# A Rat Model of Oral Mucositis Induced by Cancer Chemotherapy for Quantitative Experiments

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**Abstract.** *Background/Aim: Oral mucositis, which occurs frequently in the treatment of cancer, is a major problem. In this study, we aimed to develop a rat model of oral mucositis induced by cancer chemotherapy for quantitative measurement. Materials and Methods: A model animal of oral mucositis was prepared by injecting an acetic acid aqueous solution into the buccal mucosa of rats to which a 5-FU solution had been previously administered. The doses of 5-FU and acetic acid were examined, and a treatment experiment using Kenalog® was performed. Results: The optimal dose of the 5-FU solution and the optimal concentration of the acetic acid aqueous solution were 40 mg/kg and 25%, respectively. Treatment with Kenalog® confirmed that this model mimics immunocompromised oral mucositis. Conclusion: Compared with a mouse model, oral mucositis can be easily observed in this model and provides a large amount of oral mucosal tissue.*

Chemotherapy and radiation therapy play important roles in cancer treatment. However, they affect highly proliferating tissues and need to be considered for side effects. Oral mucositis, one of the most common side effects of these treatments, is a serious problem to be solved, because it reduces the patients' quality of life and also affects the compliance with anticancer therapies (1-3). Many model animals of oral mucositis have been studied to overcome oral mucositis (4, 5).

In our previous study, we reported a mouse model of oral mucositis induced by cancer chemotherapy. In this model, oral mucositis with good reproducibility could be produced by combining the administration of anticancer drugs and the

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production of mucosal injury. Moreover, the oral mucositis persisted for two weeks and the therapeutic effect could be easily evaluated by observing the changes in the area because the range of the ulcer was clearly identified (6). We reported experiments on the treatment of oral mucositis using this model. Therapeutic efficacy of rebamipide-loaded poly(DL-lactide-co-glycolide) nanoparticles coated with chitosan hydroxypropyltrimonium chloride and GGsTop, a newly developed selective gamma-glutamyl transpeptidase (GGT) inhibitor, was evaluated (7, 8). Since rebamipide has pharmacological effects such as promotion of prostaglandin (PGE2) synthesis (9), induction of mucus secretion (10), and free radical scavenging action (11), it is expected to be applied for the prevention and treatment of oral mucositis (12). GGsTop is one of the promising oral mucositis treatment substances that suppress reactive oxygen species by GGT inhibition, induces expression of collagen and elastin synthesis, heat shock protein 47, and keratinocyte induction of human skin fibroblasts (8, 13-15). However, since the amount of oral mucosal tissue obtained from the model mouse was insufficient for quantitative measurement, the evaluation of the therapeutic effect was limited to observation of tissue sections and measurement of changes in the ulcer area.

In this study, we planned to develop an animal model of oral mucositis induced by cancer chemotherapy using rats. Since rats are larger than mice, it was expected that a sufficient amount of tissue could be obtained for quantitative measurement. In addition, it was considered that the ease of injecting acetic acid into the buccal mucosa and observing the ulcer in the oral cavity was also important for the use of this simple animal model using a combination of an anticancer drug and mucosal injury. To establish a rat model of oral mucositis, optimal dosages of anticancer drugs and acetic acid were studied. The evaluation of the immunosuppressive state by the anticancer agent and the produced oral mucositis was performed by measuring the white blood cell count and the ulcer area, respectively. As in the previous report, 5-fluorouracil (5-FU) was used as the anticancer drug (6).

## Materials and Methods

*Materials*. 5-FU ($C_4H_3FN_2O_2$, purity >98.5%) was purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Isoflurane for the animal was purchased from Mylan Inc. (Pittsburgh, PA, USA). Medetomidine hydrochloride (Domitol®), which is an alpha 2 adrenoceptor agonist, was purchased from Nippon Zenyaku Kogyo Co., Ltd. (Koriyama, Japan). Midazolam, which is a benzodiazepine derivative, was purchased from Teva Takeda Pharma Ltd. (Nagoya, Japan). Butorphanol tartrate (Vetorphale®) was purchased from Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). Triamcinolone acetonide (Kenalog®) was obtained from Bristol-Myers Squibb (New York, NY, USA). Other chemicals were of the highest reagent grade commercially available.

