131 - VERIFICATION OF THE EFFECTIVENESS OF LOW LEVEL LASER THERAPY IN THE TREATMENT OF EDEMA IN IMMUNOSUPPRESSED RATS

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INTRODUCTION

The Achilles tendon usually suffers traumatic injuries as a structure responsible for the transmission of muscle strength to bone during activities of daily living (BUTLER; JUNCOSA; DRESSLER, 2004; LIN; CARDENAS; SOSLOWSKY, 2004). When the tendon is subjected to a tension, a deformation will occur and can lead to failures and tendon injuries (GROSS, 1992). These lesions lead to acute inflammation, which dilutes and removes harmful agents of the site, increased capillary permeability and vasodilation, facilitating tissue oxygenation and nutrition (GROSS, 1992; BUTLER; JUNCOSA; DRESSLER, 2004; POSTEN et al., 2005; BILATE, 2007; ENWONWU; RITCHIE, 2007). However, this leads to vasodilation, edema, which is the result of severe fluid loss and accumulation in the interstitial tissue (RESENDE; PEREIRA; CASTRO, 2005).

After an injury, the immune system is responsible for defending the body. When intact, it should be able to respond to different types of aggression (JUN; YANG, 2007). However, the impairment of humoral and cellular immunity can occur on several occasions, as in the case of immunodeficiency associated with infection by human immunodeficiency virus, advanced age, diabetes, heart disease and chronic kidney diseases (BRICKS, 1998). So with a compromised immune system, there is a consequent low immune response (LANGFORD; ANANWORANICH; COOPER, 2007).

Furthermore, it has been suggested that several chronic diseases, noncommunicable diseases, including cardiovascular disease, diabetes mellitus, among others, are associated with chronic inflammation of low intensity. Thus, there may be a delay in recovering tissues (GERALDO; ALFENAS, 2008).

Thus, for the repair of tissue injury is more rapid, one of the methods used is the low level laser therapy. This, especially at low doses, produces an increase in vasodilation, the transport of nutrients and oxygen to cells damaged by facilitating the repair and removal of cell debris (MARTIN, 2003; LOBATO et al., 2005). Moreover, there is an increased concentration of collagen, increased granulation tissue, increase of endothelial cells and neovascularization at the site of healing (LAMAS, 1999; KNAPPE; FRANK; ROHDE, 2004; POSTEN et al., 2005; CARRINHO et al., 2006).

This study was used rats with immunosuppression, aiming to verify whether the low level laser therapy is effective for the treatment of edema in immunosuppressed rats undergoing tendon trauma.

MATERIAL AND METHODS

Experimental groups

We used 23 male rats, Wistar, obtained from the Central Animal Vivarium at the State University of West Parana – Unioeste and maintained in own vivarium in the Laboratory of the Study Group of Injuries and Physical Therapy Resources. The animals were housed in polypropylene cages containing, grouped four in number, with ambient temperature of 25° C and a photoperiod of 12 hours light / dark control, receiving water and food ad libitum. The project was conducted in accordance with International Standards on Ethics in Animal Experimentation (ANDERSEN et al., 2004), was approved by the Ethics Committee on Animal Experiments and Practical Sessions of Unioeste, under Protocol 6909.

The animals were randomly divided into three groups:

- Control group (CG, n=7) – subjected to traumatic injury in the right Achilles tendon;
- Placebo group (PG, n=8) – underwent immunosuppression, traumatic lesion in the right Achilles tendon and the placebo treatment with laser;
- Laser group (LG, n=8) – underwent immunosuppression, traumatic lesion in the right Achilles tendon and the laser treatment 2 J/cm2.

Model of immunosuppression in rats

Immunosuppression was accomplished through the use of the drug Cyclosporin A generic laboratory Germed®, and initiated the administration of the drug five days before the trauma. We used 10 mg/kg daily for five days, orally by orogastric catheter. On the sixth day, there was the trauma in the Achilles tendon (BOREL et al., 1976).

