PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

JONAS DANTAS BATISTA

EFEITOS DA TERAPIA LASER DE BAIXA POTÊNCIA SOBRE O REPARO ÓSSEO: AVALIAÇÃO DO OSSO SUBMETIDO À RADIOTERAPIA E DO EFEITO SISTÊMICO

Porto Alegre
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Tese apresentada como requisito para obtenção do Título de Doutor pelo Programa de Pós-Graduação em Odontologia, Área de Concentração em Cirurgia e Traumatologia Buco-Maxilo-Facial da Faculdade de Odontologia, Pontifícia Universidade Católica do Rio Grande do Sul.

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2011

Dedico esta tese aos meus pais, Manoel Ulisses Batista (*in memoriam*) e minha mãe Yolita Dantas Batista e meus irmãos André e Ana Regina. Meu PAI, um exemplo de vida, de caráter e perseverança, que me apoiou muito durante esse período, mas infelizmente não chegou a ver meu trabalho concluído. Com amor e muito compreensão, me ajudaram a realização desse sonho.

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E, enfim, a todos que contribuíram na execução desse trabalho que marca minha vida profissional e pessoal.

EPÍGRAFE

"... e nunca considerem seu estudo como uma obrigação, mas sim como uma oportunidade invejável de aprender, sobre a influência libertadora da beleza no domínio do espírito, para seu prazer pessoal e para o proveito da comunidade à qual pertencerá o seu trabalho futuro."

ALBERT EINSTEIN

(1879 - 1955)

RESUMO

RESUMO

A laserterapia de baixa potência (LTBP) tem sido utilizada para acelerar o processo de reparo no tecido ósseo com resultados positivos; entretanto, não existem estudos que avaliem seu efeito sobre o osso comprometido pela radioterapia. Outra questão importante a ser investigada é a existência de um possível efeito sistêmico sobre o reparo ósseo quando o laser é aplicado distante da área do defeito ósseo cirúrgico. A presente pesquisa teve por objetivo investigar: 1- O efeito da LTBP sobre o reparo ósseo de fêmures previamente submetidos à radioterapia. 2- O efeito sistêmico (dose distante do defeito) da LTBP sobre o reparo ósseo. No primeiro estudo, vinte ratos machos (*Rattus norvegicus*, Wistar) foram distribuídos em 4 grupos com 5 animais em cada um: grupo I, submetidos à osteotomia no fêmur (OF); grupo II, submetidos à OF e LTBP; grupo III, submetidos à radioterapia no fêmur (RDT) e após 1 mês, à OF e grupo IV, submetidos à RDT e após 1 mês, OF e LTBP. Os grupos II e IV foram submetidos à LTBP (Arsenato de Gálio e Alumínio - GaAlAs, λ= 830nm; DE = 6J/cm^2, P = 50mW, t = 120s, e diâmetro do feixe de 0.04cm^2) durante a cirurgia e no período pós-operatório, a cada 48 horas. Os animais foram submetidos à eutanásia 7 dias após a cirurgia, o osso avaliado histomorfometricamente com microscopia de luz. O reparo ósseo foi observado somente nos grupos I e II, com um aumento significante no grupo II em comparação com o grupo I (ANOVA, teste de Bonferroni, P<0,001). Esse resultado demonstra o efeito positivo da LTBP no osso normal. Entretanto, não foi possível observar efeito benéfico no osso comprometido pela radiação ionizante. No segundo estudo, quarenta e cinco ratos machos foram distribuídos em 3 grupos com 15 animais em cada: grupo I submetidos à osteotomia no fêmur (OF); grupo II, submetidos à OF e LTBP no fêmur contralateral; grupo III, submetidos à OF e LTBP em ambos os fêmures. Os grupos II e III foram submetidos à mesma LTBP já descrita. Os animais foram eutanasiados nos períodos de 7, 15 e 21 dias após a cirurgia e o osso foi avaliado histomorfometricamente com microscopia de luz. A análise histomorfométrica mostrou no período de 7 dias, um aumento significante de osso neoformado no grupo III em comparação com os grupos I e II (ANOVA, teste de Bonferroni, P<0.005). Nos períodos de 15 e 21 dias a análise histomorfométrica não mostrou diferença entre os grupos. Podemos concluir que a LTBP apresentou um efeito local positivo sobre o reparo ósseo, porém, não foi observado efeito sistêmico.

Palavras-chave: Lasers. Osso. Radioterapia. Anormalidades Induzidas por Radiação

SUMMARY

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The low level laser therapy (LLLT) is used to accelerate bone repair with positive results; however, there is no study that evaluate its effect on bone compromised by ionizing radiation (IR). Another issue to be investigated is the existence of systemic effects on bone healing when the laser is applied distant from the area of the surgical bone defect. This study aimed to investigate: 1 - The effect of LLLT on bone healing in femurs previously irradiated by radiotherapy. 2 - The systemic effect of LLLT on bone repair. In the first study, twenty Wistar rats were divided into four groups: group I (control, n = 5) which was submitted only to surgical perforation (SP) of the bone; group II, submitted to SP and LLLT (n = 5); group III, submitted to IR and then SP (n = 5); and group IV, submitted to IR, SP and LLLT (n = 5). Groups II and IV received punctual laser application (DE = 6J/cm2, P = 50mW, t = 120s, and beam diameter of 0.04cm2) immediately postoperatively, and then three times at every other 48h. Animals were euthanized at 7 days after surgery, and bone sections were evaluated morphometrically with conventional microscopy. Bone repair was only observed in non-irradiated bone, with significantly improvement in group II in comparison to group I. This result demonstrates a positive local biostimulative effect of LLLT in normal bone. However, it was not able to revert the metabolic damage associated to ionizing irradiation. In the second study, forty-five Wistar rats were submitted to osteotomy on left femur and randomly allocated into three different groups: group I, control (n = 15) submitted to confection of the bone defect only; group II, laser applied in the right femur (n = 15); group III, laser applied locally on bone defect and also on the right femur (n = 15). Laser groups (II and III) received the same laser protocol described above. Five animals of each group (n = 5) were euthanized 7, 15 and 21 days after surgery. The histomorphometric analysis showed at 7 days a significant increase of bone formation in group III compared to group I and II. At day 15 and 21, histomorphometric analysis showed no significant differences among the groups. Laser therapy presented a positive local biostimulative effect in early stage of bone healing, but a systemic effect was not observed.

Keywords: Lasers. Bone. Radiotherapy. Abnormalities, Radiation-Induced

LISTA DE SIGLAS E ABREVIAÇÕES

RDT – Radioterapia

IR – *Ionizing radiation* (radiação ionizante)

LTBP – Laser Terapia de Baixa Potência

LLLT- *Low level laser therapy* (terapia laser de baixa potência)

GaAlAs - Arsenato de Gálio e Alumínio

INCA - Instituto Nacional do Câncer

ANOVA - Análise de Variância

SP – *surgical perfuration* (perfuração cirúrgica)

DE - densidade de energia

HE hematoxilina-eosina

HeNe hélio-neônio

IP Intraperitoneal

LISTA DE SÍMBOLOS

J Joule

W Watt

cm centímetro

cm^2 centímetro quadrado

E energia

h hora

J/cm2 joule por centímetro quadrado

m metro

mg miligrama

min minutos

ml Mililitro

mm milímetro

mW miliwatts

mm micrômetro

n.° número

nm nanômetro

p probabilidade de erro

P Potência

s segundo

W/cm2 Watts por centímetro quadrado

λ comprimento de onda

β Beta

Ø diâmetro da secção transversal da fibra óptica

t tempo

g grama

G Grupo

Kg kilograma

Gy Gray

SUMÁRIO

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INTRODUÇÃO

1-INTRODUÇÃO

Entre as moléstias que acometem o ser humano, as neoplasias malignas merecem atenção dos profissionais da saúde por se tratarem de doenças que apresentam prognóstico sombrio e cujas modalidades de tratamento apresentam efeitos colaterais e sequelas indesejáveis (INCA 2011). A radioterapia (RDT) é um método consolidado e empregado como opção terapêutica para controle e cura de várias neoplasias malignas. Sua ação biológica ocorre por efeitos diretos e indiretos sobre as células, ou seja, destruindo as células neoplásicas ou bloqueando novas mitoses.

O osso, quando submetido às altas doses de radiação, pode apresentar um desequilíbrio da atividade osteoblástica e osteoclástica, com favorecimento à reabsorção óssea (MAEDA et al., 1988; CUNHA et al., 2007; PELISSER et al., 2007), aumento da lise celular (CUNHA et al., 2007), alteração da rede de canais ósseos (RABELO; BELETTI; DECHICHI, 2010) e redução da resistência biomecânica (MAEDA et al., 1988). A osteogênese e a vascularização também ficam prejudicadas após radioterapia (MUHONEN et al., 2004). Desta forma, após o tratamento radioterápico em região de cabeça e pescoço, procedimentos cirúrgicos e reabilitadores apresentam maior risco de complicações no processo de reparo ósseo (MONIER et al., 2011).

Efeitos colaterais no tecido ósseo pós-radioterapia já foram demonstrados por meio de análises histológicas, histomorfométricas, bioquímicas e biomecânicas; no entanto, a complexidade das mudanças no metabolismo ósseo pós-radiação ainda não está totalmente esclarecida. Um dos principais inconvenientes da radioterapia sobre o tecido ósseo é a ocorrência da osteorradionecrose (GRIMALDI et al., 2005; MONIER et al., 2011).

Vários recursos têm sido testados para minimizar os riscos de osteorradionecrose decorrente da radioterapia, tais como: o uso de oxigenação hiperbárica (CLARK et al., 2006),

retalhos microcirúrgicos vascularizados (LEHNER et al., 2004), fatores de crescimento associados aos enxertos ósseos (AGHALOO et al., 2006), porém, novas formas de tratamento ainda se fazem necessárias.

A laserterapia de baixa potência (LTBP) em tecidos moles tem sido usada para acelerar o reparo de feridas e controlar a dor. Em tecido ósseo, por sua vez, a laserterapia de baixa potência tem-se mostrado efetiva na modulação da inflamação, acelerando a proliferação celular e o processo de reparo. No entanto, o mecanismo pelo qual a radiação laser interfere na formação óssea ainda não foi completamente esclarecido. É provável que a regeneração óssea seja dependente não apenas da dose de energia total da radiação laser, mas também, do tempo e da forma de aplicação (SAITO; SHIMIZU, 1997; PINHEIRO et al., 2006).

