radiation model (Bowen et al., 2011). The mouse radiation model, in which the tongue and snout are exposed to radiation, was developed by Dörr et al. (2000, 2002) and is useful in investigating the mucosal response to treatment. The hamster oral mucositis model, developed and used by Sonis and colleagues (Sonis et al., 1990; Ara et al., 2008; Murphy et al., 2008), has the advantage because hamsters have buccal cheek pouches, which enable us to administer test agents topically to the buccal mucosa—many agents have already been tested using this model.

In addition to mouse and hamster models, rat radiation models have recently been developed and reported (Cassatt et al., 2002; Rezvani and Ross, 2004; Yeh et al., 2007; Kitagawa et al., 2008; Li et al., 2011). An advantage of rat models is the size of the tongue, which can be handled and quantified more easily than that of the mouse. In rat irradiation models (Li et al., 2011), oral mucositis was induced by direct X-ray irradiation of the tongue, fixed outside the body with adhesive tape, an approach that carries a high risk of causing artificial damage during the procedure. Instead of the tongue, the buccal mucosa is sometimes selected as the site for induction of oral mucositis by X-ray irradiation. It is, however, difficult to define a constant area of irradiation. Therefore, in this study, we tried to develop a sophisticated, noninvasive rat irradiation model that could be shown to induce more reproducible, quantitative damage in the form of X-ray irradiation–induced glossitis.

ABBREVIATIONS: ANOVA, one-way analysis of variance; CCL2, chemokine (C-C motif) ligand 2; CXCL1, chemokine (C-X-C motif) ligand 1; IL-1β, interleukin-1β; IL-6, interleukin-6; KGF, keratinocyte growth factor; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; rhKGF, recombinant human KGF; TNFα, tumor necrosis factor α.
Oral mucositis is defined as inflammation of the oral mucosa resulting from radiotherapy and/or chemotherapy. The pathologic progression of mucositis has been described as consisting of five phases: initiation, upregulation and generation of messenger signals, signaling and amplification, ulceration, and healing (Sonis, 2004). Histologic investigations have been reported in previous studies (Kitagawa et al., 2008; Li et al., 2011), but time-dependent alterations of pathologic and molecular status during the five phases are still largely unknown. In this study, we also investigated tongue gene expression, focusing on proinflammatory cytokines and chemokines that are believed to play a pivotal role in triggering inflammation and development of the tissue damage seen in radiation-induced oral mucositis; we also performed quantitative evaluation of macroscopic injury. Understanding the pathogenesis of oral mucositis will contribute to the invention of an effective strategy for the prevention and treatment of oral mucositis during radiotherapy in patients with head and neck cancer. No medication is approved for the prevention of oral mucositis associated with radiotherapy. Experimental therapies have been investigated, including epidermal growth factor (Sonis et al., 1992; Ryu et al., 2010), keratinocyte growth factor (KGF) (Dorr et al., 2005a,b; Kilic et al., 2007), amifostine (Cassatt et al., 2002; Cassatt et al., 2003; Fleischer and Dörr, 2006), and so on. Of these therapies, palifermin, a recombinant human KGF (rhKGF), demonstrates constant preventive effects in models of irradiation-induced oral mucositis. Palifermin is approved for prophylaxis against severe oral mucositis associated with hematopoietic stem cell transplantation in hematologic malignancies (Spiegelberger et al., 2004; Blazar et al., 2006). Recently, it was shown in randomized, placebo-controlled trials that palifermin reduces severe oral mucositis in patients undergoing chemo-radiotherapy (Le et al., 2011) and postoperative radio-chemotherapy (Henke et al., 2011) for locally advanced head and neck cancer. By use of our sophisticated, noninvasive, X-ray irradiation–induced rat glossitis model, we investigated tongue gene expression to understand the pathogenesis of oral mucositis and also examined the preventive effect of palifermin to support the validity of this animal model.

Materials and Methods

Animals. Specific-pathogen–free male Sprague-Dawley rats, 6 weeks old and weighing 200–210 g, were purchased from Charles River Japan (Osaka, Japan) and were acclimated for 1 week before the experiments. The animals were maintained on a 12-hour light/12-hour dark cycle with free access to water and standard laboratory food (CRF-1; Oriental Yeast Co., Ltd., Osaka, Japan). The care and handling of the animals were in accordance with the Guidelines for Animal Care and Use of Otsuka Pharmaceutical Co, Ltd. (Tokushima, Japan).

