21 - PULSED THERAPEUTIC ULTRASOUND IN MUSCULAR LESION IN WISTAR RATS

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INTRODUCTION

The muscular lesion is one of the most common injured that affect athletes and it can delay the return to the sporting activities in weeks or months (JÄRVINEN et al., 2005). Seeking to study the repair capacity of the musculoskeletal system, several lesion induction methods comes being used, as contusion (MINAMOTO; BUNHO; SALVINI, 2001), electrical stimulation and myotoxin injections (HILL; WERNIG; GOLDSPINK, 2003).

The clinical manifestation of the lesion will depend on the severity and of the nature of the same, could be classified in three categories: the) light (degree I): it presents little injured fibers, with small edema and discomfort, besides minimum force and movement loss; b) moderate (degree II): it presents a larger number of fibers injured with loss of force, edema and more intense discomfort; c) severe (degree III): it presents extensive muscular lesion, resulting in total muscle function loss (JÄRVINEN et al., 2005).

In the injured muscle, the pain is associated to the structural lesion and the edema to the destruction of the sarcolemma and of the basal membrane integrity. The pain results in inability and it has as purpose the organism protection, staying this condition can result in muscular atrophy, disuse habits and to the conscious or unconscious protection, taking to the muscular function loss. The edema is defined as a swelling formed by the elements extravasation for the vessel exterior and the intensity of this extravasation will be correlated with the tissue aggression kind and degree (RESENDE; PEAR TREE; I CASTRATE, 2005; TEIXEIRA; MARCON; FIGUEIRÓ, 2001).

The therapeutic ultrasound is used thoroughly inside of the physiotherapy, seeking alteration in inflammatory processes and pain reduction, in spite of consent absence its respect and even of the ways for obtaining of the same ones (FRANK et al., 2005; FYFE, 1979; GAM; JOHANNSEN, 1995; KRAMER, 1987).

The aim of this study consisted of analyzing the effects of the pulsed therapeutic ultrasound, in the pain and immediate edema, in rats submitted to the gastrocnemius lateral muscle experimental lesion.

MATERIAL AND METHODS

Study Design and Sample

Twelve male rats were used in the experimental research, of the lineage Wistar, with 14 ± 2 weeks of age, obtained in Central Vivarium of the State University of the West of Parana UNIOESTE, Cascavel/PR. These were housed in polypropylene contention cages, contained in number of four, with ambient temperature of 25º C and cycle of 12 hours clear / darkness, receiving ration and water *ad libitum*. The project was driven 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 two groups, being:

•Control Group (CG, n = 6) injured and no treated;

•Therapeutic Ultrasound Group (TUG, n = 6) injured and treated with pulsed ultrasound.

Experimental Lesion Production

The rats were sedated, previous to the lesion procedure, with ethyl ether. After the right sural triceps area trichotomy, 0,1 mL of formalin 5% was introduced, in the two groups, in the lateral gastrocnemius muscle belly. To standardize the lesion place was measured 10 mm of the posterior right joint interline with caliper metallic, Mitutoyo/Stainless-Hardened®.

Functional Inability Test (Paw Elevation Time)

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

In this test, the animals are placed in a cylinder in movement (30 cm of diameter; 3 rpm) for periods of 1 minute, and a computerized device measures the total time in that the right paw is without contact with the cylinder, that is to say the paw elevation time (PET). According to Borato, Ciena and Bertolini (2008), rats with muscular lesion present significant variations, with PET increase, in posterior times to the lesion.

The rats were assessed, 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, metallic caliper was used, that it was positioned in the area of medium third of the posterior rat leg, to 1 cm of distance below the popliteal fossa, used about reference point, accomplishing the measures in lateral-medial sides. (BORATO; CIENA; BERTOLINI, 2008). The data collections were accomplished before the lesion, soon after the lesion, after the treatment, and with 2, 8 and 24 hours.

Ultrasound Equipment

A clinical model of the Ibramed® mark was used, with 1 cm^2 ERA transducer, 1 MHz, RNF 30%, pulsed 5%, 100 Hz modulated frequency, which went calibrated previous to the experiment beginning, for used dose certification that was of 0,5 W/cm^2 SATP.