Estudos recentes têm sugerido que parâmetros de densidade de energia e intensidade da radiação laser são fatores independentes e contribuem diretamente para o sucesso ou fracasso da laserterapia de baixa potência (PINHEIRO et al., 2006). O papel da laserterapia na promoção do reparo em tecido ósseo submetido à radioterapia oncológica ainda não foi estabelecido.

Tendo em vista o grande número de procedimentos odontológicos que envolvem o tecido ósseo, é de grande interesse o estabelecimento de protocolos clínicos que visem uma melhoria da regeneração óssea, especialmente em osso comprometido pela radioterapia. Para tanto, propõe-se no presente estudo, uma avaliação “in vivo”, dos efeitos da laserterapia de baixa potência em lesões ósseas promovidas em fêmures de ratos previamente irradiados por radiação ionizante.

Além dos efeitos locais, a ocorrência de efeitos sistêmicos da terapia com laser de baixa potência tem sido relatada (KANA; HUTSCHENREITER; HAINA, 1981; ROCKHIND et al., 1989; BRAVERMAN; MCCARTHY; IVANCOLICH, 1989). Diversos estudos que avaliaram o efeito da LTBP no reparo ósseo utilizaram animais distintos para o grupo experimental e controle em função de um possível efeito sistêmico do laser aplicado em uma área bem definida, como

descrito em estudos anteriores. (PINHEIRO et al., 2003a; PINHEIRO et al., 2003b; NICOLA et al., 2004; KHADRA et al., 2004; GERBI et al., 2005; TORRES et., 2008; MÁRQUEZ MARTÍNEZ; PINHEIRO; RAMALHO, 2008). Outros estudos que avaliam o efeito da LTBP no reparo ósseo em modelo animal também utilizam um grupo controle distinto (LIRANI-GALVÃO; JORGETTI; SILVA, 2006; CARVALHO et al., 2006; LOPES et al., 2007; PRETEL; LIZARELLI; RAMALHO, 2007; BLAYA et al., 2008; GERBI et al., 2008; PINHEIRO et al., 2009; DINIZ et al., 2009) apesar dessa informação sobre a interferência do efeito sistêmico não estar explícita.

Efeitos sistêmicos podem explicar a ausência de ações biomoduladoras do laser em trabalhos que utilizaram feridas contralaterais como controle, para verificação dos efeitos do laser em feridas no mesmo animal (ANNEROTH et al., 1988; BRAVERMAN; MCCARTHY; IVANCOLICH, 1989; ROCHKIND et al., 1989; DAVID et al., 1996). Entretanto, estudos que utilizaram controle interno (mesmo animal) tiveram bons resultados da LTBP no reparo ósseo (MERLI et al., 2005). De fato, nenhum estudo avaliou a existência de efeito sistêmico da LTBP no reparo ósseo, sendo necessários mais estudos para elucidar esses questionamentos.

O presente estudo compreende dois trabalhos apresentados sob a forma de artigos científicos. O primeiro teve como objetivo avaliar o efeito da terapia laser de baixa potência (Arsenato de Gálio e Alumínio - GaAlAs, λ= 830nm) no reparo ósseo de fêmures de ratos previamente submetidos à radioterapia oncológica. O segundo artigo descreve o experimento, cujo objetivo foi investigar a existência de efeito sistêmico desse mesmo protocolo laser no reparo ósseo nos fêmures de ratos.

ARTIGO 1

2. ARTIGO 1

O artigo a seguir intitula-se **"Effect of Low Level Laser Therapy on Repair of Bone Submitted to Radiotherapy"** e foi formatado de acordo com as normas do periódico *Lasers in Medical Science* - fator de impacto: 2.311 (Anexo A).

Effect of Low Level Laser Therapy on Repair of Bone Submitted to Radiotherapy

(Lasers in Medical Sciences – impact factor: 2.311)

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Abstract

Radiotherapy is a widely used modality of treatment against many types of cancer, but high doses of ionizing radiation (IR) can directly affect healthy tissues, specially related to the reduced metabolism observed in the irradiated bone. Positive biological effects of low level laser therapy (LLLT) on bone repair have been well demonstrated. However, this effect on surgical defects of bone previously compromised by radiotherapy has not been evaluated. The aim of this study was to investigate the influence of LLLT (λ = 830nm) on repair of bone that was previously submitted to ionizing radiation. Twenty Wistar rats were divided into four groups: group I (control, n = 5) which was submitted only to surgical perforation (SP) of the femur; group II, submitted to SP and LLLT (n = 5); group III, submitted to IR and then SP (n = 5); and group IV, submitted to IR, SP and LLLT (n = 5). Groups II and IV received punctual laser application (DE = 6J/cm2, P = 50mW, t = 120s, and beam diameter of 0.04cm2) immediately postoperatively, and then three times at every other 48h. Animals were euthanized 7 days after surgery, and bone sections were evaluated histomorphometrically with conventional microscopy. *Results:* Bone repair was only observed in non-irradiated bone, with significantly improvement in group II in comparison to group I. This result demonstrates a positive local biostimulative effect of LLLT in normal bone. However, it was not able to revert the metabolic damage derived associated to ionizing irradiation.

Keywords: Laser, Bone, Radiotherapy, Morphometry, LLLT

Introduction

Radiotherapy (RDT) is a well-established method used as a therapeutic option for cure and control of certain types of cancer [1]. Its biological action occurs through direct and indirect effects, which can destroy cancer cells or block further mitoses, paralyzing the neoplastic growth. Depending on the location of the tumor, normal tissues underlying the radiation field also suffer RDT action, which may limit cancer treatment temporarily or permanently.

Physiological changes in bone tissue due to radiotherapy demand longer periods to be evident because of its slow response to ionizing irradiation. The irradiation causes decrease in blood supply [2], modification in microstructure of bone channels network [3], and reduction of osteocytes counts, osteoblastic activity [4] and bone matrix [3]. These alterations interfere in bone metabolism [2] and compromise the strength and healing in irradiated bone [3].

The reconstructive surgeries and prosthetic rehabilitation of patients after treatment of malignant tumors in head and neck have been associated with significant risk of complications in the repair process due to ionizing radiation, especially high risk of osteoradionecrosis resulting from handling irradiated bone [6]. Several therapeutic treatments have been studied to minimize risks and achieve success in these patients such as the use of hyperbaric oxygenation [7], vascularized grafts [8,9], platelet rich plasma associated with grafts [10], some of these, expensive and invasive [7,8]. Thus, new alternative methods need to be investigated.

The low level laser therapy (LLLT) has been used to accelerate wound repair and pain control and has also shown to be effective in modulating inflammation [11], cell proliferation and accelerating bone repair [12]. Several studies have confirmed the positive effects of LLLT on soft tissues submitted to radiotherapy, as well as treatment and prevention of oral mucositis [13,14]. However, there are only few studies evaluating laser effects on bone previously submitted to radiotherapy [4]. However, no study evaluates laser influence in repair of standardized surgical

bone defects previously compromised by ionizing radiation. Thus, we can infer that the low level laser therapy beneficially alter the repair process in bone irradiated by ionizing radiation. In this perspective, the present study was designed to investigate the effect of low-level laser therapy in healing of bone previously submitted to ionizing irradiation in rats.

Material and Methods

Animals

Twenty healthy male Wistar rats, weighing 300 to 350g and housed five animals per cage, were maintained at appropriate facilities, with a controlled photoperiod of 12h, controlled humidity and fed with balanced diet and water *ad libitum*.

This study was previously submitted and approved by the Animal Ethics Committee from Pontifícia Universidade Católica of Rio Grande do Sul, Brazil (10/00169), and it was performed in accordance with the Brazilian College for Animal Experimentation (COBEA).

Experimental groups

Each group was composed of five animals randomly divided into four groups, as follows:

- Control group (GI): Animals were only submitted to surgical perforation (SP) of the femur (SP).
- Laser group (GII): SP and then LLLT.
- Radiotherapy group (GIII): Animals were previously submitted to ionizing radiation and then to SP.
- Radiotherapy and Laser group (GIV): LLLT applied after SP of bone previously submitted to ionizing radiation.

Procedures

Radiotherapy

Radiotherapy was executed in groups III and IV. Before irradiation, the animals were anaesthetized by an intraperitoneal injection of 100mg/kg ketamine plus 3mg/kg xylazin hydrochloride and placed in supine position. The left leg was positioned laterally and fixed in this position using wood stick and adhesive tape. A bolus made of wax with 1.5cm of thickness was positioned over the left leg. Both femur and tibia was irradiated by a single anterior field (Fig 1). The beam was individually collimated and irradiation was delivered using linear accelerator (*Varian* Clinac® 600C S/N 0310) delivering a total dose of 30Gy in one session. The interval between radiotherapy and femur surgery was 4 weeks.

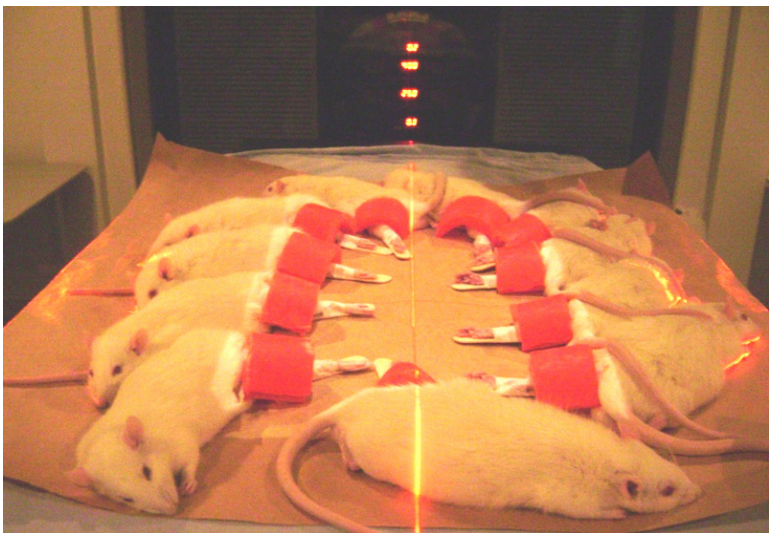

FIGURE 1 Animals anesthetized and positioned for radiotherapy session. Bolus made of red wax was placed over the femur area of left leg.

