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Animal Model of Cisplatin-Induced Oral Mucositis: Dose Optimization

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Abstract

Objective. The present study aimed to develop and validate an animal model of chemotherapy-induced oral mucositis due to cisplatin administration. **Materials and Methods.** Oral mucositis was induced in Wistar rats by cisplatin. Twenty healthy male Wistar rats were divided into four groups: a control group, and cisplatin 3 mg/kgBW (D1), cisplatin 5 mg/kgBW (D2), and cisplatin 6 mg/kgBW groups (D3). The D1, D2, and D3 groups received the cisplatin intraperitoneally on days 1, 3, and 5, whereas the control group did not receive anything. On day 7 and day 14 the entire experiment was terminated in all groups and the changes in body weight, oral mucositis grades, and histopathological scores were evaluated. **Results.** Cisplatin administration created a strong oral mucositis effect on groups D2 and D3. All the cisplatin doses decreased the rats' body weight by day 14. The worst oral mucositis grades and histopathological scores resulted from the administration of cisplatin at a dose of 5 mg/kgBW. **Conclusions.** In conclusion the cisplatin 5 mg/kgBW administered on days 1, 3, and 5 by intraperitoneal administration was the optimum dose to induce oral mucositis.

Key Words: Cisplatin ▪ Oral mucositis ▪ Rats ▪ Histopathology.

Introduction

The specific first-line therapy for cancer can vary widely depending on the type and stage of the disease. It may involve a combination of treatments, such as surgery, chemotherapy, radiation therapy, targeted therapy, or immunotherapy (1). Chemotherapy is the one of the first line therapy to treat cancer, however chemotherapy induces several side effects, including tissue damage reactions along the epithelium of the mouth and gastrointestinal tract (GIT) (1, 2). Chemotherapy-induced oral mucositis (CIOM) is a serious side effect of cytotoxic drugs (3). Patients with head and neck cancer are most affected by CIOM, with a risk of roughly 40% cases (4). Combination cancer treatment with radiation increases the chance of CIOM up to 100%. Cisplatin is the first-line chemotherapy with reported incidences of induced CIOM (5–7). Animal models enable highly regulated experimental circumstances, precise insights into the oral organs, standardized, clinically appropriate treatment regimens, and the development of new biomarkers to aid our understanding of the progression of CIOM, and how to avoid or treat it (8). The adverse effects of CIOM are not well managed, due to the lack of an understanding of the mechanism of its formation, so appropriate therapy cannot be provided (9). Therefore, in this study we developed and validated an animal model of chemotherapy-induced oral mucositis.

A previous study reported that cisplatin induced reactive oxygen species (ROS) and immune depression lead to erythema, edema, and CIOM

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(10–12). Furthermore, high levels of ROS cause apoptosis due to induction of DNA damage, and lead to increasing numbers of CIOM cases (13). The deterioration of CIOM results in forced disruption of treatment, leading to a loss of consciousness (3). These symptoms reduce the patient's quality of life. Therefore, preventing CIOM or treating it quickly brings improvement to the patient's quality of life, and reduces the need to interrupt treatment. Currently, various treatments are used, including antiinflammation drugs, however they are not adequate and have little preventive effect (14, 15). A novel drug for CIOM is needed. To develop new drugs, development of animal models is important.

A previous study reported on a CIOM mouse model induced by acetic acid injection into the oral mucosa (16). However, the study did not specifically measure the pain associated with oral mucositis, as it can induce pain in various body regions. A previous study that used acetic acid injection also did not assess the macroscopic picture of tissue damage in the oral mucosa, but only described changes in body weight and the area where CIOM formed. In addition, the induction used in the study did not describe the CIOM formed due to chemotherapy. In this study, we tried to prepare a CIOM animal model using rats in which CIOM was induced by cisplatin chemotherapy. Most animal models of CIOM induced by chemotherapy have reported CIOM along with intestine or gastric ulcers (17, 18). Surprisingly, despite considerable research, no CIOM model has led to the development of appreciable ulcers in the oral cavity.

Therefore, in this study we developed an animal model for chemotherapy-induced oral mucositis to determine the optimal dose of chemotherapy by measurement of the ulcerated area, histopathological epithelial specimens, and the CIOM grade.

