Experimental Model of Thermally Induced-Tongue Ulcer in Mice

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Abstract

Tongue ulcer is a common condition, which persists for days or weeks and causes pain and discomfort. Experimental animal models are crucial in investigating the efficacy and safety of novel drugs and therapeutic approaches for the treatment of tongue ulcer. In this report, we provide thorough information on the procedures and the ulcer pathology progress by clinical and histological observation on each main ulcer stages, to establish an experimental model of thermally induced-traumatic ulcer in tongue mucosa for utilization in future alternative therapy studies.

The procedure was performed on *Mus Musculus*, and the mice were divided into the control and thermal groups. An 80℃-ball pointed instrument used for ulcer induction was placed on the lateral left mucosa of the tongue for 5s. The day when the ulcer formed and healed was observed. Clinical assessment was conducted by observing the ulcer diameter, redness, swollen membrane, and weight of mice. Histological examination was performed by observing for epithelial disintegration, vasodilatation of blood vessels, and infiltration of inflammatory cells on days 1, 8, and 9 after thermal application.

Ulcer was observed on day 0 right after the induction, and it reached its peak on days 1-3. The average physiologic healing time was on days 8 and 9. The mice with ulcer in the lateral tongue experienced weight loss but recovered after the ulcer diminished. Ulcer treated with triamcinolone acetonide 0.1% diminished 2-3 days earlier than the ulcer that was not treated. Our thermal model of tongue ulcer in mice provides a simple and standardized method that establishes a uniform tongue ulcer model in mice. This model was designed as the standard method for tongue ulcer due to thermal injury.

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Introduction

Ulcers can be caused by mechanical, physical, and chemical trauma. Those caused by mechanical trauma are often observed in areas between the teeth as well as in the lower lip, tongue, and buccal mucosa. This condition can be caused by various factors, such as rough surface of dentures, malposition of teeth, and errors during dental treatment (exposure to high temperature devices). Chemical agents that are too acidic or alkaline may also cause ulcers because such agents can act as a local irritant or allergen. Although rare, ulcers can also be caused by heat, as in the case of eating hot food or beverages.[1-3] Another cause of oral ulcers is a temporary immune system response to flu, hormonal changes, and stress. Predisposition to ulcer in the oral cavity is caused by low levels of vitamin B12, folic acid, and iron in the body.[2,3]

Traumatic ulcers in the oral cavity are relatively common, and they are often caused by mechanical injury. These ulcers are usually found on non-keratinized tissues, such as the mucosa of the cheek, tip of the tongue, gingiva, hard palate, and soft palate. The clinical features of an ulcer may be similar to a yellowish white necrotic tissue bounded by a wide erythema area.[4] Although ulcers are self-limiting, they often cause persistent pain and

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discomfort, consequently, prompting patients to seek out treatment for a fast recovery.[5-8] The size of ulcers can exceed 10 mm in diameter and can put individuals at high risk of scarring. This type of ulcer is commonly found in the tongue, and it may persist up to 6 weeks.[3,8]

At the present time, no standard therapy for tongue ulcers and existing drugs has been unsatisfactory.[9] Both in vitro and in vivo studies on new drugs for tongue ulcers must be conducted. Therefore, a standardized method that can be utilized to establish a tongue ulcer model must be used in subsequent research, which is urgently needed. Animal models are required in testing the efficacy and safety of a new drug in the biological tissues of living things. The use of mice is extremely useful in the study of diseases in humans since their tissues are extremely similar to those of humans.[10,11] To date, studies that used experimental models of tongue ulcer caused by thermal trauma in mice are limited. Hence, this study aimed to establish a standard model for ulcer caused by thermal trauma in the tongue of mice and to obtain information on trauma exposure, type of trauma, instruments used, temperature of the thermal trauma, and duration of exposure.

Materials and methods

Animals

This study used 8-12-week-old male Swiss Webster mice (n=20) weighing 25-35g. The mice were obtained and maintained in animal cages in the Experimental Animal Laboratory Center of Biomedical and Basic Technologies of Health, Indonesia, according to the standard procedure. They were randomly selected for the experiment and divided into the experimental and control groups. The ethics committee of the Faculty of Medicine, Universitas Indonesia approved the research project (No. 590/UN2.F1/ETIK/2017).