*5-FU and acetic acid dosages for the preparation of a model rat with oral mucositis*. Nine-week-old male Wistar rats were purchased from Japan SLC Inc. (Tokyo Japan). All animal care was conducted under the Guidelines for Animal Experimentation of Tokyo University of Science, which are based on the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. Rats were housed in stainless-steel cages under standard environmental conditions (23±1°C, 55%±5% humidity and a 12/12 h light/dark cycle) and maintained with free access to water and a standard laboratory diet (carbohydrates 30%; proteins 22%; lipids 12%; vitamins 3%) *ad libitum* (Nihon Nosan Kogyo Co., Yokohama, Japan).

To replicate the immunosuppression induced by the anticancer drug, rats were administered an intraperitoneal injection of 5-FU (6). The 5-FU solution (8.0 mg/ml) was prepared by dissolving 360 mg of 5-FU in 45 ml of physiological saline. On days -5, -3, and -1 of the experiment, rats were administered the 5-FU solution at a dose of 20, 30, 40, or 50 mg/kg body weight. The control group received 1.0 ml of physiological saline instead of the 5-FU solution. The optimal 5-FU dose was determined by examining changes in body weight and white blood cell (WBC) count. We also determined the optimal acetic acid concentration for the production of oral mucositis in rats. Rats were administered the 5-FU solution on days -5, -3, and -1 of the experiment. On day 0, they were anesthetized by intraperitoneal administration of a combination of anesthetics, which was prepared with 0.3 mg/kg of medetomidine hydrochloride, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol, under isoflurane anesthesia (16), and then 25.0 μl of the acetic acid aqueous solution was injected into the mucous membrane of the right cheek. The concentration of the acetic acid aqueous solution was 20, 25, or 30%. The effect of the acetic acid concentration in the aqueous solution on the rat was confirmed by examining changes in the oral ulcer area. Moreover, to confirm the effect of 5-FU on the ulcer area of oral mucositis, the changes in the ulcer area produced by acetic acid were compared between rats administered 5-FU on days -5, -3, and -1 of the experiment and rats not administered 5-FU. All ulcer area measurements were performed using an image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA) (6-8).

*Histological evaluation*. Histological evaluation of oral mucosal tissues was performed to confirm the effect of the combined use of a 5-FU solution and an acetic acid aqueous solution in the preparation of oral mucositis model rats. On day 3, the tissue sections were produced using control rats, 5-FU solution-administered rats, acetic acid aqueous solution-administered rats, and 5-FU solution- and acetic acid aqueous solution-administered rats. Preparation of frozen tissue sections was performed based on the method of Kawamoto (17); the obtained tissue sections were compared after hematoxylin-eosin staining (8).

*Evaluation of a model rat with oral mucositis after treatment with Kenalog®*. The prepared model rats with oral mucositis were divided into two groups: a no-treatment group and a treatment group using Kenalog® once daily after the acetic acid injections. The treatment group was anesthetized by intraperitoneal administration of the combination anesthetic, and then 2 mg of Kenalog® was applied directly to the ulcer part of the oral cavity. After the treatment, the rats were allowed to sleep for several hours until the effects of anesthesia ceased. Then, changes in WBC counts and ulcer area were measured.

## Results

The effect of 5-FU administration on rats is shown in Figure 1. As shown in Figure 1A, the administration of 5-FU decreased the bodyweight of rats. On day 0, the body weight was decreased to approximately 95-98% of that on day 5 of the experiment. This weight loss was dose-dependent. Figure 1B shows the change in WBC counts in the rat blood. Similar to body weight, the reduction in WBC counts was confirmed to be dose-dependent. In the 50 mg/kg 5-FU solution administration group, 40% of rats died within a few days after 5-FU injection. Figure 2 shows changes in ulcer area when an acetic acid aqueous solution was administered to the buccal mucosa of rats. In the 20, 25, and 30% acetic acid aqueous solution administration groups, the maximum ulcer area was 14.4, 19.8, and 23.5 $mm^2$, respectively, and the period required for spontaneous healing was 13, 16, and 19 days, respectively. In the 30% acetic acid aqueous solution administration group, 33% of rats died within a few days after acetic acid injection. As shown in Figure 3, the ulcer area and the period required for spontaneous healing were significantly increased in the group administered with the 5-FU solution and acetic acid aqueous solution as compared with the group administered with acetic acid aqueous solution alone. In the acetic acid aqueous solution administration group and the 5-FU solution and acetic acid aqueous solution combination administration group, the maximum ulcer area was 14.4 and 20.1 $mm^2$, respectively, and the period required for spontaneous healing was 13 and 16 days, respectively.

