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MARCELLO PIACENTINI

EFEITO TÓPICO DA DOXICICLINA E DA DEXAMETASONA EM ÚLCERAS CONFECCIONADAS NA LÍNGUA DE RATOS: ANÁLISE CLÍNICA E HISTOLÓGICA

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PÓS-GRADUAÇÃO - STRICTO SENSU

Pontifícia Universidade Católica
do Rio Grande do Sul

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Dissertação apresentada à Escola de Ciências da Saúde da Pontifícia Universidade Católica do Rio Grande do Sul como parte dos requisitos para obtenção do título de Mestre em Odontologia, área de concentração em Estomatologia Clínica.

Orientadora: Profa. Dra. Fernanda Gonçalves Salum

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EPÍGRAFE

"Só sei que nada sei, e o fato de saber isso, me coloca em vantagem sobre aqueles que acham que sabem alguma coisa".

Sócrates

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Agradeço à **Deus** por me conceder o bem mais precioso que é a vida. Por me dar saúde e força para vencer os desafios de cada dia.

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RESUMO

RESUMO

Lesões ulceradas são frequentes na mucosa bucal e podem ser causadas por diversos fatores como traumatismos, substâncias químicas, distúrbios autoimunes, processos infecciosos dentre outros. O controle do processo inflamatório é desejável e pode otimizar o reparo dessas lesões. Corticosteróides tópicos como a dexametasona, têm sido comumente utilizados em algumas dessas lesões, entretanto, podem acarretar efeitos adversos. A doxiciclina é um antibiótico do grupo das tetraciclinas, que possui importantes propriedades anti-inflamatórias. Estudos investigando seus efeitos em lesões da mucosa bucal são ainda escassos. No primeiro artigo desta dissertação foi realizada uma revisão da literatura, investigando o efeito da doxiciclina em lesões bucais ulceradas. Foram selecionados quatro ensaios clínicos controlados e randomizados nos quais a doxiciclina foi utilizada no tratamento da ulceração aftosa recorrente e em pacientes com herpes labial. A doxiciclina promoveu remissão clínica mais rápida das lesões e escores de dor inferiores em comparação aos controles. O segundo artigo descreve um estudo experimental em ratos Wistar (n=66), realizado com o objetivo de avaliar o efeito da aplicação tópica da dexametasona (0,5 mg/5ml) e da doxiciclina (20 mg/ml) em úlceras bucais. Uma lesão ulcerada de 5 mm de diâmetro foi mecanicamente induzida no ventre lingual dos animais e os fármacos foram aplicados na forma de gel, duas vezes ao dia, durante três ou sete dias. Após a eutanásia, foi analisada a área de úlcera remanescente e as línguas foram seccionadas para análise histológica. Clinicamente não foram observadas diferenças significativas entre os grupos dexametasona, doxiciclina e controle quanto à área da úlcera remanescente em três (P=0,798) ou em sete (P=0,074) dias de tratamento. No exame histológico,

também não foram observadas diferenças entre os grupos quanto à área de tecido epitelial neoformado (P=0,211; P=0,57) ou quanto ao infiltrado inflamatório (P=0,147; P=0,881) em três ou em sete dias de tratamento. Considerando a metodologia empregada, a dexametasona e a doxiciclina não reduziram o tempo de cicatrização, nem a intensidade do infiltrado inflamatório em úlceras mecanicamente induzidas na mucosa bucal.

Palavras-chave: Úlceras orais. Doxiciclina. Dexametasona. Cicatrização.

ABSTRACT

ABSTRACT

Ulcerative lesions of the oral mucosa are frequent and can be caused by factors such as trauma, chemical substances, autoimmune disorders and infectious processes, among others. Control of the inflammatory process is desirable and may optimize the repair of ulcerative lesions on the oral mucosa. Topical corticosteroids, such as dexamethasone, have been commonly drugs used for this purpose, but they may have some adverse effects. Doxycycline is an antibiotic of the tetracycline group, which has important anti-inflammatory properties. Studies investigating its effects on oral mucosal lesions are still scarce. In the first article, a literature review was carried out investigating the effect of doxycycline on ulcerative oral lesions. Three randomized and controlled clinical trials were selected in which doxycycline was used in the treatment of recurrent aphthous ulcerations and one in patients with herpes labialis. Doxycycline promoted faster clinical remission of lesions and lower pain scores compared to controls. In the second article, an experimental study was performed in Wistar rats (n=66), in which ulcers were mechanically induced on the ventral tongue, to compare the topical effect of 0.5 mg/5 ml dexamethasone and 20 mg/ml doxycycline. The drugs were applied as a gel twice a day for three or seven days. After euthanasia, the area of residual ulcer was analyzed and the tongue was sectioned for histological analysis. No significant differences were observed among dexamethasone, doxycycline and control groups regarding to area of residual ulcer at three (P=0.798) and seven (P=0.074) days of treatment. Histological analysis also showed no differences among the groups regarding newly formed epithelium area (P=0.211, P=0.57), or inflammatory infiltrate (P=0.147, P=0.881) at three and seven days. Taking into account the experimental model, dexamethasone and doxycycline

did not accelerate the repair of mechanically induced ulcers in the oral mucosa nor did they reduce the intensity of the inflammatory infiltrate.

Key words: Oral Ulcer. Doxycycline. Dexamethasone. Wound healing.

LISTA DE ILUSTRAÇÕES

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

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EGF - Fator de crescimento epidérmico

HE - Hematoxylin-eosine

MMPs - Metaloproteinases de matriz / Matrix Metalloproteinases

PDGF - Fator de crescimento derivado de plaquetas

PAR2 - Protease-Activated Receptor 2

RAP2 - Receptor de Ativação de Proteases 2

TGF- β - Fator Transformador de Crescimento-β

SUMÁRIO

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1 INTRODUÇÃO

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Lesões ulceradas são frequentes na mucosa bucal e podem ser causadas por diversos fatores como traumatismos, doenças autoimunes, processos infecciosos dentre outros. Dessas lesões destacam-se as úlceras traumáticas uma vez que a mucosa bucal é constantemente submetida a danos de natureza química ou física. Clinicamente, podem apresentar bordos ligeiramente elevados, são recobertas por pseudomembrana necrótica branco-amarelada e provocam sintomatologia dolorosa, o que causa prejuízos funcionais como disfagia e disfonia (GILVETTI; PORTER; FEDELE, 2010). Além disso, traumatismos contínuos e infecções secundárias podem torná-las persistentes e exacerbar o quadro inflamatório (MORTAZAVI et al., 2016).

O processo de reparo das úlceras traumáticas ocorre por segunda intenção e, na maioria dos casos, se dá em aproximadamente dez dias (MORTAZAVI et al., 2016). Após a ocorrência do dano tecidual, uma série de eventos ocorre em cascata, a fim de permitir o processo de cicatrização da ferida, iniciando-se pela hemostasia, seguida de inflamação, proliferação e remodelamento (ENOCH; LEAPER, 2008; YOUNG; MCNAUGHT, 2011). Após o trauma tecidual, ocorre uma lesão microvascular e extravasamento de sangue para dentro da ferida. Inicia-se então a cascata de coagulação a fim de promover hemostasia, com agregação plaquetária e formação do coágulo. As plaquetas liberam grânulos alfa, que secretam vários fatores de crescimento, incluindo o fator de crescimento derivado de plaquetas (PDGF), fator de crescimento epidérmico (EGF), fator transformador de crescimento β (TGF- β) e fator plaquetário- IV (ENOCH; LEAPER, 2008; CHIQUET; KATSAROS; KLETSAS, 2015).

O processo inflamatório inicia com vasodialatação, mediada pela degranulação dos mastócitos, que liberam histamina e serotonina, aumentando assim a permeabilidade vascular e, consequentemente, edema e dor por aumento da pressão tissular. Além disto, a lesão tecidual ativa a enzima fosfolipase A_2, que atua nos fosfolipídeos da membrana celular, liberando ácido araquidônico, o qual é muito instável e sofre ação de dois grupos de enzimas, a cicloxigenase e a 5-lipoxigenase. A ação da cicloxigenase no ácido araquidônico produzirá prostaglandinas pró-inflamatórias, causando também dor e edema. A ação da 5-lipoxigenase sobre o ácido araquidônico irá produzir leucotrienos, dentre eles o leucotrieno B4, potente quimiotático de neutrófilos (ANDRADE et al., 2014). Os neutrófilos são as primeiras células atraídas para o local da ferida nas 24-48 horas iniciais, sendo as células predominantes nesta fase. Neste momento, partículas e bactérias são fagocitadas, enzimas degradantes e radicais livres são liberados. Progressivamente os neutrófilos vão sofrendo apoptose e sendo substituídos por monócitos (ENOCH; LEAPER, 2008; YOUNG; MCNAUGHT, 2011).

