Allopurinol Gel Mitigates Radiation-induced Mucositis and Dermatitis

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It has not been verified whether allopurinol application is beneficial in decreasing the severity of radiation-induced oral mucositis and dermatitis. Rats were divided into 4 groups and received 15 Gy irradiation on the left whisker pad. Group 1 received only irradiation. Group 2 was maintained by applying allopurinol/carrageenan-mixed gel (allopurinol gel) continuously from 2 days before to 20 days after irradiation. Group 3 had allopurinol gel applied for 20 days after radiation. Group 4 was maintained by applying carrageenan gel continuously from 2 days before to 20 days after irradiation. The intra oral mucosal and acute skin reactions were assessed daily using mucositis and skin score systems. The escape thresholds for mechanical stimulation to the left whisker pad were measured daily. In addition, the irritated tissues at the endpoint of this study were compared with naive tissue. Escape threshold in group 2 was significantly higher than that in group 1, and mucositis and skin scores were much improved compared with those of group 1. Concerning escape threshold, mucositis and skin scores in group 3 began to improve 10 days after irradiation. Group 4 showed severe symptoms of mucositis and dermatitis to the same extent as that observed in group 1. In the histopathological study, the tissues of group 1 showed severe inflammatory reactions, compared with those of group 2. These results suggest that allopurinol gel application can mitigate inflammation reactions associated with radiation-induced oral mucositis and dermatitis.

INTRODUCTION

Radiotherapy is indispensable as a form of therapy for mitigating malignant tumors in the oral region. However, oral mucositis and facial dermatitis are common acute side effects of radiotherapy, and they are the typical dose-limiting factors of radiotherapy.\textsuperscript{1-6} When patients receive radiotherapy to head and neck malignancies, oral mucositis is accompanied by severe pain, and as a result, have substantial effects limiting food intake.\textsuperscript{3,4,6} Dermatitis induced by irradiation leads to acute reaction in the skin, such as erythema, epilation, dry desquamation with or without hyperpigmentation, moist desquamation, and erosion.\textsuperscript{5} Both mucositis and dermatitis arose from radiotherapy for head or neck are the most common side effects leading to severe discomfort caused by intense pain, swallowing disorder, and impairment in eating, speaking and ingestion.\textsuperscript{6,7} These two side effects may have adverse effects on radiotherapy of cancer and patient survival.

In addition to radiation treatment, oral mucositis is frequently observed after administration of the anti-cancer drug 5-fluorouracil (5-FU) in patients with a variety of malignancies. At present, various therapeutic agents, involving antioxidant, anti-inflammatory and cytoprotective drugs, are used to treat radiation-induced oral mucositis in the orofacial region.\textsuperscript{8-12} Allopurinol, which is known to inhibit xanthine oxidase as well as proteases, is an efficient remedy against oxidase as well as proteases, is an efficient remedy against the reduction in 5-FU-induced oral mucositis, and studies on its effects are mostly based on the reduction in 5-FU-induced oral mucositis.\textsuperscript{13-17} In a previous study, allopurinol mouthwash regime has no protective effect on 5-FU-induced oral mucositis.\textsuperscript{18} However, recent studies have been reported that a mouthwash containing allopurinol is used as a clinical remedy for both the radiation-induced and 5-FU-induced oral mucositis.\textsuperscript{19} Furthermore, a compress containing allopurinol or an allo-
purinol solution is applied to radiation-induced dermatitis, and such treatments are considered to mitigate both erythema and dermatitis-related pain. However, there are no studies to examine the effects of allopurinol gel on radiation-induced mucositis and dermatitis. Indeed, there is evidence that allopurinol/carrageenan-mixed gel (allopurinol gel) had a potential to release allopurinol over 8 hr.\textsuperscript{29}

This study was designed to examine the efficacy of allopurinol gel on radiation-induced oral mucositis and facial dermatitis, in which rats who received 15 Gy of irradiation to the left face were used as the radiated model. We assessed skin reactions using the skin score system as well as the extent of oral mucositis using the mucositis system.\textsuperscript{21,22} We also evaluated the extent for escape threshold regarding the dermatitis-related pain, in which mechanical stimuli using von Frey filaments were applied to the radiation-induced facial dermatitis. In addition, assessments of skin and mucosal reactions were examined histopathologically after irradiation without and with allopurinol gel treatment.