X-ray Irradiation for Induction of Glossitis. Eighty-seven rats were used for pathophysiological and initial-phase investigations. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 45 mg/kg. For constant irradiation around the snout alone, the face of the rat was covered with a custom-built face mask made from a plastic bottle, and then the rat with the face mask in place was shielded twice with 0.5-mm-thick lead plates to protect the body from irradiation, apart from the snout. Four animals or fewer were placed on a turntable in a shielded box, lying right-side-down as demonstrated in Fig. 1. The rats received a single dose of 15 Gy of X-rays with a vertical beam to their exposed snout using a small animal X-ray irradiation system (160 KeV, 6.3 mA, dose rate 2.18 Gy/min; model CP-160; Faxitron Biopics LLC, Tucson, AZ). The dose rate was checked on a regular basis with an ionization chamber (model TN31013; PTW, Freiburg, Germany), which was connected to a dosimeter (model 35040; Fluke Biomedical, Everett, WA).

For the pathophysiological investigation, 44 animals received X-ray irradiation (set as day 0) and were divided into 11 groups. Fifteen nonirradiated animals were divided into five groups. Body weights were measured throughout the experiment. Four irradiated animals were euthanized by exsanguination after blood collection under isoflurane anesthesia, and tongue specimens were collected on days 1, 3, 5–10, 14, 21, and 28. Three nonirradiated animals were treated similarly to the irradiation groups, and tongue specimens were collected on days 1, 7, 14, 21, and 28. To investigate initiation-phase events in this model, 24 animals that underwent X-ray irradiation were divided into six groups and had tongue specimens collected at 1, 2, 4, 6, 8, and 24 hours. Four nonirradiated rats were used as controls.

Quantification of Tongue Injury during the Experiment. The tongues were excised by incision at the root; rinsed with saline, with removal of excess water using a paper towel; and then flattened and photographed using a digital camera, with a scale. The digital images were analyzed with image analysis software (WinROOF; Mitani Co., Tokyo, Japan), and the following three areas were measured: total area, which is the area from the front of the intermolar eminence to the tip (“Tongue surface in Fig. 2); total injured area, which is the discolored area (“Total injured” in Fig. 2); and ulcer-like area, which is the brilliant red-colored area (“Ulcer-like” in Fig. 2). The injured and ulcer-like areas underwent confirmatory histologic observation (Fig. 2).

Hematologic Analysis. Under isoflurane anesthesia, blood samples were collected into an EDTA tube from the abdominal inferior vena cava on each sampling day. Hematologic analysis was carried out using an automated hematologic analyzer (XT-2000i; Sysmex Corporation, Kobe, Japan).

Fig. 1. Method of X-ray irradiation to rats. Four rats were simultaneously exposed with X-ray at the same time. Anesthetized rats were placed on the turntable and irradiated around the left-side snout alone after being covered with a custom-built plastic bottle and lead plates as shown in the picture. The rats received a single dose of 15 Gy of X-rays to their exposed snout.
Histopathological Observation of the Tongue Injury. The animals were euthanized by exsanguination after blood was collected. The left half of the tongue was separated by a longitudinal cut along the midline and used for histopathological evaluation. The tongue specimens were fixed with 10% buffered formalin and embedded in paraffin. Sections (3 μm) were stained with hematoxylin and eosin and with Azan, using standard methods for morphologic analyses and for the detection of interstitial collagen deposition, respectively.