Surgery

A Two point 3mm standardized bone defects were created in each animal of all groups, with the same anesthesia protocol, under antibiotic prophylaxis (cephalosporin 30mg/kg, IP). The animal was fixed in the right lateral decubitus and the left femur was exposed through a 2 cm

continuous longitudinal incision. Then, an osteotomy was created in the lateral cortical bone using a n°8 round bur, coupled to an electric motor with 1000 rpm, under continuous saline solution irrigation. The depth of drilling was limited by contact with the inner face of the medial cortical bone (approximately 2 mm). The suture was performed using nylon 4-O.

Laser therapy

The animals of Groups II and IV were submitted to sessions of laser therapy using an infrared laser diode (GaAlAs -Flash Lase III, DMC®), with a wavelength of 830nm and potency of 50mw. The application was punctual, with a 6J/cm2 dose per session in the bone defect area during 2 minutes, with the laser tip positioned over and perpendicular to the long axis of the bone. The first session was applied immediately after drilling and before soft tissue repositioning. In the postoperative period, laser was applied every 48 hours for seven days, in a total of four sessions. The animals were euthanized seven days after surgery using saturated potassium chloride in conjunction with general anesthesia**.**

Histological procedure

The bone defect area and attached soft tissue were removed and immediately fixed in 10% phosphate buffered formaldehyde solution during 48 hours. Thereafter, the tissue blocks were decalcified in EDTA 4,13% for 4 weeks, dehydrated with graded ethanol and embedded in paraffin. Longitudinal histological sections of 5μm were obtained from the midline of the surgical defect and stained with Mallory Trichrome.

Histomorphometric Analysis

The bone neoformation was quantified by the same operator blinded for the status of each specimen. For this analysis, histological images of bone defects were captured at 4× magnification, using an Olympus BX 40 binocular microscope coupled with Olympus OLY 200 camera. The screen shots were merged, areas of soft tissue were erased using Photoshop CS2 software, and finally converted to binary images with HL Image 2005 software (Western Vision, Salt Lake City, UT, USA). The area of interest within the bone defect was delineated with four straight lines from the edges of the cortical bone to the opposite cortex (Fig. 2). The percentage of neoformed bone within the area of interest was calculated with the measure tool of the HL Image 2005.

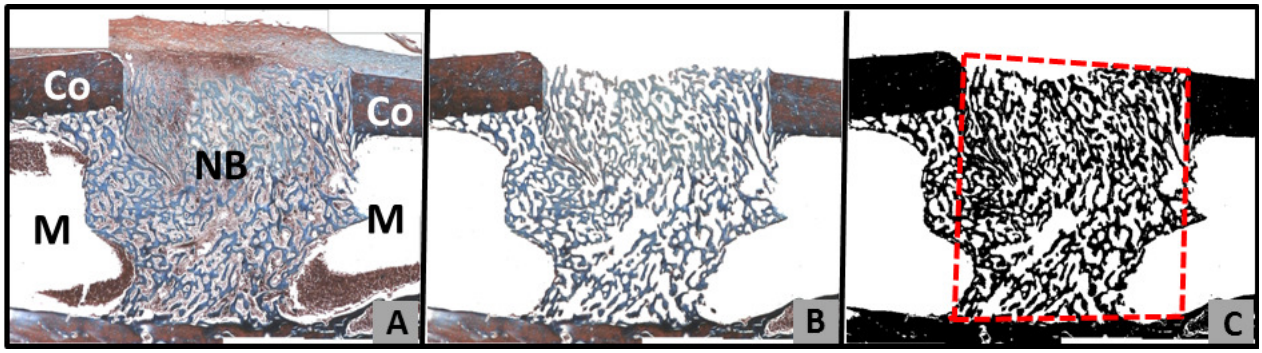

FIGURE 2: (A) - Photomicrograph of longitudinal femur section showing: cortical (Co), marrow (M) and new bone (NB) formation. (B) - Image treatment after digital removal of soft tissue. (C) - Image treatment after binary conversion and delineation of the area of interest for analysis (red dotted line). ×4. Mallory trichrome

Statistical analysis

Fisher exact test was performed to compare categorical parameters. Quantitative results were submitted to normality test and analyzed using Analysis of Variance (ANOVA) and Bonferroni´s post-hoc test. Differences were considered statistically significant if $p < 0.05$.

Results

Expected bone neoformation was evident in all the animals from Groups I and II, but in none of those from Groups III and IV, as shown in Fig. 3. In these later, marrow space was mainly occupied by reticular fibrin with frequent vacuolization, eventual fat cells and hemorrhage, sometimes intermingled by moderate quantities of monocytes and rare neutrophils. In animals of Groups I and II, repair was evident from the perforated cortex extending through medullar until the opposite cortical. It was composed by primary bone with trabecular arrangement delimiting small cavities, filled with loose connective tissue, fibroblasts, peripheral osteoblasts and blood vessels.

As shown in Figure 4, in group II bone neoformation beyond the limits of the area of interest and also the vertical extension of the bone touching the opposite cortex was usually more evident than in group I, although such distribution was not significant.

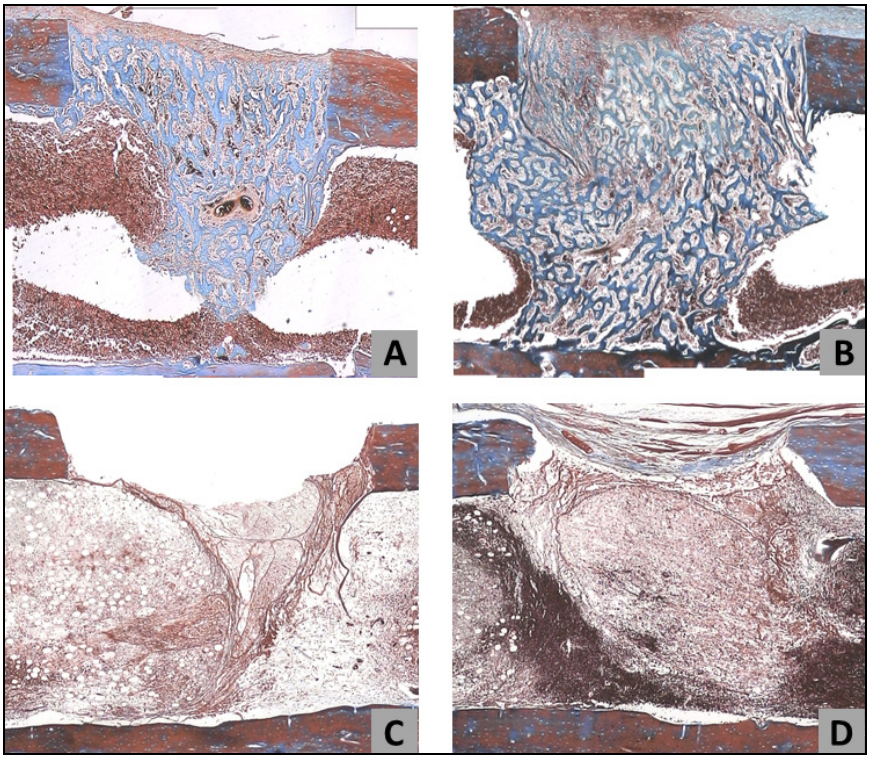

FIGURE 3: Photomicrograph of femur longitudinal section of all groups: A – Control Group (GI); B – Laser Group (GII); C - Radiotherapy Group (GIII) and D – Radiotherapy + Laser Group (GIV). ×4. Mallory Trichrome

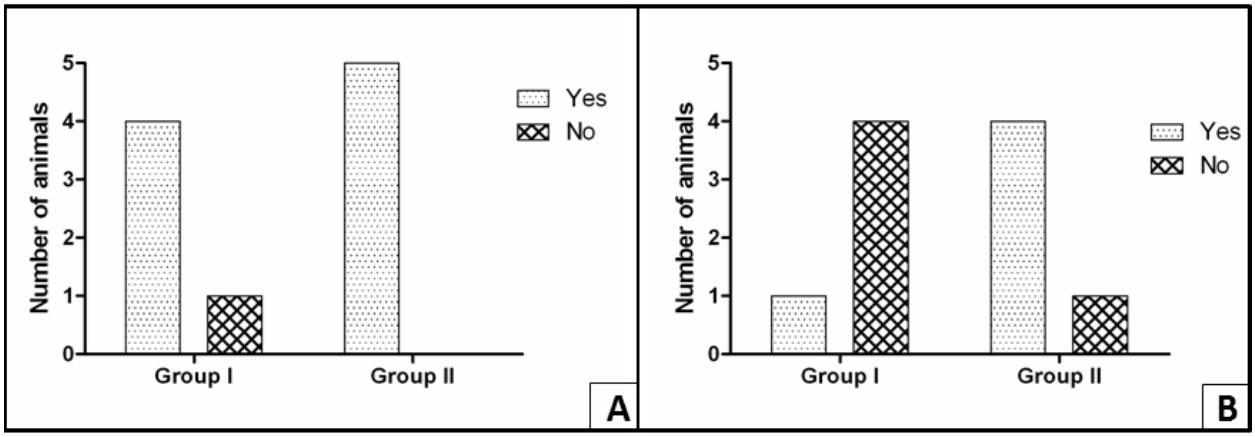

FIGURE 4: Histological paramether analysed: (A) Vertical bone neoformation reaching the opposite cortex (p>0,05). (B) – Lateral bone neoformation extending beyond the defect area (P>0,05).

The histomorphometric analysis revealed a significant increase in percentage of bone formation in group II in comparison to group I (Fig. 5). Groups III and IV did not present any bone formation, as stated before, and therefore were also significantly different from Groups I and II.

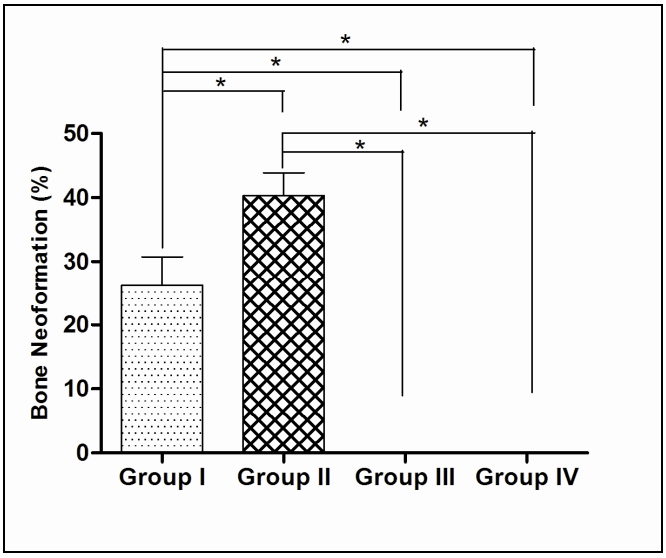

FIGURE 5. Bone formation percentage, evidencing bone increase in Group II (*p<0.05.)