**MATERIALS AND METHODS**

**Animals**

Experiments were performed with 27 male Sprague-Dawley rats, weighing 180–230 g in total. For radiation experiments, 24 rats were used. Of these, 4 rats died before the finish of experiments due to unknown reasons. For the histopathologic study, 3 naïve rats were used as control. Rats were raised under pathogen-free conditions and fed ad libitum. They were housed individually and maintained according to the 12 hr light/12 hr dark schedule. The room temperature was kept at 23°C.

The allopurinol gel was made from mixing allopurinol powder (Wako, Osaka, Japan) with κ-carrageenan powder (Wako, Osaka, Japan) at a weight ratio of 1:10, and then added to physiological saline.

The rats were randomly divided into 4 groups (each number = 5). All rats were immobilized by anesthetization with pentobarbital sodium (50 mg/kg, i.p.; Dainippon Pharma Co., Ltd., Osaka, Japan), lain on their side (the left of the body toward the top) and received a single dose of 15 Gy of irradiation with vertical beam (source: x-ray, source-to-surface distance: 80 cm, the size of the radiation field: 3 × 3 cm, dose rate: 0.68 Gy/min, dose calculation: central axis at a depth of 1 cm) to the entire left whisker pad using an x-ray irradiation system (MBR-1520R-3: Hitachi, Tokyo, Japan). These conditions were decided by previous reports.\textsuperscript{21,22} The animal’s body except for the radiation field was covered with lead during irradiation. Treatment of the 4 groups of rats before and after irradiation were as follows: group 1 received no treatment; those of group 2, application of the allopurinol gel to the left whisker pad 2 days before irradiation and the continuous application of the allopurinol gel to the left whisker pad daily for 20 days after irradiation; those of group 3, continuous application of the allopurinol gel to the left whisker pad for 20 days after irradiation; and those of group 4, continuous application of the carrageenan gel, which was prepared by the addition of κ-carrageenan powder to physiological saline, to the left whisker pad from 2 days before irradiation to 20 days after irradiation. The oral mucositis and skin reaction were assessed daily by using the mucositis score system and the skin score system, respectively.\textsuperscript{21,22} These assessments for the tissue reactions were kept score in awake conditions.

The allopurinol gel or carrageenan gel (quantity: one tea spoonful of the gel for whisker pad, half tea spoonful of the gel for intraoral region) was applied daily to the skin of the left whisker pad using a toothbrush as well as being applied to the intraoral region using a cotton swab. The quantity of gel was enough for applying to the inflammatory lesions. These gels were used at room temperature (23°C approximately). Application of the gels to the left whisker pad and intraoral regions was performed after measurement of the mucositis and skin scores as well as after behavioral examination.

Experiments were approved by the Animal Use and Care Committee of the Nippon Dental University and were consistent with the ethical guidelines of the International Association for the Study of Pain.\textsuperscript{21}

**Mucositis score system**

The mouths of the rats in all groups were observed daily to assess the sign of both mucositis and erythema. The extent of mucositis was gauged according to the mucositis score system\textsuperscript{22} as follows: Score 0 = normal, Score 0.5 = slightly pink, Score 1 = slightly red, Score 2 = severe reddening, Score 3 = focal desquamation, Score 4 = exudation or crusting covering less than one-half of lip, and Score 5 = exudation or crusting covering more than one-half of lip. This score was recorded daily from 2 days before irradiation to 20 days after irradiation.

**Skin score system**

Whisker pad skin reactions of rats in all groups were assessed daily for 20 days following irradiation. In this study, the skin score system described previously\textsuperscript{22} was used. Namely, Grade 0 = normal, Grade 0.5 = slight epilation, Grade 1 = epilation of about 50% of the area, Grade 1.5 = epilation of more than 50%, Grade 2 = complete epilation, Grade 2.5 = complete epilation with definite edema or dry desquamation in the area exceeding 50%, Grade 3 = moist desquamation in a small area, and Grade 3.5 = moist desquamation in of the majority of the area.