Induction of tongue ulcer

The mice were anesthetized with ketamine 10% and xylazine 2% (2:1) (0.12 mL/100 g body weight). Tongue ulcer was induced by exposure to thermal trauma using an 80ºC ball pointed instrument that is 1 mm in diameter (Dentica, Toronto-Ontario, Canada) for 5 s without pressure on the left lateral mucosa of the tongue (Figure 1). Three mice were used as negative controls, and a room temperature (RT) ball pointed instrument was applied on the lateral mucosa of the tongue. Three mice were utilized as positive controls, and they were administered with triamcinolone acetonide 0.1% (Taisho Pharmaceutical, Depok, Indonesia) topically on the ulcer area after induction on day 0. The mice were sacrificed on the designated days (days 1, 8, and 9) for histological examination.

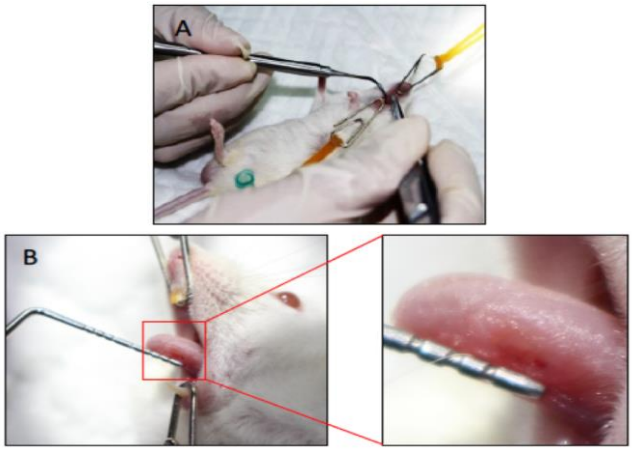

Figure 1. (A) Application of a ball pointed instrument on the lateral tongue mucosa. (B) A dental pocket probe was used to measure the ulcer area.

Clinical assessment

The researchers assessed for body weight of the mice, redness, swelling, and ulcer diameter. To evaluate the maximum ulcer diameter, a dental pocket probe with a millimeter marking (ASA Dental, Massarosa, Italy) was used for the measurement (Figure 1). Clinical assessment was performed on days 0, 1, 3, 5, 8, and 9.

Histology

On days 1, 8, and 9, the specimens of the tongue mucosa tissues were isolated, fixated with 10% formalin, sectioned, and stained with hematoxylin and eosin. Epithelial disintegration, blood vessel dilatation, and presence of immune cells were observed under a microscope.

Results

On day 0, after the application of the 80°C ball pointed instrument, the ulcer on the lateral tongue mucosa of the mice in the experimental group was observed immediately. Signs of inflammation (redness and swelling) were also noted immediately on day 0. The inflammation persisted for 1 week after the application, which reached its peak on days 1-3. The inflammation gradually subsided and disappeared completely on day 8. On the contrary, signs of inflammation were not observed in the control mice (Table 1). The ulcer appeared with a distinct border to its surrounding after induction starting on day 0. On day 1, swelling was more apparent than the previous day (Figure 2).

Groups	Presence (+) or absence (-) of redness and swelling				
	Day 0	Day 1	Day 3	Day 5	Day 8
Control	-	-	-	-	-
Experimental	+	+	+	+	-

Control group: room temperature (RT) ball pointed instrument; Experimental group: 80ºC ball pointed

Table 1. Clinical Observation of Redness and Swelling in the Tongue Ulcer Area.

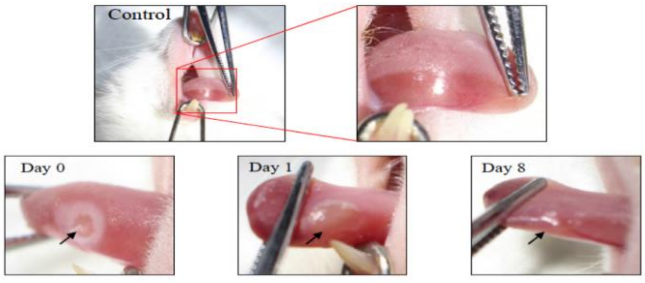

Figure 2. Clinical observation of the ulcerated area on the lateral tongue lateral. A room temperature (RT) ball pointed instrument was applied on the right lateral tongue mucosa of the control group. An ulcer was observed on the left lateral tongue mucosa after applying a 80ºC ball pointed instrument for 5s (black arrow: ulceration area).

After the application of the 80°C ball pointed instrument, the average size of the ulcer on the induction day was 3 mm, and it reached its peak (6 mm) on days 1 and 2 after induction. On day 5, the ulcer size decreased to 3 mm, and it diminished completely on day 8. In the experimental model, the control group treated with triamcinolone acetonide 0.1% right after the induction of ulcer (day 0) experienced accelerated healing than the experimental group. The ulcer was not observed on day 5, and signs of inflammation subsided completely on day 7 (Table 2).

