UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

VIVIAN PETERSEN WAGNER

COMPARAÇÃO DO EFEITO DE DOIS PROTOCOLOS DE LASER DE BAIXA POTÊNCIA NO REPARO DE ÚLCERAS BUCAIS EM RATOS: ESTUDO CLÍNICO E HISTOLÓGICO

Porto Alegre
2012

VIVIAN PETERSEN WAGNER

COMPARAÇÃO DO EFEITO DE DOIS PROTOCOLOS DE LASER DE BAIXA POTÊNCIA NO REPARO DE ÚLCERAS BUCAIS EM RATOS: ESTUDO CLÍNICO E HISTOLÓGICO

Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Cirurgião-Dentista.

Orientadora: Prof[a]. Dr[a]. Manoela Domingues Martins

Porto Alegre

2012

CIP- Catalogação na Publicação

Wagner, Vivian Petersen
Comparação do efeito de dois protocolos de laser de baixa potência no reparo de úlceras bucais em ratos : estudo clínico e histológico / Vivian Petersen Wagner. – 2012.
32 f. : il.

Trabalho de Conclusão de Curso (Graduação) – Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Curso de Graduação em Odontologia, Porto Alegre, BR-RS, 2012.

Orientadora: Manoela Domingues Martins

1. Cicatrização. 2. Reparo. 3. Laser. 4. Laser de diodo. 5.Úlcera. I. Martins, Manoela Martins. II. Título.

Elaborada por Ida Rossi - CRB-10/771

Ao meu pai, Flávio, por estar sempre disposto a me ajudar, ensinar, orientar e apoiar. Por ser meu exemplo na vida pessoal e profissional.

À minha mãe, Rejane, pelo carinho, dedicação e amor. Por me ensinar a enxergar o mundo sempre com bons olhos e seguir meu coração em minhas decisões.

Aos meus irmãos, Renan e Bruno, pelo companheirismo e cumplicidade de todos esses anos. Por todos os momentos maravilhosos que compartilhamos e ainda vamos compartilhar.

AGRADECIMENTOS

À minha família, por sempre acreditar em mim, apoiar minhas escolhas e torcer pelo meu sucesso. Sem o apoio de vocês nada seria possível.

Às minhas amigas da faculdade por tornaram os anos de faculdade mais prazerosos e divertidos. Aprendemos juntas e construímos uma amizade sólida e verdadeira que tenho certeza será para a vida toda. Agradeço pela cumplicidade acima de tudo.

Aos meus colegas da Patologia, ICs, mestrandos, doutorandos e professores, por tornarem o ambiente de trabalho um local alegre de fácil convivência e por toda ajuda e apoio em todos os momentos.

À Ale e à Chris por toda a dedicação neste trabalho, ajuda com a fase experimental no hospital e nos demais momentos do trabalho.

À minha orientadora Manô pela imensa dedicação neste trabalho. Agradeço pelo tempo investido, visitas ao hospital, reuniões na patologia, reuniões em sua casa. Agradeço pela descontração constante, fazendo com que realizar este trabalho de conclusão fosse sempre prazeroso. Agradeço pelos ensinamentos passados que foram muito importantes e valiosos para minha formação. Por fim, agradeço principalmente pela amizade e companheirismo construído.

RESUMO

WAGNER, Vivian Petersen. **Comparação do efeito de dois protocolos de laser de baixa potência no reparo de úlceras bucais em ratos:** estudo clínico e histológico. 2012. 32f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2012.

O processo fisiológico do reparo de feridas compreende uma cascata de eventos celulares e moleculares que interagem para que ocorra a restauração da integridade tecidual, podendo esse processo ser dividido em fase inflamatória, proliferativa e de remodelamento. Lesões ulceradas na mucosa bucal são extremamente comuns e diversos protocolos terapêuticos vêm sendo utilizados, buscando aliviar a sintomatologia dolorosa e acelerar o reparo. O objetivo do presente estudo foi avaliar clínica e histopatologicamente a ação de duas densidades de energia distintas do laser de baixa potência (LBP) no reparo de úlceras bucais em ratos. Foram utilizados 72 ratos machos Wistar divididos aleatoriamente em 3 grupos experimentais: Grupo Controle 0J/cm^2 (n=24), Grupo Laser 4J/cm^2 (n=24), Grupo Laser 20J/cm^2 (n=24). Foram realizadas úlceras traumáticas na língua dos animais utilizando instrumento *punch* de 3 mm. A irradiação foi realizada uma vez ao dia em contato com a ferida utilizando a técnica pontual utilizando o laser de InGaAlP (660nm, energia 40mW, *spot size* 0,04cm^2) em dois pontos por ferida. O grupo controle foi tratado em condições idênticas exceto pelo fato que o equipamento de laser foi mantido desligado. Seis animais foram mortos em cada grupo nos dias 1, 5, 10 e 14 utilizando câmera de CO_2. A língua foi removida e após a análise clínica (área da úlcera e percentual de reparo) foi fixada em uma solução de 10% de formalina tamponada durante 48 horas e em seguida corada com hematoxilina-eosina para análise histológica (reepitelização e grau de inflamação). A análise estatística foi realizada pelo teste generalizado equação de estimativa (GEE), seguida de um teste *post-hoc* de Bonferroni, quando necessário. O nível de significância foi de 5%. O tratamento com 4J/cm^2 mostrou influenciar positivamente a reparo de feridas e a maioria das alterações significativas foram observadas após 5 dias de irradiação. Este grupo apresentou uma redução do tempo de cicatrização da úlcera associada à aceleração da cicatrização epitelial, diminuição do infiltrado neutrofílico e aumento da inflamação crônica com proliferação de fibroblastos. Com base neste estudo, concluiu-se que a terapia utilizando o LBP utilizado com a mesma potência tem capacidade de influenciar de maneira diferente a cicatrização de feridas em função da densidade de energia utilizada, e que a densidade de energia mais baixa (4J/cm^2) teve melhor resposta na aceleração da reepitelização e cicatrização tecidual.

Palavras-chave: Cicatrização. Reparo. Úlcera. Laser. Laser de diodo.

ABSTRACT

WAGNER, Vivian Petersen**. Comparison between the effect of two low level laser therapy protocols on the repair of oral ulcers in rats:** clinical and histological study. 32f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2012.

The physiological process of wound healing comprises a cascade of cellular and molecular events that interact in order to restore tissue integrity. This process can be divided in three phases: inflammatory, proliferative and remodeling. Ulcerated lesions of oral mucosa are extremely common, and different treatment protocols have been used, aiming to relieve painful symptoms and accelerate the repair. The aim of this study was to evaluate clinical and histopathological aspects of oral ulcer healing after two different power densities of low level laser treatment in rats. 72 male Wistar rats were used, randomly divided into 3 experimental groups: Control Group 0J/cm^2 (n=24), Laser Group 4J/cm^2(n=24), Laser Group 20J/cm^2(n=24). Traumatic ulcers were made on the tongue using a punch with 3 mm of diameter. Laser irradiation was performed once a day in contact with the injury using the punctual technique by using the InGaAlP laser (660 nm, output power of 40 mW, spot size 0,04 cm^2) in two points per wound. The energy density used was 4J/cm^2 and 20J/cm^2, during 4 and 20 seconds respectively. The control group was treated under identical conditions except that the laser equipment was kept off. Six animals were killed in each group at 1, 5, 10 and 14 days using a CO_2 chamber. The tongue was removed and after clinical analysis (ulcer area and healing percentage) was fixed in a 10% buffered formalin solution for 48 h and then stained with hematoxylin-eosin for histopathological analysis (reepithelialization and inflammation). Statistical analysis was performed by the generalized equation estimation test (GEE) followed by a post-hoc Bonferroni test when necessary. The significance level was 5%. The 4J/cm^2 positively influence the wound healing with most significant changes observed after 5 days of irradiation. This group exhibited a reduction in time of ulcer healing associated to acceleration of epithelial healing, decrease of neutrophilic infiltrate and increase of chronic inflammation with proliferating fibroblasts. Based on the conditions of this study, we concluded that LLLT used with the same power is capable to influence in a different way the wound healing according to the energy density used, and the lower energy density (4J/cm^2) had better response in accelerating reepithelialization and tissue healing.