Discussion

Deleterious effect of ionizing radiation on bone repair has been well documented in previous study, and this compromising has been reported to extend for long periods since abnormal healing without ossification lasts for up to 18 weeks after surgical bone perforation in rats [15]. The present study was performed to investigate whether low-level laser therapy was able to improve precocious healing of bone submitted to radiotherapy.

The beneficial effects of LLLT on soft tissues and bones are more expressive when it is applied in the first seven post-surgical days [16,17]. So the present study evaluated bone healing after this period. The choice of dose of 30 Gy was also based on previous studies, which showed significant alterations in bone morphology using this dose [4,15]. The single dose rather than fractionated doses was used to reduce the risk of death and stress of animals during induction of anesthesia in each session of radiotherapy. The linear accelerator is a modern resource, widely used nowadays. It has a bild up of 1.2 cm, so in that depth of penetration of tissue, starts the largest concentration of energy [18]. As the leg of rat has a thin thickness, it is necessary to use a material that assists in the superficiality of the absorbed dose; otherwise the higher dose would be concentrated after the animal leg. Then we used 3 slices of red wax (0.5 cm each) as bolus and in fact, the femur bone damage by ionizing radiation was significant**.** In laser protocol, the wavelength of 830nm has a capacity to penetrate the superficial tissues, reaching the bone [19], since the local effect of the laser was also evaluated (group III). The dose of 6 J/cm2 was based in previous studies, that used laser protocols for bone healing in rat model with doses ranging from 0.3 [17] to 16 J/cm2 per session [19], with positive results in bone formation.

In the present model, the damaging effect of ionizing radiation was clearly evident. No bone formation was observed in both groups submitted to radiotherapy, the bone repair was absent and the defect was filled by loose tissue poorly organized. This fact could be due the reduced viable undifferentiated mesenchymal cells [20], osteoblasts and blood vessels in the irradiated area needed for repair [21]. Noxious effect of ionizing radiation also led to an important problem in this model, since some animals submitted to ionizing radiation had to be replaced due to bone fracture during their manipulation on the LLLT sessions (*not shown*). The absence of appropriate repair may have facilitated the fracture of the femur. In addition, studies of irradiated bone in animal models observed biomechanical properties changes in different periods of assessment, making it more susceptible to fractures [5,22].

For animals that were not submitted to ionizing radiation, the positive effect of laser therapy was well evidenced (Group I *versus* II). This increase in bone formation observed may be due to local effects of laser stimulating the differentiation of mesenchymal cells and also osteoblasts and fibroblasts proliferation [23]. These events may explain the extensive bone neoformation, invading the medullar area, observed in group II. Garavello-Freitas et al. (2003) evaluating the influence LLLT on the repair of surgically produced tibia damage in rats also evidenced that maximal laser-stimulated bone growth was achieved after 1 week of laser application.

However, LLLT was not able to recover adequate healing of bone in animals submitted to radiotherapy (Group IV). There is a single experimental study that evaluated repair in compromised bone due to radiotherapy [15], as well as another one that had evaluated the use of laser in bone submitted to radiotherapy [4]. Da Cunha et al. (2007) described positive effects LLLT on bone submitted to the radiotherapy. However, this study was not attained to repair of surgical wounds, used different laser protocol, longer period of radiotherapy with cobalt-60 as

source of ionizing radiation, and sacrificed experimental animals only six weeks after radiotherapy.

Conclusion

This result demonstrates a positive local biostimulative effect of this LLLT protocol in the bone of animals not submitted to radiotherapy, but it was not able to revert the metabolic damage associated to ionizing irradiation in early stages of tissue repair. Future investigations using different LLLT protocol and its evaluation in longer periods of healing are required to improve the present knowledge on this question.

Acknowledgments

The authors would like to thank Pró-Reitoria de Pesquisa e Pós Graduação of Uberlândia Federal University (PROPP-UFU) for the research grants 049/2010 and also Dr. Vitor Carvalho Lara and Dr. Mizael Carlos de Alencar of School of Medicine, Federal University of Triângulo Mineiro. Prof. Sérgio Cardoso is research fellow of the Brazilian National Council for Research and Development (CNPq, 304597/2010-4).

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ARTIGO 2

3. ARTIGO 2

O artigo a seguir intitula-se **"Systemic Effect Evaluation of Low Level Laser Therapy on Bone Repair"** e foi formatado de acordo com as normas do periódico *Photomedicine and Laser Surgery* – fator de impacto:1.633 (Anexo A).

Systemic Effect of Low Level Laser Therapy on Bone Repair

Systemic Effect of LLLT on Bone Repair

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ABSTRACT

Objective: The aim of this study was to investigate the possible systemic effect of low level laser therapy (LLLT) (λ = 830 nm) on repair of surgical bone defects in rats. *Background data:* The biological effects of local therapy with lasers on bone repair have been well demonstrated, but the possible systemic effect on bone repair has not been evaluated. *Methods:* Forty-five Wistar rats were submitted to an osteotomy on the left femur and randomly allocated into three different groups: group I, control (n = 15) submitted to bone osteotomy (BO) in left femur; group II, BO in left femur + LLLT in the right femur (n = 15); group III, BO in the left femur + LLLT in the right femur + LLLT in the left femur (n = 15). Laser groups (II and III) received applications within a 48-h interval in one point per session of DE = 6 J/cm2, P = 50 mW, t = 120 sec, and beam diameter of 0.04 cm2. Five animals of each group (n = 5) were euthanized 7, 15 and 21 days after surgery. *Results:* The histomorphometric analysis showed a significant increase of bone formation in group III compared to group I and II at 7 days. At days 15 and 21, histomorphometric analysis showed no significant differences between them. *Conclusion:* Laser therapy presented a positive local biostimulative effect in early stages of bone healing, but no systemic effect was observed.

INTRODUCTION

The laser is a non-ionizing highly concentrated radiation that, when in contact with different tissues, results in photothermal and photochemical effects. Unlike others forms of therapeutic radiation, low level laser radiation is a noninvasive method and is well tolerated by the tissues[1].

The low power laser is used in medical and dental treatments due to its therapeutic actions on different tissues. Experimental studies, *in vitro*[2] and *in vivo*[3], suggest that low level laser therapy (LLLT) modulates various biological processes after exposure to some type of trauma[4]. The LLLT stimulates tissue repair and regeneration by promoting cell proliferation, hence improving tissue healing[5]. It also accelerates formation of granulation tissue[6], synthesis of collagen fiber[7], and increase of ATP synthesis[5]. Besides, LLLT reduces acute pain by directly inhibiting peripheral nociceptors[8].The LLLT anti-

inflammatory effect was also observed by its action on prostaglandins, inhibiting the enzyme cyclooxygenase and activating lymphocytes[9].

The use of lasers in biomodulation of bone repair has been studied as a method of stimulating osteogenesis in bone defects[3], associated with biomaterials[10, 11] or with bone morphogenetic protein[12]. The laser promotes an increase of osteoblastic activity in bone submitted to radiotherapy[13], and reduces the time of consolidation in bone fractures[4]. In addition to local effects, the occurrence of systemic effects of therapy with low power laser has been reported[14-18].

The systemic effect of laser reported in the literature were observed in soft tissue healing[14,16- 18]. Based on these data, several studies that evaluated the effect of LLLT on bone healing used different animals for experimental and control groups, because of the possibility of systemic effect interferences in results[10,19-24]. Subsequent studies assessing the effect of LLLT on bone healing in rats also used a separate group of animals for a control group, probably to avoid a possible systemic effect[12,25-29], although this was not reported in the paper.

Some authors have suggested that systemic effects may explain the absence of laser biomodulator effects in studies that used the same animal as experimental and control subject[14,16,18]. However, other studies that used internal controls had positive results of LLLT on bone[3,30] and cutaneous healing[31]. Considering these conflicting results and the fact that no previous study had evaluated the existence of a systemic effect of LLLT on bone repair, the aim of this study was to investigate the systemic effect of low level laser therapy (λ = 830 nm) on repair of surgical bone defects in rats at 7, 15, and 21 days.

MATERIAL AND METHODS

Animals

Forty-five (n=45) healthy male Wistar rats weighing 300 to 400 grams each were randomly selected and distributed into three groups of 15 animals each (Table 1): control group (GI), systemic LLLT group (GII), and local and systemic LLLT group (GIII). Animals were acquired and maintained at the Federal University of Uberlândia facilities, with balanced diet and water drinking *ad libitum*. This

study was approved by the Animal Ethics Committee from Pontifícia Universidade Católica of Rio Grande do Sul, Brazil (10/00189) Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil.

TABLE 1. ANIMAL DISTRIBUTION IN CONTROL AND EXPERIMENTAL GROUPS

Groups	*Animals*	*Description*	*Evaluation periods*
I	15	Surgery (control)	7 days (n=5), 15 days (n=5), 21 days (n=5)
II	15	Surgery + Systemic laser	7 days (n=5), 15 days (n=5), 21 days (n=5)
III	15	Surgery + Local and systemic laser	7 days (n=5), 15 days (n=5), 21 days (n=5)

Surgery

All animals were submitted to an osteotomy on their left femurs for bone defect creation. All were anaesthetized with an intraperitoneal injection of 100 mg/kg ketamine and 3 mg/kg xylazin hydrochloride. They were positioned on the right lateral decubitus, and the bone access was achieved through a 2 cm continuous longitudinal incision exposing the mid-diaphysis of cortical bone. A standardized 2.3 mm diameter osteotomy was performed with a round bur, coupled to an electric motor at 1000 rpm, under abundant saline solution irrigation. The depth of drilling limit was the disruption of femur cortical bone. Then the soft tissues were repositioned and the suture was performed in muscular and cutaneous layer using nylon 4-0. The postoperative period was uneventful, and all animals presented good recovery periods.