Experimental model of trauma in the Achilles tendon

The animals were sedated prior to the procedure, with thiopental, 75 mg/Kg. Then were placed in left lateral position, exposing the lateral region of the right Achilles tendon to injury. For the production of traumatic injury, we used a device designed by the Unioeste Department of Civil Engineering. The experiment consisted of a weight (575g) always starting from the same initial height (7 cm), falling on the side of the right Achilles tendon of each animal, making an impact energy of about 0.40 J (BERTOLINI et al. 2008).

Edema evaluation

To quantify edema in the region of experimental injury, we used a metal caliper positioned mid-laterally, to check the diameter in centimeters of the right Achilles tendon. Evaluations were performed before experimental injury, after recovery from anesthesia the animal, and with two, four, eight, 24 and 48 hours after injury (BERTOLINI et al., 2008).

Treatment protocol with low level laser therapy

After the evaluation was conducted post-injury treatment with laser, Ibramed®, with a wavelength of 660 nanometers (nm), 30 mW of power, energy density 2 J/cm2, one point, specifically on the site of trauma. The animals were kept in contensor PVC thermoplastic during laser application. The placebo group received the same procedure, but when off. The laser equipment was calibrated prior to use. Laser treatment was then performed to evaluate post-injury, and after 24 and 48 hours the day of

injury.

Statistical analysis

Data were evaluated by the Kolmogorov-Smirnov test. Due to the normality of them, were analyzed by repeated measures ANOVA test for comparison within groups, and one-way ANOVA for comparison between groups. As post-hocTukey was used. In all cases the level of significance was 5%.

RESULTS

In the assessments using a caliper, it was observed that the CG, the values compared between pre-injury and four hours after injury and 48 hours after injury, the results were not significant. Thus, all other ratings, these values were significant, revealing the formation of edema in the injured site. In comparisons post-injury, no results were significant (Fig. 1A).

Comparisons between the PG, the values of pre-injury, it was observed significant increase in the animals tendons diameter at the same moments of ratings than CG. Already in the values of post-injury a significant decrease in diameter between the tendons after injury and four, 24 and 48 hours after injury (Fig. 1B).

For GL, comparisons with the pre-injury was significant post-injury, two, four and eight hours after injury. In comparison with the post-injury, the results were significant with 24 and 48 hours after injury (Fig. 1C).

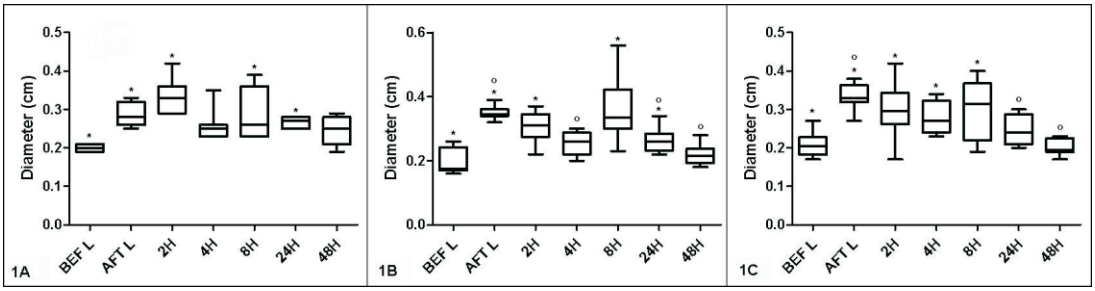

Figure 1 – Graphical representation of the caliper. 1A (CG): * Significant increase in the diameter of the tendon of the animals in ratings between pre-injury and post-injury, pre-and 2 hours after injury, pre-injury and after 8 hours and pre-injury and 24 hours after injury. 1B (GP): * Significant increase in the diameter of the tendon in ratings between pre-injury and post-injury, pre-and 2 hours after injury, pre-injury and after 8 am and pre-injury and 24 hours after injury. No significant decrease in ratings between the diameter after injury and after 4h, and 24h post-injury and post-injury after and 48h after injury. 1C (GL): * Significant increase in the diameter of the tendon in ratings between pre-injury and post-injury, pre-and 2 hours after injury, pre-injury and after 4h and 8h before injury and after injury. º Significant decrease in ratings between the diameter of post-injury and 24 hours after and 48 hours after injury and after injury

