

Healing properties of papain-based gel on oral ulcers

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Abstract

The oral mucous membrane is prone to developing ulcers originating from traumatic or immunological processes. **Aim:** The aim of the present study was to perform a histological evaluation of the antiinflammatory and healing properties of Papacarie™ applied to oral ulcers. **Methods:** Fifty adult female albino Wistar rats were randomly separated into two groups: control and Papacarie™. The animals were anesthetized, placed in prone position and ulcers were induced in the middle dorsum of the tongue through a 3-mm-diameter punch. The control group received no treatment thereafter, while those in the Papacarie™ group received an application of the gel twice a day for 14 days. Five animals from each group were euthanized on days 1, 3, 5, 7 and 14. The tongues were removed, fixed, routinely processed for hematoxylin-eosin staining and analyzed by two blind, calibrated pathologists for the presence or absence of ulcer, inflammatory infiltrate and neutrophils. Data were submitted to statistical analysis by the Fisher's exact test. **Results:** On day 1, both groups exhibited ulceration and dense acute inflammatory infiltrate. On day 5, reepithelization and scanty chronic inflammatory infiltrate were observed. On day 14, all animals were healed. There were no statistically significant differences between groups with regard to ulceration ($p=0.81$), inflammation ($p=0.55$) or neutrophils ($p=0.53$). **Conclusions:** Oral ulcers treated with a papain-based gel exhibited the same inflammatory reaction and healing aspects as those of the non-treated control group.

Keywords: papain, oral ulcer, Wistar, wound healing.

Introduction

The oral mucous membrane is prone to developing ulcers originating from traumatic or immunological processes. These episodes of oral trauma or aphthous attacks are characterized by painful lesions that range from the size of a pinhead up to several centimeters in diameter. There have been many attempts over the years to find an effective treatment for oral ulceration and localized topical regimens are considered to be the standard treatment for mild cases of oral ulcers. Standard topical treatment options that provide relief from symptoms include analgesics, anesthetics, antiseptics, anti-inflammatory agents, steroids, sucralfate, tetracycline suspension and silver nitrate. Dietary modifications may also support therapeutic measures¹.

Despite its size, an oral ulcer can cause mild to severe pain. In a recent study on oral health-related quality of life (OHRQoL) measures for oral diseases, Hapa et

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al. (2010) found that patients with recurrent aphthous stomatitis reported a lower OHRQoL than the general population².

Papain is a purified protein extracted from the latex of the papaya tree (*Carica papaya*) and is an archetypal nonspecific proteinase widely used by Brazilian nurses in traditional medicine. It can be used as phytotherapeutic agent to remove damaged or necrotic tissue in the treatment of pressure ulcers, gangrene and eschars and as a debriding chemical agent. Papain digests necrotic tissue by breaking down fibrins in the presence of sulfhydryl groups (e.g., cysteine) without digesting collagen. Papain liquefies the eschar, thereby facilitating the migration of viable cells from the wound edge to the wound cavity. It is also useful in reducing the bacterial load, decreasing exudates and increasing the formation of granulation tissue³⁻⁴.

To improve its healing properties, papain has been associated with other substances. Certain formulations contain urea, which is a chaotropic agent that facilitates the action of papain by solubilizing proteins. Others contain a copper chlorophyllin complex, which has been clinically shown to promote the formation of healthy granulation tissue, control inflammation and diminish odor⁵.

Papacarie™ is a gel containing papain, chloramine and toluidine blue that has been used to chemically soften carious dentin tissue⁶⁻⁹. Besides the papain bacteriostatic, bactericide and antiinflammatory properties, the gel presents chloramine, a compound that contains chlorine and ammonia and has bactericide and disinfectant properties. The purpose of this study was to perform a histological evaluation of the antiinflammatory and healing properties of this papain-based gel applied to oral ulcers.

Materials and methods

Animals

The sample consisted of 50 adult female albino Wistar rats (*Rattus norvegicus albinus*), with body weight ranging from 250 to 300 g. The animals were obtained from the central animal lodging facility of Nove de Julho University (UNINOVE), Brazil and were maintained at controlled light and temperature conditions with standard chow and water available *ad libitum*. All experimental procedures were carried out in compliance with the guidelines of the Brazilian College for Animal Experimentation. This study received approval from the UNINOVE Ethics Committee (process number 19/2007/CEA/UNIDERP).

Ulcer induction

After weighing, each animal received a single intramuscular injection of 80mg/kg of xylazine (Anasedan, Vetbrands, Jacareí, SP, Brazil) and 10mg/kg of ketamine (Dopalen, Vetbrands). The animal was then placed in prone position. A 3-mm-diameter punch was made to induce injury by removing a circular area from the dorsum of the tongue in such a way that the injury was located on the middle portion of the median sagittal plane. To control the depth of

the induced ulcers, a rubber stop was placed on the punch in order to produce ulcers with 5 mm of depth.

