J Oral Med Oral Surg 2023;29:8
https://doi.org/10.1051/mbcb/2023001

https://www.jomos.org

JOMOS
Journal of Oral Medicine and Oral Surgery

Original Research Article

The impact of Lidocaine gel on TNF-α expression in surgically induced oral mucosal ulcers: an immunohistochemical analysis in rabbits

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(Received: 21 September 2022, accepted: 23 December 2022)

Keywords:
Lidocaine / tumor necrosis factor (TNF-α) / oral ulcer / wound healing

Abstract – Background: Besides being a local anesthetic agent lidocaine is a promising anti-inflammatory agent with limited studies on its effect on the mucosa. **Aim**: Assess the anti-inflammatory effect of lidocaine following surgical induction wound in the oral mucosa as assessed by tumor necrosis factor-α (TNF-α) expression. **Materials and methods:** The study was conducted on 32 albino rabbits that were categorized into 2 equal groups of 16 rabbits: In the control group an oral wound was surgically induced and left without treatment and in the treatment group an oral wound was surgically induced and received topical Lidocaine gel. Euthanasia of animals was carried out on days 1, 3, 7, and 10, and sample sites were processed for histopathological and immunohistochemical staining for TNF-α. **Results**: In the histological observations, it was noticed that the healing process was more rapid and convenient in the test group compared to the control group. For Immunohistochemical assessment, the TNF-α started to express clearly at 1 day and gradually decreased and disappeared at 10 days with a superior effect of the lidocaine group over the control group. **Conclusion:** Lidocaine seems to have anti-inflammatory reactions by lowering TNF-α levels and preventing the production of pro-inflammatory cytokines.

Introduction

Inflammatory pain is one of the most prevalent clinical complaints, and it affects anywhere from 25.35% to 50.75% of individuals in the major European countries [1–4]. The bulk of nonsteroidal anti-inflammatory drugs (NSAIDs) makes up the therapeutic alternatives currently available for pain accompanied by inflammation. On the other hand, the use of NSAIDs for an extended time is linked with an increased possibility of gastrointestinal bleeding, acute renal injury, and heart problems [5]. Therefore, it is essential to research and develop new medications that are both safe and capable of relieving inflammatory pain. Lidocaine is an amide local anesthetic medical agent broadly used in dental maneuvers. Because these medications reduce sodium ion permeability and aid in minimizing membrane depolarization, they cure pain by limiting neuronal transmission [6,7]. Repair and regenerative activities begin immediately after the damage occurs. Proliferation, modification of mediators and extracellular matrix, and circulatory blood cells are all initiated by different growth factors [8]. Inflammation, immune mechanisms, and cell-to-cell communication are all influenced by cytokines. Several stimuli, including trauma and inflammation, trigger the production of interleukins and Tumor necrosis factor-α (TNF-α) [9,10]. TNF-α, an inflammatory cytokine, and collagen synthesis are responsible for a wide range of cellular signaling activities and play a crucial part in wound healing specifically in the acute stage of inflammation. TNF-α also plays a role in regulating the immune system [11]. Besides being a local anesthetic agent lidocaine is considered a promising anti-inflammatory agent yet limited studies on its effect on mucous epithelium are available in the literature [12,13]. To the best of our knowledge, no experimental studies investigating the anti-inflammatory effect of lidocaine intraoral has not been previously reported. Therefore, the current study was conducted on rabbits to establish a rabbit model of inflammation. This study aimed to clarify the role of lidocaine gel against inflammation via examining the histological and immunohistochemical expression of TNF-α in the oral mucosa after induction wound by surgery in rabbits.

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Table I. Wilcoxon Signed Ranks Test for histopathological readings

Days	1- day	3-days	7-days	10-days
Mean rank	2.5	2.5	3.00	3.00
P-value	0.063	0.063	0.038	0.038

*Statistical significant at $P \leq 0.05$.

Materials and methods

This experiment observed animal functions according to the National Institutional Health Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985). The ethical committee accepted the study after measuring ethical criteria at all stages of performing operations and handling animals [Approval letter No. UOM.Dent/A.L.85/21]. Thirty-two healthy albino male rabbits, whose weight was about 2 ± 0.5 kg, were purchased from the animal house, College of Veterinary Medicine, University of Mosul. Animals were housed in convenient animal circumstances, with ten rabbits per cage. Food and water were *ad libtium* with exposure to 12 hours' light–dark cycle, adequate ventilation, and a temperature of 22–25 °C.

