12 - USE OF THE LASER InGaAlP, 670 nm, IN WISTAR RATS MUSCULAR LESION

ÉMERSON BORATO; ADRIANO POLICAN CIENA;
GLADSON RICARDO FLOR BERTOLINI.
Universidade Estadual do Oeste do Paraná, Cascavel - Paraná - Brasil.
gladsonricardo@gmail.com

INTRODUCTION

The muscular lesion is one of the most common traumas found in athletes and it can put back the sporting activities return in weeks or months (JÄRVINEN et al., 2005). Seeking to study the musculoskeletal system repair capacity, researchers come using experimental lesion induction methods as contusion (MINAMOTO; BUNHO; SALVINI, 2001), electrical stimulation and myotoxin injections (HILL; WERNIG; GOLDSPINK, 2003).

When it happens a lesion, damaged cells are not substituted by new cells, but there is the satellites cells recruitment that multiplies and they are founded with the damaged fibers. This kind of cells aid in the skeletal muscle regenerative capacity that it happens for intrinsic mechanism, reestablishing the contractile function (JÄRVINEN et al., 2005).

The low level laser therapy produces cellular enzymatic content increase, due to increase in the mitochondrial metabolism, what induces the cellular biostimulation (GRECO et al., 2001). According to Oron et al. (2001), the irradiation laser modulates several biological processes, could cause increase of the mitochondrial breathing and ATP synthesis, to accelerate the wounds healing, to promote the skeletal muscle regeneration, to decrease the inflammation and to promote angiogenic stimulus. They affirm that the laser therapy induces regulatory proteins synthesis in the satellites cells, of the skeletal muscle, for cellular cycle activation.

The low level laser therapy use can be considered in the inflammation treatment and pain relief due to its effect anti-inflamatory, in the improvement or acceleration of the regeneration (SHIBATA et al., 2005). According to Amaral, Parizzotto and Salvini (2001), the low level laser therapy application in the muscular tissue regeneration process after experimental lesion, results in changes, as mitochondrial density and muscular fiber increase, when compared with the control group. They concluded that the laser therapy effect can be dose-specific.

The aim of this study was to evaluate the effects of the two treatment doses irradiation with low level laser therapy, in the analgesia and edema, of rats submitted to the experimental lesion of the lateral gastrocnemius muscle, with formalin 5%injection.

MATERIALS AND METHODS

Animals

Male rats Wistar (n=20), with 12 ± 2 weeks of age, and 290 ± 50 g of weight, obtained in Central vivarium of the State University of the Paraná West - UNIOESTE, Cascavel/PR. The rats were housed in a 12 h light-dark cicle, four rats per cage, with free access to standard rat food and water. The experiment was carried according to the ethical precepts defined by the Brazilian School of Animal Experimentation (COBEA) (ANDERSEN et al., 2004).

Experimental groups

The rats were randomly distributed in three groups, being:

- Control Group (GC, n = 6) - injuried rats and treated with turned off equipment (sham);
- 10 Group (G10, n = 7) - injuried rats and irradiated with 10 J/cm²;
- 20 Group (G20, n = 7) - injuried rats and irradiated with 20 J/cm².

Experimental Lesion Production

The rats were sedate, previous to the procedure of the lesion, with ethyl ether. After trichotomy of the right triceps sural area, 0,1 ml of formalin 5% was introduced, in the three groups, in the lateral gastrocnemius muscle girth. To standardize the lesion place it was measured with metallic caliper (Mitutoyo/Stainless-Hardened®) 10 mm of the knee right posterior joint line.

Test of Functional Inability (Paw Elevation Time)

The test was characterized basically by a metallic cylinder in movement, and a computer software with connection to an adapted metallic boot to the rat paw, described originally by Tonussi and Ferreira (1992).

In this test, rats are placed on a revolving cylinder (30 cm diameter; 3 rpm) for 1-min periods and a computer-assisted device measures the total time that a specific hind paw was not in contact with the cylinder surface, i.e. the paw elevation time (PET). According to Bressan, Cunha and Tonussi (2006), animals that didn't suffer any intervention invasiva, and they don't present march alterations, the PET it is about of 10 s, considering than this value only increases in the affected limb.

The rats were evaluated, with relationship to PET, before the experimental lesion, immediately after the lesion, after the treatment, 2, 8 and 24 hours after the lesion.

