Abstract. Background: Oral mucositis (OM), one of the side-effects induced by chemotherapy, has 40% incidence and the incidence rate increases to approximately 100% in combination with radiotherapy. We describe OM in ICR mice induced using 5-fluorouracil (5-FU) and 20% acetic acid. Materials and Methods: We optimized the dose of 5-FU and 20% acetic acid and validated the efficacy of standard therapies for OM. Results: All mice developed OM after administration of 5-FU and 20% acetic acid. Application of Kenalog® reduced maximum ulcer area and the duration of spontaneous recovery in a dose-dependent manner. Conclusion: We succeeded in developing a mouse model of OM induced by cancer chemotherapy. New drugs for OM induced by anticancer drugs can be evaluated simply by monitoring the WBC count in this mouse model. This model is expected to contribute to development of new drugs and elucidation of the mechanisms of ameliorating stomatitis as a side-effect of anticancer drugs.

Surgical treatment, chemotherapy, radiotherapy, and immunotherapy are the common means of cancer treatment (1). In chemotherapy, cancer cells are injured doing specific cell-cycle phases (2). Normal healthy cells, such as oral epithelial cells, gastric epithelial cells, hematopoietic cells and root hair cells, are injured as well as cancer cells. As a result, such damage to normal cells leads to various side-effects such as oral mucositis (OM), diarrhea, immunosuppression, and hair loss (3).

One of the side-effects induced by chemotherapy, OM, is reported to have 40% incidence and the rate increases to approximately 100% in combination with radiotherapy (4, 5). Direct mucous membrane damage and secondary infection in the mouth cause OM (3, 6). The former is caused by reactive oxygen species produced as a result of anticancer drugs and the latter is due to immune depression based on myelosuppression (7). OM caused by anticancer drug treatment is exhibited as erythema, edema or ulceration that can be accompanied by alterations ranging from mild burning sensation to large and painful ulcers that have a wide range more than general stomatitis (8, 9). Symptoms such as eating and sleep disorders, communication difficulties, as well as the sharp pain, develop and reduce the patient’s quality of life (10). It is also reported that loss of consciousness occurred due to deterioration of OM and forced cessation of treatment (11). Therefore, preventing symptoms of OM or treating it quickly brings improvement of a patient’s quality of life, and reduces the need for treatment interruption. At present, various treatments (oral cryotherapy, brushing, administration of steroidal anti-inflammatory drug, disinfectant mouthwash, etc.) are used (3, 10) but they do not seem to be adequate and have little preventive effect (12). A novel drug for OM is needed. In order to develop new drugs, development of animal models is important.

Considerable attention has been paid to using hamsters or rats as animal models for the development of OM induced by anticancer drug treatment (13-16), radiation (13, 17, 18), scratch wound (19) or application of acetic acid to the mucous membrane (14). However, hamsters and rats are not of low cost or easily handled. Moreover, institutions which have radiation equipment are limited. On the other hand, there are few reports on development of mouse models for OM. Moreover to our knowledge, nothing has been reported on mouse models developed using acetic acid injection into the oral mucosa. Surprisingly, despite considerable research attention, no OM model leads to the development of appreciable ulcers in the oral cavity. In general, OM manifests as erythema, edema or ulceration that can be accompanied by alterations ranging from mild burning sensation to a wide range of large and painful ulcers. From
this point of view, these OM models are irrelevant as mimics of OM induced by anticancer treatment.

In this study, we tried to prepare an OM animal model using mice in which OM was induced by direct application of acetic acid to their mucous membranes after treatment with the anticancer drug 5-fluorouracil (5-FU). Although 5-FU has been widely used for the treatment of various cancer types, such as gastrointestinal, breast, and head and neck cancer (20-22), it causes OM as a side-effect. Most animal models of OM reported previously were induced by 5-FU (14-16). Furthermore, we evaluated the effectiveness of this model by measurement of the ulcerated area and white blood cell (WBC) count. The aims of this study were (i) to develop an animal model for OM induced by an anticancer drug with lower cost and easier handling than conventional models, and (ii) to produce appreciable ulcers to precisely estimate the effects of drugs for therapy of OM.

**Materials and Methods**

**Animals.** Nine-week-old male ICR mice, weighing 30-40 g, were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in groups of five per cage in a room maintained under standardized light (12:12 h light-dark cycle) at an ambient temperature of 23±2˚C, humidity of 60±10˚C with free access to food pellets and drinking water, and were acclimated for 7 days prior to use in experiments. The experimental protocol used in this study was in accordance with the guidelines of Tokyo University of Science (approval number Y14048).

**Chemicals and drug preparation.** Acetic acid (99.7%) obtained from Wako Pure Chemical Industries (Osaka, Japan) was diluted to 20% with distilled water (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan). 5-FU obtained from Wako Pure Chemical Industries was chosen in this study, and diluted with distilled water to 8 mg/ml. Turk reagent was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Isolflurane was obtained from Intervet Inc. (Millsboro, DE). Kenalog® was obtained from Bristol-Myers Squib (Tokyo, Japan). All other chemicals were of reagent grade.

**OM induction.** In order to replicate immunosuppression induced by anticancer drugs, mice were administered an intraperitoneal injection of 5-FU on days -5, -3, and -1 of the experiment at a dose of 30, 40, 50, or 60 mg/kg body weight (n=5 at each dose). Subsequently, on day 0, under isoflurane anesthesia, the left cheek of the mouse was extended outside the oral cavity. Subsequently, mucosal ulcers were induced by injection of 20% acetic acid (5, 10, 15, or 20 μl) using a microsyringe with a 31-G needle (n=5). In order to estimate this model’s OM induced by 5-FU, the ulcerated areas of mice administered 5-FU and acetic acid were compared with those of mice treated only with acetic acid (n=5).