DISCUSSION

In a study using the same model of tendon trauma and treatment with low level laser therapy in animals immune system unchanged, found a significant edema reduction, with 2 J/cm2 dose after two hours of laser irradiation (BERTOLINI et al., 2008). Thus, this study aimed to verify the laser action in immunosuppressed animals.

In this study, there was edema formation after tendon trauma, the significant increase in diameter made in the study and control groups, which showed edema in the same periods. In the LG was no decrease in the diameter of the Achilles tendon after 24 and 48 after injury. Thus, it appears that the low level laser was effective to decrease local edema. However, in four hours after injury in CG and PG were back to baseline, but not for GL, it demonstrates that in the initial phase the laser produced an increase of edema, which may have occurred by the release of histamine by mast cells, which is elicited by laser application (KNAPPE; FRANK; ROHDE, 2004; POSTEL et al., 2005).

In all groups, there was restoration of the values of swelling after 48 hours, but LG just showed such a result within 24 hours. PG showed significant edema reduction, when compared with the time post-trauma, 4, 24 and 48 h, this fact was not observed for CG. It is inferred that possibly because of reduced inflammatory reaction, this could have been observed in immunosuppressed animals (LANGFORD; ANANWORANICH; COOPER, 2007).

This finding is confirmed by similar results reported by Albertini et al. (2004), who observed a significant reduction in paw edema of rats after carrageenan-induced through the application of low power laser, with doses of 1 and 2.5 J/cm2, after two and four hours of their induction. The best result was found after two interventions being carried out at least one hour after the edema induction.

In another study, by Elwakil (2007), there was less amount of granulomatous reaction after the use of low power laser and dose 1 J/cm2. Also Bjordal, Lopes-Martins and Iversen (2006) found a decrease in concentration of prostaglandin (PGE2) after one hour of treatment with laser, 904 nm and a dose of 5.4 J/cm2. For the placebo group, concentrations of PGE2 increased gradually during the period after treatment. Thus, it can be observed that the laser promoted a reduction of inflammation by decreasing the PGE2 concentrations.

No studies were found that related animals immunosuppressed with treatment of low level laser therapy, a fact which prevented a more detailed discussion on the subject. Therefore, we used studies with samples of the immune system unchanged. In the present study, we observed that the depression of the immune system of animals did not alter the response front of the laser therapy.

It can be seen as limitations of this study, which were not correlated the findings on the formation of edema, with histological or biochemical changes. Thus, future studies may relate these variables, the characteristics of these changes.

With this study, we can conclude that the low level laser therapy is effective to reduce edema in rats subjected to immunosuppressed tendon trauma, after 24 hours.

REFERENCES

ALBERTINI, R.; AIMBIRE, F. S. C.; CORREA, F. I.; RIBEIRO, W.; COGO, J. C.; ANTUNES, E. et al. Effects of different protocol doses of low power gallium-aluminum-arsenate (GaAlAs) laser radiation (650nm) on carrageenan induced rat paw oedema. **Journal of Photochemistry and Photobiology**, B: Biology, v. 74, n.2/3, p.101-107, 2004.

ANDERSEN, M. L.; D'ALMEIDA, V.; KO, G. M.; KAWAKAMI, R.; MARTINS, P. J. F.; MAGALHÃES, L. E; et al.

Princípios éticos e práticos do uso de animais de experimentação. São Paulo: UNIFESP – Universidade Federal de São Paulo, 2004.