Methods

Material and Study Design

This post-test only control group study design was conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, Indonesia, from January - March 2023.

Chemical and Drug Preparation

The cisplatin 0.5 mg/ml injections were obtained from PT. DANKOS FARMA (A Kalbe Company) Jakarta, Indonesia.

CIOM Induction

Twenty male Wistar rats (250 g ± 20 g) were used in this study. The rats were maintained at a controlled room temperature (21 °C ± 2), humidity at approximately 55% ± 10, light and dark cycles every 12 h, and no restriction of food and drink. After a week of acclimation, the rats were randomly divided into the following four groups: control/untreated, and D1 (Cisplatin 3 mg/kgBW), D2 (Cisplatin 5 mg/kgBW), and D3 (Cisplatin 6 mg/kgBW) groups. Cisplatin was administered intraperitoneally (i.p.) on days 1, 3, and 5 (19-22). The control group did not receive anything. The rats were sacrificed on day 14 by guillotine decapitation.

Epithelial Specimen Analysis

The buccal oral mucosa tissue was taken out and fixed with 10% formalin for 24 hours to create a paraffin block preparation. Tissue preparations 5 µm thick were removed and stained with hematoxylin-eosin.

Mucositis Grade Analysis

A modified version of the National Cancer Institute Common Terminology Criteria for Adverse Events was used to grade CIOM in the rats on days 7 and 14, as follows: Grade 0, normal mucosa; Grade 1, redness of the mucosa with punctate ulcers or a pseudo membrane; Grade 2, confluent ulceration or a pseudo membrane with no bleeding following slight stimulation; Grade 3, confluent ulceration or a pseudo membrane with bleeding following a slight stimulation; and Grade 4, tissue necrosis or spontaneous bleeding (23).

Mucositis Score Analysis

Buccal samples were collected from rats for histopathological analysis on days 7 and 14 after cisplatin administration. Specimens were fixed in the 10% neutral-buffered formalin, dehydrated, and embedded in paraffin. Five-micrometer-thick sections were obtained for hematoxylin and eosin staining, and examined under a light microscope (×100). Histological parameters were assessed in a single-blind manner and graded as follows (24): Score 0, normal epithelium and connective tissue who no vasodilatation, cellular infiltration, hemorrhagic areas, ulceration, or abscesses; Score 1, scattered vasodilatation, areas of reepithelization, diffuse cell infiltration with multiple mononuclear leukocytes, and absence of bleeding, edema, ulcers and abscesses; Score 2, moderate vasodilatation, epithelial hydropic degeneration (vacuolization), moderate cell infiltration dominated by polymorph nuclear leukocytes, the presence of hemorrhagic areas, edema and rarely small ulcers but absence of abscesses; Score 3, marked vasodilation, cell infiltration with multiple polymorph nuclear leukocytes, the presence of hemorrhagic sites, the presence of edema and ulceration, and the absence of abscess; Score 4, severe vasodilatation and inflammatory infiltration, characterized by neutrophils, abscesses and diffuse ulcers (21).

Ethical Considerations

The study was approved by the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University (N0. 399/X/2022).

Statistical Analysis

The data are presented as mean ± standard deviation (SD). Normal distribution was assessed using the Shapiro-Wilk test, and homogeneity was examined via the Levene's test. Furthermore, data analysis used one-way ANOVA and continued with the Least Significant Difference (LSD) test with $P<0.05$ under SPSS version 23.

Result

Body weight decreased after the development of oral mucositis in all the rats in the three groups (Figure 1). Significant differences were observed in body weight on day 14 between groups D1, D2, and D3 compared to the control group ($P<0.05$). Significant differences were also noted between groups D1 and D3, D2 and D3, and D2 and D3.

Mucositis grades were highest on day 14 in the D3 group. The grades worsened over 14 days in all treated groups compared to the control group (Figure 2). The mucositis grades of groups D1, D2, and D3 were 1.4, 2.2, and 3.2 on day 7, respectively.