Edema Evaluation

To quantify the edema in the experimental lesion area, caliper was used, it was positioned in fact in the rat posterior leg medium third area, to 1 cm of distance below the popliteal fossa, used about reference point, accomplishing the measures in lateral-medial sides. The data collections were accomplished before the lesion, soon after the lesion, after the treatment, and with 2, 8 and 24 hours.

Laser Equipment

A clinical model of the Bioset® mark was used, InGaAlP, 670 nm, and with 30 mW potency, which went calibrated previous to the experiment beginning, for used dose certification.

For the low level laser therapy application in the experimental lesion place, the rats were immobilized previously in a container, made of thermoplastic-PVC material for being non-toxic and inert, described previously by Lirani (2004). The treatment was accomplished in the punctual form, in direct contact of the probe on the lesion area in 90 degrees angle. The laser therapy was accomplished after the accomplishment of the lesion, soon after the PET and edema reevaluation.

Statistical Analysis

The data were showed by the descriptive statistics (average, deviation-pattern) and analyzed within the groups with paired, two-tailed Student *t* test, and one way ANOVA for inter-groups analysis. Being accepted the significance level p <0,05.

RESULTS

The results obtained for PET are presented in the table I.

Tabela I - the values observed in the different moments of PET evaluation, for the Control group (CG), treated with laser 10 J/cm^2 (G10) and 20 J/cm^2 (G20). The values are presented on the average and standard-deviation of the paw time in the air, in seconds.

	Before lesion	After lesion	After treatment	2 hours	8 hours	24 hours
GC	9,72±2,05	14,46±2,78	14,19±2,12	15,78±1,48	14,89±1,42	15,98±1,18
G10	10,30±1,27	15,68±1,98	15,24±2,39	14,75±1,94	15,25±1,75	15,26±2,15
G20	11,19±1,07	15,08±2,18	16,06±2,32	14,43±1,61	16,52±1,57	14,90±2,03

After the experimental lesion accomplishment in GC, there was significant increase of PET soon after the lesion (48,48%; p=0,0198), and this increase stayed after sham treatment (46,06%, p=0,0124) and after 2 hours (62,43%, p=0,0023), concluding with significant increase in 8 and 24 hours (53,27%, p=0,0004 and 64,49%, p=0,0026, respectively).

G10 presented, in similar way to GC, significant increase of PET soon after the formalin injection (52,23%, p=0,0001), which stayed significant during whole the experiment, in the different appraised moments (47,96%, p=0,0009; 43,20%, p=0,0027; 48,06%, p=0,0014; and, 48,16%, p=0,0009).

For G20, in similar way to for G10, it happened significant increase of PET soon after the formalin injection (34,76%, p=0,0005), which stayed significant during every moment of evaluation (43,52%, p=0,0029; 28,95%, p=0,0167; 47,63%, p=0,0002; and, 33,15%, p=0,0040).

In all the analyzed groups, when comparing the values in the before lesion moment, with the other moments, there was significant variation (p <0,05), indicating that there was increase in PET and in any moment after the treatment it happened restoration to the initial values; and when comparing the moments after treatment, 2, 8 and 24 hours, with the moment after lesion, there was not significant variation (p>0,05), indicating that in any moment after the lesion, it happened significant decrease of the values of PET. When accomplishing comparisons among the groups, in any moment there was significant difference (p>0,05).

The results obtained in the edema evaluation with caliper, in the area of the triceps sural are shown in the table II.

Table II - the values observed in the different moments of edema evaluation, for the control group (GC), treated with laser 10 J/cm^2 (G10) and 20 J/cm^2 (G20). The values are presented on the average and standard-deviation of the diameter, evaluated in centimeters.

	Pré-lesão	Pós-lesão	Pós-tto	2 horas	8 horas	24 horas
GC	1,17±0,06	1,41±0,11	1,40±0,22	1,41±0,21	1,43±0,16	1,38±1,11
G10	1,13±0,07	1,37±0,07	1,54±0,11	1,51±0,11	1,53±0,11	1,45±0,12
G20	1,15±0,06	1,34±0,07	1,53±0,15	1,51±0,15	1,53±0,10	1,41±0,11

With the experimental lesion accomplishment in the GC right posterior limb, there was significant edema production soon after the lesion (20,39%; p=0,0067), with after sham increase tendency (19,71%, p=0,076) and after 2 hours (21,08%, p=0,0563), concluding with significant increase in 8 and 24 hours (22,37%, p=0,0172 and 18,25%, p=0,0068, respectively).