BERTOLINI, G. R. F.; SILVA, T. S.; CIENA, A. P.; TRINDADE, D. L. Efeitos do laser de baixa potência sobre a dor e edema no trauma tendíneo de ratos. **Revista Brasileira de Medicina do Esporte**, v.14, n.4, p.362-366, 2008.

BILATE, A. M. B. Inflamação, citocinas, proteínas de fase aguda e implicações terapêuticas. **Temas de Reumatologia Clínica**, v.8, n.2, p.47-51, 2007.

BJORDAL, J. M.; LOPES-MARTINS, R.A. B.; IVERSEN, V. V. A randomised, placebo controlled trial of low level laser therapy for activated Achilles tendinitis with microdialysis measurement of peritendinous prostaglandin E2 concentrations. **British Journal of Sports Medicine**, v.40, n.1, p.76-80, 2006.

BOREL, J. F.; FEURER, C.; GUBLER, H. U.; STÄHELIN, H. Biological effects of cyclosporine A: a new antilymphocytic agent. **Agents and Actions**, v.6, n.4, p.468-475, 1976.

BRICKS, L. F. Indicação de vacinas e imunoglobulinas em indivíduos que apresentam comprometimento da imunidade. **Revista de Saúde Publica**, v.32, n.3, p.281-294, 1998.

BUTLER, D. L.; JUNCOSA, N.; DRESSLER, M. R. Functional efficacy of tendon repair processes. **Annual Review of Biomedical Engineering**, v.6, p.303-329, 2004.

CARRINHO, P. M.; RENNO, A. C. M.; KOEKE, P.; SALATE, A. C. B.; PARIZOTTO, N. A.; VIDAL, B. C. Comparative study using 685nm and 830nm lasers in the tissue repair of tenotomized tendons in the mouse. **Photomedicine Laser Surgery**, v.24, n.6, p.754-758, 2006.

ELWAKIL, T. F. An in-vivo experimental evaluation of He-Ne laser photostimulation in healing Achilles tendons. **Lasers in Medical Science**, v.22, n.1, p.53-59, 2007.

ENWONWU, C. O.; RITCHIE, C. S. Nutrition and inflammatory markers. **The Journal of American Dental Association**, v.138, n.1, p.70-73, 2007.

GERALDO, J. M.; ALFENAS, R. C. G. Papel da dieta na prevenção e no controle da inflamação crônica – evidências atuais. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v.52, n.6, p.951-967, 2008.

GROSS, M. T. Chronic tendinitis: pathomechanics of injury, factors affecting the healing response, and treatment. **Journal of Orthopaedic & Sports Physical Therapy**, v.16, n.6, p.248-261, 1992.

JUN, Y.; YANG, H. M. Modelando a interação entre sistema imunológico inato e bactérias estreptococos e estafilococos. **Tendências em Matemática Aplicada e Computacional**, v.8, n.3, p.413-422, 2007.

KNAPPE, V.; FRANK, F.; ROHDE, E. Principles of lasers and biophotonic effects. **Photomedicine Laser Surgery**, v.22, n.5, p.411-417, 2004.

LAMAS, M. C. S. 1999. **Laser a diodo de arseneto de gálio (As-Ga) aplicado às fraturas do terço médio do rádio de cães.** 49p. Dissertação (Mestrado em Medicina Veterinária). Escola de Veterinária da UFMG, Belo Horizonte, 1999.

LANGFORD, S. E., ANANWORANICH, J. & COOPER, D. A. 2007. Predictors of disease progression in HIV infection: a review. **AIDS Research and Therapy**, v.4(11). Disponível em: <http://www.aidsrestherapy.com/content/4/1/11>. Acesso em: 24/08/2010.

LIN, T. W.; CARDENAS, L.; SOSLOWSKY, L. J. Biomechanics of tendon injury and repair. **Journal of Biomechanics**, v.37, n.6, p.865-877, 2004.