Keywords: Healing. Repair. Ulcer. Laser. Diode laser.

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

Na clínica odontológica lesões como a úlcera traumática e a ulceração aftosa recorrente podem ser observadas frequentemente e se caracterizam pela perda do revestimento epitelial associada à exposição do tecido conjuntivo. Estas lesões são sintomáticas e usualmente reparam num período de 7 a 10 dias. Em boca, vários modelos experimentais utilizando animais e estudos clínicos em humanos vêm sendo realizados na busca de protocolos que contribuam para o alívio da dor e aceleração do reparo de feridas. Diversos protocolos vêm sendo utilizados, porém nenhum tratamento específico foi estabelecido. Entre estes, podemos citar o uso de antissépticos, analgésicos, antibióticos, imunomoduladores e antiinflamatórios, fitoterápicos além de tratamentos locais específicos como remoção cirúrgica, debridamento, cauterização química, ultrassom de baixa densidade e laser de baixa potência (LBP) (SCULLY; SHOTTS, 2000; FIELD; ALLAN, 2003; LEÃO; GOMES; PORTER, 2007; MIZIARA, 2009; MARTINS et al., 2011; FERNANDES et al., 2010; MARTINS et al., 2009).

O processo de reparo de lesões, como as úlceras, envolve fenômenos bioquímicos e fisiológicos que buscam a restauração tecidual (MANDELBAUM; SANTIS; MANDELBAUM, 2003). Este processo pode ser dividido em três fases dinâmicas e interrelacionadas denominadas inflamatória, proliferativa e remodelativa, que se sobrepõem de forma contínua e temporal. Nestas fases ocorrem eventos como migração celular, vasodilatação, angiogênese, formação de tecido de granulação e deposição de matriz extracelular (ARAÚJO et al., 2010; MENDONÇA; COUTINHO-NETTO, 2009).

A fase inflamatória se inicia após a ação do agente agressor, que desencadeia a liberação de mediadores químicos inflamatórios (histaminas, citocinas, complemento) que promovem alterações vasculares e exsudativas no sítio inflamatório. A migração de células inflamatórias para o tecido intersticial tem como papel principal eliminar agentes agressores e fagocitar componentes do tecido conjuntivo danificados, além de preparar o tecido para a próxima fase através da liberação de fatores de crescimento (MENDONÇA; COUTINHO-NETO, 2009). A fase proliferativa se caracteriza por fenômenos como a reepitelização, fibroplasia e angiogênese, sendo estes dois últimos os responsáveis pela formação do tecido de granulação. Este tecido representa o arcabouço do reparo tecidual, pois ocupa a

área da lesão e é responsável pelo fechamento da lesão propriamente dita (MENDONÇA; COUTINHO-NETO, 2009). A formação de uma rede vascular no tecido de granulação contribui para que o processo de reparo ocorra de forma mais eficaz e mais rápida, permitindo um maior fluxo de nutrientes para a região e a saída de metabólitos da ferida (GONÇALVES et al., 2010). A fase de remodelamento é marcada pela maturação dos elementos teciduais e alterações na matriz extracelular, com a deposição de proteoglicanas e colágeno, na tentativa de recuperação da estrutura tecidual normal. Durante esta fase a maioria dos vasos, fibroblastos e células inflamatórias desaparecem por processos de emigração, apoptose ou processos de morte celular desconhecidos (MENDONÇA; COUTINHO-NETO, 2009).

O laser (*light amplification by stimulated emission of radiation*) se caracteriza como um feixe de luz monocromático (emissão de apenas uma cor), com coerência espacial e coerência direcional (ZEZELL, 2004). O LBP corresponde à emissão de luz laser com potência abaixo de 500mW, onde a irradiação propriamente dita vai gerar um efeito biológico no tecido sem aquecimento, uma vez que a energia absorvida pelos fótons não vai ser transformada em calor e sim em efeitos fotoquímicos, fotofísicos e/ou fotobiológicos (LINS et al., 2010; BAPTISTA et al., 2010).

As baixas densidades de energia e comprimentos de onda dos LBP fazem com que haja capacidade da luz laser penetrar em profundidade nos tecidos (HENRIQUES; CAZAL; CASTRO, 2010). A dosimetria, ou seja, a dose de irradiação ionizante à qual o indivíduo está exposto, depende de inúmeros parâmetros e tem um papel muito importante na terapia de LBP, uma vez que existem respostas distintas entre as células de acordo com cada padrão de irradiação por laser, e estas respostas vão depender do comprimento de onda, energia, potência, densidade de potência, densidade de energia, tempo de irradiação, frequência de irradiação, intervalo entre as irradiações, área do feixe e modo de irradiação. Entretanto, até o presente momento, a relevância destes parâmetros no reparo não está totalmente esclarecida. Estudos prévios mostram resultados contraditórios que podem ser explicados, em parte, pela variedade na combinação de parâmetros, assim como na dificuldade de medir de forma objetiva os possíveis efeitos do tratamento (PEPLOW; CHUNG; BAXTER, 2010). Estudos prévios demonstram que a densidade de energia apresenta a maior relevância na resposta ao tratamento com LBP, sendo assim a

maioria dos protocolos terapêuticos utilizados variam em função deste parâmetro que é mais importante do que a dose total (PEPLOW et al., 2010; PEREIRA et al, 2002; von BREUGHEL, DOP BARR, 1992)

Endre Mester et al. (1971) foram os primeiros a demonstrar que quantidades de baixa intensidade de luz são capazes de produzir reparo no tecido e alívio da dor. Desde então, foram desenvolvidos estudos na busca de explicar o mecanismo de interação do LBP com as células. Karu (1988) apontou que o efeito do laser seria resultado da absorção da luz por flavinas e citocromos da cadeia respiratória mitocondrial, que através de alterações na transferência de elétrons geraria espécies reativas de oxigênio. Estas levariam à modificação da permeabilidade da membrana celular, aumentando o fluxo Ca^{2+} e por consequência aumentando a proliferação celular. Smith (1991) propos que a radiação infravermelha poderia ativar diretamente os canais de Ca^{2+} através de modificações fotofísicas, iniciando sua ação diretamente ao nível da membrana celular. Outro aspecto importante que deve ser levado em conta é que a resposta celular ao tratamento com LBP depende do estado fisiológico em que a célula irradiada se encontra. O laser tem a capacidade de induzir a proliferação tecidual apenas em células que estejam com a taxa de proliferação diminuída no momento da irradiação, ou seja, é importante considerar o fato de que o LBP não trará efeitos terapêuticos se a célula irradiada estiver em seu nível normal de funcionamento (KARU, 1989; SMITH, 1991).

A terapia utilizando LBP é conhecida por modular processos biológicos, um fenômeno conhecido por fotobiomodulação (RIBEIRO et al., 2009; REDDY et al., 2004). Seu objetivo é promover, através dos mecanismos já descritos anteriormente, o aumento do metabolismo celular, podendo assim induzir diversos efeitos teciduais, como analgésico, antiinflamatório e reparador (BOURGUIGNON-FILHO et al., 2005). Conhecendo estes efeitos, o LBP aparece como uma alternativa na área da saúde para prevenir, modular e/ ou tratar doenças que possuam na sua fisiopatologia o desenvolvimento de um processo inflamatório/imunológico (EDUARDO et al., 2007; BAPTISTA et al., 2010).