Componentes da coagulação, fragmentos de imunoglobulina G, citocinas e produtos de degradação de colágeno e elastina atraem os monócitos, que sofrem alteração fenotípica e chegam ao local da lesão como macrófagos teciduais nas 48-72 horas seguintes. Estas células são fundamentais para o reparo, realizando fagocitose e liberando fatores de crescimento e proteases como a colagenase, que são responsáveis pela proliferação celular e debridamento da ferida, respectivamente (ENOCH; LEAPER, 2008; YOUNG; MCNAUGHT, 2011). Cerca de 72 horas após a instalação da ferida, o perfil histológico apresenta uma nova característica, com infiltração celular predominantemente linfoplasmocitária, típica de inflamação crônica, além da proliferação de fibroblastos e vasos sanguíneos,

sinalizando o início do reparo. Os fibroblastos são responsáveis pela produção de fibronectina, proteínas da matriz, ácido hialurônico e, posteriormente, colágeno e proteoglicanos. Estes componentes ajudam a construir a nova matriz extracelular, que suporta ainda mais o crescimento celular interno e é essencial para o processo de remodelamento. Esta fase proliferativa pode durar de duas a quatro semanas e a etapa final desta fase é a epitelização (ENOCH; LEAPER, 2008; CHIQUET; KATSAROS; KLETSAS, 2015).

O controle da inflamação em lesões ulceradas bucais é recomendado, pois além de favorecer o reparo impede a cronificação da úlcera (MORTAZAVI et al., 2016). Drogas como corticosteróides, antibióticos, anestésicos, anti-inflamatórios, anti-histamínicos, antissépticos entre outras têm sido utilizadas de forma tópica a fim de favorecer o processo de reparo e diminuir a sintomatologia dolorosa dos pacientes (GILVETTI; PORTER; FEDELE, 2010; LIU et al., 2012; RENNICK et al., 2016).

A dexametasona é um glicocorticóide sintético que apresenta um potente efeito anti-inflamatório, com meia-vida plasmática de três a quatro horas e meia-vida biológica de 36 a 54 horas (KIM et al., 2009). Esta droga induz a síntese e secreção da proteína anexina-1, que inibe a atividade da enzima fosfolipase A_2. A inibição desta enzima reduz a disponibilidade do ácido araquidônico e, consequentemente, de prostaglandinas e leucotrienos, mediadores do processo inflamatório, causando diminuição da dilatação capilar, do número de linfócitos, neutrófilos e monócitos (SUDLOW et al., 1996). Por outro lado, causa diminuição da proliferação de fibroblastos, podendo retardar a síntese do colágeno (KIM et al., 2009).

Ao aplicar dexametasona em ulcerações aftosas recorrentes, Liu et al. (2012) observaram redução da dor, do tamanho das úlceras e do tempo de duração em

relação ao placebo. No entanto, existe uma certa preocupação com o uso de corticóides de média e alta potência na cavidade bucal, caso sejam usados de forma prolongada, devido ao risco de causar atrofia na mucosa e aumentar a suceptibilidade à proliferação de microorganismos oportunistas como a *Candida albicans*, (QUIJANO; RODRÍGUEZ, 2008; LIU et al., 2012).

A doxiciclina é um antibiótico quimicamente derivado das tetraciclinas de 1ª geração. Possui ação bacteriostática de amplo espectro, inibindo a síntese proteica bacteriana ao segmentar a subunidade ribossomal 30S, tanto de bactérias gram positivas, quanto de gram negativas (NELSON; LEVY, 2011). Esta droga também age na subunidade 40S dos ribossomos, que são próprias de células dos mamíferos, implicando em falta de especificidade (FONSECA, 2008). Em 1983 Golub et al., demostraram que as tetraciclinas apresentam uma potente ação anti-inflamatória, independente de sua ação antimicrobiana, inibindo a atividade da colagenase no tecido gengival de ratos. Desde então, estudos a respeito destes mecanismos vem sendo realizados, demonstrando que a doxiciclina em doses sub-antimicrobianas, de 20 a 40 mg por dia, possui atividade anti-inflamatória. Assim, este fármaco passou a ser prescrito no tratamento de doenças inflamatórias, auto-imunes, periodontais, granulomatosas e acne. Algumas características como regulação de citocinas, antioxidação, inibição da produção de ácido araquidônico, inibição do receptor de ativação de proteases 2 (RAP2), das metaloproteinases de matriz (MMPS), da quebra do colágeno e da quimiotaxia de leucócitos têm sido apontadas como responsáveis pelo seu efeito anti-inflamatório (MONK; SHALITA; SIEGEL, 2011).

Vijayabala et al. (2013), em um ensaio clínico com 50 pacientes portadores de ulcerações aftosas recorrentes menores, obtiveram resultados significativos em

relação ao alívio da dor e remissão clínica das lesões com a aplicação tópica de hiclato de doxiciclina.   Di Caprio et al. (2015), em um estudo *in vitro*, avaliaram a redução de citocinas envolvidas na patogênese de doenças inflamatórias cutâneas, utilizando doxiciclina em doses altas (100 ou 200 mg/dia) e baixas (20-40 mg/dia) e observaram que ambas modularam diretamente a expressão de mediadores inflamatórios. No entanto, os resultados foram mais efetivos quando as doses menores foram utilizadas. Estudos em lesões traumáticas na mucosa bucal não foram encontrados na literatura.

A intervenção nos sete primeiros dias após o trauma reduz a sintomatologia dolorosa e a duração das lesões ulceradas, evita infecções secundárias e otimiza a capacidade funcional do sistema estomatognático. Sendo assim, o presente estudo teve como objetivos realizar uma revisão da literatura investigando o efeito da doxiciclina sobre lesões ulceradas bucais. Além disso, foi realizado um estudo experimental com o objetivo de avaliar o efeito da aplicação tópica da doxiciclina e da dexametasona na cicatrização de lesões ulceradas produzidas mecanicamente na língua de ratos.

2 PROPOSIÇÃO

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2.1 Objetivo Geral

Avaliar o efeito da aplicação tópica de doxiciclina (20 mg/ml) e dexametasona (0,5 mg/ 5 ml) no reparo de úlceras mecanicamente induzidas na língua de ratos.

2.2 Objetivos Específicos

- Realizar uma revisão da literatura, investigando a eficácia da doxiciclina no tratamento de lesões bucais ulceradas.

- Avaliar clinicamente o efeito da aplicação tópica de doxiciclina (20 mg/ml) e dexametasona (0,5 mg/ 5 ml) no reparo de úlceras traumáticas produzidas mecanicamente na língua de ratos.

- Avaliar, por meio de análise histológica, o efeito desses agentes nos componentes celulares do processo inflamatório e na neoformação de tecido epitelial em úlceras traumáticas produzidas mecanicamente na língua de ratos.

3 ARTIGO DE REVISÃO DA LITERATURA

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DOXYCYCLINE: AN OPTION IN THE TREATMENT OF ULCERATED ORAL LESIONS?

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DOXYCYCLINE: AN OPTION IN THE TREATMENT OF ULCERATED ORAL LESIONS?

Doxycycline on oral mucosa lesions

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ABSTRACT

WHAT IS KNOWN AND OBJECTIVES: In addition to its antimicrobial effect, doxycycline has potent antiinflammatory activity. In view of these pharmacological characteristics, its use in the management of inflammatory, autoimmune and granulomatous diseases has been proposed. The objective of this study was to investigate, through a systematic literature review, the effect of doxycycline in the treatment of ulcerated lesions of the mouth.

METHODS: An electronic search was performed in accordance with PRISMA guidelines in PubMed, Cochrane Central Register, Web of Science, Bireme/LILACS and Scopus databases. Controlled, randomized clinical trials were selected. The concentration of doxycycline, frequency of application, symptom relief and clinical improvement of the lesions were analyzed.

RESULTS AND DISCUSSION: According to the inclusion criteria, four articles were selected. In three of these studies, doxycycline was used in the treatment of aphthous stomatitis, and in one study, it was used in the treatment of herpes labialis. In all studies, the drug was used topically, both as a hydrogel and as a crushed tablet (along with a prosthetic adhesive). The groups treated with doxycycline showed faster clinical remission of lesions and lower pain scores compared to controls.

WHAT IS NEW AND CONCLUSION: The present study demonstrated that topical doxycycline has a positive effect on the treatment of recurrent aphthous ulceration and herpes labialis. Experimental animal studies and double-blind randomized clinical trials should be performed on other oral lesions, such as traumatic ulcers and mucositis.