G10 presented, in similar way to GC, significant edema production soon after the formalin injection (21,26%, p=0,0002), which stayed significant during whole the experiment, in the different appraised moments (36,40%, p=0,0002; 33,66%, p=0,0002; 35,87%, p=0,0001; and, 28,43%, p=0,0012). For G20, in similar way to G10, it happened the edema formation (16,07%, p=0,0045), which stayed significant during every evaluation moment (p=33,01%, p=0,0018; 30,93%, p=0,0026; 33,28%, p=0,0002; and, 22,50%, p=0,0053).

For all the groups, when comparing the before lesion moment values, with the other moments, there was significant variation (p <0,05), or as in the GC case (in the moments after sham and 2 hours) tendency increases it, indicating that there was edema increase and in any moment after the treatment it happened initial values restoration; and when comparing the moments after treatment, 2, 8 and 24 hours, with the moment after lesion, there was not significant variation (p>0,05), indicating that in any moment after the lesion, it happened significant PET values decrease. Again, when accomplishing comparisons among the groups, in any moment there was significant difference (p>0,05).

DISCUSSION

Although the musculoskeletal repair process is studied thoroughly, doubts stay on the treatments effect that it stimulate the process of muscular regeneration. The low level laser therapy effect in the tissue treatment is controversial and its effectiveness to stimulate the cellular proliferation and to promote the regeneration process it is debatable in the literature. Even so, the irradiation laser non-thermic effects on the satellite cells can alter the musculoskeletal regeneration process in rats (AMARAL; PARIZZOTTO; SALVINI, 2001).

Recent researches show the low level laser therapy positive effect in the right and left anterior tibial muscular regeneration, in rats myotoxin induced lesion (AMARAL; PARIZZOTTO; SALVINI, 2001), in the experimental chronic infarct scar process due to the modulation of several biological processes (ORON et al., 2001), *in vitro* cellular proliferation (PINHEIRO et al., 2002). The low level laser therapy has indication in muscular lesions, because it presents anti-inflamatory effects, how TNFá liberation decrease (AIMBIRE et al., 2006), COX-2 (ALBERTINI et al. 2007) and PGE_2 (MIZUTANI et al., 2004).

Morrone et al. (1998) using a laser AsAlGa, 780 nm, on the skeletal muscle after 4 days of injury, told important biological effects, such as: blood flow increase, improvements in capillary hydrostatic pressure, emptying of intertissue spaces and stimulation of electrolyte exchange. They concluded that the laser therapy use is important for the injuried muscular tissue regeneration speed and with evident advantages related with the functional recovery.

Albertini et al. (2004) researched the laser GaAlAs, 650 nm, 1 J/cm² and 2,5 J/cm² function, in the acute inflammation induced by carrageenin injection in rats paw. The results showed anti-inflamatory effect in both doses, possibly for the stimulation and adrenal corticosteroids hormonal liberation.

On the other hand, Oliveira et al. (1999) they studied the effect of different doses (3 and 10 J/cm²) of laser GaAs (904

nm) in the rats anterior tibial muscle regeneration. The irradiation was repeated daily by five days in a row, with the first application being done two hours after the experimental lesion induction. They didn't observe differences among the treated groups with 3 J/cm² and 10 J/cm² and in the sham group. They just observed in the group of 10 J/cm^2 corporal weight increase.

The present study used the laser InGaAlP, with 670 nm wavelength, with 10 J/cm² and 20 J/cm², in the muscular repair after experimental lesion with formalin 5% in the rats right lateral gastrocemius muscle. The irradiation laser happened soon after the lesion. The animals were appraised with relationship to the pain through the paw time of elevation (PET) and with relationship to the edema in the periods before lesion, after lesion, 2, 8 and 24 hours.