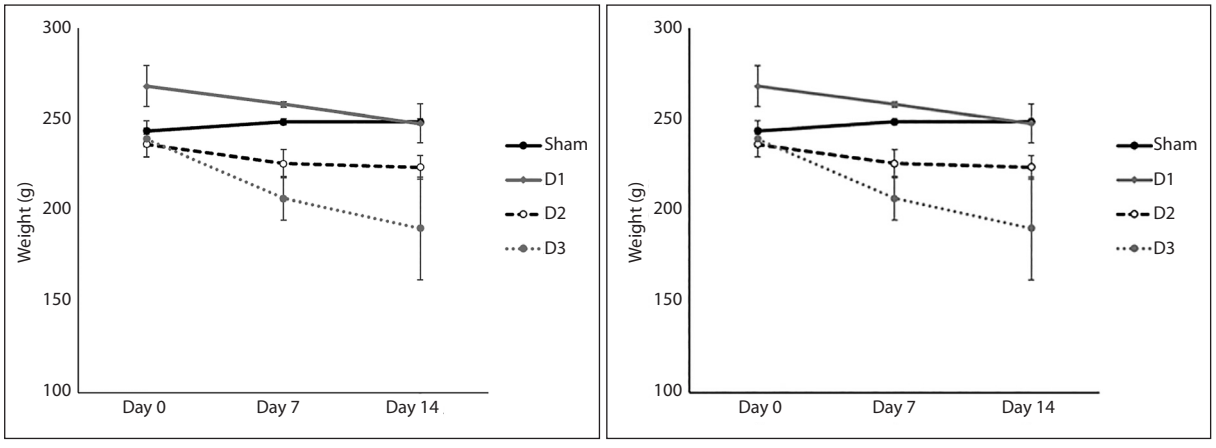

Figure 1. Evaluation of the rat cisplatin-induced oral mucositis model. Changes in body weight of the rats in the four groups.

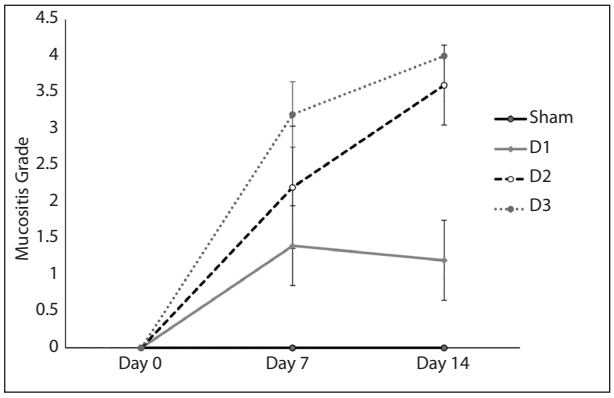

Figure 2. Evaluation of the rat cisplatin-induced oral mucositis model. Mucositis grades in the four groups of rats.

Interestingly, the mucositis score worsened on day 14 in groups D2 and D3. The mucositis grades in D1, D2, and D3 were 1.2, 3.6, and 4, respectively. In group D1 the mucositis grades improved on day 14. This phenomenon indicates that the administration of cisplatin at doses of D2 and D3 successfully induced mucositis consistently.

The histopathological scores were highest on day 14 in groups D2 and D3. In group D1 the histopathological scores were improved on day 14 (Figure 3). Intragroup comparisons revealed no significant differences between D2 and D3 groups on day 14. The findings from the histopathological