Key Words: doxycycline, oral ulcer, aphthous stomatitis, herpes labialis

WHAT IS KNOWN AND OBJECTIVES

Ulcerated lesions are frequent in the oral mucosa and can be caused by trauma, drugs, chemicals, hypersensitivity reactions, autoimmune disorders and infectious processes, among others.[1-3] Usually, they are covered by a yellowish-white pseudomembrane and surrounded by erythematous halo, and they can cause substantial painful symptomatology. According to duration and frequency, oral ulcers are classified as acute, chronic and recurrent, and may be single or multiple.[3] The variety of etiological factors often makes its clinical management challenging.[4] The time of repair is variable and dependent on the etiological nature. However, continuing trauma and secondary infections may exacerbate the inflammatory condition and make it persistent.[3] Interventions to prevent such clinical episodes are necessary. Drugs such as corticosteroids, antibiotics, anesthetics, antiinflammatories, antihistamines and antiseptics have been used topically and/or systemically to promote the healing process and decrease the painful symptoms of patients.[5-8]

Doxycycline is an antibiotic chemically derived from 1st generation tetracyclines. It has broad-spectrum bacteriostatic action, inhibiting bacterial protein synthesis by binding to the 30S ribosomal subunit of both gram-positive and gram-negative bacteria.[9] This drug also acts on the 40S ribosomal subunit, which is specific to mammalian cells, implying lack of specificity.[10] In 1983, Golub et al.[11] demonstrated that tetracyclines possess potent antiinflammatory activity independent of their antimicrobial action, inhibiting collagenase activity in rat gingival tissue. Since then, studies on these mechanisms have been conducted, demonstrating that doxycycline at sub-antimicrobial doses, from 20 to 40 mg per day, has antiinflammatory activity. Thus, this drug is now prescribed in the treatment of inflammatory, autoimmune, periodontal, granulomatous and acne diseases. Some

properties such as regulation of cytokines, antioxidation, inhibition of protease-activated receptor 2 (PAR2), inhibition of matrix metalloproteinases (MMPS), inhibition of collagen breakdown and chemotaxis of leukocytes have been reported as being responsible for its antiinflammatory effect.[12]

Di Caprio et al.[13] evaluated the in vitro antiinflammatory properties of doxycycline at low (20 to 40 mg/day) and high (100 to 200 mg/day) doses to investigate whether this drug could reduce cytokines involved in the pathogenesis of inflammatory diseases. The study provided evidence that both low and high doses of doxycycline are able to directly modulate the expression of inflammatory mediators. The lower doses were more effective in modulating these mediators and did not trigger antibacterial resistance.

Some studies have suggested the use of doxycycline in the treatment of ulcerated lesions of the mouth, but a consensus has not yet been reached on its efficacy on this type of lesions.[14,15] We carried out a systematic review to investigate effectiveness of doxycycline on ulcerated lesions of the mouth.

METHODS

We carried out a systematic review on studies investigating the effect of topical and systemic use of doxycycline in the treatment of oral lesions such as traumatic ulcers, herpes simplex, erosive lichen planus, recurrent aphthous stomatitis, oral mucositis, benign mucosal pemphigoid and pemphigus vulgaris. An electronic search was conducted according to PRISMA guidelines in the PubMed, Cochrane Central Register of Controlled Clinical Trials, Web of Science, Bireme/LILACS and Scopus databases. We used the MeSH terms: "Doxycycline"

AND "Oral Ulcer" OR "Stomatitis, Aphthous" OR "Lichen Planus, Oral" OR "Oral Mucositis" OR "Stomatitis, Herpetic" OR "Pemphigus" OR "Pemphigoid, Benign Mucous Membrane."

A PICO was stablished:

P – Patients with oral ulcers and animal models with oral ulcers.

I – Topical and/or systemic doxycycline.

C – *Sham* or other drug.

O – Symptoms relief, clinical improvement and histological changes.

The last search was conducted on May 2018.

Inclusion and Exclusion Criteria

The software EndNote 7 was used to aid in the selection of articles. We included controlled studies that used doxycycline topically or systemically for the treatment of ulcerated oral lesions. The concentration of doxycycline, the frequency with which it was applied, the substance used as control, pain relief (human studies), clinical improvement and histological changes (animal studies) had to be described. Case reports, reviews, case series, author comments and letters to the editor were excluded.

Study Design

Initially, the search for articles was carried out by two independent authors in the databases described. After the initial search, the exclusion of articles was carried out by reading the titles and abstracts. At this point duplicate articles were also removed. If there was not enough information in the abstract, or if there was a

divergence in the inclusion and exclusion criteria among the researchers, the article was read completely until a consensus was reached.

Data extraction was then performed independently and duplicated by the two reviewers. The following data were collected: study groups, drug concentrations, route of administration, type of oral lesion, duration of follow-up and results. Figure.1 shows the flowchart of the search strategy and the selection of articles.
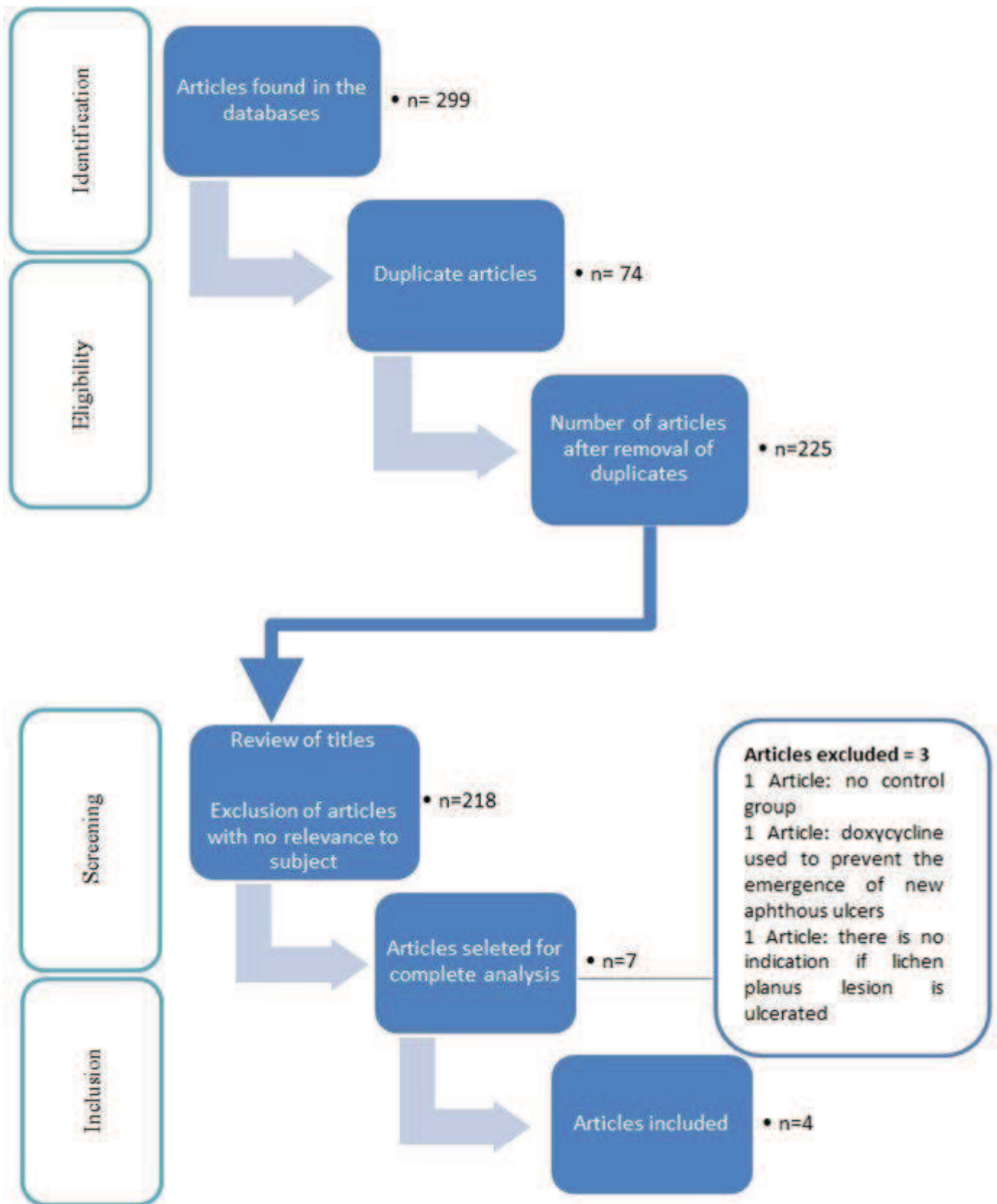
Figure 1. Flowchart of selection of articles

RESULTS

Four controlled clinical trials were included. In three of these, doxycycline was used in the treatment of minor aphthous stomatitis [15-17] and one in the treatment of herpes labialis.[18] Patients with systemic comorbidities were excluded of the studies. No studies were found in which doxycycline had been used for the treatment of oral traumatic ulcers, mucositis, erosive lichen planus, pemphigus vulgaris or benign mucosal pemphigoid. Nor were there studies found in animal models. Although it was proposed as an inclusion criterion, we found no study that used doxycycline systemically in the treatment of ulcerated lesions of the oral mucosa.