LOBATO, D. A.; DEL CARLO, R. J.; VILORIA, M. I. V.; MONTEIRO, B. S.; SILVA, P. S. A.; MARCHESI, D. R. et al. Efeitos da aplicação do laser diodo de arseneto de gálio na osteoartrite experimental em coelhos. **Revista Ceres**, v.52, n.304, p.875-886, 2005.

MARTIN, R. Low level laser therapy (LLLT) precipitates a complex set of physiological interactions at the celular level that reduces acute inflammation, reduces pain, and accelerates tissue healing. **Practical Pain Management**, v.3, n.6, p.20-25, 2003.

POSTEN, W.; WRONE, D. A.; DOVER, J. S.; ARNDT, K. A.; SILAPUNT, S.; ALAM, M. Low-level laser therapy for wound healing: mechanism and efficacy. **Dermatologic Surgery**, v.31, n.3, p.334-340, 2005.

RESENDE, M. A.; PEREIRA, L. S. M.; CASTRO, M. S. A. Proposta de um modelo teórico de intervenção fisioterapêutica no controle da dor e inflamação. **Fisioterapia Brasil**, v.6, n.5, p.268-271, 2005.VERIFICATION OF THE EFFECTIVENESS OF LOW LEVEL LASER THERAPY IN THE TREATMENT OF EDEMA IN IMMUNOSUPPRESSED RATS

VERIFICATION OF THE EFFECTIVENESS OF LOW LEVEL LASER THERAPY IN THE TREATMENT OF EDEMA IN IMMUNOSUPPRESSED RATS

ABSTRACT:

Introduction: The Achilles tendon is commonly affected by traumatic injuries that lead to inflammation and edema. When the immune system is altered, there is a low immune response, which can lead to a slowing of tissue recovery. Thus, the low level laser therapy can be used for presenting beneficial effects in this type of injury. Thus, the goal of this study was to determine whether the low level laser therapy is effective for the treatment of edema in rats immunosuppressed undergoing tendon trauma. Materials and Methods: 23 male rats of Wistar strain were divided randomly into control group, placebo and laser. The animals were immunosuppressed and subjected to a lesion in the right Achilles tendon. For treatment, we used low level laser therapy, 660 nm, 30 mW and dose 2 J/cm2. Results: There was a significant decrease in the diameter of the tendons in the GP, between post-injury and four, 24 and 48 hours after injury and in the GL, the results were significant between post-injury, 24 and 48 hours after injury. Conclusion: The low level laser therapy is effective to reduce edema in immunosuppressed rats underwent tendon trauma.

KEYWORDS: Low-Level Laser Therapy, Immunosuppression, Achilles Tendon.

VERIFICATION DU NIVEAU EFFICACITE DE LA THERAPIE LASER DE BASSE DANS LE TRAITEMENT DE L'ŒDEME CHEZ LES RATS IMMUNODEPRIMES

RÉSUMÉ:

Introduction: Le tendon d'Achille est souvent affectée par les blessures traumatiques qui conduisent à l'inflammation et l'œdème. Lorsque le système immunitaire est altéré, il ya une faible réponse immunitaire, qui peut conduire à un ralentissement de la récupération des tissus. Ainsi, la thérapie laser de faible niveau peut être utilisé pour la présentation des effets bénéfiques dans ce type de blessure. Ainsi, l'objectif de cette étude était de déterminer si la thérapie faible niveau laser est efficace pour le traitement de l'œdème chez les rats immunodéprimés subissant un traumatisme du tendon. Matériels et Méthodes: 23 rats mâles de souche Wistar ont été répartis au hasard dans le groupe contrôle, placebo et laser. Les animaux ont été immunodéprimés et soumis à une lésion du tendon d'Achille droit. Pour le traitement, nous avons utilisé la thérapie au laser à

faible niveau, 660 nm, 30 mW et la dose de 2 J/cm2. Résultats: Il y avait une diminution significative du diamètre des tendons dans le GP, entre post-traumatiques et quatre, 24 et 48 heures après l'accident et dans le GL, les résultats ont été significatifs entre les post-traumatique, 24 et 48 heures après l'accident . Conclusion: Le traitement de bas niveau laser est efficace pour réduire l'œdème chez les rats immunodéprimés a subi un traumatisme du tendon.