For the therapeutic ultrasound 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 transducer direct contact on the lesion area, in 90 degrees angle, with use of water soluble gel as coupler half. The therapy was accomplished after the lesion, soon after the PET and edema reevaluation.

Data Analysis

The data were presented by the descriptive statistics (average, standard-deviation) and analyzed with the Analysis of Variance with Repeated Measures (ANOVA), with post-hoc test of Tukey, for intra-groups analysis. Being accepted the significance level p <0,05.

RESULTS

Functional Inability Test (Paw Elevation Time)

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

Table I values observed in the different moments of PET evaluation, for the control group (CG) and treated with pulsed therapeutic ultrasound (TUG). The values are presented on the average and standard-deviation of the paw time in the air, in seconds.

	Prÿ-lesÿo	Pÿs-lesÿo	Pÿs-tto	2 horas	8 horas	24 horas
GC	9,72ÿ2,05	14,46ÿ2,78	14,19ÿ2,12	15,78ÿ1,48	14,89ÿ1,42	15,98ÿ1,18
GUT	4,25ÿ0,12	15,86ÿ1,96	20,88ÿ3,34	17,84ÿ0,73	13,23ÿ2,36	13,12ÿ1,0

After the experimental lesion accomplishment in CG, there was significant PET increase soon after the lesion (p<0,01), and this increase stayed post-placebo (p<0,01) and after 2 hours (p<0,001), concluding with significant increase in 8 and 24 hours (p<0,001). When comparing the values of the moment post-lesion with the following ones, pain significant alteration was not observed (p>0,05).

The treated group with pulsed therapeutic ultrasound presented, in similar way to CG, significant PET increase soon after the formalin injection, which stayed significant during whole the experiment, in the different appraised moments (p<0,001). When it was compared the moment post-lesion with the post-treatment, there was significant increase of the PET values (p<0,001) indicating pain increase. For the following moments there was not significant variation (p>0,05).

Edema Evaluation

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

Table II values observed in the different moments of edema evaluation, for the control group (CG) and treated with pulsed therapeutic ultrasound (TUG). The values are presented on the average and standard-deviation of the diameter, evaluated in centimeters.

	Prÿ-lesÿo	Pÿs-lesÿo	Pÿs-tto	2 horas	8 horas	24 horas
GC	1,17ÿ0,06	1,41ÿ0,11	1,40ÿ0,22	1,41ÿ0,21	1,43ÿ0,16	1,38ÿ1,11
GUT	1,48ÿ0,10	1,80ÿ0,11	1,74ÿ0,08	1,78ÿ0,11	1,36ÿ0,05	1,38ÿ0,11

With the experimental lesion accomplishment in the CG right posterior paw, there was production of significant edema soon after the lesion (p<0,05), and it continued increased post-placebo, 2 hours and 8 hours (p <0,05), with return at the initial levels after 24 hours of the lesion (p>0,05). When comparing the values after lesion with the following moments, it was not observed in any moment significant variation (p>0,05).

The treated group presented, in similar way to CG, significant edema production soon after the formalin injection (p<0,001), which stayed significant after the treatment (p<0,01) and 2 hours (p<0,001), but there was return to the initial values in 8 and 24 hours after the lesion (p>0,05). When it was compared the values after the lesion, with the following moments, it was observed that there was not significant values decrease after the treatment and 2 hours (p>0,05), even so with 8 and 24 hours of lesion, there was significant edema reduction (p<0,001).

DISCUSSION

The therapeutic ultrasound is an extremely common form for lesions treatment in soft tissue, because of its thermal and not thermal effects, which include cavitation and acoustic microstreaming, that it can alter the cellular membrane function and permeability, accelerating the tissue recovery (SPEED, 2001). Some studies explore the ultrasound use about the muscular tissue regeneration front to the lesion, with conflicting results, like Rantanen et al. (1999) that accomplished contusion in rats' gastrocnemius, and treatment with pulsed ultrasound 20%, 3 MHz and intensity of 1,5 W/cm^2, they observed cells satellites proliferation increase, but non myotubes production alteration.