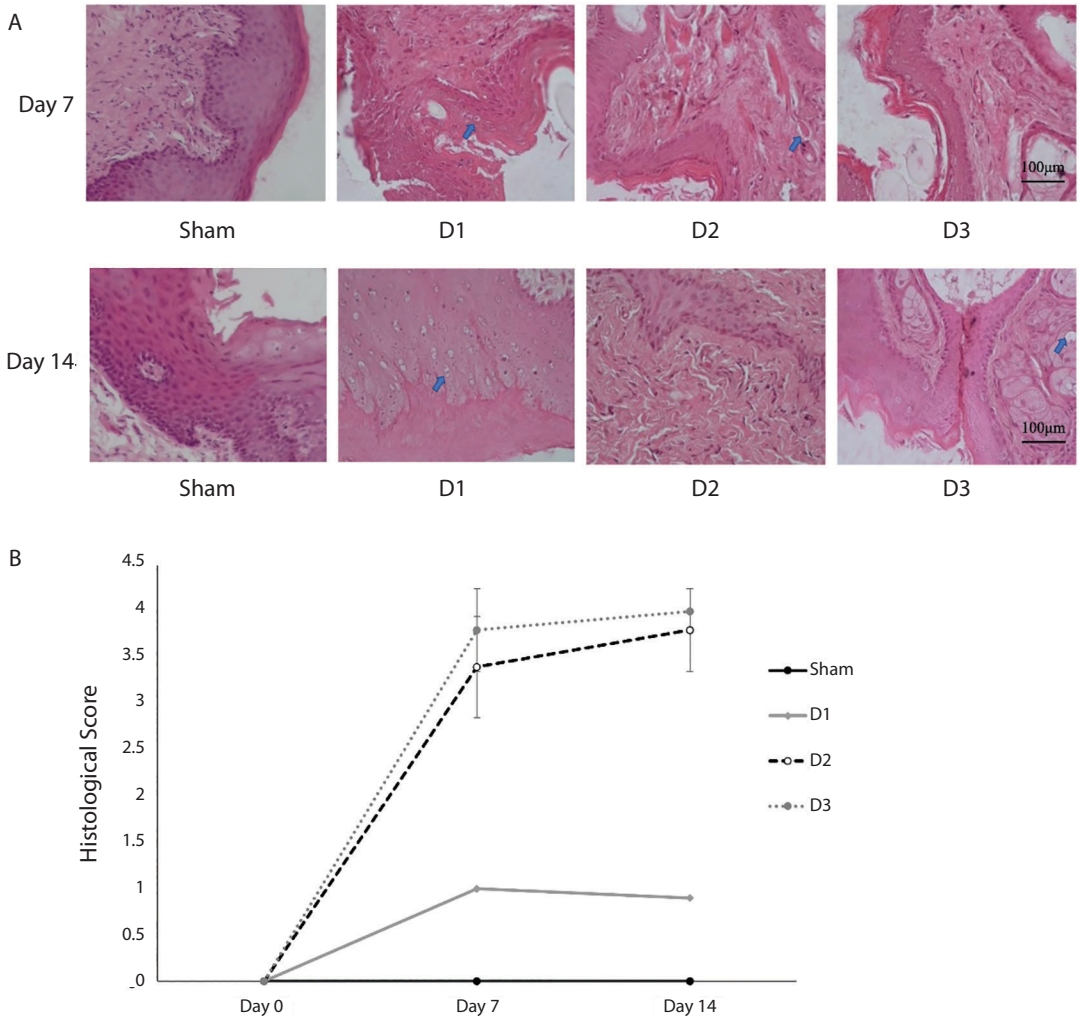

Figure 3. Evaluation of the rat cisplatin-induced oral mucositis model. (A) Histological morphology of buccal on days 7 and 14 after cisplatin administration. (B) Changes in the mucositis scores of the four groups of rats. D1 (Cisplatin 3 mg/kgBW), D2 (Cisplatin 5 mg/kgBW), and D3 (Cisplatin 6 mg/kgBW) groups.

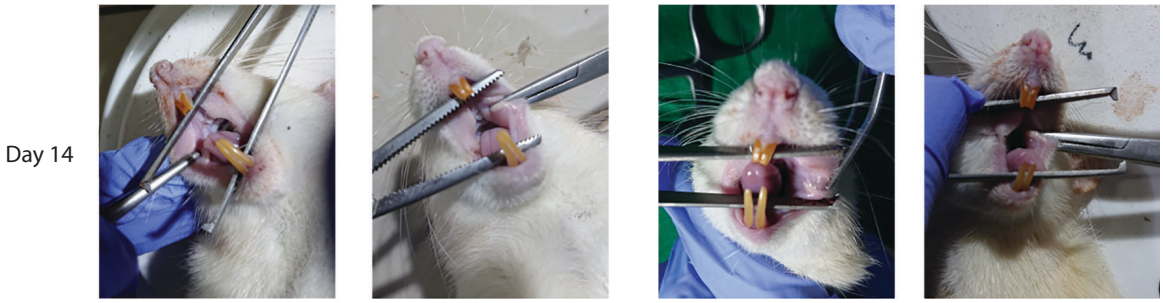

Figure 4. Macroscopic changes in the oral cavity of the rat cisplatin-induced oral mucositis model. D1 (Cisplatin 3 mg/kgBW), D2 (Cisplatin 5 mg/kgBW), and D3 (Cisplatin 6mg/kgBW) groups.