Study design and surgical procedure

According to the study materials, this was a single-blinded randomized trial in which animals were divided into two groups of 16 rabbits each on a random basis: Group A had a 1 cm intra-oral mucosal buccal incision on the right side that was left untreated. In contrast, Group B had a 1 cm intra-oral mucosal buccal incision on the left side that was treated with lidocaine gel 5%. (LidoGel, China). For anesthesia, each animal received an intramuscular injection of ketamine hydrochloride 4 mg/kg in 50 mg/ml (Gracure Pharmaceuticals Ltd, Bhiwadi, India) and xylazine base 5 mg/kg in 20 mg/ml (Interchemi Co, Holland) in the thigh muscle. The anesthesia integrity was tested after 10–15 min by loss of ear pinch reflex. About 1 cm intra-oral mucosal buccal incision was operated on each side of the buccal mucosa of the cheek of rabbits intra-orally with a surgical blade no. 15. For standardization, the amount of gel was measured by a disposable syringe (1 ml).

Collection of specimens and scoring

Depending on the time of sacrifice, the animals in each group were separated into four subgroups, each of 4 rabbits; **G1:** Day 1 post-surgery; **G2:** Day 3 post-surgery; **G3:** Day 7 post-surgery; **G4:** Day10 post-surgery.

Tissue preparation for histological study and immunohistochemistry analysis

Following euthanization of rabbits, the selected site of study of buccal mucosa was fixed for histological examination and was observed under a light microscope using a scoring system recorded by many authors with minor modifications [14,15]. All collected data were subjected to statistical analysis and the percentage of nuclear and cytoplasmic expression in the connective tissue is allocated into four scores [16,17]: 0: no positive cells; 1 Mild: Positive cells in a percentage of 1–33%. 2 Moderate: Positive cells in a percentage of 34–66%. 3 Intense: Positive cells in a percentage of 67–100%.

Statistical analysis

Collected data underwent statistical analysis using "Statistical Package for Social Statistics" (SPSS) version 25 for Windows software. The "Wilcoxon Signed Ranks Test" was used to assess histologic and immunohistochemical alteration between the two groups. Statistical significance was set at $P \leq 0.05$.

Results

Histological assessment

The differences between the control and treatment groups regarding inflammatory reactions at a given time interval of healing are shown in Table I.

The impact of the use of lidocaine used in this study was noticed on day one and continued up to 10 days as revealed by a statistical significance in Wilcoxon Signed Ranks. Although the inflammatory reaction on days 1, 3, 7 and 10 appeared to have a higher importance in which *P*-value (0.063, 0.063, 0.038, 0.038), respectively, the healing process and reepithelization were more rapid and convenient in the treatment group when compared with the control group as shown in Figure 1.

Immunohistochemical assessment

The expression of TNF-α via immunostaining was conducted in this work to clarify the cytoplasmic alterations in this marker produced by connective tissue cells throughout the healing periods among rabbits from day one to day 10 with and without lidocaine, as shown in Table II. TNF-α started to express clearly at one day and gradually decreased and disappeared at 10 days with a superior effect of the lidocaine group over the control group as shown in Figure 2.

Discussion

Lidocaine has been used for years in dental practice and is considered a safe and efficient well-known local anesthetic agent. Since the 1970 s, its pharmacology has been thoroughly studied. Besides its use in infiltration and block anesthesia, it