McBrier et al. (2007) they examined the ultrasound influence (3 MHz, 0,3 W/cm^2) on the MGF expression after muscular trauma in rats' gastrocnemius, and they observed reduction in the MGF mRNA expression in the treated limb. Markert et al. (2005) when observing the ultrasound effects (3 MHz, 0,1 W/cm^2) on injured rats' gastrocnemius, didn't observe evidences that the ultrasound increased the muscular regeneration after contusion.

However Karnes and Burton (2002), evaluating the rats' muscle repair, injured by repeated eccentric contractions and treated with therapeutic ultrasound (1 MHz, 0,5 W/cm^2), they observed significant improvement in the force production for those treated, compared with the non treated. Fisher, Hiller and Rennie (2003) analyzing the rats' gastrocnemius repair injured by impact, they observed that with pulsed ultrasound the muscles answered favorably in the repair process, with larger amount of contractile protein, than animals treated with ultrasound continuous or untreated.

Since the presented above, in the present study it intended to analyze the pulsed ultrasound use, on two aspects of the inflammatory process, occurred after rats' lateral gastrocnemius lesion, that is to say, the pain and the edema. In the pain analysis of the rat posterior limb, through the paw elevation time, significant presence of the same was observed soon in all the groups after the experimental lesion production, indicating the lesion production in all the groups. The pain, presented by the rats, stayed during the first 24 hours for the two groups, not being observed pain decrease in the treated groups with pulsed therapeutic ultrasound and in the control group. But, for the treated group, when comparing the moment post-lesion with the moment post-treatment, there was significant pain increase that didn't occurred with the control group, demonstrating like this pain increase effects with the therapeutic ultrasound. Such a discovery is contrasting with the Madiman, Weasel and Fisher (1995) study, they tell that the ultrasound presents analgesic effect for increasing the mechanical pain threshold. Since when walking on the metallic surface the rats presented larger air paw time, indicating larger mechanical pain after the treatment with therapeutic ultrasound.

With relationship to the inflammatory process edema, Franco et al. (2005) they told absence of therapeutic ultrasound effect, in 0,3 W/cm^2 intensity. However, Fyfe (1979) also evaluating inflammatory processes induced in rats, it tells that the therapeutic ultrasound presents important action in the edema reduction. Fact also observed in the present study after 8 hours of

the lesion. In the edema analysis of the rat right posterior limb, significant presence of the same was observed soon in all the groups after the experimental lesion production. For the control group there was decrease of the same after 24 hours of the lesion, fact also observed for the treated group, but besides this moment there was also significant reduction effect in 8 hours, demonstrating like this important effect in the edema reduction for the treatment with ultrasound.

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

The immediate effects of the pulsed ultrasound treatment were not significant to reduce the pain, besides producing larger increase than the control group, but it produced significant effects in the edema reduction, in rats submitted to the experimental lesion of the lateral gastrocnemius muscle, with formalin 5%.

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REFERENCES

ANDERSEN, M. L.; D'ALMEIDA, V.; KO, G. M.; KAWAKAMI, R.; MARTINS, P. J.; MAGALHÃES, L. E.; TUFIK, D. **Princípios éticos e práticos do uso de animais de experimentação.** São Paulo: UNIFESP, 2004.

BORATO, E.; CIENA, A. P.; BERTOLINI, G. R. F. Use of the laser InGaAlP, 670 nm, in wistar rats muscular lesion. **The FIEP Bulletin**, v. 78, p. 50-53, 2008.

FISHER, B. D.; HILLER, C. M.; RENNIE, S. G. A. A comparison of continuous ultrasound and pulsed ultrasound on the soft tissue injury markers in the rat. **Journal of Physical Therapy Science**, v. 15, p. 65-70, 2003.

FRANCO, A. D.; PEREIRA, L. E.; GROSCHITZ, M.; AIMBIRE, F.; MARTINS, R. Á. B. L.; CARVALHO, R. A. Análise do efeito do ultra-som no edema inflamatório agudo estudo experimental. **Fisioterapia em Movimento**, v. 18, n. 2, p. 19-24, 2005.