Preparation of Tongue RNA and Real-Time Polymerase Chain Reaction. According to the five-phase theory of Sonis et al. (Sonis, 2004; Sonis et al., 2004), reactive oxygen species (ROS) and inflammatory cytokines play pivotal roles in cancer therapy–induced oral mucositis. In our study, changes in the expression of mRNA for inflammatory cytokines were determined during the appearance and disappearance of X-ray irradiation–induced glossitis as well as during the initial phase before the appearance of glossitis. The tip of the right half of the tongue was obtained by incision ~5 mm from the tip, immediately immersed in RNAlater (Ambion/Life Technologies, Austin, TX), and then stored at −20°C. Each of the normal and irradiated tongue specimens, approximately 100 mg in weight, was immersed in RNAlater and transferred into a plastic tube (3-ml volume) with 600 μl of RLT buffer (Qiagen, Valencia, CA) and stainless steel beads (Yasui Kikai Corporation, Osaka, Japan). The sample was automatically crushed at 1900 rpm for 1 minute using a cell disruptor (Multi-beads shocker; Yasui Kikai Corporation) according to the manufacturer’s instructions. The total RNA was extracted from the supernatant of the crushed mixture with an RNeasy Mini Kit (Qiagen). The quality and concentration of total RNA was checked by spectrophotometer. cDNA was synthesized with MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA), and real-time polymerase chain reaction (PCR) was performed using Applied Biosystems 7500 Fast Real-time PCR System. The reaction mixture was prepared with TaqMan Gene Expression Primer and Probe (Applied Biosystems) according to the manufacturer’s protocol. The thermal cycling conditions were at 95°C for 20 seconds, followed by 40 cycles of amplification at 95°C for 3 seconds and 60°C for 30 seconds. The expressions of mRNA for interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), chemokine (C-X-C motif) ligand 1 (CXCL1) and chemokine (C-C motif) ligand 2 (CCL2) were standardized to β-actin mRNA, and the relative expression of each gene was quantified by the ddCt method as ratios to the mean value for normal tongue tissues.

Preventive Effects of Palifermin on X-ray Irradiation-Induced Rat Glossitis. Palifermin (tradename Kepivance), a human recombinant keratinocyte growth factor (rhKGF), was purchased from Amgen (Thousand Oaks, CA) and was dissolved in sterilized phosphate-buffered saline (PBS; pH 7.4; Sigma-Aldrich, St. Louis, MO) at concentrations of 3 and 10 mg/ml. Palifermin solution was divided among cryotubes and stored at −20°C until usage. Thirty-two animals were allocated to four groups: subcutaneous administration of PBS (Control-1) or 10 mg/kg palifermin once on the day before irradiation (Palifermin-1); and subcutaneous administration of PBS (Control-2) or 3 mg/kg palifermin once a day for 3 days (Palifermin-2) before X-ray irradiation, according to a previous report (Gibson et al., 2005).

Glossitis was induced by 15 Gy X-ray irradiation to the snout as described above. Seven days after irradiation, the rats were euthanized by exsanguination under isoflurane anesthesia, and the tongue specimens were collected. The preventive effects of palifermin were evaluated by comparing the total injured area ratio and the ulcer-like area ratio with those of the corresponding control groups, calculated by the following formula:

Total injured area ratio (percentage) = injured surface area (indicated as “Total Injured” in Fig. 2)/tongue surface area (indicated as “Tongue surface” in Fig. 2) × 100

Ulcer-like area ratio (percentage) = ulcer-like area (indicated as “Ulcer-like” in Fig. 2)/tongue surface area × 100

Statistical Analysis. Data are presented as the mean ± S.E.M. The one-way analysis of variance (ANOVA) Dunnett’s test was used to determine the significance of differences in the tongue injury area and the levels of mRNA for proinflammatory cytokines and chemokines in the time-course studies. Statistical significance between two groups was determined by a t test using SAS software (Release 9.1; SAS Institute, Tokyo, Japan) in the study of the preventive effects of palifermin. P < 0.05 was considered significant.

Results

Changes in Body Weight after X-ray Irradiation and Hematologic Findings. Six days after X-ray irradiation to the snout, a decrease in body weight was observed (day 6); this began to recover from day 10 (Fig. 3). In parallel with the period of decreasing body weight, food consumption and water intake were also reduced; these reductions continued until day 14 (data not shown).

White blood cell counts in the irradiation group dropped transiently on day 7, with full recovery after day 14. During the experiment, no significant changes were observed in red blood cell count, hemoglobin concentration, or hematocrit (data not shown).