Experimental Groups

The animals were randomly separated into two groups. The control group received no treatment. These animals were handled in the same manner as the other group, but without the application of gel and the ulcers were left to heal without interference. The animals in the papain group received the application of Papacarie™ (Fórmula & Ação, São Paulo, SP, Brazil) twice a day with a cotton swab for fourteen days. The lesions were accompanied daily.

Histological analysis

For the histological analysis, 5 animals from each group were euthanized with an overdose of anesthesia on days 1, 3, 5, 7 and 14. The tongue was removed, fixed with 10% paraformaldehyde, embedded in paraffin and routinely processed for hematoxylin-eosin staining. The specimens were analyzed by two blind, calibrated pathologists who evaluated the presence or absence of ulcer, inflammatory infiltrate and neutrophils.

Statistical analysis

Data were subjected to statistical analysis by the Fisher's exact test. The nature of the variables studied and the variability of the means was considered using the Bioestat 5.0 software program. The significance level was set at 5%.

Results

Data from the histological analysis are presented in Table 1. On day 1, both groups exhibited ulceration, dense acute inflammatory infiltrate and neutrophils. On day 3, one animal of each group had no ulcer, only acute inflammation, while the others exhibited ulceration and acute inflammation (Figures 1 A and B). On day 5, only one animal from each group exhibited ulceration and acute inflammation, while the others exhibited reepithelization and scanty chronic inflammatory infiltrate. On day 7, none of the specimens exhibited ulcer, but a few exhibited chronic inflammatory infiltrate (Figures 1 C and D). On day 14, all animals were healed. There were no statistically significant differences between groups with regard to ulceration ($p = 0.81$), inflammation ($p = 0.55$) or neutrophils ($p = 0.53$).

Discussion

From the results of the present study, it was observed that oral ulcers treated with Papacarie™ gel exhibited the same inflammatory reaction and healing process as the control group, with ulceration until the third day and remodeling of the connective tissue within 14 days. These results corroborate previous findings demonstrating that this gel is biocompatible and may be used in clinical practice.

Table 1. Histopathological findings in the control or Papacarie™ groups according to the day of experiment and histological parameter.

		Control				Papacarie™					
		D1	D3	D5	D7	D14	D1	D3	D5	D7	D14
Ulcer	yes	5	4	1	0	0	5	3	2	0	0
	no	0	1	4	5	5	0	2	3	5	5
Inflammatory process	yes	5	5	4	1	3	5	5	4	3	1
	no	0	0	1	4	2	0	0	1	2	4
Neutrophils	yes	5	4	1	0	0	5	5	2	1	0
	no	0	1	4	5	5	0	0	3	4	5

The numbers represent the amount of animals of each group. Notice that the healing process was similar to both groups.