Laser protocol

The animals of groups II and III were submitted to sessions of laser therapy (λ = 830 nm). The animals of group II were irradiated on the right femur (systemic dose), and group III in both femurs (local and systemic doses). The equipment used was a laser diode infrared (GaAlAs -Flash Lase III, DMC®), with a wavelength of 830nm, potency of 50 mW and dose of 6 J/cm^2 per session. The application was punctual, with a 6J/cm^2 dose per session in the bone defect area during 2 minutes, with the laser tip positioned over and perpendicular to the long axis of the bone. The first session was applied immediately after drilling and before soft tissue repositioning. In the postoperative period, applications were taken

every 48 hours for 7, 15, and 21 days, resulting in 4, 8, and 11 sessions, according to each subgroup. Animals were euthanatized at 7, 15, and 21 postoperative days through the administration of saturated potassium chloride under general anesthesia**.**

Histological procedure

The bone defect area and the attached soft tissue were removed and immediately fixed in 10% phosphate buffered formaldehyde solution for 48 hours. Thereafter, the tissue blocks were decalcified in EDTA 10% for 4 weeks, dehydrated with graded alcohols, and embedded in paraffin. From the central region of the defect, histological sections of 5μm were obtained and stained in Mallory Trichrome.

Histomorphometric Analysis

In the histomorphometric analysis, the percentage of bone neoformation was quantified by the same examiner in a blind manner. Histological images of the bone defect area were captured at X4 magnification, using an Olympus BX 40 binocular microscope (Olympus BX 40—Shinjuku-ku, Tokyo, Japan) coupled with Olympus OLY 200 camera (OLY 200—Center Valley, PA, USA) linked to a PC computer through a 3153 Data Translation digitizer plate (Data Translation 3153—Marlboro, MA, USA). From each histological section, the entire bone defect area were digitalized using HL Image 2005 program (Western Vision, Salt Lake City, UT, USA). The screen shots were merged, and then all areas of soft tissue were removed using Photoshop CS2 software. Images were transformed into binary images, and the area of each bone defect was delimited from the edges of the cortical bone to the opposite cortex (Figure 1). The parameters analyzed were the bone defect area (BDA), consisting of the total area of lesion and the neoformation area (NA). The percentage of bone neoformation was calculated by the formula NA /BDA x100.

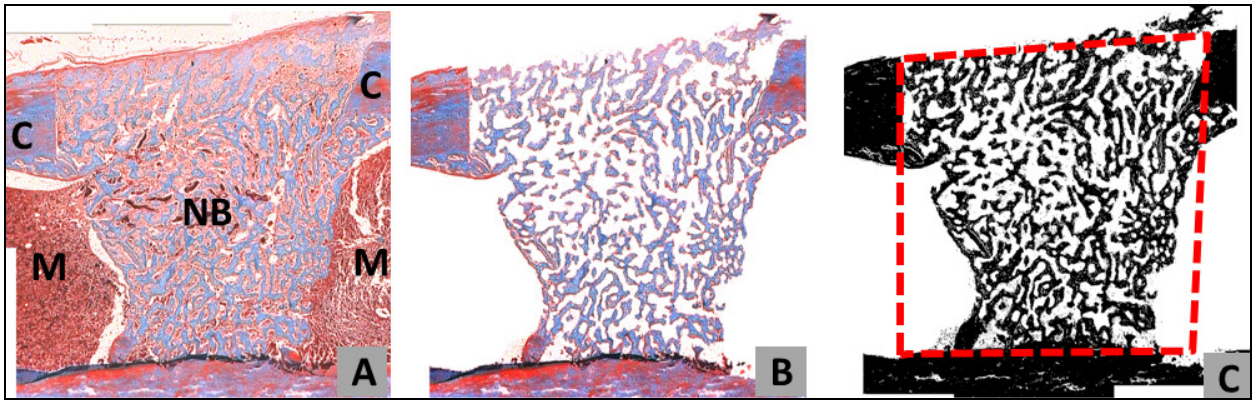

Figure 1.A. Photomicrograph of femur longitudinal section showing cortical (C), marrow (M), and area of new bone formation (NB). 1.B. Image after the removal of soft tissue. 1.C. Image after conversion to binary image and delimitation of the total area of bone defect (red line). X4. Mallory Trichrome.

Statistical analysis

The results were submitted to normality test and analyzed using analysis of variance (ANOVA) and Bonferroni. Differences were considered statistically significant if $p<0,05$.

RESULTS

Histological results

Histologic analysis in all groups showed bone neoformation at cortical extending through medullar until the opposite cortical. The new bone tissue was primary type with trabecular arrangement delimiting small cavities, filled with cells, blood vessels, and collagen fibers. The histomorphometric analysis revealed a significant increase in the percentage of bone formation in group III compared to group II and the control group at 7 days (TABLE 2). There was no difference between group I and II in this period (Figure 2).

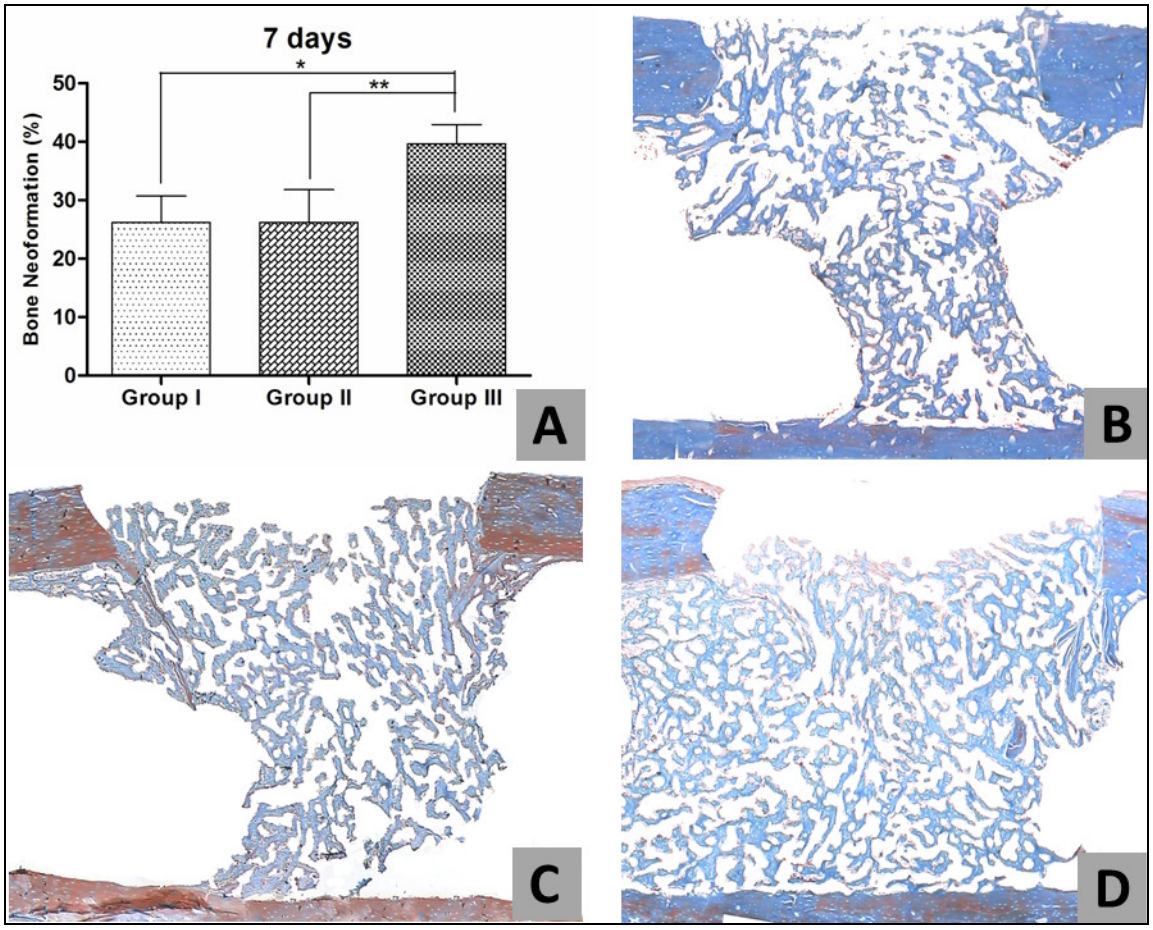

Figure 2. (A) Percentage of new bone in defect area after 7 days (*p<0.05; ** p<0.005). Photomicrograph of the groups I (B), group II (C), and group III (D) in 7-day period showing the new bone formation beyond the area of cortical bone surrounding the marrow area in group. X4. Mallory Trichrome.

After 15 days, bone remodeling with a decrease of bone neoformation in the marrow area was observed, compared with the 7-day period in all groups (Figure 3.). At this period, the histomorphometric analysis revealed no significant differences of new bone formation between group I, group II, and group III, although the histologic images showed more bone density in group III.