Groups	Average Ulcer Diameter (mm)				
	Day 0	Day 1	Day 3	Day 5	Day 8
Control	3	6	6	0	0
Experimental	3	6	6	3	0

Control group: 80ºC ball pointed instrument followed by the administration of triamcinolone acetonide 0.1%; Experimental group: 80ºC ball pointed instrument

Table 2. Diameter of the Tongue Ulcer.

A microscopic analysis of the specimen obtained from the control mice showed an intact epithelium with no sign of inflammation (Figure 3A). On day 1 after the induction of ulcer, the epithelial layer appeared disintegrated (Figure 3B). Infiltration of Inflammatory cells was apparent underneath the ulceration (Figure 3B1), and a dilated blood vessel was evident (Figure 3B2). On day 8, the dilated blood vessel was still observed with a complete reintegration of the epithelium (Figures 3C and 3C1). On day 9, the mucosa of the tongue recovered completely with a slightly dilated blood vessel (Figure 3D).

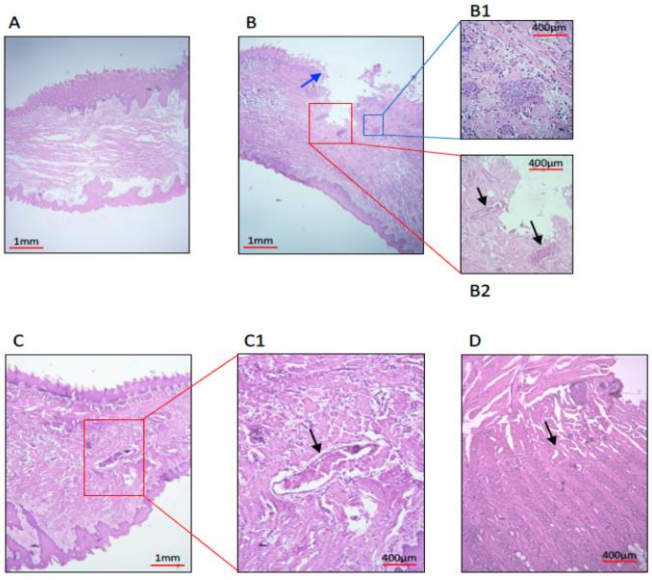

Figure 3. Lateral tongue mucosa sections stained with hematoxylin and eosin. (A) Normal lateral tongue mucosa. (B) Day 1 after the induction of ulcer. Epithelial disintegration is represented in blue arrow.

(B1) Presence of immune cells around the ulcer area. (B2) Dilatation of blood vessels around the ulcer area as shown in black arrow. (C) Day 8 after the induction of ulcer. (C1) Dilatation of blood vessels are shown in black arrow. (D) Day 9 after the induction of ulcer. The dilatation of blood vessels (black arrow) is smaller in size compared with the previous day.

Weight loss was observed on day 1 after the induction of ulcer and was still noted until day 5 in the mice from the experimental group. The mice started to recover from weight loss on day 8, followed by a significantly reduced inflammation on the tongue mucosa. Meanwhile, as expected, the control mice gained weight with time (Table 3).

Group	Average Body weight (gram)				
	Day 0	Day 1	Day 3	Day 5	Day 8
Control	30	31	31	33	34
Experimental	30	30	27	27	30

Control group: Room temperature (RT) ball pointed instrument; Experimental group: 80ºC ball pointed instrument

Table 3. Body Weight of the Mice During the Ulcer Period.

Discussion

This present study started with a preliminary research to determine the optimum temperature and instrument that should be used in the induction of ulcer in the tongue mucosa of mice, compatible exposure time, and application technique. Early exposure temperature was set based on the research of Meyerholz et al. that used 100℃ 2 cm x 2 cm custom-made aluminum branding iron to induce burn injuries on the skin of rats.12 A preliminary study has used a burnisher dental instrument that was heated to 100℃. Necrosis was observed on the tongue of the mice, and eventually, the mice died 2 days after exposure to thermal trauma. The second preliminary study has used a 1-mm-diameter ball pointed instrument, and the exposure temperature started at 50℃ (the lowest temperature) with 5s exposure without any pressure. An ulcer formed; however, it healed a day after the induction. In the next experiment, the temperature was increased to 60℃. An ulcer formed; however, it healed 3 days after exposure to trauma. In thermal trauma, an exposure temperature of 70℃ also obtained similar results to an exposure temperature of 60℃.