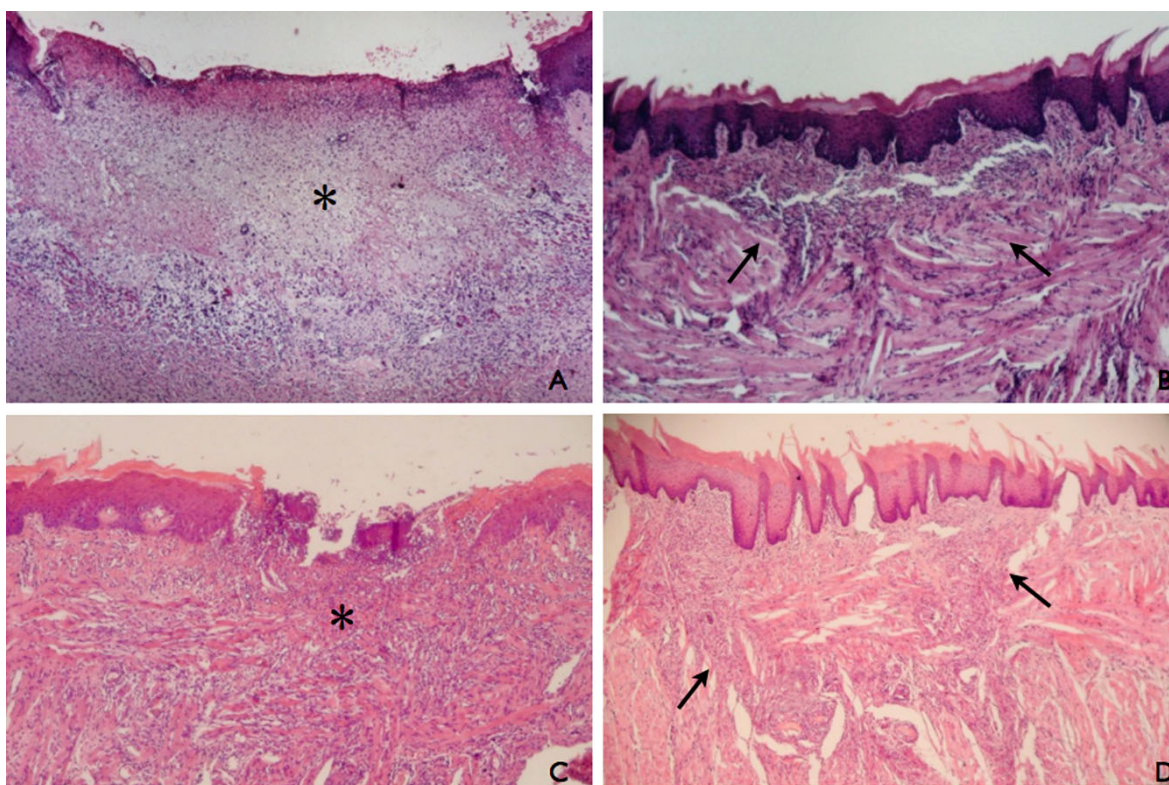


Fig. 1. Comparison of histological features of healing in control group and Papacarie™ group. A: lingual mucosa of rat from control group on day 3, with ulceration, inflammation and edema (*); B: healing process on day 7, with complete reepithelization, formation of epithelial ridges and granulation tissue (arrows); C and D: Papacarie™ group on days 3 and 7, respectively, with similar histological features (hematoxylin-eosin; original magnification: 100x)

Papacarie™ has been used on humans as a mechanical-chemical method for the removal of carious tissue, as it unites the cleaning and healing properties of papain with the disinfectant characteristics of chloramine. It also helps soften carious dentin tissue, thereby facilitating its removal, as the solution stains only the degraded portion and it does not affect the healthy tissue surrounding the lesion. Clinical studies have demonstrated favorable results after 1 year of follow-up on 60 teeth in children from 5 to 9 years of age and 30 molars in adolescents and adults up to 23 years of age⁶.

Myagi et al.¹⁰ tested the *in vitro* cytotoxicity of Papacarie™ on human pulp fibroblasts (FP5 cell line). The gel was applied to round glass coverslips and left in contact with confluent cultures. Cell viability percentages were obtained 50 s and 24 h after the cell contact with Papacarie™. The results showed that direct contact with the gel for 50 s

yielded lower cell viability percentages than those of control cells (viable control cells: 100%; viable cells in Papacarie™ group: 80%). After 24 h, this substance achieved the same results as those found in the control group¹⁰.

Mastrantonio (2007) analyzed the biocompatibility of papain/chloramine gel on the dorsum of rats and observed a moderate inflammation process in the initial days, comparable to that of the control group, followed by the normal course of healing. These results were reproduced in the present study, in which reepithelization was observed in three days and moderate inflammatory infiltrate lasted the first five days, followed by the formation of granulation tissue and remodeling of the connective tissue¹¹.

The hypothesis of the present study was that Papacarie™ would accelerate wound closure due to the healing properties of papain and the disinfecting action of chloramine and based

on previous favorable clinical results found *in vitro*, *in vivo* and on inflamed human pulp tissue⁶⁻¹². Against expectations, however, the Papacarie™ group behaved like the control group, with no improvement in the oral ulcer healing process. We used a literature well-established methodology for the study of oral ulcers, so the size of the ulcer is adequate¹³. A possible explanation for this outcome is that chloramine in combination with papain alone is not the most adequate agent for the mucosal healing process and perhaps another substance should be added to improve the characteristics of chloramine.

There are a variety of known organic chloramines that have proven useful in organic synthesis. When combined with amino acids, chloramines exhibit interesting biological properties. For example, taurine chloramine has been shown to inhibit the production of superoxide anion (O₂⁻) and nitric oxide (NO) in phagocytes by direct inhibition of the signaling pathways of Ras activation, ERK1/2 phosphorylation and NF-kappaB activation. These effects appear to provide protection from the inadvertent cytotoxicity caused by the overproduction of O₂⁻ and NO¹⁴⁻¹⁵.

Another hypothesis to explain the lack of statistical difference between the groups may be that the Papacarie™ vehicle was not adequate to be used in the oral environment and probably did not stay enough time in the wound. An alternative would be to use an adhesive vehicle as an orabase gel.

In conclusion, the findings of the present study show that the Papacarie™ gel is biocompatible but, with the present formulation, it is not useful to improve the healing of oral ulcers.

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