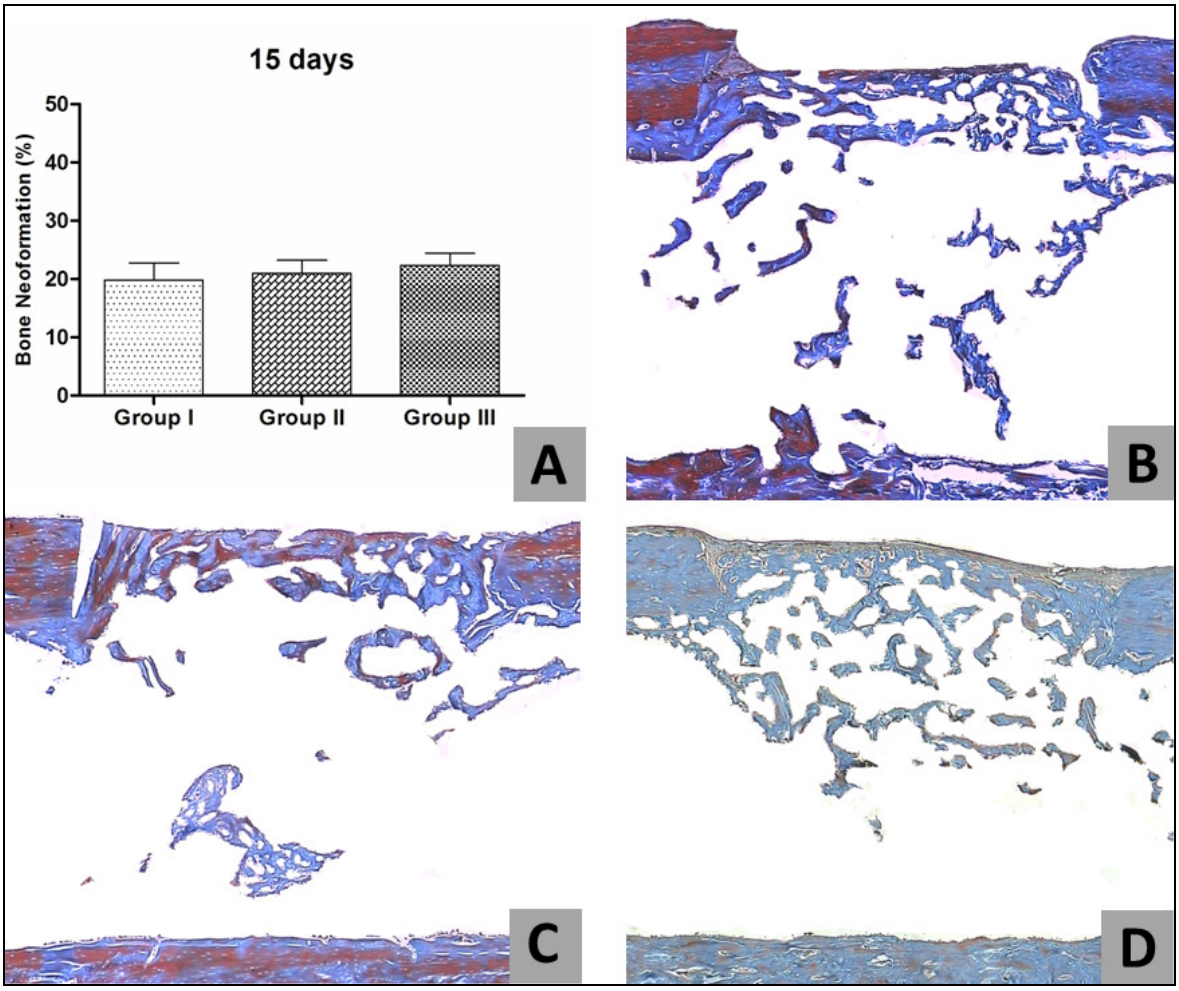

Figure 3. (A) Percentage of new bone in defect area after 15 days. Photomicrograph of the groups I (B), group II (C), and group III (D) in 15-day period showing bone remodeling with a decrease of new bone in marrow area X4. Mallory Trichrome.

After 21 days, advanced bone remodeling with new bone mostly located in cortical area was observed. The cortical bone defect was almost filled by primary bone. Little or no bone was observed in the marrow area (Figure 4.). At this point, the histomorphometric analysis revealed no significant differences of new bone formation between all groups.

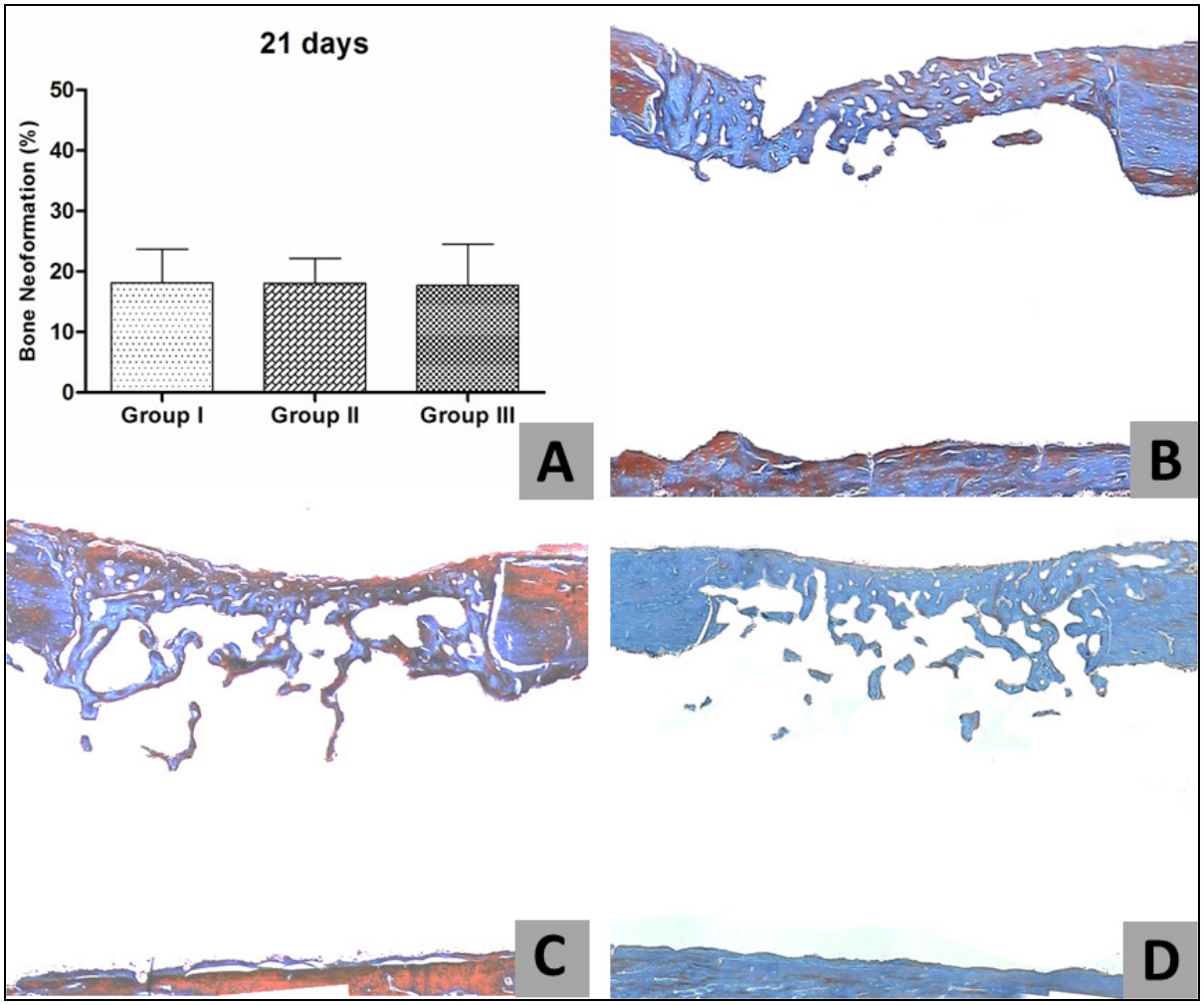

FIGURE 4: (A) Percentage of new bone in defect area after 21 days. Photomicrograph of the groups I (B), group II (C) and group III (D) in 21 days period showing the increase of bone density in cortical defect area and almost completed bone remodeling in medular area. X4.Mallory Trichrome

TABLE 2. BONE NEOFORMATION IN ALL GROUPS IN THE EVALUATED PERIODS

	Bone Neoformation (%)					
	7 days		*15 days*		*21 days*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Group I	26,20	4,547	19,86	2,906	18,10	5,589
Group II	26,24	5,587	21,02	2,205	18,00	4,182
Group III	35,66*	3,294	22,34	2,105	17,68	6,824

*(p<0,005)

Regarding bone healing over the course of time, histomorphometric analysis showed a significant decrease of bone percentage in all groups when compared at 7 and 21 days ($p<0.05$), being more evident in group III ($p<0.005$). Group III also showed a significant decrease of bone when compared to the 7 and 15-day analysis ($p<0.005$).

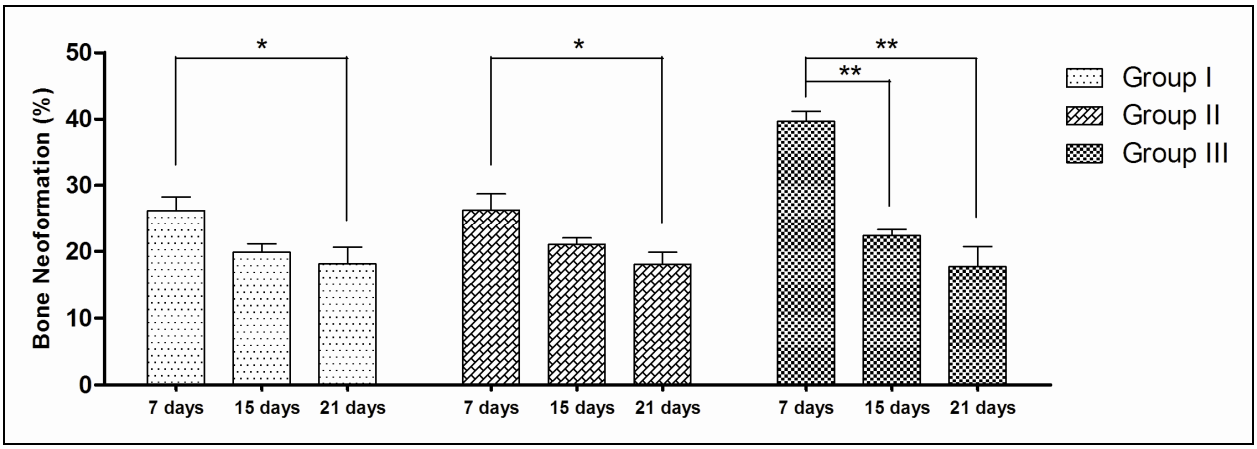

Figure 5. Bone formation percentage during experimental periods showing a bone reduction from 7 to 21 days. The most evident bone reduction was in group III *$p<0.05$; ** $p<0.001$.

DISCUSSION

This study evaluated the possible induction of systemic effects of LLLT on bone repair using a laser protocol in an animal model. For this evaluation, we used histomorphometric analysis to quantify new bone formation in surgical defects that were submitted to local LLLT doses (local effects) and LLLT doses that were distant from the bone defect (systemic effects). This purpose stemmed from limited research about the systemic effects of the laser, whereas local effects on tissue repair are well documented.

The study of laser therapy should consider aspects such as wavelength and radiation dose. The wavelength of 830nm (used in this study) penetrates the tissue surface (skin), reaching the underlying bone (femur). Thus, it was also possible to observe the local effect of LLLT, in addition to possible systemic effects (group III). The application of $6J/cm^2$ was used based on previous studies that found positive results on bone healing and neoformation with doses from 0.3 [32] to 16 J/cm^2 per session[10,11].