Figure 4 shows images of the hematoxylin-eosin-stained oral mucosal tissue section. It was confirmed that the tissue was hollowed out by the administration of the 5-FU solution. In addition, it was shown that the administration of the acetic acid aqueous solution destroyed the surface layer and excessive proliferation of cells.

Figure 5 shows the results of the treatment of a model rat with oral mucositis using Kenalog®. As shown in Figure 5A, in the no-treatment group and the treatment group, the
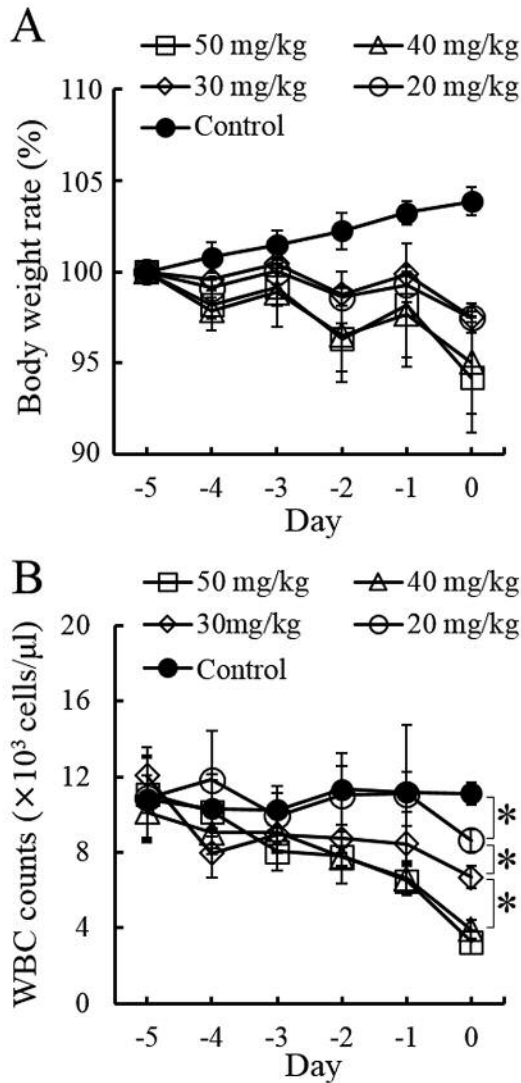
A



B



Figure 1. *Changes in body weight (A) and white blood cell (WBC) count (B) when 5-FU solution was administered to rats (mean±S.D., n=5). The doses of 5-FU were 20, 30, 40, and 50 mg/kg of body weight.*



Figure 2. *Changes in the ulcer area after injection of acetic acid aqueous solution into the buccal mucosa of rats on day 0 (mean±S.D., n=5). Acetic acid concentrations in the aqueous solution were 20, 25 and 30%.*



Figure 3. *Changes in the ulcer area when 25% acetic acid aqueous solution and 5-FU were administered in combination (white circle) (mean±S.D., n=5, \*p<0.05, t-test).*