Os estudos até então conduzidos mostram que os resultados obtidos com a terapia com LBP estão baseados no aumento da proliferação celular (PEREIRA et al., 2002; PEPLOW et al., 2010), promoção da síntese de pré-colageno e colágeno (PEREIRA et al., 2002; SOUZA et al., 2011), regulação da proliferação de miofibroblastos e células linfóides (RIBEIRO et al., 2009), potencial antiinflamatório

(LIM et al., 2007; AIMBIRE et al., 2006), aumento da neo-vascularização (CORAZZA et al., 2007; PEREIRA et al., 2010) e liberação de fatores de crescimento (DAMANTE et al., 2008).

Os efeitos bioestimuladores do LBP no reparo de úlceras vêm sendo comprovados em diversos estudos, porém a maioria dos trabalhos realizados em animais utilizam o modelo de reparo em pele, onde o procedimento de úlcera é realizado no dorso do animal (PUGLIESE et al., 2003; MEDRADO et al., 2003; NASCIMENTO et al., 2004; GAL et al., 2006; VIEGAS et al., 2007; CORAZZA et al., 2007; MEDRADO et al., 2008; REIS et al., 2008; RIBEIRO et al., 2008; PEREIRA et al., 2010; GONÇALVES et al., 2010). Estes estudos buscam compreender os mecanismos através dos quais o efeito bioestimulador é alcançado, além de estabelecer o protocolo ideal de utilização deste recurso terapêutico. A literatura é escassa no que se refere a utilização do LBP no reparo de úlceras orais. Assim, neste estudo pretendeu-se avaliar clínica e histopatologicamete a ação da terapia com LBP no reparo de úlceras bucais em ratos, e se esta ação é dose-dependente.

2 ARTIGO CIENTIFICO

INFLUENCE OF DIFFERENT ENERGY DENSITIES OF LOW LEVEL LASER THERAPY ON CLINICOPATHOLOGICAL ASPECTS OF ORAL WOUND HEALING IN RATS

VIVIAN PETERSEN WAGNER, undergraduate student,[1] MARCO ANTONIO TREVIZANI MARTINS, D.D.S, Ph.D.,[1] LUISE MEURER, M.D, Ph.D.,[3] CHRIS KREBS DANILEVICZ,[1] ALESSANDRA MAGNUSSON,[1] MÁRCIA MARTINS MARQUES, D.D.S, Ph.D.,[2] MANOEL SANT'ANA FILHO, D.D.S, Ph.D.,[1] MANOELA DOMINGUES MARTINS, D.D.S, Ph.D.[1]

1. Department of Oral Pathology, School of Dentistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.
2. Department of Dentistry, School of Dentistry, University of São Paulo, São Paulo, São Paulo, Brazil.
3. Department of Pathology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Corresponding Author:

Manoela Domingues Martins

Faculdade de Odontologia - UFRGS

Departamento de Odontologia Conservadora

Ramiro Barcelos, 2492/ 503 Bom Fim, ZIPCODE 90035-003

Brazil

e-mail: manomartins@gmail.com

Este trabalho de conclusão de curso está escrito em forma de artigo e seguiu as normas da revista Photomedicine and Laser Surgery.

Abstract

Objective: The aim of this study was to evaluate clinical and histopathological aspects of oral ulcer healing after two different power densities of low level laser treatment in rats. **Methods**: A prospective blind study was made of adult male Wistar rats (n=72) randomly divided into three groups: Control Group 0J/cm^2 (n=24), Laser Group 4J/cm^2 (n=24), Laser Group 20J/cm^2 (n=24). Traumatic ulcers were made on the tongue dorsum using a punch with 3 mm of diameter. Laser irradiation was performed once a day in contact with the wound using the punctual technique by using the InGaAlP laser (660 nm, output power of 40 mW, spot size 0,04 cm^2) in two points per lesion. The energy density used was 4J/cm^2 and 20J/cm^2, during 4 and 20 seconds respectively. The control group was treated under identical conditions except that the laser equipment was kept off. Six animals were killed in each group at 1, 5, 10 and 14 days using a CO_2 chamber. The tongue was removed and after clinical analysis (ulcer area and healing percentage) was fixed in a 10% buffered formalin solution for 48 h and then stained with hematoxylin-eosin for histopathological analysis (reepithelialization and inflammation). Statistical analysis was performed by the generalized equation estimation test (GEE) followed by a post-hoc Bonferroni test if necessary. The significance level was 5%. **Results**: Clinical and histopathological analysis revealed differences between the 4J/cm^2 and the other groups in relation to ulcer area, healing percentage and degree of reepithelialization and inflammation. The 4J/cm^2 positively influence the wound healing with most significant changes observed after 5 days of irradiation. This group exhibited a time reduction of ulcer healing associated to acceleration of epithelial healing, decrease of neutrophilic infiltrate and increase of chronic inflammation with proliferating fibroblasts. **Conclusion:** Based on the conditions of this study, we concluded that LLLT used with the same power is capable to influence in a different way the wound healing according to the energy density used, and the lower energy density (4J/cm^2) had better response in accelerating reepithelialization and tissue healing.

Introduction

Oral ulcers, originating from traumatic or immunological processes, are one of the most common complaints of oral mucosa. These lesions are characterized by the damage of both the epithelium and lamina propria and usually repair few days after the etiological factor is removed. Despite the size, which can range from a few millimeters up to several centimeters, an oral ulcer often cause mild to severe pain. The ulcer treatment aims to accelerate the repair and relieve symptoms, improving quality of life. In some types of oral ulcers, like recurrent aphthous stomatitis and mucositis, patients report a lower quality of life related with oral health compared to general population.[1,2]

The wound healing process, as in oral ulcers, consists in a dynamic and complex process that involves biochemical and physiologic phenomenon in order to guarantee the restoration of the tissue.[3] This process involves three main overlapping phases: inflammatory, proliferative and remodeling.[4] Different therapeutic protocols have been tested in literature in order to accelerate this process and reduce pain.[5-10]

Low level laser therapy (LLLT) is known to modulate several biological processes, a phenomenon known as photobiomodulation.[11,12] The LLLT increases cell metabolism and may induce different tissue effects such as analgesic, anti-inflammatory and reparative.[13] Endre Mester[14] was the first to describe that LLLT can improve healing and relieve pain. Studies conducted so far show that the results obtained with LLLT are based on increasing cell proliferation,[15,16] promoting collagen synthesis,[15,17] regulating proliferation of myofibroblasts and lymphoid cells,[11] anti-inflammatory potential,[18,19] increasing neo-vascularization,[20,21] and release of growth factors.[22]

Several parameters have an influence on LLLT results once it exists distinct responses between cells according to the protocol used. However, the relevance of these parameters of laser irradiation on improving the oral ulcer healing process remains unclear until the present time. Previous studies in dorsal skin ulcer model or in burn made lesions show contradictory results that may be in part explained by a variety of parameters combination as well as the inability to measure accurately the possible effect of irradiation.[23]

The biostimulator effect of LLLT has been proven in many studies that seek to understand the mechanisms by which this effect is achieved and also to establish the ideal protocol for therapeutic use of this resource. The aim of this study was to evaluate clinical and histopathological aspects of oral ulcer healing after two different energy densities of LLLT in rats.

Methods

A prospective and blind study was performed.