examination align with the macroscopic observations of the oral cavity, indicating that the alterations in the oral cavity of both groups D2 and D3 did not demonstrate significant disparities. This was substantiated by the presence of a whitish hue in the cheek region of the mice, particularly notable in groups D2 and D3 (Figure 4).

Discussion

The present results revealed markedly lower body weights in groups D1, D2, and D3, suggesting that cisplatin inhibits appetite. Decreased food intake was noted after the onset of oral mucositis, along with a corresponding reduction in body weight, and these changes were attributed to the pain associated with oral mucositis. This result supported a previous study showing that the body weight of a oral mucositis animal model had significantly decreased on days 9 and 11 after 5-FU administration (25).

The inhibition of the growth of granulation tissues is an important step in oral mucositis (4). Inhibition of fibroblast also plays the most important role in the formation of un-granulation tissues (15). The administration of cisplatin in D2 and D3 significantly increased confluent ulceration or the formation of a pseudo membrane, with bleeding. Interestingly, on day 14 in group D3 the oral mucosa of the animals was necrotic with spontaneous bleeding. However, in group D1 the mucositis grades had improved on day 14. Furthermore, macroscopic, and histopathological findings indicated more rapid tissue damage in groups D2 and D3 than in D1 and the control group. The tissue damage of the oral mucosa was exacerbated by the inhibition of the cell migration-promoting effect of cisplatin. These results indicate that the doses in D2 and D3 did not show any significant differences. Therefore, the D2 dose was sufficient to induce oral mucositis caused by cisplatin chemotherapy. In this condition, it was also confirmed that the D2 dose was able to maintain the condition of oral mucositis until day 14, while in D1 there was improvement without treatment. A previous study also reported that the administration of 5-FU induced vasodilatation and inflammatory infiltration on days 9 and 11 (25). On the basis of these results, we concluded that the cisplatin 5 mg/kgBW administrated on days 1, 3, and 5 by intraperitoneal administration was the best dose to induce oral mucositis. However, future studies are needed to investigate the molecular mechanism of cisplatin-induced oral mucositis in more detail.

Taken together, this finding holds significant implications for research pertaining to oral mucositis and related therapies. By establishing the optimal dosage and a reliable induction method, subsequent research can be more directed towards intervention studies and the development of therapies to address this condition. This research may serve as a foundation for testing pharmaceuticals or medical procedures aimed at preventing or reducing oral mucositis in a rat population, expediting progress in this field and potentially yielding benefits for the treatment of oral mucositis in humans.

Conclusion

Cisplatin administered intraperitoneally at a dose of 5 mg/kgBW produced histopathological oral mucositis without death, and was validated and shown to be optimal.

What Is Already Known on This Topic:
Chemotherapy-induces oral mucositis is a serious side effect of cytotoxic drugs. However, the adverse effects of chemotherapy-induced oral mucositis are not well managed, due to a lack of understanding of its mechanism of formation. At present, there is no established method for creating an animal model of oral mucositis induced by chemotherapy. Many studies have primarily focused on gastric mucositis, and no standardized approach has been developed for oral mucositis induced by chemotherapy in animal models.

What This Study Adds:
In this study, we developed an animal model of chemotherapy-induced oral mucositis. Additionally, we validated the conditions of oral mucositis using several inflammation parameters. Through the findings of this research, valuable information was obtained regarding the establishment and validation of a method for inducing oral mucositis through chemotherapy induction.

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Authors' Contributions: Conception and design: OK and DGT; Acquisition, analysis, and interpretation of data: BW and OK; Drafting the article: OK; Revising it critically for important intellectual content: DGT and MS; Approved final version of the manuscript: DGT.

Conflict of Interest: The authors declare that they have no conflict of interest.

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