MOTS-CLÉS: traitement au laser, de bas niveau, immunosuppression, du tendon d'Achille.

VERIFICACIÓN DE LA EFICACIA DE LASER DE BAJA POTENCIA EN EL TRATAMIENTO DEL EDEMA EN RATAS INMUNOSUPRIMIDAS

RESUMEN:

Introducción: El tendón de Aquiles suele verse afectado por las lesiones traumáticas que conducen a la inflamación y el edema. Cuando el sistema inmunológico se altera, hay una baja respuesta inmune, que puede conducir a una desaceleración de la recuperación de los tejidos. Así, el láser de baja potencia se puede utilizar para la presentación de efectos beneficiosos en este tipo de lesiones. Así, el objetivo de este estudio fue determinar si la terapia láser de baja escala es eficaz para el tratamiento del edema en ratas inmunosuprimidas con pacientes sometidos a traumatismo del tendón. Materiales y Métodos: 23 ratas macho de la cepa Wistar se dividieron aleatoriamente en grupo control, y el láser placebo. Los animales fueron inmunodeprimidos y sometidos a una lesión en el tendón de Aquiles derecho. Para el tratamiento, se utilizó láser de baja potencia, 660nm, 30mW y 2J/cm2 dosis. Resultados: Se observó una disminución significativa en el diámetro de los tendones en el Gran Premio, entre después de la lesión y cuatro, 24 y 48 horas después de la lesión y en el GL, los resultados fueron significativas entre después de la lesión, 24 y 48 horas después lesión. Conclusión: La terapia láser de baja escala es eficaz para reducir el edema en ratas inmunosuprimidas sufrió un traumatismo del tendón.

PALABRAS-CLAVE: Terapia por Láser de Baja Intensidad, Inmunosupresión, Tendón Calcáneo.

VERIFICAÇÃO DA EFICÁCIA DO LASER DE BAIXA POTÊNCIA NO TRATAMENTO DO EDEMA EM RATOS IMUNOSSUPRIMIDOS

RESUMO:

Introdução: O tendão calcâneo é comumente acometido por lesões traumáticas que levam à inflamação e formação de edema. Quando o sistema imunológico está alterado, há uma baixa resposta imunológica, que pode levar a um retardamento da recuperação tecidual. Assim, o laser de baixa potência pode ser utilizado por apresentar efeitos benéficos nesse tipo de lesão. Dessa forma, o objetivo de estudo foi verificar se o laser de baixa potência é eficaz para o tratamento do edema em ratos imunossuprimidos, submetidos a trauma tendíneo. Materiais e métodos: Foram utilizados 23 ratos, machos, da linhagem Wistar, divididos aleatoriamente em: grupo controle, placebo e laser. Os animais foram imunossuprimidos e submetidos à lesão no tendão calcâneo direito. Para o tratamento, utilizou-se laser de baixa potência, 660nm, 30mW e dose de 2J/cm2. Resultados: Observou-se que houve diminuição significativa no diâmetro dos tendões, no GP, entre pós-lesão e quatro, 24 e 48 horas após a lesão e, no GL, os resultados foram significativos entre pós-lesão e 24 e 48 horas após a lesão. Conclusão: o laser de baixa potência é eficaz para reduzir o edema em ratos imunossuprimidos submetidos a trauma tendíneo.

PALAVRAS-CHAVE: Terapia a laser de baixa intensidade, imunossupressão,