Animals

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals[24], and the experiment was approved by the Hospital de Clinicas de Porto Alegre Ethics Committee in Research and by the Ethics Committee on Animal Use of HCPA (protocol 12-0338). Seventy-two male Wistar rats weighing 150-200g were kept in a number of 5 animals per cage under standard conditions of temperature (20-24^{o}C) and light/dark cycle, with solid chow and water *ad libitum*. Animals were randomly divided into three groups of 24 animals each: control group 0J/cm^2, Laser group 4J/cm^2, Laser group 20J/cm^2.

Wound procedure

Under aseptic conditions the animals were anesthetized with intraperitoneal administration of ketamine (0,1ml/100g) and xylazine (0,05ml/100g). Traumatic ulcers were surgically made on the tongue dorsum using a punch with 3 mm of diameter (Figure 1a and b).

FIG. 1. Clinical aspect (a and b) of oral ulcer after punch incision. Low level laser irradiation (c).

Laser Irradiation

Laser irradiation was delivered with InGaAlP lasers (MM Optics Ltd., São Carlos, SP, Brazil). Irradiations were performed in contact, using the punctual irradiation mode in a 0,04cm^2 area, in two points per wound (Figure 1c). The 660 nm laser was applied with output power of 40mW. The energy densities used were 4J/cm^2 and 20J/cm^2 with 4 and 20 seconds exposure time in each point of the ulcer respectively (Table 1). The irradiation was held by the same investigator, once a day under inhalatory anesthesia with isoflurane until the day of death. The control groups were treated under identical conditions except that the laser equipment was kept off.

TABLE 1. EXPERIMENTAL GROUPS AND FLUENCE PARAMETERS USED IN THE STUDY.

	Wave-length (nm)	Output power (mW)	Power density (mW/cm^2)	Energy density (J/cm^2)	Irradiation time (s)	Beam spot (cm^2)
0J/cm^2	-	-	-	-	-	-
4J/cm^2	660	40	1	4	4	0.04
20J/cm^2	660	40	1	20	20	0.04

Clinical analysis

Six rats in each group were killed in CO_2 chamber after 1, 5, 10 and 14 days after surgical procedure. Two clinical analyses were performed: ulcers area and healing percentage. For that, the length and width of the ulcers of all groups were measured using a digital caliper and photographed. These measurements were multiplied to calculate the area in square centimeters.

The healing percentage of the wound and the healing time were recorded as described by Khorasani et al.[25] The healing percentage in the wound = [(area at first moment – area in this second time) / (area at first moment)] X 100.

Histopathological analysis

The tongue was removed and fixed in a 10% buffered formalin solution for 48 hours. After washing with water, the specimens were dehydrated and embedded in paraffin. The tissue specimens were sectioned into 5µm-thick slices and stained with hematoxylin-eosin for routine histological analysis.

Initially a descriptive analysis of each time/experimental group was done, followed by a semi-quantitative analysis using scores. The degree of

reepithelialization was measured using the criteria described by Sinha, Gallaher:[26] Grade 0: re-epithelialization at the end of the wound; Grade 1: reepithelialization covering less than half of the wound; Grade 2: reepithelialization covering more than half of the wound; Grade 3: reepithelialization covering the entire wound with irregular thickness; Grade 4: reepithelialization covering the entire wound with normal thickness. The degree of inflammation was evaluated by the phase resolution of the inflammatory process described by Kumar et al.,[27] and used by Camacho-Alonso, López-Jornet:[28] Grade 1: acute inflammation (pyogenic membrane); Grade 2: prevalence of acute diffuse inflammation; Grade 3: prevalence of chronic inflammation (proliferating fibroblasts); Grade 4: resolution and healing (reduction or disappearance of chronic inflammation despite some inflammatory cells persist).

Statistical analysis

Results were expressed as means and standard deviations. Using SPSS Statistics 18.0, groups, time and the interaction between group and time were compared by the generalized equation estimation test (GEE) followed by a post-hoc Bonferroni when necessary. The significance level was set at 5%.

Results

Clinical analysis

The clinical evaluation of ulcer area revealed that on Day 1 the mean was similar between all groups revealing no detectable signal of repair at this time. On Day 5 a decrease in the mean area was observed in all groups. However the 4J/cm^2 group exhibited a significant lowest value, followed by 20J/cm^2 and 0J/cm^2 groups. These results indicated that 4J/cm^2 irradiation was associated with minor ulceration on clinical analysis (Figure 2). At Day 10, all animals of the 4J/cm^2 group exhibited clinically repaired lesions, whereas in the other groups this clinical situation was only observed at the end of the experimental time (Day 14). On Days 10 and 14 no statistical significant difference was observed between groups (Table 2).

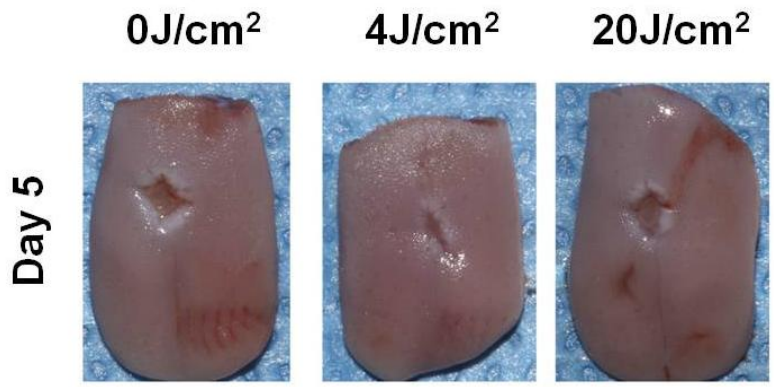

FIG. 2. Clinical aspect of experimental groups' oral ulcer at Day 5. Note that the 4J/cm^2 exhibited a smaller ulcer area.

TABLE 2. CLINICAL EVALUATION OF THE MEAN AND STANDARD ERROR OF AREAS (in mm^2)

Group	Day 1	Day 5	Day 10	Day 14
0J/cm^2	3.21 ±0.38 ^{a}A	1.79 ±0.35 ^{a}A	0.03±0.02 ^{b}A	0.00±0.00 ^{b}A
4J/cm^2	2.94± 0.31 ^{a}A	0.48± 0.16 ^{b}B	0.00±0.00 ^{c}A	0.00±0.00 ^{c}A
20J/cm^2	3.67± 0.40 ^{a}A	0.95±0.15 ^{b}A	0.25±0.20 ^{c}A	0.00±0.00 ^{c}A

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, GEE test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, GEE test).

The clinical analyses of healing percentages of the oral wounds are described in Table 3. On Day 1 no significant difference was observed between all groups. On Day 5, the 4J/cm^2 group exhibited a significant superior value of healing percentage. On Days 10 and 14 no significant differences were observed.

TABLE 3. CLINICAL EVALUATION OF THE PERCENTAGE OF THE WOUND HEALING (%)

Group	Day 1	Day 5	Day 10	Day 14
0J/cm^2	54.43 ±5.38 ^{a}A	74.59 ±5.07 ^{b}A	99.55±0.41 ^{c}A	100±0.00 ^{c}A
4J/cm^2	58.35± 4.43 ^{a}A	93.12± 2.33 ^{b}B	100±0.00 ^{c}A	100±0.00 ^{c}A
20J/cm^2	48.53± 5.52 ^{a}A	86.46±2.21^{b}A	96.41±2.95 ^{c}A	100±0.00 ^{c}A

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, GEE test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, GEE test).

Histopathological analysis

Descriptive analysis

At Day 1 all groups presented discrete or no migration of epithelial cells to the center of the wound presenting exposure of connective tissue. Intense diffuse acute inflammation (polymorphonuclear infiltrate) could be noticed, sparsely collagen fibers and hyperemia were present in the wound area.