The local positive effect of laser therapy was well evidenced in animals with the laser applied directly over the bone defect area (Group III) at day 7. This increase in bone formation observed may be

due to local effects of the laser stimulating the differentiation of mesenchymal cells and also osteoblasts and fibroblasts proliferation[11]. These events may explain the extensive bone neoformation, invading the medullar area, observed after 7 days in group III. Gál et al. (2006) evaluated the effect of laser use in wound repair and reported that most significant morphological changes occurred during the first 7 days of healing, with more glycoproteins, proteoglycans, and collagen fibers synthesized by fibroblasts. Garavello-Freitas et al. (2003) considered the influence LLLT on the repair of surgically produced tibia damage in rats and also showed that maximal laser-stimulated bone growth was achieved after 7 days of irradiation.

An interesting observation was that the bone remodeling in group III was faster than in groups I and II. The greater initial bone formation (7 days) was followed by accelerated bone resorption, so no difference was found in the percentage of bone between the 3 groups at 15 and 21 days. This interpretation is also based on the significantly reduction ($p<0,005$) in the percentage of bone in group III between 7 and 15 days and also 7 and 21 days. This finding suggests that low-level laser irradiation would stimulate osteoclast activity to promote bone resorption and remodeling. Garavello-Freitas et al. (2003) evaluated LLLT in a rat model and also observed smaller areas of trabeculae in the 14-day group compared to the 7-day-irradiated group of rats submitted to the same dose of laser.

The present study did not observe systemic effects of low level laser therapy in bone healing, considering that laser application distant from the lesions did not interfere with bone defect healing. The assessment of the systemic effect on bone tissue is not described in English indexed literature. Previous studies evaluating the systemic effect of LLLT only assessed this effect in the healing of soft tissue wounds[14,17,18]. Braverman et al. (1989) examined the systemic effect of laser use in cutaneous wound repair in rabbits and showed significant effects only on tensile strength evaluation of wounds; however, no statistically significant difference was observed in the histological evaluation of samples. Schindl et al. (2002) studied the effect of lasertherapy in skin blood circulation by means of temperature in patients with diabetic microangiopathy and observed an increase of temperature in distant areas of laser application.

They reported that this systemic effect is due to the release of cytokines and growth factors into the systemic blood stream, causing vasodilation and neoangiogenesis.

In the current study, the laser application point was in the contralateral leg of the defect, and the release cytokines and growth factors may not have reached the bone defect in sufficient concentrations to interfere with the repair of bone tissue. Another explanation is that the laser scope is wider than the application site, which could be called a *regional effect*, because in some studies that evaluated the systemic effect on soft tissue, the cutaneous injuries (control and experimental) were relatively close[14,15,18].

The results of this work may allow us to obtain relevant data about the potential efficacy of local LLLT, but not the systemic effect. However, the reasons for the laser stimulatory effect and also the absence of define parameters to clinical use warrant further investigation.

CONCLUSION

In the present study, although the application using this LLLT protocol was locally effective at the early stage of bone repair, the induction of systemic effects on bone repair did not demonstrate positive results.

Author Disclosure Statement

No conflicting financial interests exist.

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DISCUSSÃO GERAL

4. DISCUSSÃO GERAL

Segundo dados do Instituto Nacional do Câncer (INCA) no Brasil, as estimativas para o ano de 2011 são de 489.270 casos novos de câncer. Dentre os tipos mais incidentes, o câncer de boca se encontra na sétima posição em ambos os sexos (INCA, 2011). O tratamento desse tipo de câncer é na maioria dos casos cirúrgico, geralmente seguido por radioterapia, dependendo do estágio da doença. (FURNESS et al., 2011). A radioterapia é uma modalidade terapêutica que utiliza radiações ionizantes no combate a neoplasias com objetivo de atingir células malignas, impedindo sua multiplicação e determim a morte celular (INCA, 2011). Contudo, os tecidos normais subjacentes ao campo de irradiação também sofrem ação da RDT, podendo causar complicações orais importantes tais como: mucosite, cáries de radiação, xerostomia, trismo, perda do paladar, infecções secundárias, osteomielite, limitando o tratamento oncológico temporária ou permanentemente (ALMEIDA et al., 2004). O tecido ósseo, por possuir baixa atividade proliferativa é caracterizado como tecido de resposta lenta e, quando irradiado apresenta alterações por um período mais prolongado. Neste contexto, criamos a hipótese que a laserterapia de baixa potência poderia ser uma alternativa terapêutica para otimizar o reparo tecidual em defeito cirúrgico de ossos previamente expostos à radiação ionizante. Entretanto, nenhum efeito benéfico no processo de reparo ósseo foi observado nos animais do grupo radioterapia submetido à LTBP, enquanto que essa mesma terapia laser foi efetiva nos animais não irradiados. Neste grupo, houve um aumento da área de matriz óssea formada na região do defeito ósseo, o que está de acordo com estudos anteriores (GARAVELLO-FREITAS et al., 2003). Como os efeitos deletérios da radioterapia permanecem por vários meses (LAROUXEL et al., 2009), novos estudos avaliando períodos mais longos são necessários. A princípio, o projeto englobava outros períodos de avaliação; entretanto, devido à perda considerável de animais

causada por fraturas diagnósticas no período pós-operatório, houve a necessidade de redução dos grupos, permanecendo o período de 7 dias.

A grande diversidade e falta de padronização nos protocolos de laserterapia também dificultam a comparação de resultados, pois existe uma grande variação no comprimento de onda, doses, número de sessões e intervalos de aplicação. Ao realizar uma busca avançada sobre pesquisas em laserterapia na língua inglesa utilizando a base de dados PUBMED, com as palavras-chave: *bone, laser* e *rat*, foram encontrados 204 estudos, sendo selecionados 16 estudos tendo como critério de seleção os estudos que avaliaram a LTBP no reparo de defeitos ósseos, utilizando o rato como modelo animal. Nestes estudos, foram observados 7 diferentes comprimentos de onda (633, 650, 735, 780, 830, 880, 904 e 1024nm) , 9 doses (0.28, 1.4, 2, 3, 4, 5, 10.2, 15 e 16 J/cm2), 5 tipos de intervalos entre as doses (dose única, 24 horas, 48 horas, 3 vezes por semana, 5 vezes por semana) (BARUSHKA; YAAKOBI; ORON, 1995; DAVID et al., 1996; SAITO; SHIMIZU, 1997; GARAVELLO; BARANAUSKAS; DA CRUZ-HÖFLING, 2004; KHADRA et al., 2004; KHADRA, 2005; GERBI et al., 2005; NISSAN et al., 2006; RENNO et al., 2006; CARVALHO et al., 2006; SILVA; CAMILLI, 2006; PRETEL; LIZARELLI; RAMALHO, 2007; NINOMIYA et al., 2007; DENADAI et al., 2009; AKYOL; GÜNGÖRMÜŞ, 2010; SAAD et al., 2010)

Na mesma base de dados, não foi encontrado estudo que avaliasse o efeito da LTBP sobre o reparo de defeitos cirúrgicos em osso comprometido pela radiação ionizante da radioterapia. Apenas o estudo conduzido por Da Cunha (2007) avaliou o efeito da LTBP no osso

comprometido pela radiação ionizante, onde foi utilizada a mesma dose de radiação (30 Gy) também em dose única, entretanto, não foi realizado defeito ósseo. Outro estudo avaliou o reparo de defeito ósseo em fêmur de ratos utilizando duas doses de radiação (30 e 45 Gy), entretanto, não foi utilizado laser nesse estudo (LEROUXEL et al., 2009). Até o presente momento apenas nosso estudo avaliou o efeito da LTBP no reparo do osso comprometido pela radioterapia oncológica.

A avaliação de um possível efeito sistêmico do laser sobre o reparo ósseo também foi objeto desse estudo, uma vez que apenas investigações no reparo de tecido mole avaliaram esse efeito (KANA; HUTSCHENREITER; HAINA, 1981; MESTER et al., 1985; ROCHKIND et al., 1989; BRAVERMAN; MCCARTHY; IVANCOLICH, 1989; SCHINDL et al., 2002; RODRIGO et al., 2009).

Kana e colaboradores (1981) não observaram diferença na velocidade de cicatrização (aumento na síntese de colágeno) entre feridas contralaterais do grupo submetido à LTBP (sendo uma irradiada e a outra não irradiada), contudo, houve diferença quando comparadas com as feridas dos animais não irradiados (grupo controle independente). Mester e colaboradores (1985) relataram que a LTBP no tratamento de lesão na córnea estimulava a cicatrização da córnea contralateral não irradiada. Rochkind e colaboradores (1989), utilizando um delineamento experimental similar ao de Kana e colaboradores (1981) avaliaram o efeito sistêmico da LTBP no reparo de feridas cutâneas e também observaram que feridas contralaterais não irradiadas tiveram um reparo mais acelerado que feridas de animais não irradiados pelo laser. Braverman e colaboradores (1989), avaliando o efeito da LTBP no reparo de feridas cutâneas em coelhos, encontraram aumento da resistência do tecido à tração nas feridas irradiadas e não irradiadas quando comparadas com feridas de animais não irradiados; entretanto outros parâmetros avaliados tais como: temperatura cutânea e retal, taxa de cicatrização cutânea não apresentaram

diferenças estatisticamente significantes. Schindl e colaboradores (2002), avaliando o efeito da LTBP na microcirculação de pacientes portadores de microangiopatia diabética, observaram que a aplicação transcutânea do laser causava um aumento da temperatura tanto no membro afetado pela patologia submetido à laserterapia, quanto no membro contralateral.

Diversos estudos que avaliam o efeito da LTBP sobre o reparo do tecido ósseo em modelo animal recomendam a utilização de um grupo controle independente devido a esse possível efeito sistêmico (NICOLA et al,. 2003; PINHEIRO et al,. 2003a; PINHEIRO et al,. 2003b; KHADRA et al., 2004; GERBI et al., 2005; TORRES et., 2008; MÁRQUEZ MARTÍNEZ; PINHEIRO; RAMALHO, 2008). Entretanto, não existem estudos que tenham avaliado se existe e qual o impacto desse efeito no reparo do tecido ósseo. Esse estudo não achou evidências de efeito sistêmico em relação à área de neoformação óssea, o que vai de encontro a estudos que utilizaram controle interno avaliando a LTBP sobre o reparo do osso (MERLI et al., 2005) e de tecido mole (GÁL., et al 2006), os quais observaram diferenças entre os lados irradiados e não irradiados.