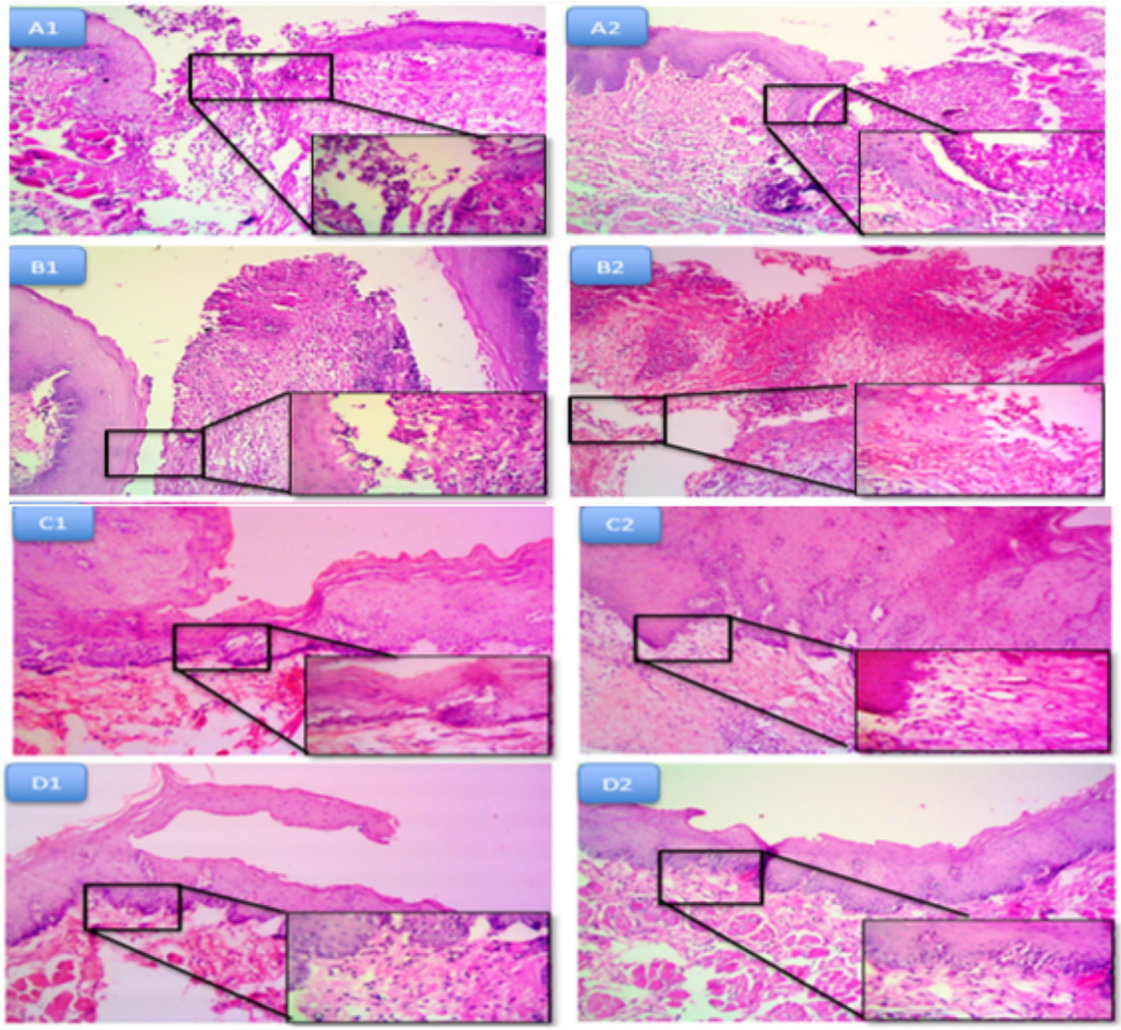

Fig. 1. Photomicrographs of rabbit oral mucosa for histopathological examination. A1&2: Control and treatment group respectively at one-day interval reveals heavy infiltration of inflammatory cells. B1&2: Control and treatment group respectively at three-day interval reveals slightly decrease of inflammation in treatment group compared to control group. C1&2; D1&2: Control and treatment group at seven-day interval and control and treatment group at 10-day interval respectively show superior healing process and decreasing of inflammatory reaction and formation of granulation tissue more regular than control group. H&E stain, 100×, 400×.

is widely used topically to achieve anesthesia of skin or mucosa [18]. Other therapeutic effects of lidocaine include antiarrhythmic, antinociceptive, antithrombotic, and anti-inflammatory. This is due to its decreasing effect on inflammatory mediators [19]. Its anti-inflammatory action remains unclear; yet, it is suggested that lidocaine's anti-inflammatory effects can be seen at concentrations much lower than those needed to block sodium channels [20], Another theory has illustrated its effects on migration, exocytosis, phagocytosis, cell metabolism and by hampering membrane-ion transporters there is dysregulation of cellular pH levels with eventual depression of cytokine release [21]. One of these inflammatory cytokines is TNF-α. The receptors for this cytokine are expressed in a variety of tissues. Its main impact is on the interactions between leukocytes and blood vessel cells which start inflammatory processes with various spatial, temporal, and anatomical presentations using adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [22]. In the current study, the aim was to evaluate the anti-inflammatory effect of lidocaine after topical treatment of the intraoral buccal wound with lidocaine via the histological and immunohistochemical study of the level of expression of the TNF-α. In the lidocaine group, the inflammatory reaction is obviously reduced at three days, there is decreasing in the number of inflammatory cells and formation of regular granulation tissue compared with the control group, these effects continue up and around ten days of healing periods. Inhibiting the expression of pro-inflammatory cytokines, the metabolic activity of leukocytes, and the release of histamine is how lidocaine achieves its anti-inflammatory effects, as has been well documented in the literature [23,24]. Triggered endothelial cells, macrophages, neutrophils, monocytes, B lymphocytes, neurons, dendritic cells, and astrocytes secrete the broadly bioactive peptide substance (TNF-α) [25],