Pathophysiological Observation of X-ray Irradiation-Induced Glossitis. Pathophysiological changes in the tongue and buccal mucosa were evaluated macroscopically and histologically. No significant macroscopic changes were observed in the irradiated tongue, buccal mucosa, or whisker pads until day 4. Five days after irradiation, reddening became apparent at the tip of the tongue, and this color change was associated with slight loss of fur and dull fur over the whisker pads. We chose to further examine tongue injuries because macroscopic injuries to the buccal mucosa were slight during this investigation. The area of tongue injury increased gradually, reaching a peak on day 7, associated with the appearance of an
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Fig. 3. The time course of body weight after X-ray irradiation. Each bar represents mean ± S.E.M. The rats received a single dose of 15 Gy X-ray irradiation on day 0. Nonirradiated group: day 0 (n = 15), day 3 (n = 12), day 5 (n = 12), day 6 (n = 12), day 7 (n = 12), day 8 (n = 9), day 9 (n = 9), day 10 (n = 9), day 15 (n = 6), day 20 (n = 6), day 27 (n = 3). X-ray group: day 0 (n = 48), day 3 (n = 44), day 5 (n = 40), day 6 (n = 36), day 7 (n = 32), day 8 (n = 28), day 9 (n = 24), day 10 (n = 20), day 15 (n = 8), day 20 (n = 8), day 27 (n = 4).

Fig. 4. The time course of changes in tongue surface area, total injured area and ulcer-like area after X-ray irradiation. The tongue was excised at the root and photographed using a digital camera with a scale. Three areas were measured with image analysis software: tongue surface area, total injured area, and ulcer-like area. Tongue surface area of nonirradiated group was also shown as a control. Each bar represents mean ± S.E.M. Normal group, n = 3. X-ray group, n = 4.

ulcer-like injury (Fig. 4). After day 7, both the area of total injured and ulcer-like areas decreased gradually and had completely disappeared on day 28. Tongue surface areas (“Tongue surface” in Fig. 2) did not change in the nonirradiated group, but those of the irradiation group decreased in parallel with the tongue injury, indicating tongue atrophy. To avoid underestimation of the tongue injury due to this atrophic change, we used ratios rather than absolute areas of total injury and ulcer-like injury, in evaluating markers of the preventive effects of the drug. Histologic observation showed thinning of the keratin layer and squamous epithelium of the tongue on day 5 and almost entire disappearance of the epithelial layers on day 7 (Fig. 5A). Marked infiltration by inflammatory cells was also seen deep to the near-absent epithelial layers on day 7 (Fig. 5B). Subsequently, re-epithelialization gradually started to cover the injured mucosa with almost complete regeneration of the epithelium by day 21.

Changes in Gene Expression for IL-6, IL1β, TNFα, CXCL1, and CCL2. Following the X-ray irradiation of the rats’ snouts, gene expression of inflammatory cytokines (IL-6, IL-1β, and TNFα) and chemokines (CXCL1 and CCL2) was measured in the tips of their tongues. After irradiation, expression of all mRNAs tested for gradually increased until day 6 and then became markedly elevated on day 7, approximately 950-fold for IL-6, 330-fold for IL-1β, 10-fold for TNFα, 200-fold for CXCL1, and 12-fold for CCL2, compared with the nonirradiated group (Fig. 6). Subsequently, IL-6 mRNA expression decreased rapidly on day 8, with gradual decreases in IL-1β, TNFα, CXCL1, and CCL2. Twenty-eight days after irradiation, macroscopic tongue injuries had disappeared; however, the levels of expression of genes for cytokines and chemokines were slightly elevated, with the exception of IL-6.

To investigate the initial phase of X-ray irradiation, the expression levels of the same genes were determined during the 24 hours after irradiation. IL-6 mRNA expression gradually increased and peaked at 4 hours after irradiation at 30 times the normal level (Fig. 7). In contrast, the levels of expression of mRNA for the other cytokines and chemokines were elevated 1 hour after irradiation.

Preventive Effects of Palifermin on X-ray Irradiation-Induced Rat Glossitis. Although there is currently no established form of prevention and/or treatment of radiation therapy-induced oral mucositis, palifermin, an rhKGF, is the most likely candidate, as it has been shown to reduce severe oral mucositis during chemo-radiotherapy for patients with head and neck cancer in two randomized, controlled clinical trials (Henke et al., 2011; Le et al., 2011), and many preclinical studies have shown positive results.