Considerando a diversidade atual de estudos sobre o efeito da LTBP, especialmente no reparo do tecido ósseo, novas pesquisas que investiguem interações específicas entre osso e a laserterapia poderão indicar protocolos de LTBP mais efetivos e, assim, contribuir para nortear uma abordagem terapêutica mais eficaz desse tratamento em osso comprometido.

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ANEXOS

ANEXO A

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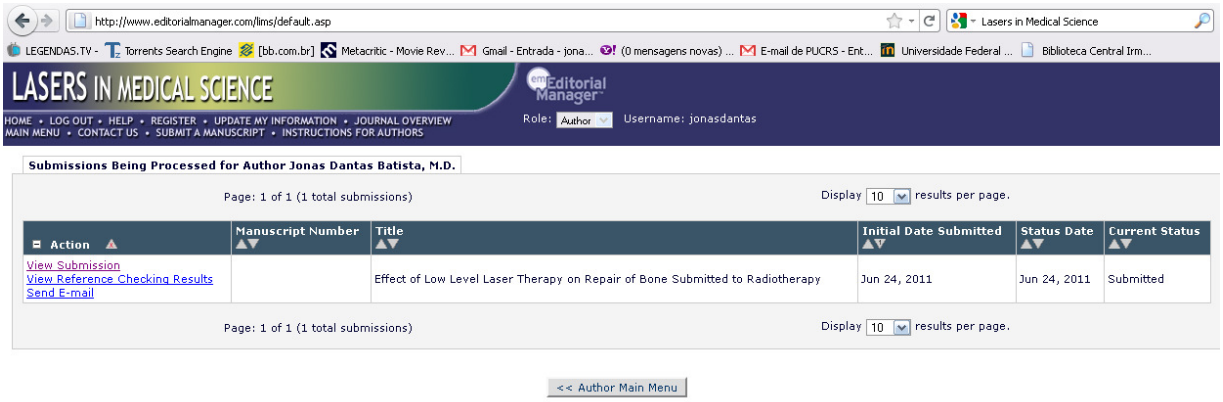

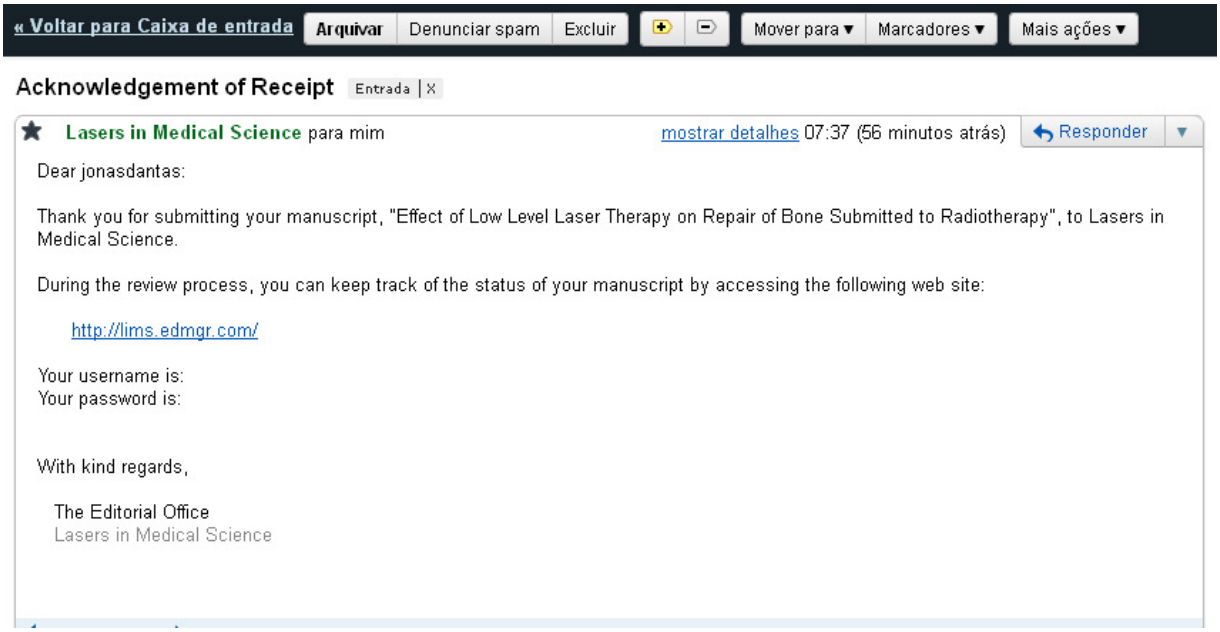

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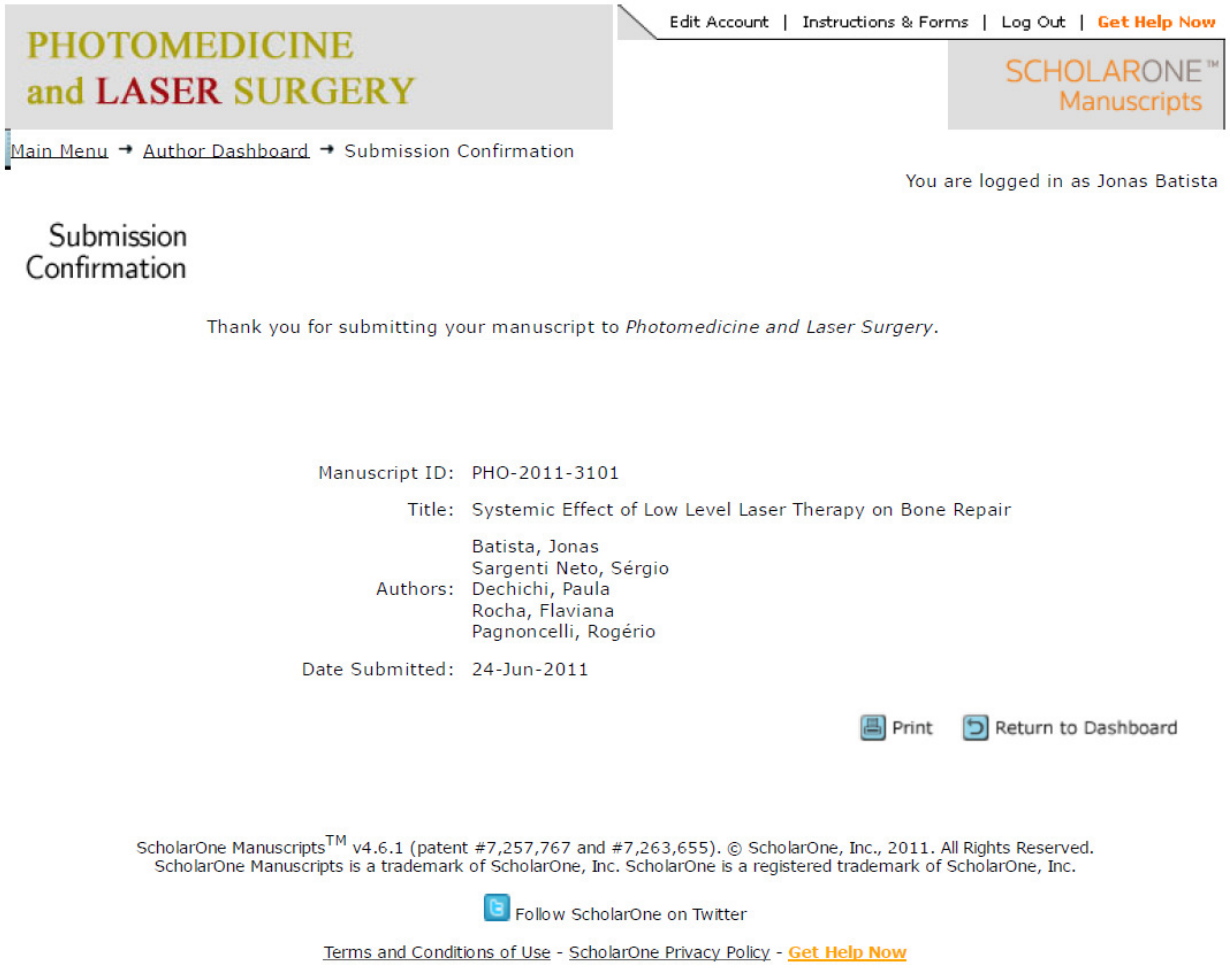

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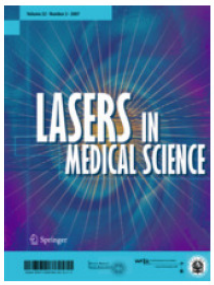

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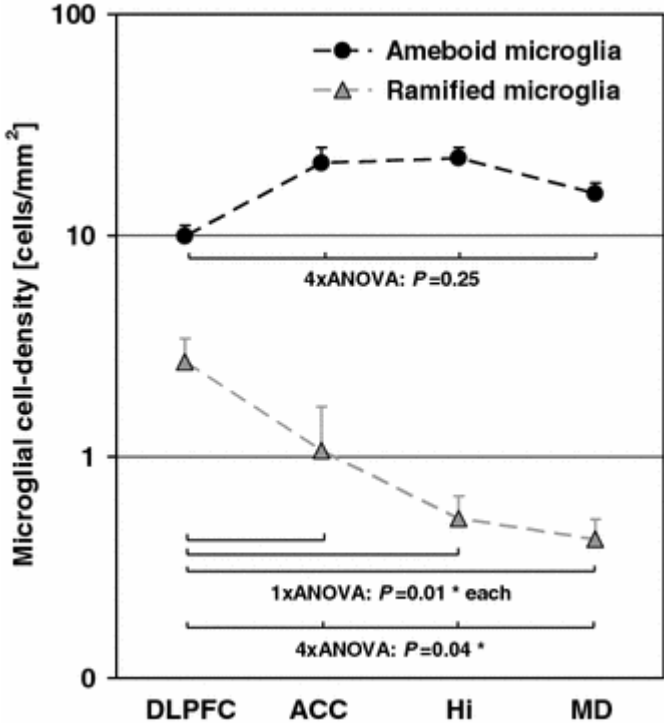

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