Table 1 describes the selected clinical trials. In these studies, doxycycline was used topically in the form of crushed tablet or hydrogel. Monocaprin hydrogel and placebo in the form of hydrogel and crushed tablet were used for the control. Of the four articles selected, two used conventional doses of doxycycline (100-150 mg),[15,17] and two used subdoses (1.5 mg/g - 0.05%).[16,18] The four studies showed positive results for doxycycline, with faster clinical improvement[16-17] and lower pain scores than controls.[15-17] Two studies were randomized single-blind.[15,17] In contrast, the other two selected studies were randomized double-blind.[16,18]

The selected studies totaled a sample of 280 participants, 130 with recurrent aphthous ulceration and 150 with herpes labialis. In the studies that used crushed doxycycline along with prosthetic adhesive, the drug was applied by the health care professional in a single dose. [15,17] This form of application may entail some difficulty in performing the treatment, as well as caution regarding the adhesiveness of the pharmaceutical formulation. On the other hand, in the studies that used the drug in

the form of a hydrogel, the applications were performed by the patients themselves four to five times a day.[16,18]

Table 1. Controlled randomized clinical trials investigating the topical use of doxycyline for treatment of oral ulcerated lesions.

Author/year	Sample	Type of lesion	Drug used/concentration /frequency of use	Duration of study/ follow-up	Outcomes
Ylikontiola et al. 1997.[15]	31 patients	Minor aphthous stomatitis	- Crushed tablet of 150 mg doxycycline + prosthetic adhesive Placebo + prosthetic adhesive Single application	10 days	The pain was significantly less in the doxycycline group (P<0.05) compared to placebo. Variables of clinical improvement were not described in this study. Pain was measure by VAS.
Skulason et al. 2009[16]	49 patients	Minor aphthous stomatitis	-1.5 mg/g doxycycline hydrogel -Placebo 4 times a day	3 days	68% of ulcers healed in 3 days of treatment in the doxycycline gel group, while in the placebo group, 25% of lesions regressed in this period (P<0.05). Pain scores (VAS) were also significantly lower in the doxycycline group (P<0.05). Healing was determined by the subjects as the time when they no longer aware of the presence of the ulcer.
Vijayabala et al. 2013[17]	50 patients	Minor aphthous Stomatitis	- Crushed tablet of 100 mg doxycycline + prosthetic adhesive - Placebo + prosthetic adhesive Single application	10 days	Patients treated with doxycycline had less pain at day 1 (P<0.001) and more rapid involution of lesions (P<0.001) compared to placebo. Pain also measure by VAS. A graduated periodontal probe was used to measure the ulcer size on its maximum diameter.
Skulason et al. 2011[18]	150 patients	Herpes labialis	- 0.5% monocaprin hydrogel - 0.5% monocaprin hydrogel + 0.15% doxycycline -Placebo	5 days	The mean healing time of the lesions was significantly shorter in the monocaprin+doxycycline group in relation to the others (P<0.05). Pain relief (VAS) was significantly greater in the monocaprin+doxycycline group when

	5 times a day	compared to monocaprin alone and placebo (P=0.0114). Clinical evaluation was determined by the subjects as the time when they no longer aware of the presence of the lesion.

VAS – Visual Analogic Scale

DISCUSSION

Studies show that in addition to the antimicrobial effect, doxycycline has a potent antiinflammatory effect. In view of these pharmacological characteristics, some authors have suggested the use of topical and/or systemic doxycycline for the treatment of inflammatory, autoimmune and granulomatous diseases.[11-22] Based on these properties, the present study aimed to review the literature investigating doxycycline as a therapeutic option in the management of ulcerated oral lesions.

In oral medicine, the antiinflammatory properties of doxycycline have been investigated in periodontal diseases, demonstrating effectiveness as a complementary therapy to conventional scaling treatment. [11,22-25] In this study, seven articles were found in which doxycycline was used in ulcerated oral lesions, with three being excluded because they did not meet the inclusion criteria adopted. No animal studies were found, so little research that contained histological analysis. Of the selected studies, three evaluated the effect of doxycycline in patients with recurrent aphthous ulceration[15-17] and one in patients with herpes simplex,[18] showing that doxycycline has been little explored in the management of ulcerated oral lesions.

Skulason et al.[18] proposed the combination of monocaprin, a monoglyceride of capric acid capable of inactivating HSV *in vitro*, with doxycycline to promote blocking viral replication and to optimize tissue repair. This combination promoted pain relief and reduced healing time.

Studies point to glucocorticoids as the first-line treatment for recurrent aphthous ulceration.[6,26,27] However, there is some concern about the use of medium and high potency corticosteroids in the oral cavity due to the risk of causing mucosal atrophy and predisposing to proliferation of opportunistic microorganisms if they are

used extensively.[6,27]   Three of the studies analyzed were performed on aphthous lesions and all showed promising results; however in none of these was the effect of doxycycline compared to that of corticosteroids.

Golub et al.[22] did not observe adverse effects from topical use of doxycycline for 36 weeks at subantimicrobial doses in patients with chronic periodontal disease. The studies selected in this review did not describe adverse effects; however, it should be considered that the treatment period was short, ranging from single application to five days. It was also possible to observe that both high and low doses of doxycycline were able to modulate the inflammatory process, alleviate pain and accelerate the repair process of the lesions. [15-18]

In this study, it was possible to show that, despite the properties of doxycycline, which have already been established in the literature, its use is still little explored in acute, chronic and recurrent ulcerative lesions of the oral mucosa. In the selected studies, topical use of doxycycline had a positive effect in the treatment of recurrent aphthous ulceration and herpes labialis. Experimental animal studies and double-blind, randomized clinical trials are needed with regard to other lesions, such as traumatic ulcers and oral mucositis, to provide evidence that doxycycline may be an option in the treatment of ulcerated lesions that occur the mouth.

WHAT IS NEW AND CONCLUSION

Although the scientific evidence is based on few clinical trials, the present study suggests that topically used doxycycline may be a therapeutic option in the management of ulcerated oral lesions. The antibiotic effects of doxycycline and also its antiinflammatory properties, which affect cytokine regulation, antioxidation, inhibition of MMPS and chemotaxis of leukocytes, among others, are responsible for the beneficial effects of this drug in the treatment of ulcerated lesions of mouth.

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4 ARTIGO DE PESQUISA

EFFECT OF TOPICAL DOXYCYCLINE AND DEXAMETHASONE ON INDUCED

ULCERS ON THE RAT TONGUE: CLINICAL AND HISTOLOGICAL ANALYSIS

EFFECT OF TOPICAL DOXYCYCLINE AND DEXAMETHASONE ON INDUCED ULCERS ON THE RAT TONGUE: CLINICAL AND HISTOLOGICAL ANALYSIS

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ABSTRACT

Objective: This study investigated the topical effect of doxycycline and dexamethasone in the repair of mechanically induced ulcers on tongue of rats.

Design: Sixty-six Wistar rats were randomly divided into three groups: control (glycerin), doxycycline (20 mg/ml) and dexamethasone (0.5 mg/5 ml). A 5-mm ulcer was produced with a punch on the ventral tongue. The substances were applied as a gel with a sterile swab every 12 h. Eleven animals from each group were euthanized after three days of treatment and the others after seven days. Clinical evaluation of the residual ulcers was performed. The tongues were surgically resected and prepared for histological analysis.

Results: In the clinical analysis, no significant differences were observed among the groups regarding the remaining ulcer area after three ($P=0.798$) or seven days of treatment ($P=0.074$), although the dexamethasone group showed smaller lesions than the others. No significant differences were observed among the groups for the neoformed epithelium area ($P=0.211$, $P=0.57$) or for the intensity of the inflammatory infiltrate ($P=0.147$; $P=0.881$).

Conclusions: Considering the experimental conditions, we can conclude that dexamethasone and doxycycline did not accelerate wound healing or reduce the inflammatory process of traumatic lesions of the oral mucosa.

Keywords: oral ulcers, doxycycline, dexamethasone, wound healing

HIGHLIGHTS

- The effect of doxycycline and dexamethasone on oral ulcers was evaluated.

- Clinical and histological variables were analyzed.

- The drugs had no effect on accelerating healing or reducing the inflammation.

INTRODUCTION

Ulcerated lesions are frequent in the oral mucosa and can be caused by various factors such as trauma, chemical substances, autoimmune disorders and infectious processes, among others (Mortozavi, Safi, Baharvand, & Rahmani, 2016). These lesions are usually painful and can cause functional impairments such as dysphagia and dysphonia. The variety of etiological factors often makes its clinical management challenging. In addition, continuous trauma and secondary infections may make them persistent with an exacerbated inflammatory condition (Mortazavi et al., 2016). Drugs such as corticosteroids, antibiotics, anesthetics, anti-inflammatories, antihistamines, antiseptics and others have been used topically in attempt to promote the repair process and reduce the painful symptoms of some ulcerated lesions of the oral mucosa (Gilvetti, Porter, & Fedele, 2010; Liu, Zhou, Liu, Wang, & Chen, Wang, Zhou, Dong, Xu, Wang, Guo, Lin, Wu, Du, Wei, Zeng, Wang, Wu, Li, Zhou, & Zhou, 2012; Rennick, Campbell, Naidu, Taylor, & Buschang, 2016).

