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Topical chlorhexidine, povidone-iodine and erythromycin in the repair of traumatic ulcers on the rat tongue: Clinical, histological and microbiological evaluation

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ABSTRACT

Objective: This study investigated the effect of topical application of 0.12% chlorhexidine, 10% povidone-iodine and 50% erythromycin on the optimization of healing process of traumatic ulcers made on ventral tongue of rats.
Design: Forty-Eight Wistar rats were randomly divided into four groups: control, chlorhexidine (Chx), povidone-iodine (PvI) and erythromycin (Er). An ulcer of 5 mm in diameter was made on the ventral tongue of the animals. After 24 h, a microbiological sample was taken and daily application of the substances started. Six animals each group were euthanized at 4 days and the others at 8 days postoperative, totaling three and seven days of treatment. Prior to euthanasia, a new microbiological collection was performed.
Results: The experimental groups showed less area of residual ulcer. A significant difference was seen between the PvI and Chx in relation to the control after three days of treatment ($p < 0.05$). Although the experimental groups displayed greater newly formed epithelial area, there was no significant difference compared to the control ($p > 0.05$). Er exhibed the lowest inflammation scores after seven days of treatment ($p < 0.05$). PvI showed reduction of microorganisms at both times and under aerobic ($p < 0.01$ at 3 days and $p < 0.001$ at 7 days) and microaerophilic ($p < 0.05$) conditions. Er significantly reduced the count of microorganisms in aerobic condition when compared to control group ($p < 0.05$ at 3 days and $p < 0.01$ at 7 days).
Conclusions: All drugs promoted reduction of the microorganisms at the site of the injury, which may have a direct effect on the tissue repair process.

1. Introduction

Oral mucosa is continuously subjected to physical or chemical injuries, where it becomes a common site for the occurrence of ulcerated lesions (Bascones-Martínez, Figuero-Ruiz, & Esparza-Gómez, 2005; Muñoz-Corcuera, Esparza-Gómez, González-Moles, & Bascones-Martínez, 2009). Clinically, the lesions are yellowish, surrounded by an erythematous halo; however, when they display a chronic character, the halo becomes whitish. The traumatic lesions develop in a short time and cause painful symptoms (Muñoz-Corcuera et al., 2009).

The repair of this type of injury occurs by secondary intention, and events occur in cascade, comprising the stages of inflammation, proliferation and remodeling, culminating with healing (Enoch & Leaper, 2005; Young & McNaught, 2011). However, this process becomes complex due to the specifics of the oral environment and high number of microorganisms. Studies describe that the oral cavity can harbor as many as 800 to 1000 different bacterial species (Gomez & Nelson, 2016; Mariano et al., 2015). These microorganisms produce substances that may alter or interfere with healing, delaying the repair process (Edwards & Harding, 2004). Several methods and substances have been employed in an attempt to accelerate tissue repair and prevent infection, such as low-level laser therapy, topical steroids, antiseptics, and antibiotics (Alexandratou, Yova, Handris, Kletsas, & Loukas, 2002; Belenguer-Guallar, Jiménez-Soriano, & Claramunt-Lozano, 2014; Schemel-Suárez, López-López, & Chimenos-Küstner, 2015).

Among the antiseptics, chlorhexidine stands out and is considered the gold standard; it has a broad-spectrum germicidal effect and acts for about 12 h, due to its adsorption to oral surfaces (substantivity), thereby reducing plaque at various concentrations (Charles, Mostler, Bartels, & Mankodi, 2004; Kozlovsky, Artzi, Hirshberg, Israeli-Tobias, & Reich, 2007; Ribeiro, Hashizume, & Maltz, 2007). The bactericidal effect arises from its positive charge, which produces non-specific binding

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to the negatively charged membrane phospholipids of bacteria; this causes an alteration in bacterial osmotic equilibrium, with potassium and phosphorus leakage (Hidalgo & Dominguez, 2001). In an *in vivo* study, application of 0.2% chlorhexidine on palatal wounds of rats has reduced healing time and favored wound epithelialization (Mariano et al., 2015). Nevertheless, due to its local adverse effects such as temporary staining of teeth and taste disturbances, the clinic uses of chlorhexidine in medium- and long-term is limited (Addy, 1994).

Another antiseptic with important clinical applications, particularly in pre-surgical preparation, is povidone-iodine. It has good antibacterial properties and low potential for the development of microbial resistance and adverse reactions (Kramer, 1999). However, rinses with povidone-iodine cause yellow-brown stains on the tooth surface and soft tissues, forming a film (Cherry, Daly, Mitchell, & Highfield, 2007). Its bactericidal effect is due to the inactivation of cytoplasmic substrates that are essential for bacterial viability (Khan & Naqvi, 2006). In the oral cavity, povidone-iodine has shown good clinical results in the management of oral mucositis (Rao et al., 2014; Roopashri, Jayanthi, & Guruprasad, 2011). Madan, Sequeira, Shenoy, and Shetty (2008) investigated the effect of povidone-iodine in the prevention and treatment of radiation-induced oral mucositis. Povidone-iodine was able to significantly reduce the severity and incidence of these lesions. Additionally, studies have shown that povidone-iodine has an anti-inflammatory, hemostatic effect and causes reduction in postoperative edema and trismus (Kumar et al., 2006; Kumar, Reddy, Naidu, & Pandiarajan, 2011).