At Day 5, the group 0J/cm^2 revealed reepithelialization covering more than half of the wound. Some acute inflammatory focus was still present in most of the animals, but the number of mononuclear cells present was higher than in day 1 (Figure 3 a and b). All the animals of the 4J/cm^2 group exhibited reepithelialization. Granulation tissue was evident, and no more acute inflammation focus was noted. Mononuclear infiltrate, neovascularization and proliferating fibroblasts were evident. Collagen fibers were predominantly organized and parallel to the basal layer of epithelium (Figure 3 c and d). The 20J/cm^2 group presented reepithelialization covering more than half of the wound in some animals, and others presented reepithelialization covering the entire wound with irregular thickness. Inflammatory infiltrate revealed formation of granulation tissue in most of animals, but in some cases acute inflammatory focuses could still be noted (Figure 3 e and f).

At Day 10 all groups presented reepithelialization covering the entire wound, 0J/cm^2 presented epithelium with irregular thickness and 4J/cm^2 and 20J/cm^2 with normal thickness. Formation of granulation tissue was evident in all groups, and rare polymorphonuclear cells were observed.

At day 14, reepithelialization covering the entire wound with normal thickness was observed in all groups, as reduction or disappearance of chronic inflammation showing the resolution and healing of the wound.

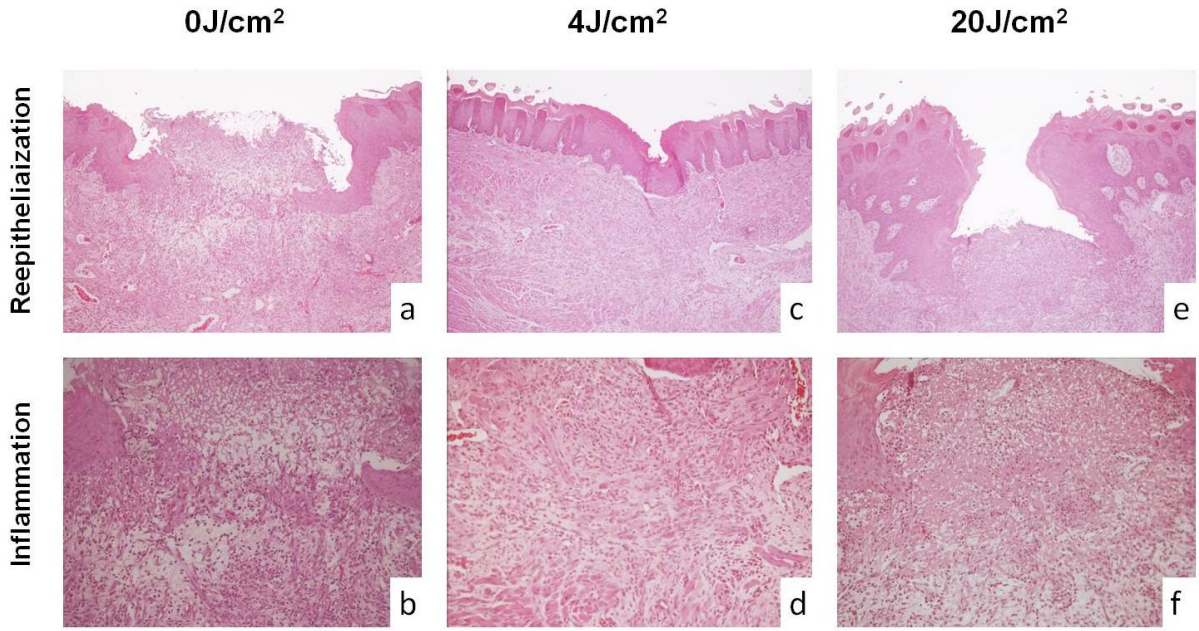

FIG. 2. Photomicrographs of experimental groups at Day 5. (a and b) 0J/cm^2 group; (c and d) 4J/cm^2 group; (e and f) 20J/cm^2 group. Note that the 4J/cm^2 group presented reepithelialization covering the entire wound and more chronic inflammatory infiltrate. (H/E, original magnification. a, c, e X 100; b, d, and f X200)

Reepithelialization degree

On Day 5, the 4J/cm^2 group presented a higher degree of reepithelialization, and this result was statistically significant. The 0J/cm^2 and 20J/cm^2 groups presented a value compatible with connective tissue exposure. On Day 10, the 20 J/cm^2 group presented a higher and statistically significant degree of reepithelialization (Table 4).

TABLE 4. HISTOPATHOLOGICAL EVALUATION OF THE MEAN AND STANDARD ERROR OF REEPITELIALIZATION DEGREE

Group	Day 1	Day 5	Day 10	Day 14
0J/cm^2	1,33 ±0,27 ^{a}A	2,00 ±0,00 ^{a}A	3,00 ±0,00 ^{b}A	3,67± 0,27 ^{b}A
4J/cm^2	1,33 ±0,27 ^{a}A	3,67 ±0,27 ^{b}B	3,67 ±0,27 ^{b}A	3,67± 0,27 ^{b}A
20J/cm^2	0,33 ±0,27 ^{a}A	2,33 ±0,27 ^{b}A	4,00 ±0,00 ^{b}B	3,67± 0,27 ^{b}A

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, GEE test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, GEE test).

Inflammation degree

Although variations were found in the pattern of inflammatory infiltrate, no statistically significant difference was found between groups (Table 5).

TABLE 5. HISTOPATHOLOGICAL EVALUATION OF THE MEAN AND STANDARD ERROR OF INFLAMATION DEGREE

Group	Day 1	Day 5	Day 10	Day 14
0J/cm^2	2,33 ±0,27 ^{a}A	2,67 ±0,27 ^{a}A	3,33 ±0,72 ^{ab}A	4,00± 0,00 ^{b}A
4J/cm^2	2,00 ±0,00 ^{a}A	3,33 ±0,27 ^{b}A	3,33 ±0,27 ^{b}A	3,00± 0,00 ^{b}A
20J/cm^2	2,00 ±0,00 ^{a}A	2,67 ±0,27 ^{ab}A	3,00 ±0,00 ^{b}A	3,33± 0,27 ^{b}A

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, GEE test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, GEE test).

Discussion

The effects of LLLT on wound healing process have been studied in several researches using specially the dorsum skin model to investigate the effect of different irradiation parameters.[11,20,21,29-33] Growing body evidence suggests that LLLT has anti-inflammatory action and accelerates tissue repair.[18,19,21,22] However, these effects are dependent on laser irradiation parameters such as wavelength, laser output power,[23,34] and energy density.[15,23] Similar parameters can have different effects on different cell types, and few studies were conducted to analyze the LLLT in rats oral mucosa wound healing.[35] It is of paramount importance to know the correct combination of parameters (e.g., wavelength, power density, and energy density) to reach the desirable effects on oral ulcer healing. For this reason, the aim of the present study was to evaluate the effects of different energy densities of LLLT on clinicopathological aspects of oral ulcers healing in rats. The main results revealed that clinical and histological findings change over time according to the laser energy density protocol, with 4J/cm^2 showing better results.