maximum ulcer area was 19.3 and 20.8 mm$^2$, respectively. From day 3 to day 13, the ulcer area was significantly decreased in the treatment group compared to the no-treatment group. Kenalog$^®$ decreased the time required to cure oral mucositis by three days compared to the no-treatment group. As shown in Figure 5B, WBC counts sharply increased on day 1, which was the day after administration of the acetic acid aqueous solution. In the no-treatment group, WBC counts approached normal with spontaneous healing of oral mucositis. In the treatment group, WBC count after day 7 was significantly decreased as compared to the no-treatment group.
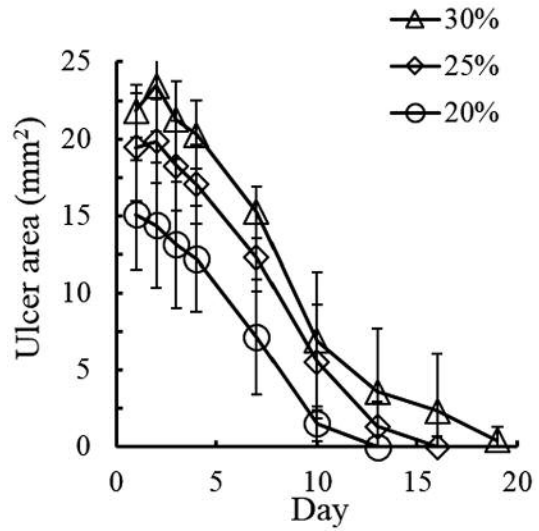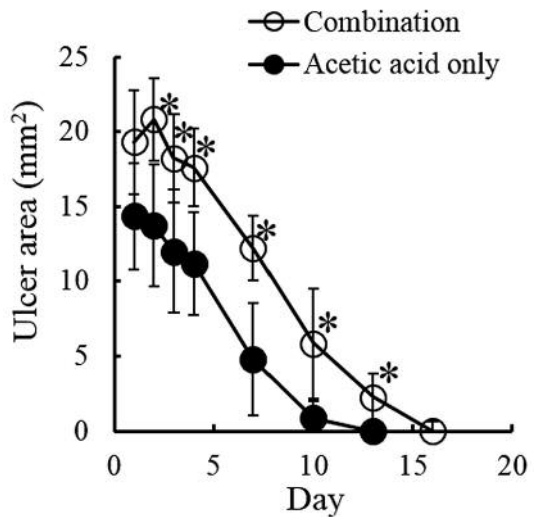
## Discussion

The 5-FU solution-administrated groups lost weight (Figure 1A), whereas the control group gained weight due to food intake. 5-FU is known to induce intestinal mucositis with severe diarrhea (18, 19). It causes anorexia, dehydration, and various systemic toxicities to cause weight loss (20). We considered that this weight loss was
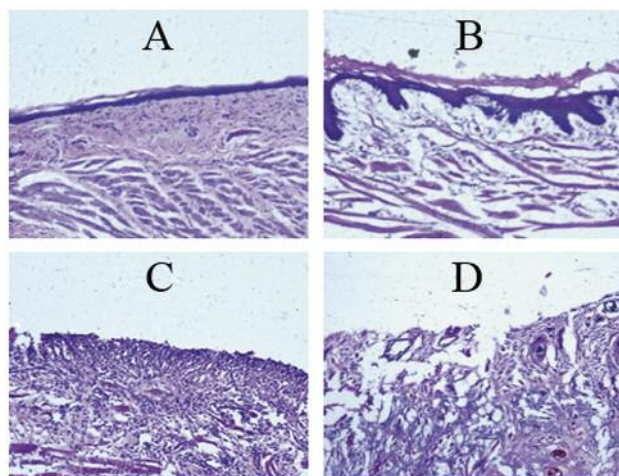
Figure 4. *Hematoxylin-eosin-stained oral mucosal tissue section of rats. (A) Normal healthy rat. (B) Rats administrated 40 mg/kg of 5-FU on days -5, -3, and -1 of the experiment. (C) Rats administrated 25% acetic acid aqueous solution on day 0 of the experiment. (D) Rats administrated 40 mg/kg of 5-FU on days -5, -3, and -1 and 25% acetic acid aqueous solution on day 0 of the experiment.*