In Control-1, the ratios of the total injured area and ulcer-like area were 39.6 and 14.0%, respectively, in the X-ray irradiation–induced glossitis model. Palifermin (10 mg/kg s.c.), administered once 1 day before irradiation, slightly reduced the ratios of these areas to 36.9 and 12.5%, respectively, but failed to show significant inhibition (Fig. 8). Conversely, 3 mg/kg palifermin per day given on each of the 3 days before irradiation significantly reduced the total injured area ratio (81.9% reduction, P < 0.01) and completely protected against ulcer-like injury (100% reduction, P < 0.01).

Discussion

Oral mucositis is a serious side effect in patients with head and neck cancer who receive radiotherapy or chemo-radiotherapy and leads to significant impairment of quality of life as well as discontinuation of cancer therapy, prolonged hospitalization, and death. A reproducible, quantitative animal model is an absolute requirement for the development of effective medications. For this purpose, we introduced X-ray irradiation–induced injury to the rat tongue as a model of oral mucositis. Before deciding on the method used in this study, many preliminary trials were undertaken to determine a precise, highly reproducible way of inducing glossitis. Our trials included creation of shielding devices, examination of the irradiation dosage, evaluation of methods for quantitation of injuries, and the timing of evaluation. Based on concepts similar to ours, Li et al. (2011) recently developed a radiotherapy-induced model...
of glossitis in rats, in which an anesthetized rat was covered with a cone-shaped lead device and the tongue was fixed to the outer surface of the device using adhesive tape. This method is an improvement in the production of reproducible glossitis that can be quantified; however, artifactual tongue damage may be unavoidable as the tongue is placed on the device using forceps and fixed with adhesive tape. Furthermore, a single 30-Gy dose of X-ray irradiation is fairly large when compared with previous reports.

In this study, we were able to induce noninvasively a quantifiable glossitis in rats without direct contact with the tongue. To achieve constant X-ray exposure to the snout, face masks were shaped using plastic bottles as described under Materials and Methods. The face was covered with this custom-built mask, and the body was shielded with lead plates except for the snout (Fig. 2). Keeping the left side upwards was also important in the induction of reproducible glossitis. When the rat is positioned on its stomach or back, the skull or jaw, respectively, prevents the tongue from receiving constant X-ray irradiation. No signs of irradiation-induced damage were observed until days 3 or 4 when an area of erythema started to appear at the tip of the tongue, accompanied by slight loss of fur and dull fur over the whisker pads.

In the present study, we were able to observe macroscopic and histologic damage continually. No change in body weight was observed in irradiated rats until day 5. It decreased between days 7 and 10 associated with decrease in the amounts of food consumption and water intake, and then gradually recovered to normal (data not shown). Although the delayed occurrence of oral mucositis has been reported in previous studies (Murphy et al., 2008; Mangoni et al., 2009; Li et al., 2011), this is the first report to demonstrate delayed weight loss. The cause of this delayed weight loss is considered to be decreased food consumption as a result of oral mucositis, as food consumption and water intake decreased in parallel with the degree of tongue damage. In addition, the absence of significant hematologic changes after irradiation suggested that irradiation with lead shielding all but the snout minimized whole body damage.

The total area of injury peaked on day 7, accompanied by appearance of an ulcer-like injury, which was confirmed by histologic assessment (Fig. 3). The timing to appearance of ulceration following radiation exposure should be associated with the cell cycle of lingual epithelial cells (Vissink et al., 2003), in which X-ray irradiation–induced DNA damage arrests cell proliferation. In the previous study of Li et al. (2011), the maximal glossitis score was observed 14 days after irradiation, related to the fusion of pre-existing ulcers. The discrepancy in timing of ulceration between the current study and previous study might be due to differences in dosage and techniques of X-ray exposure. In our study, total and ulcer-like areas of injury gradually decreased after day 7, and macroscopic injury had disappeared by day 28. These changes were confirmed by histopathological evaluation. Although it is very important to examine histologic changes when investigating the pathophysiology of oral mucositis, few researchers have studied time-dependent changes. The regenerated epithelium was much thicker than normal (Fig. 5A), which was also reported in X-ray irradiation–induced skin injury in rats (Takikawa et al., 2012). Cell proliferation and apoptotic changes should be examined in a further study.