In the present investigation, two different energy densities were tested: the 4J/cm^2 and 20J/cm^2 with the same wavelength, spot size, power, and frequency of irradiation, interval between irradiations, both performed in contact and using the punctual irradiation mode. The choice to vary only the energy density was based on a study by van Breughel and Dop Bärr[36] that indicates that this parameter appears to

be more important than total dose in wound healing. Previous researches have also shown that small doses demonstrated better results in wound healing in rats when compared to higher doses.[15,20,29,32,37] These results can be well described by the Arndt-Schultz (A.S.) curve. This curve shows that a small stimulus excites physiological activity, whereas a higher stimulus inhibits it.[38] Based on these, we used 4J/cm^2 as a small energy density and chose 20J/cm^2 as a high energy density. Our in vivo results demonstrated that LLLT at the same power density can influence differently the oral wound healing as a function of energy density. The irradiations with 660nm, 40mW, 4J/cm^2 accelerated the healing of oral ulcer when compared to 0J/cm^2 and 20J/cm^2. The 4J/cm^2 at Day 5 showed positive clinical behavior promoting decrease in the mean area of oral ulcer and higher healing percentages of the lesion when compared to control and higher energy density protocol. The histopathological analysis revealed that 4 J/cm^2 group at Day 5 is in a more advance stage of repair. This finding is supported by the degree of reepithelialization, which showed epithelium covering the entire wound in all animals and also by the histopathological aspect of connective tissue. A chronicity of the inflammatory infiltrate at Day 5 on 4 J/cm^2 group could be noticed, whereas the 0 J/cm^2 and 20 J/cm^2 groups still presented focuses of acute inflammation. These histopathological aspects can be associated with the clinical finding of reduction of area and superior value of healing percentage at Day 5, showing that the 4 J/cm^2 dose presented the best pattern of healing. Similar results were obtained by Demidova-Rice et al.[39] in excisional wound healing in mice that found a biphasic relationship, with positive effects at 2J/cm^2 and inhibitory effect at 50J/cm^2. The most part of studies appoint the inhibitory effect of higher power levels, rather than dosage *per se.*

Low level laser is absorbed by cellular photosensitizes such as cytochromes and flavins that promote a cascade event that result in CA^{++} flux, which affects the levels of cyclic nucleotides interfering on DNA and RNA synthesis, modulating cell proliferation.[40] A further increase of the dose inducts cellular antioxidant activity and can cause destruction of photoreceptors, which is accompanied by growth inhibition and cell lethality, as expected from the A.S. law.[38,40]

Previous studies with LLLT using 4 J/cm^2 in wound repair have already demonstrated to promote a decrease of polymorphonuclear infiltrate and increase of vascularization,[21,29] higher deposition of collagen fibers,[29,31,32] and elastic fibers.[32] Previous studies concerning reepithelialization have demonstrated that 4 J/cm^2 dose

can promote higher number of newly formed epithelial layers[32] and irradiation with 3 and 5 J/cm^2 on cultured epithelial cells is capable of increasing cell growth rate.[43]

Other aspects that have been described that indicate a positive effect of 4J/cm^2 dose in wound healing are: it induces the formation of more fusiform cells expressing desmin and alpha smooth muscle actin,[29] decreases the levels of IL-1β mRNA,[30] increases the populations of intact and degranulated mast cells,[21] promotes angiogenesis, collagen type I and fibronectin deposition,[31] as well as increases synthesis activity.[33]

It is difficult to compare our results with the observations of wound healing in the literature because most studies' reports were performed in other tissue models, like skin, that exhibited different repair conditions. Furthermore, these studies mentioned only the power and wavelength of the laser irradiation, while the energy density is not mentioned. Nevertheless, the increase in energy density has shown impairment of in vivo wound healing.

Wound healing is a complex physiological process divided into three main overlapping stages; inflammatory, proliferative and remodeling.[4] The tissue injury causes an inflammation leading to haemostasis and clot formation; after that the fibroplasia and neovascularisation, formation of granulation tissue, reepithelialization, and finally the formation of new extracellular matrix and tissue remodeling could occur. LLLT has been found to accelerate wound healing as well as to reduce pain and inflammatory response. In this study the 4J/cm^2 LLLT tested showed an important effect in wound healing accelerating at least two of the healing phases: the inflammatory and proliferative ones. This protocol decreases the acute inflammation and induces the fibroplasia and granulation tissue formation. However, the correlation between our animal model results and clinical outcomes is still to be established. Therefore, care should be taken before extrapolating these results to clinical practice without additional testing.

Conclusion

Based on the conditions of this study, we concluded that LLLT used with the same power is capable to influence in a different way the wound healing according to the energy density used, and the lower energy density (4J/cm^2) had better response in accelerating reepithelialization and tissue healing.

Acknowledgments

We thank Grupo de Pesquisa e Pós-Graduação do Hospital de Clinicas de Porto Alegre (GPPG/FIPE: 12-0338) for financial support.

Author Disclosure Statement

No competing financial interests exist.

References

1. Hapa, A., Aksoy, B., Polat, M., et al. (2010). Does recurrent aphthous stomatitis affect quality of life? A prospective study with 128 patients evaluating different treatment modalities. J Dermatolog Treat. 22, 380-5.

2. Cheng, K.K., Leung, S.F., Liang, R.H.S., et al. (2010). Severe oral mucositis associated with cancer therapy: impact on oral functional status and quality of life. Support Care Cancer. 18, 1477–1485.

3. Mandelbaum, S.H., Di Santis, E.P., Mandelbaum, M.H.S. (2003). Healing: current concepts and auxiliary resources - Part I. An Bras Dermatol. 78, 393-408. (in Portuguese)

4. Mendonça, R.J.D., Coutinho-Netto, J. (2009). Cellular aspects of healing. An Bras Dermatol. 84, 257-262. (in Portuguese)

5. Field, E.A., Allan, R.B. (2003). Review article: oral ulceration – etiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic**.** Aliment. Pharmacol. Ther. 15, 949-962.

6. Leão, J.C., Gomes, V.B., Porter, S. (2007). Ulcerative lesions of the mouth: an update for the general medical practitioner. Clinics (Sao Paulo). 62, 769-780.

7. Miziara, I.D. (2009). The treatment of recurrent aphthous stomatitis still intrigues. Rev. Assoc. Med. Bras**.** 55, 96. (in Portuguese)

8. Martins, M.D., Fernandes, K.P.S., Pavesi, V.C. (2011). Healing properties of papain-based gel on oral ulcers**.** Braz. J. Oral. Sci. 10, 120-123.

9. Fernandes, K.P.S., Bussadori, S.K., Marques, M.M. (2010). Healing and cytotoxic effects of *Psidium guajava* (Myrtaceae) leaf extracts. Braz J Oral Sci. 9, 449-454.

10. Martins, M.D., Marques, M.M., Bussadori, S.K. (2009). Comparative analysis between *chamomilla recutita* and corticosteroids on wound healing. An *in vitro* and *in vivo* study. Phytother. Res*.* 23, 274–278.

11. Ribeiro, M.A.G., Albuquerque, L.C., Ramalho, L.M.P., et al (2009). Immunohistochemical assessment of myofibroblasts and lymphoid cells during woung healing in rats subjected to laser photobiomodulation at 600nm. Photomed. Laser Surg. 27, 49-55.

12. Reddy, G.K. (2004). Review photobiological basis and clinical role of low-intensity lasers in biology and medicine. J. Clinic. Laser Med. Surg. 22, 141-150.

13. Bourguignon-Filho, A.M., Feitosa, A.C.R., Beltrão, G.C., et al. (2005). Use of low level laser therapy on wound healing. Literature review. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial. 46, 37-43. (in Portuguese)

14. Mester, E., Spiry, T., Szende, B., et al. (1971). Effect of laser rays on wound healing. Am. J. Surg. 122, 532 – 535.

15. Pereira, A.N., Eduardo, C.P., Maston, E., et al. (2002). Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. Lasers Surg. Med. 31, 263–267.

16. Peplow, P.V., Chung, T., Baxter, D. (2010). Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. Photomed. Laser Surg. 28, s3-s40.