**OM treatment.** In order to assess the usefulness of this model as a model of stomatitis, we prepared 20 mouse models under isoflurane anesthesia, then applied Kenalog® (15, 20, 25 μmol), an existing OM drug which is triamcinolone acetonide formulation, directly once daily after the acetic acid injections. This operation was carried out until the end of complete healing. All mice recovered during the experimental period.

**Assessments.** From day 3 to the end of treatment, once every 2 or 3 days, the mice were anesthetized with isoflurane and the left cheek was inverted and the area of mucositis measured (as mm²) using image analysis software Image J (ACTREC, Navi Mumbai, India). Furthermore, the tail venous blood was collected, diluted in Turk’s reagent and the WBC count in order to assess immunosuppression score.

**Statistical analysis.** Analysis of variance and multiple comparison tests using Dunnet’s method, t-test, and Tukey’s method were applied to determine differences. Data are expressed as the means±SD and differences were considered significant at p<0.05.

**Results**

**Efficient dose of 5-FU.** The mice were dosed with 5-FU to induce immunosuppression as an anticancer drug side-effect. WBC counts decreased in a 5-FU dose-dependent manner (Figure 1). A significant difference between the control group and the group treated with 50 mg/kg 5-FU was observed. However, the administration of 60 mg/kg 5-FU resulted in death. Furthermore, even though mice administered 5-FU at 30, 40, or 50 mg/kg remained alive, OM was not induced.
**Determination of the dose of acetic acid.** We injected acetic acid to induce OM. The ulcerated area of mucositis became larger and the duration of spontaneous recovery became longer in a dose-dependent manner (Figure 2A). WBC counts were significantly increased after the administration of acetic acid and recovered to normal values with ulcer healing (Figure 2B).

**Evaluation of the need for 5-FU administration.** In order to confirm the necessity of 5-FU treatment, we produced OM using acetic acid only and using acetic acid plus 5-FU. This experiment showed that 5-FU treatment enlarges the area of OM (Figure 3A). Furthermore, the body weight of mice given the combination of 5-FU and acetic acid significantly decreased compared to that in group treated with acetic acid.
only group on the day after treatment of 5-FU with acetic acid (Figure 3B).

**Therapy with a pre-existing drug for OM.** Application of Kenalog® reduced the maximum ulcerated area and the duration of spontaneous recovery in a dose-dependent manner (Figure 4A). The body weight was also improved by administration of Kenalog® (Figure 4B). However, WBC count significantly decreased below normal values (Figure 4C).

**Discussion**

In this study, we produced a mouse model of disease using 5-FU and acetic acid. Our aim was (i) to develop an animal model for OM induced by anticancer drug with lower cost and more safely and quickly than conventional models, and (ii) to produce appreciable ulcers to precisely estimate the effects of drugs. We succeeded in development of the model which fulfills the goals. This model is simple, inexpensive, and broadly applicable.

This model was administered 5-FU to mimic human OM. 5-FU-based animal models for OM are common models. 5-FU is an inhibitor of DNA synthesis and RNA function and damages cells undergoing active cell division such as cancer cells (2). However, normal cells in active cell division are similarly damaged. OM, one of the common side-effects of 5-FU, is caused by a decline of the mucosal barrier function due to myelosuppression and injury to mucous membrane cells of the mouth (20, 21). Several factors or genes contributing to 5-FU-induced mucositis have been studied (23-26).

WBC counts of mice administered 5-FU (50 mg/kg) were significantly reduced (Figure 1). This is important for the evaluation of immunity, and clearly shows 5-FU indeed induced immunosuppression.

Administration of 5-FU alone did not cause OM. In previous reports, animal models for 5-FU-based OM were induced not only by 5-FU but also by other stimuli (27). Therefore, we used acetic acid to induce OM. Using acetic acid, ulcers of almost the same size could be produced in all individuals. In addition, it was possible to clearly recognize the ulcer as shown in Figure 1B.

In previous clinical studies, it was reported that stomatitis as an anticancer drug side-effect continues for 1 or 2 weeks after anticancer drug treatment (8, 9). Figure 2A shows administration of 15 μl acetic acid induced OM which continued for 2 weeks. Therefore, we decided a dose of 15 μl acetic acid was appropriate.

We demonstrated that the combination of 5-FU and acetic acid is effective in inducing OM in mice (Figure 3). Administration of a combination of 5-FU and acetic acid was effective at inducing OM in hamsters (14). However, such efficacy was not reported in mice.

In the present study, we have shown that Kenalog® is able to treat ulcers of the model mice (Figure 5A). Kenalog® is a triamcinolone acetonide, which is a steroid and has an anti-inflammatory effect.
The OM model induced by 5-FU and application of acetic acid in mice make it possible to estimate the therapeutic effect of new drugs on stomatitis objectively from the size of the ulcerated area and the period of ulcer healing, and to consider the safety of anticancer drugs from the number of leucocytes. This model has high reproducibility and ease of handling. It is expected that this model will enable histological analysis by using tissue sections to elucidate the mechanisms of ulcer healing more precisely. This model for OM induced by an anticancer drug will contribute to the development of new drugs for stomatitis induced by anticancer drugs.

Conclusion

We succeeded in the development of a mouse model of OM induced by cancer chemotherapy. This model can be easily produced and evaluated by measuring the ulcerated area and the period of spontaneous recovery. The model enables us to consider if a new drug for OM is safe for using with an anticancer drug by monitoring the WBC count. This model should contribute to development and elucidation of the mechanisms of action of new drugs against stomatitis as a side-effect of anticancer drug therapy.

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References


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