It has been assumed that cancer therapy–induced mucositis develops via five pathophysiological phases: initiation, upregulation and generation of messenger signals, signaling and amplification, ulceration, and healing (Sonis, 2004). In the initial phase, free radicals and reactive oxygen species, which are generated by chemotherapy or radiotherapy, are believed to play an important role. In addition to directly damaging cells and tissues, they stimulate a number of transcription factors, which lead to the production of proinflammatory cytokines and chemokines (Logan et al., 2007). In the current study, we found that gene expression of these cytokines (IL-6, IL-1β, and TNFα) and chemokines (CXCL1 and CCL2) increased transiently and peaked within 1–4 hours after X-ray irradiation without any macroscopic or microscopic tissue damage. To our knowledge, this is the first report to show transient increases of cytokines and chemokines in the early phase of X-ray irradiation–induced glossitis in rats. This finding suggests that suppression of this transient elevation of cytokines and chemokines may be a good strategy to prevent radiotherapy-induced oral mucositis. Subsequently, marked increases in expression of genes for these cytokines and chemokines were detected on day 7. The increase coincided with the appearance of ulceration and inflammation. Among these findings, the expression of IL-6 mRNA increased more than 300 times compared with normal levels. Protein levels measured by enzyme-linked immunosorbent assay
were increased (data not shown). Since marked infiltration with inflammatory cells was observed on histologic evaluation, the IL-6 produced is thought to be derived from these inflammatory cells. IL-6 gene expression dropped sharply on day 8 and then gradually decreased until day 28. This change reflects the severity of the lesions, as it was related to the process by which ulcers and injury healed on histologic evaluation.

**Fig. 6.** Gene expression analysis of IL-6 (A), IL-1β (B), TNFα (C), CXCL1 (Gro1) (D) and CCL2 (MCP-1) (E) from days 1 to 28 after irradiation. The expressions of mRNA for IL-6, IL-1β, TNFα, CXCL1, and CCL2 were standardized to β-actin mRNA and the relative expression of each gene was quantified by the ddCt method. The mean expression levels of nonirradiated tongue tissues (N) were normalized to 1. Each bar represents mean ± S.E.M. for samples of four except for the nonirradiated group (N) where there were three samples. *P < 0.05, **P < 0.01 versus the nonirradiated group (one-way ANOVA, Dunnett’s test).
As described in the Introduction section, palifermin (recombinant human KGF) has been shown to reduce radiation- and chemo-radiation–induced oral mucositis in various experimental models and clinical settings (Dorr et al., 2005a, b; Borges et al., 2006; Jaal et al., 2010; Henke et al., 2011; Le et al., 2011). In addition, human and rat KGF are very similar with great
homology, 89.4% at the DNA and 90.2% at the protein level. Accordingly, we selected palifermin to demonstrate the utility of our newly created model of rat glossitis. Interestingly, administration of 3 mg/kg s.c. palifermin, once a day for 3 days, but not 10 mg/kg s.c. once on the third day, markedly reduced the total area of injury and completely protected against ulcer-like injury. Gibson et al. (2005) reported that 10 mg/kg palifermin given once was superior to 3 mg/kg administered three times, to reduce irinotecan-induced diarrhea in Dark Agouti (DA) rats. We are unable to explain the exact reason for this discrepancy, but our results match a report that keratinocyte growth factor administered on a daily basis three or more times resulted in a dramatic increase in the thickness of oral epithelia and protected against radiation-induced cytotoxicity (Farrell et al., 1999). As with many previous animal models, palifermin markedly reduced tongue injury in our novel rat glossitis model, and this finding supports the idea that our glossitis model is appropriate for investigation of the pathophysiology of oral mucositis induced by X-ray irradiation and in evaluating the preventive effect of pharmacological interventions.

In conclusion, by providing single X-ray irradiation of the snout in rats, we have successfully developed a novel model of oral mucositis that is highly reproducible and permits quantitation of the degree of injury. In addition, the expression of inflammatory cytokines and chemokines in the tongue increased transiently within 24 hours after irradiation and became markedly increased on day 7. The latter increase was strongly associated with the appearance of ulceration. Our model of oral mucositis is expected to be useful in the evaluation of new therapies as well as for elucidation of the pathogenesis of mucositis associated with radiation therapy or chemo-radiation therapy for head and neck cancer.

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