17. Souza, T.O.F., Mesquita, D.A., Ferrari, R.A.M., et al. (2011). Phototherapy with low-level laser affects the remodeling of types I and III collagen in skeletal muscle repair. Lasers Med Sci. 26, 803 – 814.

18. Lim, W., Lee, S.G., Kim, I., et al. (2007). The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors. Lasers Surg. Med. 39, 614–621.

19. Aimbire, F., Albertini, R., Pacheco, M.T.T., et al. (2006). Low-level laser therapy induces dose-dependent reduction of TNF-afla levels in acute inflammation. Photomed. Laser Surg. 24, 33-37.

20. Corazza, A.V., Jorge, J., Kurachi, C., et al. (2007). Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources. Photomed. Laser Surg. 25, 102-106.

21. Pereira, M.C., Pinho, C.B., Medrado, A.R.P., et al. (2010). Influence of 670 nm low-level laser therapy on mast cells and vascular response of cutaneous injuries. J. Photochem. Photobio. B. 98, 188–192.

22. Damante, C.A., Micheli, G., Miyagi, S.P.H., et al. (2008). Effect of laser phototherapy on the release of fibroblast growth factors by human gingival fibroblasts. Lasers Med Sci. 24, 885-891.

23. Peplow, P.V., Chung, T., Baxter, G.D. (2010). Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models. Photomed. Laser Surg. 28, 291-325.

24. Institute for Laboratory Animal Research; National Academies Press. Guide for the care and use of laboratory animals. Washington, D.C.: National Academies Press, 2011.

25. Khorasani, G., Hosseinimehr, S.J., Azadbakht, M., et al. (2009). Aloe versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. Surgery Today. 39, 587-591.

26. Sinha, U.K., Gallagher, L.A. (2003). Effects of steel scalpel, ultrasonic scalpel, CO2 laser, and monopolar and bipolar electrosurgery on wound healing in guinea pig oral mucosa. Laryng.113, 228-36.

27. Kumar, V., Cotran, R.S., Robbins, S.L. (2003). Robbins basic pathology. 7th ed. Imprenta Philadelphia, PA: Saunders.

28. Camacho-Alonso, F., Lopez-Jornet, P. (2007). Clinical-pathological study of the healing of wounds provoked on the dorso-lingual mucosa in 186 albino rats. Otolaryngol. Head Neck Surg., 136, 119-24.

29. Medrado, A.P., Pugliese, L.S., Reis, S.R.A., et al. (2003). Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Lasers Surg. Med. 32, 239–244.

30. Viegas, V.N., Abreu, M.E.R., Viezzer, C., et al. (2007). Effect of low-level laser therapy on inflammatory reactions during wound healing: comparison with meloxicam. Photomed. Laser Surg. 25, 467-473.

31. Medrado, A.P., Soares, A.P., Santos, E.T., et al. (2008). Influence of laser photobiomodulation upon connective tissue remodeling during wound healing. J. Photochem. Photobio. B. 92, 144–152.

32. Pugliese, L.S., Medrado, A.P., Reis, S.R.A., et al. (2003). The influence of low-level laser therapy on biomodulation of collagen and elastic fibers. Pesqui. Odontol. Bras.17, 307-313.

33. Reis, S.R.A., Medrado, A.P., Marchionni, A.M., et al. (2008). Effect of 670-nm laser therapy and dexamethasone on tissue repair: a histological and ultrastructural study. Photomed. Laser Surg. 26, 307 -313.

34. Almeida-Lopes, L., Rigau, J., Zângaro, R.A., et al. (2001). Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. Lasers Surg. Med. 29, 179-184.

35. Fahimipour, F., Nouruzian, M., Anvari, M., et al. (2011). Effect of low-level laser therapy on experimental wounds of hard palate mucosa in mice. Ind. J. Exp. Bio. 49, 357-361.

36. van Breugel, H.H.F.I., Dop Bärr, P.R. (1992). Power density and exposure time of He–Ne laser irradiation are more important than total energy dose in photo-biomodulation of human fibroblasts in vitro. Lasers Surg. Med. 12, 528–537.

37. Walsh, L.J. (1997). The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. Aust. Dent. J. 42, 247-254.

38. Lubart, R., Lavi, R., Friedmann, H., et al. (2006). Photochemistry and photobiology of light absorption by living cells. Photomed. Laser Surg. 24, 179-185.

39. Demidova-Rice, T.N., Salomatina, E.V., Yaroslavsky, A.N. et al. (2007). Low-level light stimulates excisional wound healing in mice. Lasers Surg. Med. 39, 706-715.

40. Karu, T.I. (1988) Molecular mechanisms of therapeutic effect of low-intensity laser radiation. Laser Life Sci. 2, 53-74.

41. Eduardo, F.P., Mehnert, D. U., Monezi, T. A., et al. (2003). Cultured epithelial cells response to phototherapy with low intensity laser. Lasers Surg. Med. 39, 365-372.

3 CONSIDERAÇÕES FINAIS

Baseado no resultado do presente estudo podemos concluir que a terapia de LBP utilizada com densidade de energia de 4J/cm^2 foi capaz de acelerar o reparo de ulceras bucais em ratos, através de uma reepitelização mais rápida associada a cronificação do processo inflamatório. Os resultados encontrados mostram que a fotobioestimulação está relacionada a densidade de energia e que a menor dose utilizada apresentou melhor resultado clínico e histológico.

REFERÊNCIAS

AIMBIRE, F. et al. Low-level laser therapy induces dose-dependent reduction of tnf-afla levels in acute inflammation. **Photomed. Laser Surg.**, New York, v. 24, no. 1, p. 33-37, Feb. 2006.

ARAÚJO, L.U. et al. Profile of wound healing process induced by allantoin. **Acta Cir. Bras.**, São Paulo, v. 25, n.5, p. 460-466, Sept./Oct. 2010.

BAPTISTA, J. et al. Influence of laser photobiomodulation on collagen in during skeletal muscle tissue remodeling after injury in rats. **Photomed. Laser Surg.**, New York, v. 29, no. 1, p. 11-17, Aug. 2010.

BOURGUIGNON-FILHO, A.M. et al. Utilização do laser de baixa intensidade no processo de cicatrização tecidual. Revisão de literatura**. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial**, Barcelona, v. 46, n.1, p. 37-43, 2005.

CORAZZA, A.V. et al. Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources. **Photomed. Laser Surg.**, New York, v. 25, no. 2, p. 102-106, Apr. 2007.

DAMANTE, C.A. et al. Effect of laser phototherapy on the release of fibroblast growth factors by human gingival fibroblasts. **Lasers Med. Sci.**, London, v. 24, no. 6, p. 885-891, Nov. 2008.

EDUARDO, F.P. et al. Cultured epethelial cells reponse to phototherapy with low intensity laser. **Lasers Surg. Med.**, New York, v. 39, no. 9, p. 365-272, Apr. 2007.

FERNANDES, K.P.S. et al. Healing and cytotoxic effects of Psidium guajava (Myrtaceae) leaf extracts**. Braz. J. Oral. Sci.**, v. 9, n. 4, p. 449-454, Oct./Dec. 2010.

FIELD, E.A.; ALLAN, R.B. Review article: oral ulceration – etiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic**. Aliment. Pharmacol. Ther.**, Liverpool, v. 15, no. 10, p. 949-962, Nov. 2003.

GÁL, P. et al. Histological Assessment of the Effect of Laser Irradiation on Skin Wound Healing in Rats. **Photomed. Laser Surg.,** v. 24, n. 4, p. 480-488, 2006

GONÇALVES, R.V. et al. Comparative study of the effects of gallium- aluminum-arsenide laser photobiomodulation and healing oil on skin wounds in wistar rats: a histomorphometric study. **Photomed. Laser Surg.**, New York, v. 28, no. 5, p. 597-602, Oct. 2010.