Table II. Wilcoxon Signed Ranks Test for immunohistochemical readings.

Days	1- day	3-days	7-days	10-days
Mean rank	3.00	2.5	2.5	2.00
P-value	0.038*	0.059*	0.046*	0.083*

*Statistical significant at $P \leq 0.05$.

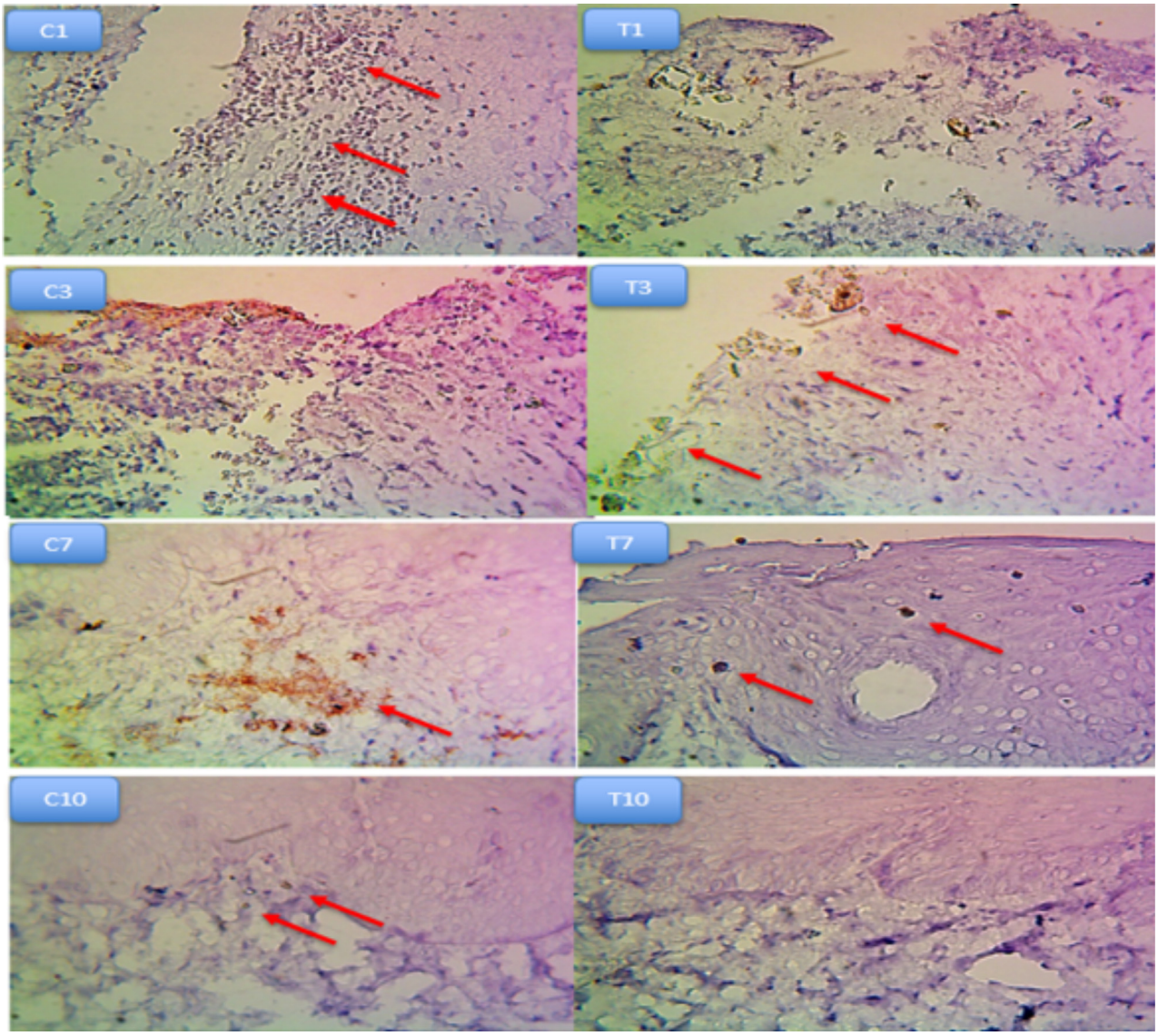

Fig. 2. Photomicrographs of rabbit oral mucosa for immunohistochemical examination of TNF-α. C1: Heavy expression of TNF-α (arrows) in control group compared to (T1) treatment group at one-day interval reveals heavy infiltration of inflammatory cells. C3: TNF-α also continue to express in control group, while reduced in treatment group (arrows) (T3) at three-day interval. C7&T7: Control and treatment group at seven-day interval, showed remission of TNF-α in (T3) in contrast to (arrows) (C7) control group (arrow). C10: Control group appeared slightly expression of TNF-α (arrows) compared to completely absence of TNF-α in (T10) treatment group at 10-day interval. 100×.

which promotes the production of IL-1β, IL-6, and other cytokines, such as IL-4 and IL-5 to attend a pivotal role in the inflammatory response [26]. Our study found that the inflammatory cytokine (TNF-α) was started to express during the first 24hr, after injury even after the application of lidocaine gel and treatment of the wound, then the level of TNF-α appeared to reduce after 3 days of healing periods and continue down-regulated up to 10 days after trauma. These results supported those of Karnina *et al.* who found that 2 mg/kg lidocaine effectively inhibited the inflammatory

process by lowering TNF- protein levels at 24 hours after lidocaine treatment in the test group compared to the placebo group by suppressing NF-k mRNA expression, NF-k protein levels, and TNF- protein levels in mice with musculoskeletal injury [27]. Furthermore, the results of the additional study suggest that lidocaine may be a useful anti-inflammatory agent in endotoxemia [28]. In a similar vein, Lin *et al.