Dexamethasone, a synthetic glucocorticoid, has been used for this purpose (Kim, Brar, Jakubowski, Kaltman, & Lopez, 2009) because of its ability to induce the synthesis and secretion of annexin-1 protein, which inhibits phospholipase A2 activity, thus decreasing the availability of arachidonic acid, prostaglandins and leukotrienes (Sudlow, Carey, Forder, & Rothwell, 1996). The suppression of each

stage of the inflammatory process means a decrease in capillary dilation and the number of lymphocytes and neutrophils. On the other hand, dexamethasone causes a decrease in the proliferation of fibroblasts, which may delay the synthesis of collagen (Kim et al., 2009). Furthermore, there is some concern about the use of medium- and high-potency corticosteroids in the oral cavity due to the risk of causing mucosal atrophy and predisposing to the proliferation of opportunistic microorganisms such as *Candida albicans* if they are used for a prolonged period (Quijano & Rodríguez, 2008; Liu et al., 2012).

Doxycycline is an antibiotic chemically derived from 1st generation tetracyclines. Golub et al. (1983) demonstrated that tetracyclines show a potent anti-inflammatory action, independent of its antimicrobial action. This feature is due to its ability to inhibit matrix metalloproteinases, collagen breakdown, and leukocyte chemotaxis (Monk, Shalita, & Siegel, 2011). Clinical studies using doxycycline for the treatment of aphthous stomatitis and herpes labialis have shown promising results in reducing the healing time and pain scores of patients (Ylikontiola, Sorsa, Häyrinen-Immonen, & Salo,1997; Skulason, Holbrook, Gunnarsson, & Kristmundsdottir, 2009; Skulason, Holbrook, Thormar, Gunnarsson, & Kristmundsdottir, 2012; Vijayabala, Kalappanavar, Annigeri, Sudarshan, & Shettar, 2013).

Many oral ulcerative lesions are treated with topical corticosteroids, which can have adverse effects on patients. Considering the anti-inflammatory and antibiotic properties of doxycycline and the few preclinical studies investigating its effects on oral mucosal lesions, the present study aimed to compare the topical effects of doxycycline and dexamethasone on the repair of ulcerative lesions produced mechanically on the rat tongue.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), under protocol No. 8321. The sample size was calculated using the software *Minitab*, aiming at the application of ANOVA test with six levels, that is three experimental groups and two study times, estimating a sample size of 22 units per group. The sample consisted of 66 male Wistar rats weighing 200-300 g. They were kept in the Center for Experimental Biological Models (CeMBE) of PUCRS, in microisolators that were equipped with air inlet and outlet filters and had controlled humidity and temperature (23±1ºC) and 12-h light-dark cycle. The animals were randomly divided into three groups of 22 animals each: Control Group (glycerin), Doxycycline Group (20 mg/mL doxycycline), and Dexamethasone Group (0.5 mg/5 mL dexamethasone). The doxycycline doses were based on Golub et al. (2016).

At the beginning of the experiment the animals were anesthetized intraperitoneally with 10% ketamine hydrochloride (75 mg/kg) and 2% xylazine hydrochloride (3 mg/kg) until loss of protective reflexes. With the aid of a disposable punch, an ulcer was produced in the middle third of the ventral tongue 3 mm from the apex, with a diameter of 5 mm and a depth of 1 mm (Teixeira, de Figueiredo, Cherubini, Garcia, de Oliveira, & Salum, 2018). Twenty-four hours after the induction of the lesions, the animals started to receive the treatment determined for each group.

The substances tested were prepared as a gel and applied topically every 12 h on the ulcers in the amount of 1 mL, with the aid of a disposable swab, under manual restraint. Feed and water were removed for 30 minutes after application to avoiding

product removal. In eleven animals of each group the applications were carried out for three days, and in the others, for seven days. The animals were euthanized on the fourth and eighth days with injection of 10% ketamine hydrochloride and 2% xylazine hydrochloride intraperitoneally. The flowchart demonstrating the steps of the study is shown in Figure 1.
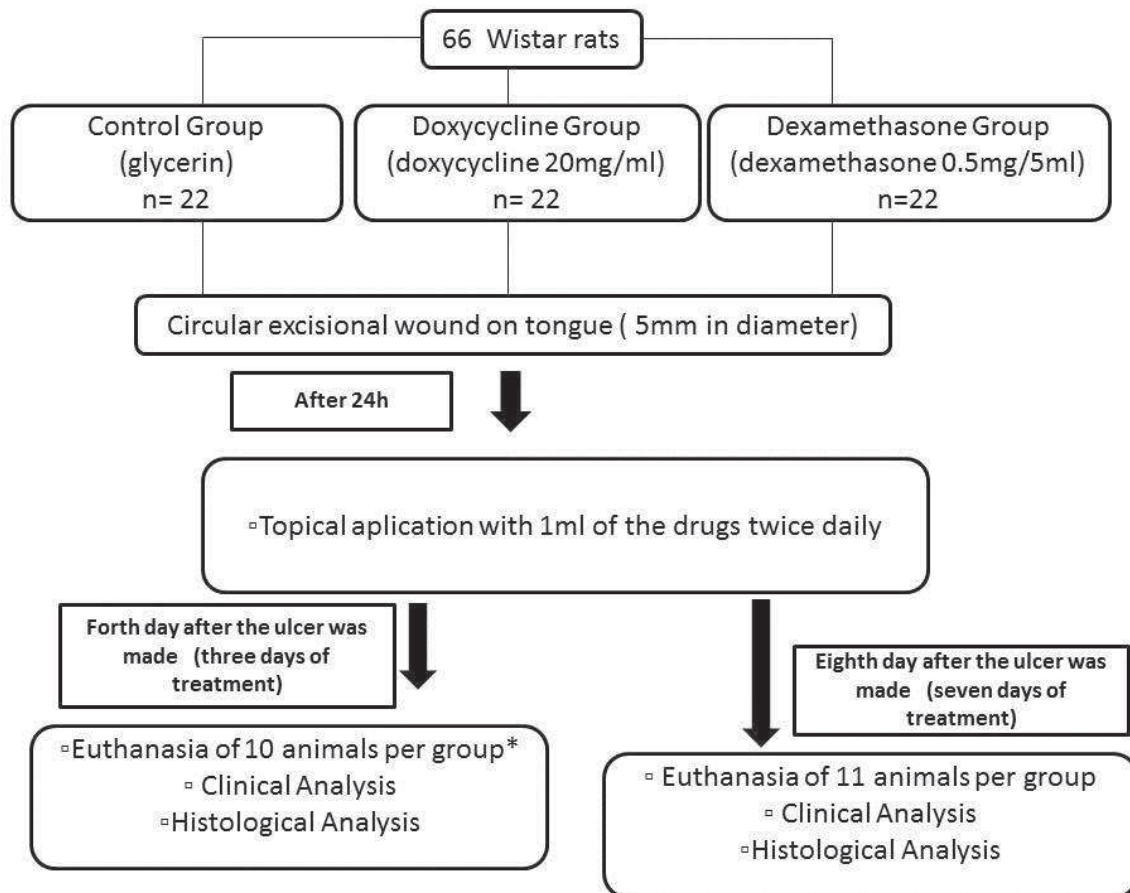
*1 animal in each group was lost during anesthesia

Figure 1 Flowchart describing the steps of the study

Clinical Evaluation

Immediately after euthanasia, all lesions were measured using a periodontal probe (mm) and a photographic record was taken (NikonCoolPixL23 camera, Japan). Images of the residual ulcers were analyzed with ImageJ 1.5 software (developed by

Wayne Rasband of the Research Services Branch, National Institute of Mental Health, Bethesda, MD) for measurement of residual ulcer (mm^2).

Preparation of Specimens and Histological Analysis

The tongue of each animal was surgically resected and immersed in a flask containing 10% buffered formalin for 24 hours. After fixation, the specimens were cross-sectioned, subjected to routine histological processing and stained with hematoxylin-eosin (HE).

All slides were examined by a single and blinded examiner under a light microscope (Olympus, model Bx50, Japan). Each slice was qualitatively analyzed throughout its length. The field with the highest intensity of inflammatory response (cellular and vascular) was captured at 200X using a microscope-coupled system (Média Cybernetics, Cool SNAP-Procf model, USA). The images were saved in TIFF format for analysis of the inflammatory response according to the following classification criteria (Figueiredo, Pesce, Gioso, & Figueiredo 2001): 0 (absence of inflammation); 1 (slight - presence of mononuclear cells); 2 (moderate - presence of mononuclear infiltrate and/or few neutrophils and eosinophils); 3 (intense - presence of polymorphonuclear infiltrate of neutrophils and eosinophils).