**Behavioral test**

We performed behavioral test using von Frey filaments to assess dermatitis-related pain. Behavioral test using von
Frey filaments has established as a method to measure pain in the study of pain. Many experiments for behavioral test using von Frey filaments have been performed. 24–26

In daily sessions, all rats were trained to keep their snout protruded through a hole in the cage wall during mechanical stimulation of the whisker pad area using von Frey filaments (Touch-Test™; North Coast Medical, Inc., CA, USA) for 1 week. After successful training, behavioral tests were performed on all groups. The mechanical escape thresholds of the left whisker pad area were measured from 2 days before irradiation to 20 days after irradiation. Each von Frey stimulation was applied 5 times. The escape threshold intensity was determined when rats moved their heads away from at least one of the 5 stimuli. The von Frey mechanical stimuli were applied to the whisker pad in ascending and descending orders during the trials in order to evaluate the escape threshold intensity. The median escape threshold intensity was calculated from the values after 2 ascending trials and 1 descending trial.

**Histopathological assessments**

For the histopathological study, some irradiated rats without and with allopurinol gel application were anesthetized with 50mg/kg ketamin HCl (Sankyo Yell Co., Ltd., Tokyo, Japan) at day 20 and the irradiated fields were obtained from whisker pad region and tongue. The tissues samples were fixed in 10% formalin. After routine processing, the tissues were imbedded in paraffin wax. Four-μm-thick slices were prepared and stained with hematoxylin and eosin for evaluation with light microscopy. The tissue damages were evaluated synthetically, using damage of the acute oral mucosal reactions (degeneration and vacuolar alteration of the basal layer, congestion and inflammatory infiltration in submucosa, and alteration of cell changes in stratified squamous epithelium) and that of the acute skin reactions (epidermal atrophy, collagen fiber loss, and hair follicle atrophy) in terms of percentages, which scored on a 5-points ordinal scale as follows; Grade 0 = Normal, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, Grade 4 = marked, and Grade 5 = Severe. These methods were referred to previous studies. 21,22

**Statistical analysis**

Statistical analysis was performed using ANOVA followed by Tukey’s test for the behavior data, skin score data and mucositis score data. Results were represented by mean ± SEM. Differences were considered as significant at $p < 0.05$.

**RESULTS**

**Mucositis score**

The finding of mucositis began in 5 of 5 rats in group 1 and 4 of 5 rats in group 4 on day 4 after irradiation. It began in 2 of 5 rats in group 2 on day 8 and 5 of 5 rats in gruppe 3 on day 6. In group 1, all 5 rats showed score 2 (severe reddening) on day 8 and score 5 (exudation or crusting covering more than one-half of lip) on day 18. In group 2, all rats showed score 0.5 (slightly pink) on day 14 and 3 of 5 rats showed score 1 (slightly red) on day 18. In group 3, 3 of 5 rats showed score 1 on day 10 and 2 of 5 rats showed score 2 on day 14. In group 4, all rats showed score 4 (exudation or crusting covering less than one-half of lip) on day 12 and score 5 on day 18.

Figure 1 shows the time course of mucositis scores in each group. The mucositis scores in group 1 and 4 started to increase on day 4, and the progression of mucositis was almost the same for the 2 groups. Some rats in group 1 and 4 showed severe intraoral conditions (exudation or crusting covering less than one-half of the lip) on day 10, and extremely severe intraoral conditions (exudation or crusting covering more than one half of the lip) were observed on day 18. The increase in the rates of the mucositis score of group 1 and 4 were significantly higher than those of group 2 and 3.

**Skin score**

The finding of dermatitis began in 3 of 5 rats in group 1, 2 of 5 rats in group 3 and 2 of 3 rats in group 4 on day 4 after irradiation. It began in 2 of 5 rats in group 2 on day 8. In group 1, all 5 rats showed more grade 1 (epilation of about 50% of the area) on day 10 and grade 2 (complete epilation) on day 16. In group 2, all rats showed grade 0.5 (slight epilation) on day 14 and 3 of 5 rats showed score 1 (slightly red) on day 18. In group 3, all rats showed grade 0.5 on day 8 and grade 1 on day 12. In group 4, all rats showed grade 0.5 on day 6 and grade 2 on day 18. We could not observe a remarkable change in the right whisker pad.

Figure 2 shows the time course of the skin scores of the
rats in all groups. The skin scores of group 1, 3 and 4 started to increase on day 2. The rate of the skin score increase for group 1, being similar to that of group 4, tended to be higher than those for group 3 and 4. Some rats in group 1 and 4 showed complete epilation on the left whisker pad area on day 16. The skin scores of the rats in group 2 began to increase on day 8, their rates being the lowest among the rats in all groups. In addition, there were significant differences in the rate of increase of the skin score between the rats in group 1 and those of group 2. Rats in group 2 showed weak epilation on day 10, and epilation in approximately 50% of those animals was observed on the left whisker pad area.