The use of 80℃ ball pointed instrument for 5s without pressure on the lateral tongue of the mice induced ulceration, and the mice physiologically recovered 8 days after the induction of ulcer. This experiment obtained results similar to that of a previous research by Cavalcante et al., which has proved that epithelial cells recovered 7 days after trauma exposure in a microscopic observation.4 It is more likely the recovery period for ulcer in mice is similar to that in humans.2,3 Therefore, in this study, we induced thermal trauma using an 80℃ ball pointed instrument for 5s without pressure on the lateral tongue of the mice.

To determine the actual time of ulcer formation and recovery period, we performed a clinical assessment on mice exposed to thermal trauma daily from the induction day to the complete physiological recovery day. The results showed that the ulcer formed right after the exposure to thermal trauma and healed on day 8. Based on this preliminary research, we could determine the ideal time to sacrifice the mice and obtain the specimens for histological analysis. The number of animals used in the study was based on the 3R principle for reducing the animal model samples, as stated in a previous publication.[13]

Mouth ulcer is a self-limiting disease. The use of proper medications on the surface of the ulcer potentially reduces pain and discomfort; moreover, it can accelerate the recovery period of the ulcer.[14] Thus, in the research on establishing ulcer models, the evaluation of the formation and recovery period of ulcer is important.

Thermal trauma was chosen for the induction of ulcer in this study due to the high incidence of burns in the mucosa due to the ingestion of hot foods or beverages or use of dental treatment instruments. The ulcer model that involved the induction of thermal trauma has never been used in previous studies. A previous study has shown the use of an ulcer model involving the induction of mechanical trauma with surgical blade no. 15 or the administration of acid.[4,15] The differences in stimulus can cause differences in the formation of and recovery period of ulcer.

In the present study, thermal trauma induced by an 80°C ball pointed instrument for

5 s produced similar ulcers in terms of size and morphology. Macroscopically, the signs of inflammation, such as redness in the ulcer area caused by the vasodilation of blood vessels and swelling caused by the high permeability of blood vessels that induced the infiltration of exudate to tissues with trauma, were observed in all of the experimental groups but not in the control group.[16,17]

In this study, the maximum ulcer diameter formed a day after exposure to thermal trauma. This formed an ulcer based on the histologic features characterized by epithelial disintegration, dilatation of blood vessels, and infiltration of inflammatory cells to the surrounding area (Tables 1 and 2; Figure 2). Thermal trauma, which was induced to the lateral tongue, may cause the occlusion of blood vessels and damage in the collagens, endothelial cells, and epithelial cells, and as a result, a crater-like ulcer is formed.[16-18]

Based on the depth of tissue breakdown, oral lesions could be erosion, excoriation, or ulcer. Ulcer could be determined via a microscopic observation, which shows epithelial disintegration reaching the lamina propria, followed by the vasodilation of blood vessels and infiltration of inflammatory cells.[4,19]

Tongue ulcers are painful, and they affect the masticatory system. However, in this study, pain sensation was not observed directly; however, weight loss in mice was noted. The pain caused by tongue ulcer potentially reduces food intake that would result in weight loss. In this research, we observed weight loss in all the mice from the experimental group for 5 days after the induction of ulcer. The mice started to recover from weight loss, followed by recovery from ulcer (Table 3).

The healing process took 9 days after the induction of ulcer. Based on the histological analysis, the healing process was extensively characterized by re-epithelization, decreased dilatation of blood vessels, and absence of inflammatory cells. Our tongue ulcer model has a similar healing time to that in a previous study (~10 days).[4]

The use of topical corticosteroid was effective and safe for the treatment of oral ulceration.[20,21] In this study, triamcinolone acetonide 0.1% was used in the positive controls. The result showed that the tongue ulcer in mice healed completely within 7 days. It was faster than the normal recovery time. Moreover, the present tongue ulcer model could be the ideal standard in developing a tongue ulcer model in which the ulceration period could extend up to 9 days. The duration of ulceration period would be beneficial for researchers in examining the efficacy of the novel treatment in the accelerating ulcer-healing process.

Conclusions

We optimized the tongue ulcer mode in mice using a controlled and efficient procedure. Thus, further studies on the treatment of oral ulcer particularly in the tongue region should be conducted.

Declaration of Interest

The authors declare no conflict of interest and thank N.Z. Djamal, N. Sabrina, A. Tjahajani, for discussion and technical assistance. This work was supported in part by a grant PDPUPT Kemenristekdikti RI 2018, No.:351/UN2.R3.1/HKP05.00/2018.

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