For measurement of the area of newly formed epithelium, we selected a field such that the wound could be histomorphometrically evaluated throughout its width. Measurement of epithelial tissue was performed by a single examiner calibrated with the help of ImageJ 1.5 software. First, a 5 mm line was drawn so that the ulcerated area occupied the central portion. The epithelial tissue contained in this region was outlined with an area measurement tool (polygonal shape) to obtain the area in mm^2

of each edge. The measurements were summed, and thus, the total area of neoformed epithelial tissue was obtained (Figure 2).
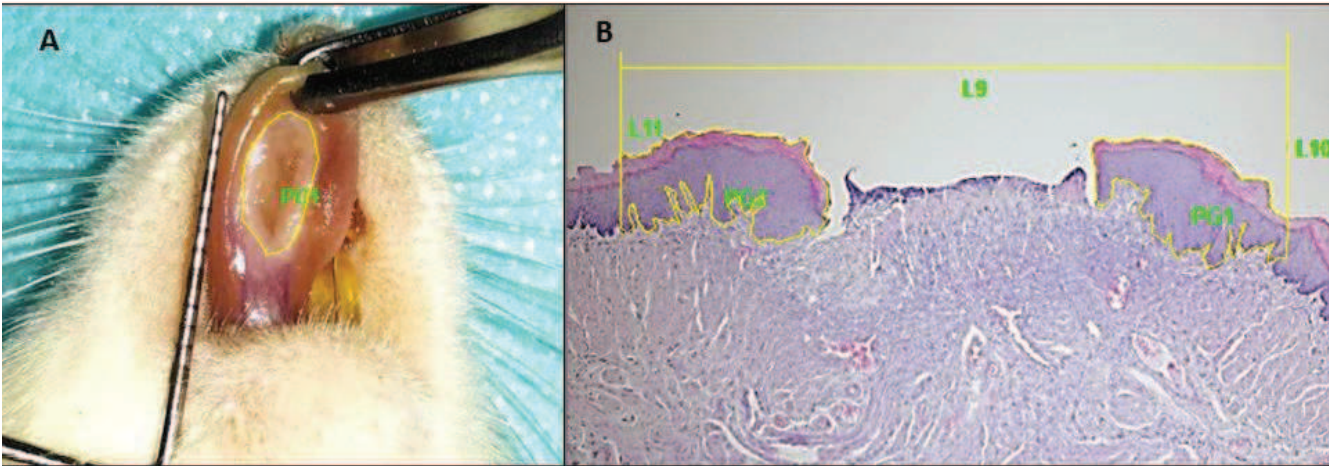
Figure 2. Measurement of residual ulcer area (A) and measurement of area of neoformed epithelial tissue (B) after three days of treatment (Control Group) using ImageJ software.

Analysis of Data

Data were initially analyzed using descriptive statistics. The Kruskal-Wallis test was used to compare the intensity of the inflammatory response, the area of residual ulcer and the area of newly formed epithelium, among the groups. The null hypothesis was rejected if $P \leq 0.05$. SPSS version 18.0 was used for statistical analyses.

RESULTS

Three animals were lost during anesthesia, one in each group of three days of treatment.

Clinical Analysis

One animal in the Control Group and one in the Dexamethasone Group showed complete healing of the ulcerative lesion after seven days of treatment. In all other animals there was residual ulcer at the end of both study times. In three (P=0.798) and seven (P=0.074) days of treatment, no significant differences were observed in relation to the residual ulcer area among the dexamethasone, doxycycline and control groups. It should be noted that there was a certain tendency towards better results in the dexamethasone group after seven days of treatment compared to the others (Table 1).

Table 1. Area of residual ulcer (mm^2) after three and seven days of treatment with doxycycline and dexamethasone (median, 25th-75th percentile).

TIME	Control Group	Doxycycline Group	Dexamethasone Group	P
3 days	12.72 (8.98-19.99)	13.5 (6.36-22.04)	14.1 (12.79-18.05)	0.798
7 days	2.33 (0.89-3.26)	2.47 (1.81-4.09)	1.49 (0.68-1.85)	0.074

Kruskal-Wallis test

Histological Analysis

Epithelial neoformation was determined by measuring neoformed epithelium on the margins of ulcers (Figure 3). There was also no significant difference among the dexamethasone, doxycycline and control groups for the area of neoformed epithelial tissue, after three (P=0.211) or seven (P=0.57) days of treatment (Table 2).
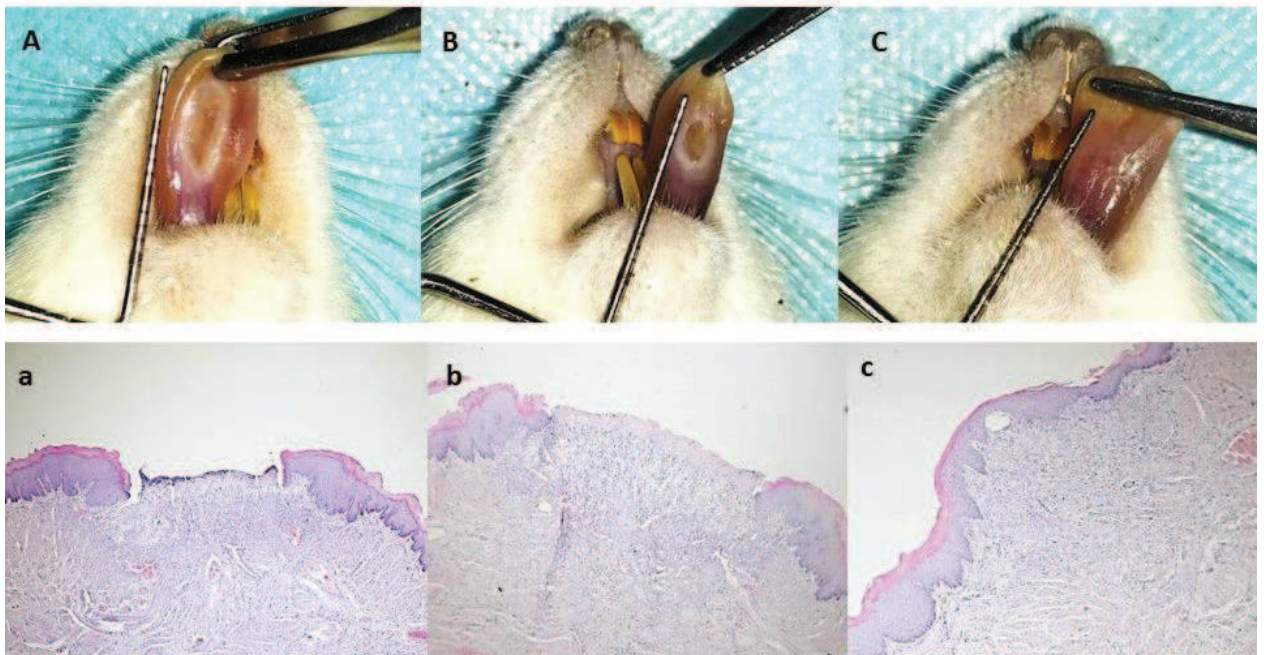
Figure 3. Clinical and histological images of tongue lesions. Animal of the Control Group after three days of treatment (A-a), animal of the Doxycycline Group after seven days of treatment (B-b), and complete regression of the ulcer in the Dexamethasone Group after seven days (C-c).

Table 2. Area of neoformed epithelium (mm²) after three and seven days of treatment with doxycycline and dexamethasone (median, 25th-75th percentile).

TIME	Control Group	Doxycycline Group	Dexamethasone Group	P
3 days	0.45 (0.15-0.77)	0.11 (0.0-0.69)	0.66 (0.22-0.71)	0.211
7 days	1.7 (1.2-2.2)	1.43 (1.3-2.08)	1.14 (1.0-1.89)	0.570

Kruskal-Wallis test

Regarding the inflammatory response (Figure 4), after three days of treatment, the Control Group had the highest number of specimens with a score 3 (intense - polymorphonuclear infiltrate of neutrophils and eosinophils) in relation to the others. However, there was no significant difference among the groups regarding this variable (P=0.147). In seven days of the treatment no significant difference was observed among the groups in the intensity of the inflammatory infiltrate (P=0.881),