**Behavioral test**

Figure 3 shows the mechanical threshold needed to evoke escape behavior as induced by mechanical stimulation of the whisker pad. Sudden decrease in the escape threshold of rats in group 1 was observed on day 4 after irradiation, and this reduction level lasted until day 20 after irradiation. Rats in group 4 showed a low escape threshold as was observed with the rats in group 1. Throughout the examination period the escape threshold of rats in group 2 decreased slowly after irradiation, in comparison with the rats of group 1. The escape threshold of rats in group 3 decreased suddenly on day 4 after irradiation, the same as that of group 1. This
reduction lasted for day 8 and the escape threshold after day 10 significantly increased slowly as compared with that of group 1. There was a significant difference between groups 2 and 3 regarding the reduction in escape threshold observed during the examination period.

**Histopathological assessments**

Figure 4 shows the typical samples of the whisker pad skin and intraoral mucosa of naïve rat (A and B), group 1 (C and D) and group 2 (E and F). The tissues of whisker pad region in group 1 damaged such as epidermal atrophy, edema and hair follicle atrophy (C). The slice of the intraoral mucosa in group 1 indicated epithelium loss, degeneration, vacuolar alteration of the basal layer, and inflammatory infiltration in submucosa (D). Whisker pad skin in group 2 damaged slight epidermal atrophy but had survival hair follicles (E). Survival epithelium and slight inflammatory infiltration were observed in the intraoral mucosa in group 2 (F). The order of tissue damages caused by irradiation was, as follows; group 1 = group 4 (synthetic histopathological value; skin: 4.5 ± 0.3, mucositis: 4.0 ± 0.6) > group 3 (skin: 2.0 ± 0.6, mucositis: 2.3 ± 0.3) > group 2 (skin: 1.3 ± 0.3, mucositis: 1.3 ± 0.3).

**DISCUSSION**

The present study, based on observation of the intraoral region and whisker pad skin of rats receiving 15 Gy of irradiation, showed that the application of allopurinol gel worked effectively in the mitigation of radiation-induced oral mucositis and facial dermatitis. In addition, the results of the escape threshold by mechanical stimulation to the irradiated whisker pad suggest that dermatitis-related pain was mitigated by allopurinol gel application, as compared with the results observed in rats without allopurinol gel treatment and those treated with carrageenan. This was further confirmed by evidence demonstrating that the tissue damages of the whisker pad region, which were represented by epidermal atrophy, edema and hair follicle atrophy, were not observed in irradiated rats with allopurinol gel pretreatment.

Radiation is known to generate reactive oxygen species, such as superoxide radicals, hydrogen peroxides and hydroxyl radicals, and they cause injury in cells, leading to mucositis occurrence. Injurious effects of radiation on the skin are resultant from the production of free radicals as well as the release of inflammatory mediator/cytokines. Allopurinol is well known to inhibit xanthine oxidase, orotidylate decarboxylase, and proteases, and also shows an antioxidant effect, consequently reducing the production of active oxygen. From histopathological experiments, these effects may inhibit either mucositis or dermatitis. Therefore, allopurinol is able to mitigate dermatitis-related pain. In this study, we used the allopurinol gel prepared by mixing allopurinol both κ-carrageenan and a physiological saline. According to this gelation, allopurinol remains more stably and for a longer duration than allopurinol solution on both the whisker pad and intraoral region.

In the present study, application of allopurinol gel mitigated radiation-induced oral mucositis and facial dermatitis as well as dermatitis-related pain in comparison with the results seen in rats without treatment with allopurinol and/or carrageenan gel. It is likely that application of allopurinol gel before irradiation was more effective for preventing mucositis, dermatitis and pain. Accordingly, allopurinol may be available for treatment of the radiation-induced tissue inflammation, particularly when its application was made before the appearance of tissue injury seen after irradiation. In conclusion, the present study clearly demonstrated that application of allopurinol in the form of allopurinol/carrageenan-mixed gel (allopurinol gel) at the point of pre-irradiation was very effective in the mitigation of radiation-induced oral mucositis and facial dermatitis as well as dermatitis-related pain.

**REFERENCES**


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