* examined the effects of lidocaine on macrophages during inflammation in endotoxemia mice and the underlying mechanism. They discovered that lidocaine protects mice from LPS-induced inflammation and in a dose-dependent fashion inhibits lipopolysaccharide (LPS)-induced production of (TNF-α) and interleukin-6 (IL-6). More so, Lidocaine can counteract the increased glycolysis and glycolytic capacity in macrophages that is caused by LPS [29]. In a previous study by Weinberg et, it was concluded that lidocaine, in supposition, is a potent anti-inflammatory agent, with properties that are often compared to non-steroidal anti-inflammatory drugs (NSAIDs) and steroids [30]. The current study's limitations were the lack of clinical trials to be compared.

Conclusion

According to the study's findings, the anti-inflammatory properties of lidocaine could be explored further from a therapeutic standpoint. However, the clinical use of this drug as an anti-inflammatory, such as that which occurs in the mouth, is not supported by sufficient, well-conducted trials at this time. More research is needed to determine whether lidocaine can be indicated in selected cases, but its potential anti-inflammatory effects make it of particular interest in dentistry when used in topical preparations.

Author contributions

Conceptualization: Ghada A. Taqa. Methodology: *Luma I. Al-Allaf*. Writing original draft: Alyaa I. Naser. Visualization, Investigation: Rayan S. Hamed. Supervision: Rayan S. Hamed. Writing- Reviewing and Editing: Alyaa I. Naser.

Funding

The research is self-funded.

Ethical approval

This research was approved by the Research Ethics Committee of the University of Mosul, College of Dentistry (UoM.Dent/A.L.85/21).

Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent

Consent form is not applicable, this article does not contain any study involving human subjects, it's an experimental study.

Author contributions

Conceptualization: Ghada A. Taqa. Methodology: *Luma I. Al-Allaf*. Writing original draft: Alyaa I. Naser. Visualization, Investigation: Rayan S. Hamed. Supervision: Rayan S. Hamed. Writing- Reviewing and Editing: Alyaa I. Naser.

Acknowledgement. We thank the Collage of Dentistry University of Mosul for their support in conducting this research.

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