HENRIQUES, A.C.G.; CAZAL, C.; CASTRO, J.F.L. Ação da laserterapia no processo de proliferação e diferenciação celular. Revisão da literatura. **Rev. Col. Bras. Cir.**, Rio de Janeiro, v. 37, n. 4, p. 295-302, jul./ago. 2010.

KARU, T.I. Molecular mechanisms of therapeutic effect of low-intensity laser radiation. **Laser Life Sci.,** Lausanne**,** v. 2, no. 1, p. 53-74, 1988.

KARU, T.I. Laser biostimulation: a photobiological phenomenon. **J. Photochem. Photobio. B.**, Lausanne, v. 3, no. 4, p. 638-640, 1989.

LEÃO, J.C.; GOMES, V.B.; PORTER, S. Ulcerative lesions of the mouth: an update for the general medical practitioner. **Clinics (Sao Paulo)**, São Paulo, v. 62, no. 6, p.769-780, Dec. 2007.

LIM, W. et al. The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors. **Lasers Surg. Med.**, New York, v. 39, no. 7, p. 614–621, Aug. 2007.

LINS, R.D.A.U. et al. Biostimulation effects of low-power laser in the repair process**. An. Bras. Dermatol.**, Rio de Janeiro, v. 85, n. 6, p.849-855, nov./dez. 2010.

MANDELBAUM, S.H.; SANTIS, E.P.D.; MANDELBAUM, M.H.S. Cicatrização: conceitos atuais e recursos auxiliares - Parte I. **An. Bras. Dermatol.**, Rio de Janeiro, v. 78, n. 4, p.393-410, jul./ago. 2003.

MARTINS, M.D. et al. Comparative analysis between chamomilla recutita and corticosteroids on wound healing. an in vitro and in vivo study. **Phytother. Res.**, England, v. 23, no. 2, p. 274–278, Feb. 2009.

MARTINS, M.D. et al. Healing properties of papain-based gel on oral ulcers**. Braz. J. Oral. Sci.**, Piracicaba, v. 10, n.2, p.120-123, Apr./June. 2011.

MEDRADO, A.P. et al. Influence of laser photobiomodulation upon connective tissue remodeling during wound healing. **J. Photochem. Photobio. B.**, Lausanne, v. 92, n. 3, p.144–152, Sep. 2008.

MEDRADO, A.P. et al. Influence of Low Level Laser Therapy on Wound Healing and Its Biological Action Upon Myofibroblasts. **Lasers Surg. Med.**, New York, v. 32, n. 3, p. 239–244, 2003

MENDONÇA, R.J.D.; COUTINHO-NETTO, J. Aspectos celulares da cicatrização. **An. Bras. Dermatol.**, São Paulo, v.84, n. 3, p.257-262, jul. 2009.

MESTER, E; SPIRY, T; SZENDE, B; TOTA, J.G. Effect of laser rays on wound healing. **Am. J. Surg**, [S.l.], v. 122, n.4, p. 532-535, Oct. 1971.

MIZIARA, I.D. O tratamento da estomatite aftóide recorrente ainda intriga**. Rev. Assoc. Med. Bras.**, São Paulo, v. 55, n. 2, p. 96, mar/apr. 2009.

NASCIMENTO, P.M. et al. A Preliminary Report on the Effect of Laser Therapy on the Healing of Cutaneous Surgical Wounds as a Consequence of an Inversely Proportional Relationship between Wavelength and Intensity: Histological Study in Rats. **Photomed. Laser Surg.,** v. 22, n. 6, p. 513-518, 2004.

PEPLOW, P.V.; CHUNG, T.; BAXTER, D. Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. **Photomed. Laser Surg.**, New York, v. 28, Suppl 1, s3-s40, Aug. 2010.

PEREIRA, A.N. et al. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. **Lasers Surg. Med.**, New York, v. 31, no. 4, p. 263–267, 2002.

PEREIRA, M.C et al. Influence of 670 nm low-level laser therapy on mast cells and vascular response of cutaneous injuries. **J. Photochem. Photobiol. B.**, Lausanne, v. 98, no. 3, p.188–192, Mar. 2010.

PUGLIESE, L.S. et al. The influence of low-level laser therapy on biomodulation of collagen and elastic fibers. **Pesqui. Odontol. Bras.**, São Paulo, v. 17, n.4, p. 307-313, 2003

REDDY, G.K. Review photobiological basis and clinical role of low-intensity lasers in biology and medicine. **J. Clin. Laser Med. Surg.**, New York, v. 22, no. 2, p. 141-150, Apr. 2004.

REIS, S.R.A. et al. Effect of 670-nm Laser Therapy and Dexamethasone on Tissue Repair: A Histological and Ultrastructural Study. **Photomed. Laser Surg.,** New York, v. 26, n. 4, p. 307 -313, 2008

RIBEIRO, M.A.G. et al. Immunohistochemical assessment of myofibroblasts and lymphoid cells during woung healing in rats subjected to laser photobiomodulation at 600nm. **Photomed. Laser Surg.**, New York, v. 27, n. 1, p. 49-55, Feb. 2009.

SCULLY, C.; SHOTTS, R. Mouth ulcers and other causes of orofacial soreness and pain. **Bmj.,** London, v. 321, no. 7254, p.162-165, July. 2000.

SMITH, K.C. The photobiological basis of low level laser irradiation therapy. **Laser Ther.**, [S.l.], v. 3, no. 1, p. 19-24, 1991.

SOUZA, T.O.F. et al. Phototherapy with low-level laser affects the remodeling of types I and III collagen in skeletal muscle repair. **Lasers Med. Sci.**, London, v. 26, no. 6, p. 803-814, Nov. 2011.

VAN BREUGEL, H.H.F.I., DOP BÄRR, P.R. Power density and exposure time of He–Ne laser irradiation are more important than total energy dose in photo-biomodulation of human fibroblasts in vitro. **Lasers Surg. Med.,** New York, v. 12, n. 5, p. 528–537, 1992

VIEGAS, V.N. et al. Effect of Low-Level Laser Therapy on Inflammatory Reactions during Wound Healing: Comparison with Meloxicam. **Photomed. Laser Surg.**, New York, v. 25, n. 6, p. 467-473, 2007

ZEZELL D, M. **Utilização Clínica do Laser.** Apostila de Workshop, IPEN/FOUSP, São Paulo, 23 a 27 de junho, 2004.

ANEXO – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão de Ética no Uso de Animais (CEUA/HCPA) analisou o projeto:

Projeto: 120338
Data da Versão do Projeto: 25/09/2012

Pesquisadores:
LUISE MEURER
URSULA DA SILVEIRA MATTE
MANOEL SANTANA FILHO
MARCO ANTONIO TREVIZANI MARTINS
ALESSANDRA SELINGER MAGNUSSON
CHRIS KREBS DANILEVICZ
VIVIAN PETERSEN WAGNER

Título: EFEITO DO LASER DE BAIXA POTÊNCIA NA ANGIOGÊNESE E NOS NÍVEIS TECIDUAIS DE CITOCINAS PRÓ-INFLAMATÓRIAS DURANTE O REPARO DE ÚLCERAS BUCAIS EM RATOS

Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08/10/2008, que estabelece procedimentos para o uso científico de animais.

- Os membros da CEUA/HCPA não participaram do processo de avaliação de projetos onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.

Porto Alegre, 16 de outubro de 2012.

Dr. Alessandro Osvaldt
Coordenador CEUA/HCPA