Pain was observed in all the groups when comparing the results before lesion with the results after lesion, indicating the experimental lesion production. In the treated groups with low level laser therapy this pain stayed during the first 24 hours, going of encounter with research accomplished by Bingöl, Altan and Yurtkuran (2005), that they observed the effect of the laser AsGa, 904 nm, in patients with shoulder arthralgia, tells improvement in some passive and active movements and in the palpation sensibility, but any significant pain improvement of the treated group compared to the control group.

In relation to the edema similar results were observed to the presented in the pain evaluation. It happened edema in all the groups, indicating the experimental lesion production. In way, contrary to the study of Morrone et al. (1998), decrease was not observed in the intertissue spaces liquid, what would contemplate in edema reduction. Besides, in a contrary way, it was observed that the increase tendency that happened for the sham group in the period after treatment and 2 hours, was confirmed for the two used laser doses, result that it can have been happening due to the immediate application of the laser.

The fact of having happened the radiation application, soon after the accomplishment of the formalin chemical irritation, it can have been producing blood extravasating increase, because according to Maegawa et al. (1998), the low level laser therapy produces arteriolar vasodilatation and blood flow increase, and owed this way, in spite of the production of PGE_2 decrease (SAKURAI et al., 2001), the combination of the vasodilatation induced by the lesion with the vasodilatation induced by the low level laser therapy, they can have been contributing to the edema increase observed.

However, it is worth to stand out as limitations of this study, the absence of lesion size and intensity visualization, as well as the not observation of inflammatory chemical mediators involved.

CONCLUSION

We concluded in this study, that with the wavelength of 670 nm, in the used doses of 10 and 20 J/cm^2, there was not decrease in the pain and edema, in animals submitted to the muscular lesion with formalin 5% injection.

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USE OF THE LASER InGaAlP, 670 nm, IN WISTAR RATS MUSCULAR LESION

ABSTRACT:

The laser of low potency induces the cellular biostimulation that could cause increase of the mitochondrial breathing and synthesis of ATP, to accelerate the cicatrization of wounds, to promote the regeneration of the skeletal muscle, to reduce the inflammatory answer and to stimulate angiogenic process. The objective of this study was to analyze the effects of the two irradiation doses of treatment with low level laser, in the analgesia and edema of rats submitted to the experimental lesion of the muscle lateral gastrocnemius. Males rats Wistar (n=20) were used, randomly distributed in three groups, being: GC- injuried animals and agreements with turned off equipment (sham); G10- injuried animals and irradiated with dose of 10 J/cm², 670 nm; G20- injuried animals and irradiated with dose of 20 J/cm², 670 nm. For the production of the experimental lesion, 0,1 ml of formalina 5% was introduced, in the three groups, in the lateral gastrocnemius muscle girth. The evaluation of the pain happened through the Time of Elevation of the Paw (TEP), and the edema was evaluated with aid of metallic caliper, both evaluations happened before the lesion, soon after the lesion, after the treatment, 2, 8 and 24 hours after the lesion. The results showed significant increase of TEP and of the edema, in every moment evaluated after the induction of the lesion. It is ended that laser, in the used parameters, did not produce decrease in the pain and edema in animals submitted to the muscular lesion with injection of formalina 5%. KEYWORDS: low level laser therapy, analgesia, edema.

USAGE DE L'InGaAlP LASER, 670 NM, DANS LÉSION MUSCLÉE DANS SOURIS WISTAR

RÉSUMÉ:

Le laser de puiss basse puissance induit le biostimulation cellulaire. L'objectif de cette étude était analyser les effets des deux doses de l'irradiation de traitement avec bas laser de niveau, dans l'analgésie et oedème de souris soumis à la lésion expérimentale du muscle gastrocnemius latéral. Les 20 souris mâles Wistar utilisés, ont été distribué dans trois groupes, en étant: GP - animaux de lesioné et traité avec l'appareil éteint (placebo); G10 - animaux lesioné irradié avec dose de 10 J/cm², 670 nm,; G20 - animaux lesioné irradié avec dose de 20 J/cm², 670 nm. Pour la production de la lésion expérimentale, 0,1 ml de formalina 5% ont été introduits, dans les trois groupes, dans le ventre du muscle gastrocnemius latérale. L'évaluation de la douleur s'est passée à travers le Temps d'Élévation de la Patte (TEP), et l'oedème a été évalué avec l'aide d'attelle-étrier métallique, les deux évaluations se sont passées avant la lésion, bientôt après la lésion, après le traitement, 2, 8 et 24 heures après la lésion. Les résultats ont montré augmentation considérable de TEP et de l'oedème, dans chaque moment évalué après installation de la lésion. Il est terminé que le laser, dans les paramètres utilisés, n'a pas produit baisse dans la douleur et l'oedème aux animaux soumis à la lésion musculaire avec injection de formalina 5%.