due to the side effects of 5-FU. Administration of the 5-FU solution also decreased WBC counts, which is the result of myelosuppression caused by 5-FU (Figure 1B). To reproduce the effects of cancer chemotherapy, it is desirable that a model rat with oral mucositis is immunosuppressed. Thus, the decrease in WBC counts indicates the usefulness of the model rat produced in this study. In the 50 mg/kg 5-FU solution administration group, which had the highest 5-FU dose, the rat mortality was high. Therefore, a dose of 40 mg/kg was appropriate. Similarly, in the acetic acid aqueous solution administration test, the mortality of rats was high in the group where the highest concentration was administered. Oral mucositis due to anticancer drugs begins around 7-10 days after the start of treatment and lasts for 2 weeks (21, 22). The period required for spontaneous healing was 13 days and 16 days in the 20% acetic acid aqueous solution administration group and the 25% acetic acid aqueous solution administration group, respectively (Figure 2). Therefore, we determined that an acetic acid concentration of 25% is appropriate. In addition to being able to reproduce an immunosuppressive state, it was shown that the administration of 5-FU was useful for producing ulcers with a large area and of appropriate duration for conducting therapeutic studies (Figure 3). Furthermore, it was shown that the administration of acetic acid aqueous solution was necessary for the destruction of the surface layer of the oral mucosa (Figure 4). From these results, it was revealed that the combined use of 5-FU and acetic
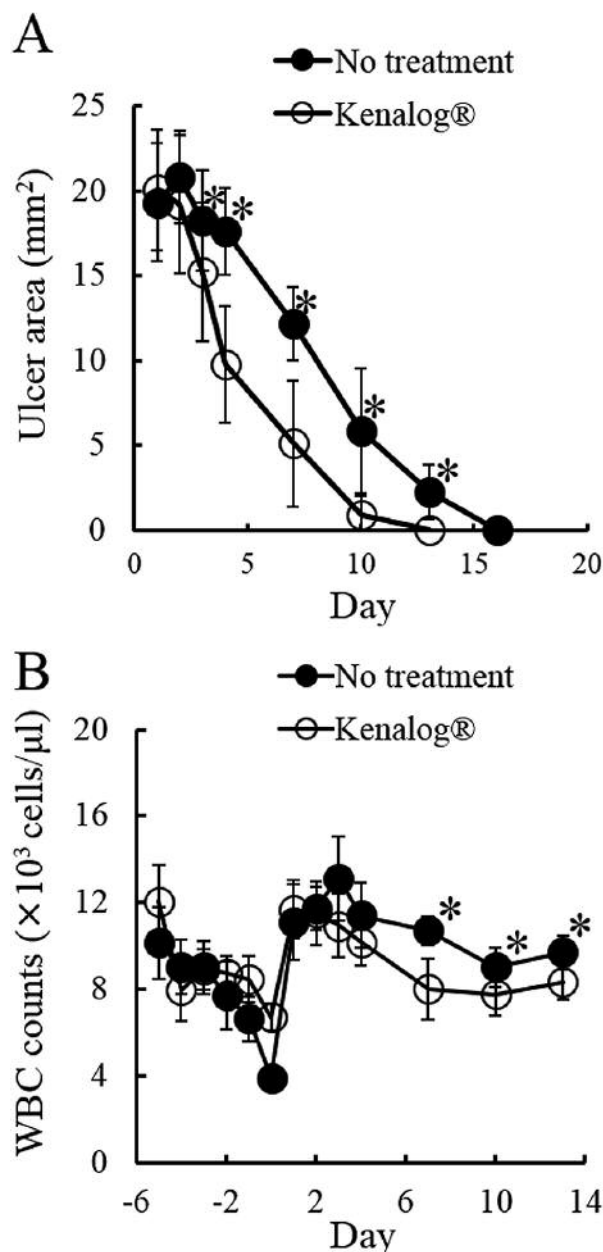


Figure 5. *Changes in ulcer area (A) and white blood cell (WBC) count (B) (mean±S.D., n=5, \*p<0.05, t-test). The group using Kenalog® once daily after acetic acid injection is represented by white circles.*

acid was effective in producing a useful rat model of oral mucositis induced by cancer chemotherapy.

The shortening of the treatment period and the decrease in the WBC count shown in the treatment study using Kenalog® confirmed that this model mimics oral mucositis in immunocompromised states. This model would be useful in the screening of drugs that are not recommended for use under immunosuppression.

## Conclusion

In this study, we succeeded in developing a rat model of oral mucositis with the use of cancer chemotherapy (5-FU) and acetic acid in combination. Since this model had a sufficient ulcer area that lasted for a relatively long period, it is possible to easily evaluate the therapeutic effect of drugs on oral mucositis. Compared to the mouse model, this model allows for an easier observation of oral mucositis and provides a large amount of oral mucosal tissue. It will contribute to the development of quantitative studies of oral mucositis induced by cancer chemotherapy.

## Conflicts of Interest

The Authors declare that they have no potential conflicts of interest regarding this study.

## Authors' Contributions

Takeuchi and R. Kawamata designed the study, and wrote the initial draft of the manuscript. K. Makino contributed to analysis and interpretation of data, and assisted in the preparation of the manuscript. All Authors approved the final version of the manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Acknowledgements

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