despite the fact that the Doxycycline Group showed the highest number of samples with a score of 1 (light – sparse mononuclear cells) (Figure 5).
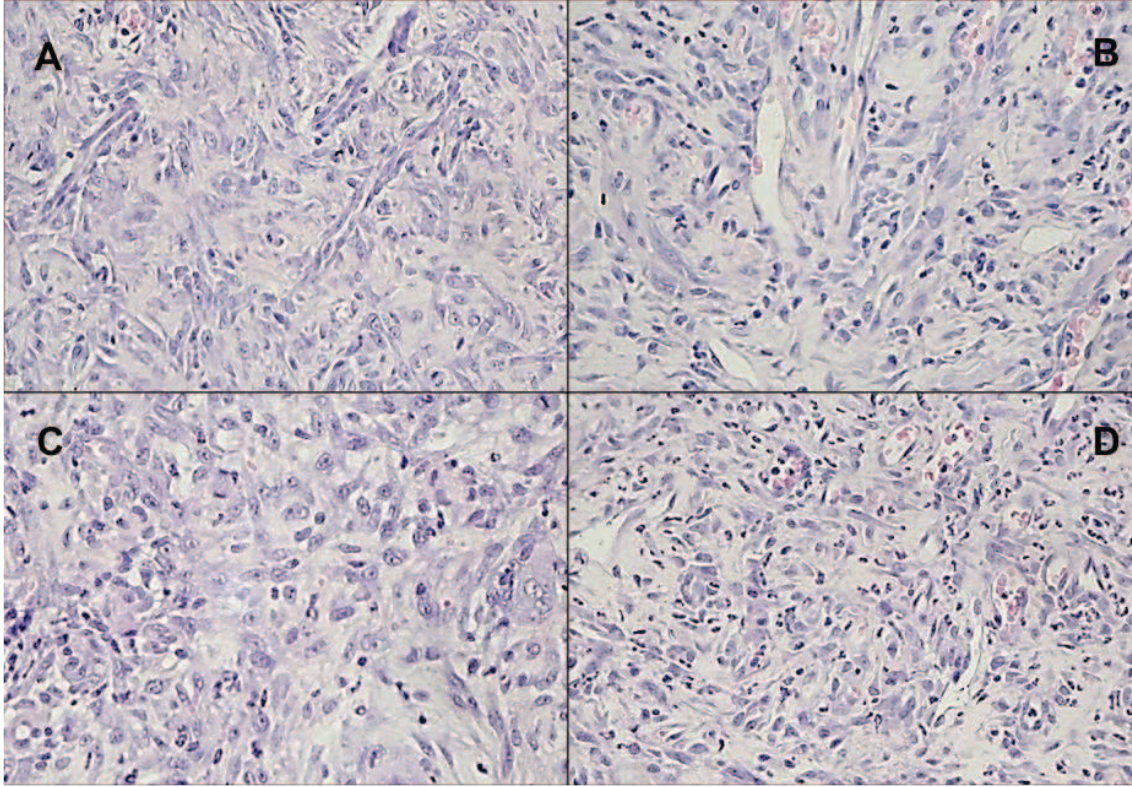
Figure 4. Histological analysis of inflammatory infiltrate (HE, 200X magnification), Doxycycline Group (A) showing sparse inflammatory cells after seven days of treatment. Presence of mononuclear infiltrate and sparse neutrophils at three days of treatment with doxycycline (B) and dexamethasone (C). Neutrophilic and eosinophilic infiltrate at three days in the Control Group (D).
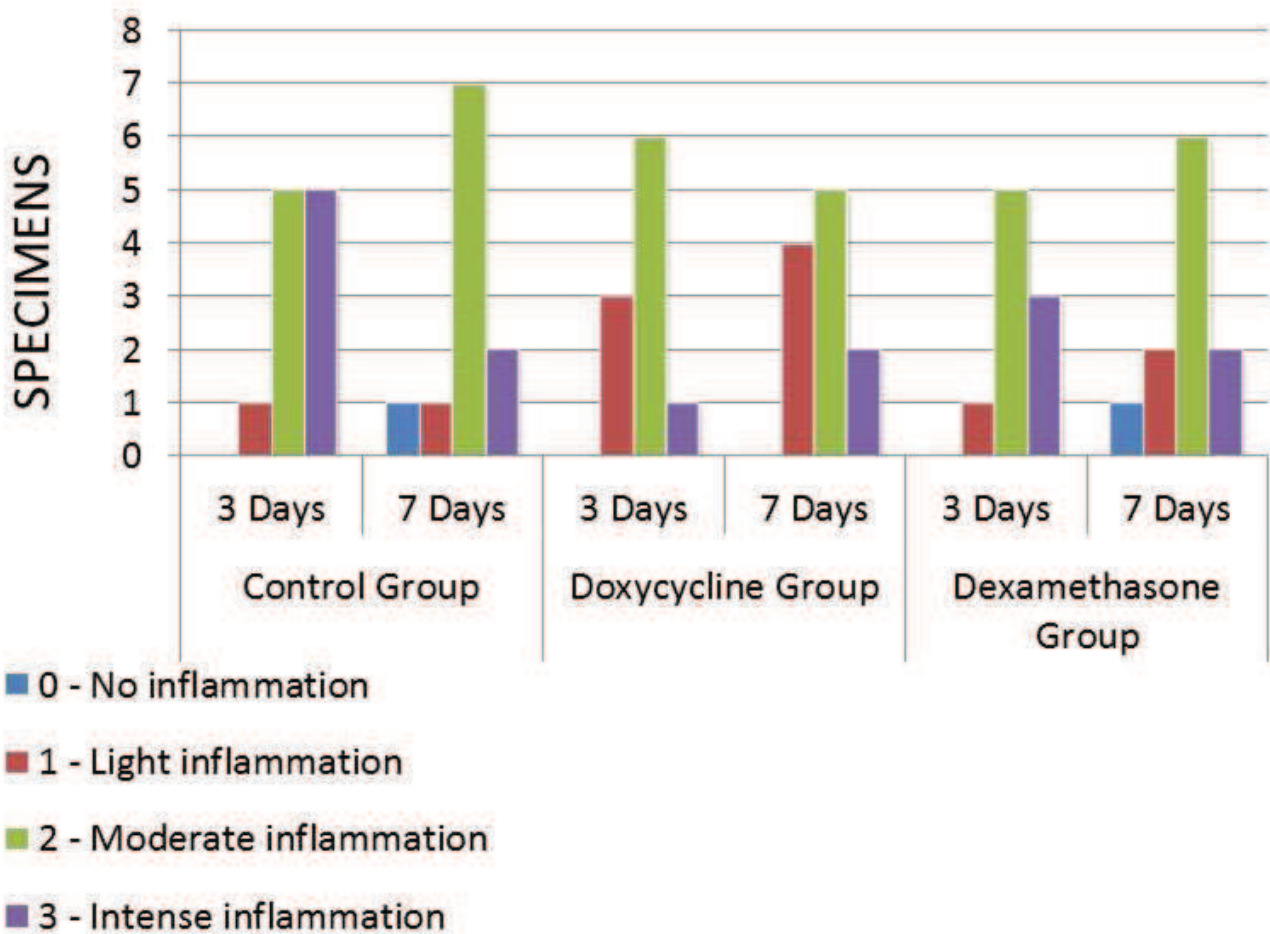
Figure 5. Intensity of inflammatory infiltrate after three and seven days of treatment with doxycycline and dexamethasone.

DISCUSSION

Since oral ulcerative lesions are usually treated with corticosteroids, and because prolonged use of these drugs may lead to adverse effects, in this study we sought a therapeutic alternative for these lesions, also investigating the topical effect of doxycycline. It was expected that when treated with doxycycline and dexamethasone, drugs with anti-inflammatory properties, ulcerative lesions would show a decrease in size, greater epithelization and reduction of the inflammatory

process. Modulation of the inflammatory response during the healing process of oral lesions is important so that the tissue repair occurs properly and in a shorter period. However, the animals treated with dexamethasone and doxycycline did not differ from the control group with regard to the variables investigated.

In the oral cavity, the effect of doxycycline has been investigated in recurrent aphthous ulcers and herpes and as adjuvant in the treatment of periodontal disease (Skulason et al., 2009; Vijayabala et al., 2013; Golub, Elburki, Walker, Ryan, Sorsa, Tenenbaum, Goldberg, Wolff, & Gu, 2016). Inhibition of leukocyte chemotaxis, MMPs synthesis and collagen breakdown has been pointed out as the main actions for the anti-inflammatory effect of doxycycline (Monk et al., 2011). Dexamethasone induces the secretion of annexin-1 protein, which blocks the cascade of prostaglandins and leukotrienes, mediators of the inflammatory process (Sudlow et al., 1996). In addition, doxycycline and dexamethasone, when used topically in the treatment of aphthous ulcers, promote a decrease in pain and faster regression of lesions (Ylikontiola et al., 1997; Skulason et al., 2009; Vijayabala et al., 2013). The divergence between the results of the present study and the literature can be explained by the difference in the etiology of these lesions. The immunological nature of the aphthous ulcers, with recruitment of neutrophils and lymphocytes, which release proteinases, explains the beneficial action of doxycycline and dexamethasone in this lesion. Faced with the inability to reproduce aphthous lesions in rats, we opted for producing mechanically induced ulcers, where the migration of inflammatory cells into the region is mediated by histamine and cytokines secreted by mast cells shortly after the rupture of the epithelial barrier. In the absence of constant noxious stimuli, these cells tend to move away from the region, allowing the healing process to occur naturally.

There are no studies in animal models testing the effect of doxycycline on oral ulcerative lesions, which makes it difficult to compare the results of this investigation. When injecting dexamethasone into oral ulcers in dogs, Alamoud et al. (2014) also did not observe significant differences in healing in relation to the control. Cavalcante et al. (2011) induced ulcers in the buccal mucosa of rats and observed their clinical and histological behavior for 10 days without treatment. There was a linear reduction in the size of the ulcers, with complete regression after 10 days. In this period, there was a gradual reduction of the inflammatory process and increase in epithelization. Considering that the present study was highly controlled and free of factors that maintain the inflammatory process, such as continuous traumas or secondary infections, therapy with the drugs tested did not have a beneficial effect on the variables investigated.