Erythromycin is a broad-spectrum macrolide antibiotic, wherein its bactericidal effect occurs by the inhibition of protein synthesis. It is used for the treatment of infections affecting the skin and mucosa; it shows immunomodulatory characteristics (Shinkai, Henke, & Rubin, 2008) and anti-osteoclastogenic activity and may inhibit the formation of bacterial biofilms (Tsang et al., 2003). However, there are few studies using erythromycin in topical form for the repair of oral wounds (Shahabooei et al., 2015).

Due to the exposed antimicrobial properties and immunomodulatory (erythromycin) and anti-inflammatory (povidone-iodine) potential and the absence of previous studies assessing the effect of the topical application of erythromycin and povidone-iodine on oral traumatic wounds, a preclinical study has been necessary. Thus, this study aimed to evaluate the effect of chlorhexidine, povidone-iodine and erythromycin on the optimization of healing process of traumatic ulcers made on ventral tongue of rats.

2. Material and methods

The study was approved by the Ethics Committee on the Use of Animals of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil, under protocol number 15/00481b. The sample consisted of seven-week-old 48 male *Wistar* rats (*Rattus norvegicus*), weighing 300–350 g. The animals were kept in the Center for Experimental Biological Models of PUCRS in temperature-controlled (23 ± 1 °C) chambers equipped with input and output air filters and with a 12 h light-dark cycle. They were housed in cages appropriate for rodents with free access to water and food. The animals were randomly divided into four groups of 12 animals each: Control Group (glycerin), Chlorhexidine Group (Chx, 0.12% chlorhexidine), povidone-iodine Group (PvI, 10% povidone-iodine), and Erythromycin Group (Er, 50% erythromycin) (Fig. 1). The substances were prepared in gel form, with glycerin as the vehicle.

The rats were anesthetized with intramuscular injection of ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (10 mg/kg). Afterwards, a circular excisional wound, 5 mm in diameter and 1 mm deep, was made in the center of ventral tongue, using a punch-out biopsy tool. Mucosa specimens were removed by sharp dissection exposing a circular area for secondary healing (Kozlovsky et al., 2007). After 24 h, the first microbiological sample was collected from the ulcer, with a sterile swab. Immediately, the animals were restrained by hand and 0.1 mL of the substance used for the treatment was applied to the ulcer area with a sterile swab, for 15 s. This process was repeated every 24 h during the experimental time, three or seven days. Water and food were removed for thirty minutes after each application to maintain the gel in contact with the region as long as possible. At the end of treatment, a second microbiological sample was collected. Six animals were euthanized at 4 days and the others at 8 days post-operative, totaling three and seven days of treatment, respectively (Fig. 1). Euthanasia was performed using a carbon dioxide chamber.

2.1. Clinical and macroscopic evaluation

Immediately after euthanasia, a clinical evaluation of the repair was performed through visual inspection and macroscopic quantification of wound closure was performed. Briefly, a periodontal probe was placed near the wound (for calibration of the software) and the images of the tongues were captured with a digital camera (Canon 60D, Tokyo, Japan). In the ImageJ 1.50i software (Wayne Rasband Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA), the ulcers were outlined manually (polygonal tool) and the area was obtained in mm^2. This analysis was performed by a single blinded and calibrated examiner.

2.2. Processing of specimens

Following clinical analysis, surgical resection of the tongue was performed and tissues fixed in 10% formalin. After 24 h, specimens were transversely sectioned in the central region of wound and subjected to routine histological processing. The specimens were embedded in paraffin and 5-μm-thick sections were obtained and stained with hematoxylin and eosin.

2.3. Histological analysis

Initially, we analyzed all of the histologic slides qualitatively and afterward selected a field such that the wound could be histomorphometrically evaluated throughout its width. Images of the histologic field were captured with a digital camera (Retiga 2000R CCD – Surrey, Canada) connected to a binocular light microscope (Axioskop 40 – Carl Zeiss, Göttingen, Germany) with an original magnification of 12.5× (objective lens – Carl Zeiss, Göttingen, Germany) and saved on a computer (TIFF format). The measurement of epithelial tissue area was performed with the aid of ImageJ 1.50i software. On the histological image, a line about 5 mm long was drawn so that ulcerated area would occupy the central portion. Epithelial tissue contained in this region was outlined by the area measurement tool (polygonal shape) to obtain the epithelial area in mm^2 of each edge. The values found were added to obtain total area of epithelium formed.

For the analysis of inflammation, the field with the greatest intensity of inflammatory infiltrate was selected (200×) and captured with an original magnification of 400× (objective lens – Carl Zeiss, Göttingen, Germany). Each ulcer was scored histologically on a 0–3 scale: 0 (no inflammation), 1 (slight inflammation, few and sparse mononuclear cells), 2 (moderate inflammation, moderate mononuclear infiltrate and/or neutrophils and sparse eosinophils) and 3 (intense inflammation, polymorphonuclear infiltrate of neutrophils and eosinophils) (Figueiredo, Pesce, Gioso, & Figueiredo, 2001).

The microscopic analysis was performed by a single blinded and calibrated examiner. Briefly, two analyses of ten slides were performed with one-week interval. The intra-examiner agreement was evaluated using the intra-class correlation coefficient, and the value obtained was 0.9 for both analyses, inflammatory infiltrate and epithelial tissue area.