MOTS-CLEF: laser de basse puissance, analgésie, oedème.

USO DEL LASER inGaAlP, 670 nm, EN LESIÓN MUSCULAR EN RATONES WISTAR

RESUMEN:

Visando estudiar la capacidade reparativa de lo sistema músculo esquelético, pesquisadores vienen utilizando métodos de indución de lesión experimental. El laser baja potencia induce la bioestimulación celular, podiendo causar el aumento de la respiración mitocondrial y síntesis de ATP, acelerar la cicatrización de heridas, promover la regeneración de lo músculo esquelético, disminuyer la perpuesta inflamatória y estimular la neoformación de vaso sanguienos. El objetivo de este estúdio fue analisar los efectos de dos dosis de laser de baja potencia en la analgesia y edema de ratones sujetos a la lesión experimental de lo músculo gastrocnemio lateral. Fueran utilizados 20 ratones Wistar machos, repartidos aleatoriamente en três grupos: GC- animales lesionados y tratados con aparatos desligado; G10- animales lesionados y irradiados com dosis de 10J/cm^2, 670 nm; G20- animales lesionados y irradiados con dosis de 20J/ cm^2, 670 nm. Para producir lesión experimental, se introdución 0,1 ml de formalina 5%, em los três grupos, em lo vientre del músculo gastrocnemio lateral. La evaluación del dolor ocurrió por médio del Tiempo de Elevación de la Pata (TEP), y lo edema fue evaluado con ayuda del paquímetro metálico, lãs evaluaciones ocurrieram pre-lesión, pos-lesión , pos-tratamiento, 2, 8 y 24 horas pos-lesión. Los resultados mostraran aumento significativo del TEP y de la evaluación con paquímetro, en todos los momentos evaluados después la indución de la lesión. Se concluye que el laser en los parâmetros utilizados, no produció en el dolor y edema, en animales sometidos a la lesión muscular con inyección de formalina 5%.

PALABRAS-CLAVES: laser de baja potencia, analgesia, edema.

USO DO LASER InGaAlP, 670 nm, EM LESÃO MUSCULAR EM RATOS WISTAR

RESUMO:

Visando estudar a capacidade reparativa do sistema músculo-esquelético, pesquisadores vêm utilizando métodos de indução de lesão experimental. O laser de baixa potência induz a bioestimulação celular, podendo causar aumento da respiração mitocondrial e síntese de ATP, acelerar a cicatrização de feridas, promover a regeneração do músculo esquelético, diminuir a resposta inflamatória e estimular a neoformação de vasos sanguíneos. O objetivo deste estudo foi analisar os efeitos de duas doses de laser de baixa intensidade, na analgesia e edema, de ratos submetidos à lesão experimental do músculo gastrocnêmio lateral. Foram utilizados 20 ratos Wistar machos, distribuídos aleatoriamente em três grupos, sendo: GC - animais lesionados e tratados com aparelho desligado (simulacro); G10 - animais lesionados e irradiados com dose de 10 J/cm², 670 nm; G20 - animais lesionados e irradiados com dose de 20 J/cm², 670 nm. Para a produção da lesão experimental, introduziu-se 0,1 ml de formalina 5 %, nos três grupos, no ventre do músculo gastrocnêmio lateral. A avaliação da dor ocorreu através do Tempo de Elevação da Pata (TEP), e o edema foi avaliado com auxílio de paquímetro metálico, ambas as avaliações ocorreram pré-lesão, pós-lesão, pós-tratamento, 2, 8 e 24 horas pós-lesão. Os resultados mostraram aumento significativo do TEP e da avaliação com paquímetro, em todos os momentos avaliados após a indução da lesão. Conclui-se que laser nos parâmetros utilizados, não produziu diminuição na dor e edema, em animais submetidos à lesão muscular com injeção de formalina 5%.

PALAVRAS-CHAVE: laser de baixa potência, analgesia, edema.