Teixeira et al. (2018) carried out clinical, histological and microbiological analyses of traumatic ulcers on the tongue of rats after subjecting them to topical treatment with chlorhexidine, iodopovidone and erythromycin. After seven days of treatment, even the placebo group showed a prevalence of the non-harmful resident microbiota and an unremarkable presence of pathogenic bacteria, obtaining similar results in the repair process between the groups. In our study, the microbiota was not evaluated, because the main objective was to analyze the effect of both drugs on the repair process, and the inflammatory infiltrate as well. In view of the similarity in methods with the study by Teixeira et al. (2018), it is suggested that the microbiota present in our study was similar to that found by these authors, since the repair process was also the same among the groups after seven days of treatment.

Cavalcante et al. (2011) observed complete healing in oral ulcers of rats after 10 days. In the present study, we opted for shorter experimental times, since with

longer periods the wound could be completely healed, making comparison between the groups impossible. In addition, as histological characteristics vary gradually over days, we chose two study times, three and seven days.

Studies that treated aphthous ulcers with doxycycline used conventional doses and subdoses of the drug (Ylikontiola et al., 1997; Skulason et al., 2009; Vijayabala et al., 2013). In this study, we used subantimicrobial doses to exclusively explore its anti-inflammatory effect. Regarding dexamethasone, the concentration chosen was the same commercially available as mouthwash. The drugs were applied in gel form twice a day, to promote greater adhesiveness. Considering the short duration of treatment, no adverse effects were observed such as mucosal atrophy and candida proliferation (Quijano & Rodríguez, 2008; Liu et al., 2012).

Clinical studies have demonstrated that doxycycline and dexamethasone act to control diseases with inflammatory characteristics (Skulason et al., 2009; Vijayabala et al., 2013; Liu et al., 2012). It was believed that by inhibiting MMPs, the doxycycline group would show an increase in the formation of collagen fibers and consequently a more favorable repair process, compared to the others, even in the face of traumatic ulcers. However, in our study, this effect was not observed.

CONCLUSIONS

Considering the experimental conditions, we can conclude that dexamethasone and doxycycline have no effect on accelerating healing or reducing the inflammatory process of traumatic lesions of the oral mucosa.

ACKNOWLEDGEMENTS

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5 DISCUSSÃO COMPLEMENTAR

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Não existe um tratamento padrão-ouro para as lesões ulceradas da mucosa bucal, uma vez que podem ser causadas por diversos agentes físicos, químicos, infecciosos ou ainda apresentar natureza imunológica. O trauma mastigatório e a contaminação secundária por micro-organismos muitas vezes exacerbam o processo inflamatório, a sintomatologia dolorosa e a duração das lesões. A busca por novas modalidades de tratamento tem sido impulsionada pelo desejo de proporcionar uma melhor qualidade de vida aos pacientes por meio de medidas que otimizem o processo de reparo, diminuam a sensibilidade dolorosa e tenha mínimos efeitos adversos.

No primeiro artigo desta dissertação foi realizada uma revisão da literatura com o propósito de avaliar o efeito da doxiciclina frente a lesões ulceradas da cavidade bucal, visto que esta droga tem sido utilizada no tratamento de doenças inflamatórias, auto-imunes, periodontais, granulomatosas e acne, devido sua capacidade de inibir MMPs e a quimiotaxia de leucócitos (MONK et al., 2011). Os resultados demonstraram que a doxiciclina é ainda pouco utilizada no tratamento de lesões bucais ulceradas. Foram encontrados somente quatro estudos controlados, que demonstraram efeitos benéficos do fármaco no alívio da sintomatologia dolorosa e na remissão clínica das lesões em pacientes com ulcerações aftosas e herpes labial (SKULASON et al., 2012; VIJAYABALA et al., 2013). Embora as lesões de herpes labial estejam localizadas em semi-mucosa, optamos por incluir este estudo, tendo em vista que o herpes simples pode se manifestar e causar ulcerações na cavidade bucal. Diante destes resultados, observa-se a necessidade de mais estudos com o intuito da avaliar os efeitos do uso tópico ou sistêmico da doxiciclina

em outras lesões bucais, como úlceras traumáticas, pênfigo, líquen plano e mucosite.

Tais evidências estimularam a realização de um estudo avaliando o efeito tópico da doxiciclina e da dexametasona em úlceras mecanicamente induzidas na língua de ratos. As substâncias utilizadas no presente estudo foram formuladas na forma de gel, para que sua adesividade e tempo de ação fossem otimizados, assim como nos estudos de Skulason et al. (2009) e Skulason et al. (2012). Optou-se pela concentração subantimicrobiana da doxiciclina (20 mg/ml), explorando suas propriedades anti-inflamatórias, pois o efeito deste fármaco foi comparado ao de um corticosteroide (McCARTY; FIVENSON, 2014; GOLUB et al., 2016).

Os animais foram eutanasiados nos 4º e 8º dias após a confecção das úlceras, ou seja, após três e sete dias de tratamento. A escolha desses períodos foi baseada em estudos anteriores (CAVALCANTE et al., 2011; ALAMOUDI et al., 2014) e realizada com o intuito de avaliar uma fase mais inicial do processo inflamatório e outra em que o processo de cicatrização estivesse em um estágio mais avançado. As características histológicas variam gradualmente, e em períodos de dez dias ou mais as úlceras traumáticas poderiam estar completamente cicatrizadas (CAVALCANTE et al., 2011; ALAMOUDI et al., 2014).

Ao contrário do descrito na literatura em relação às ulcerações aftosas e herpes labial, no presente estudo não foram observados resultados satisfatórios com o uso tópico de doxiciclina ou dexametasona nas lesões ulceradas induzidas na língua de ratos. As variáveis testadas: área da úlcera remanescente, área de tecido epitelial neoformado e intensidade de infiltrado inflamatório não apresentaram diferenças significativas entre os grupos doxiciclina, dexametasona ou controle. É possível que a utilização de outros métodos como análise por meio de marcadores

imunoistoquímicos específicos para macrófagos (CD68) e linfócitos (CD3) trouxesse dados adicionais sobre o processo inflamatório. Além disso, essa divergência entre os resultados do presente estudo com a literatura, pode ser justificada pela diferença na etiologia dessas lesões. A natureza imunológica da afta, com recrutamento de neutrófilos e linfócitos, os quais liberam proteinases, explicam a ação benéfica da doxiciclina e da dexametasona nesta lesão. Diante da impossibilidade de reproduzir lesões aftosas em ratos, optamos pela confecção de úlceras induzidas mecanicamente. Nestas, a migração de células inflamatórias para a região é mediada por histamina e citocinas, secretadas pelos mastócitos, logo após o rompimento da barreira epitelial. Na ausência de estímulos nocivos constantes, essas células tendem a se afastar da região, permitindo que o processo de cicatrização ocorra naturalmente. Portanto, nessas úlceras traumáticas a inflamação não é o fator determinante para o desenvolvimento e manutenção da lesão.

Estudos clínicos demonstram que a doxiciclina e a dexametasona atuam no controle de lesões ulceradas bucais com características inflamatórias (SKULASON et al, 2009; VYJAIABALA et al, 2013; LIU et al, 2012). Acreditava-se que ao inibir MMPs, o grupo doxiciclina apresentaria aumento na formação de fibras colágenas e consequentemente um processo de reparo mais favorável em relação aos demais. No entanto, considerando as condições experimentais, a dexametasona e a doxiciclina não apresentaram efeito em acelerar a cicatrização ou em reduzir o processo inflamatório de lesões traumáticas produzidas na mucosa bucal de ratos.

6 CONCLUSÕES

6 CONCLUSÕES

Com base nos resultados deste estudo, pode-se concluir que:

Considerando as condições experimentais, a dexametasona e a doxiciclina não apresentaram efeito em acelerar a cicatrização ou em reduzir o processo inflamatório de lesões traumáticas da mucosa bucal.

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ANEXO

APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA PUCRS

SIPESQ

Sistema de Pesquisas da PUCRS

Código SIPESQ: 8321   Porto Alegre,22 de novembro de 2017

Prezado(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou o Projeto de Pesquisa "EFEITO TÓPICO DA DOXICICLINA E DA DEXAMETASONA EM ÚLCERAS TRAUMÁTICAS EM VENTRE LINGUAL DE RATOS: ANÁLISE CLÍNICA, HISTOLÓGICA E IMUNOISTOQUÍMICA" coordenado por FERNANDA GONCALVES SALUM.

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está autorizada a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Duração do Projeto: 22/11/2017 - 22/02/2018

Nº de Animais	Espécie
66	Rattus norvegicus
Total de Animais: 66	

Atenciosamente,

Comissão de Ética no Uso de Animais(CEUA)

APÊNDICE
FICHA DE COLETA DE DADOS

FICHA DE ANÁLISE CLÍNICA   E HISTOLÓGICA

Número da Lâmina:..... ....

Grupo:...

Área da úlcera (mm^2):.................................

Área de epitelização (mm^2):............................

Infiltrado Inflamatório

( ) Ausente (ausência de inflamação)

( ) Leve (células mononucleares esparsas)

( ) Moderada (infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos

( ) Intensa (infiltrado polimorfonuclear de neutrófilos e eosinófilos)