FYFE, M. C. A study of the effects of different ultrasonic frequencies on experimental oedema. **The Australian Journal of Physiotherapy**, v. 22, n. 5, p. 205-207, 1979.

GAM, A. N.; JOHANNSEN, F. Ultrasound therapy in musculoskeletal disorders: a meta-analysis. **Pain**, v. 63, p. 85-91, 1995.

HILL, M.; WERNICK, A.; GOLDSPINK, G. Muscle satellite (stem) cell activation during local tissue injury and repair. **Journal of Anatomy**, v. 203, p. 89-99, 2003.

JÄRVINEN, T. A. H.; JÄRVINEN, T. L. N.; JÄRVINEN, M. K.; Kalimo, h.; JÄRVINEN, M. Muscle Injuries: Biology and Treatment. **American Journal of Sports Medicine**, v.33, n. 5, p. 745-764, 2005.

KARNES, J. L.; BURTON, H. W. Continuous therapeutic ultrasound accelerates repair of contraction-induced skeletal muscle damage in rats. **Archives of Physical Medicine and Rehabilitation**, v. 83, p. 1-4, 2002.

KRAMER, J. F. Sensory and motor nerve conduction velocities following therapeutic ultrasound. **The Australian Journal of Physiotherapy**, v. 33, n. 4, p. 235-243, 1987.

LIRANI, A. P. R. **Estudo comparativo dos efeitos do Ultra-som e do Laser de baixa intensidade, no reparo ósseo de tíbia de ratos.** 109p Dissertação (Mestrado). Escola de Engenharia de São Carlos / Faculdade de Medicina de Ribeirão Preto. Universidade de São Paulo. 2004.

MADIMAN, S.; WEASEL, J.; FISHER, B. The effect of ultrasound on the mechanical pain threshold of healthy subjects. **Physiotherapy**, v. 81, n. 12, p. 718-723, 1995.

MARKERT, C. D.; MERRICK, M. A.; KIRBY, T. E.; DEVOR, S. T. Nonthermal ultrasound and exercise in skeletal muscle regeneration. **Archives of Physical Medicine and Rehabilitation**, v. 86, p. 1304-1310, 2005.

McBRIER, N. M.; LEKAN, J. M.; DRUHAN, L. J.; DEVOR, S. T.; MERRICK, M. A. Therapeutic ultrasound decreased mechno-growth factor messenger ribonucleic acid expression after muscle contusion injury. **Archives of Physical Medicine and Rehabilitation**, v. 88, p. 936-940, 2007.

MINAMOTO, V. B.; BUNHO, S. R.; SALVINI, T. F. Regenerated rat skeletal muscle after periodic contusions. **Brazilian Journal of Medical and Biological Research**, v. 34, n. 11, p. 1447-1452, 2001.

RANTANEN, J.; THORSSON, O.; WOLLMER, P.; HURME, T.; KALIMO, H. Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury. **The American Journal of Sports Medicine**, v. 27, n. 1, p. 54-59, 1999.

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, p. 268-271, 2005.

SPEED, C. A. Therapeutic ultrasound in soft tissue lesions. **Rheumatology**, v. 40, p. 1331-1336, 2001

TEIXEIRA, M. J.; MARCON, R. M.; FIGUEIRÓ, J. A. B. **Dor, epidemiologia, fisiopatologia, avaliação, síndromes dolorosas e tratamento.** São Paulo: Moreira Jr; 2001.

TONUSSI, C. R.; FERREIRA, S. H. Rat knee-joint carrageenin incapacitation test: an objective screen for central and peripheral analgesics. **Pain**, v. 49, p. 421-427, 1992.

End: Rua Universitária, 2069. Jardim Universitário, Cascavel Pr. CEP: 85819-110. CP: 711.

PULSED THERAPEUTIC ULTRASOUND IN MUSCULAR LESION IN WISTAR RATS

ABSTRACT:

The therapeutic ultrasound is used thoroughly in the physiotherapy, its action is highlighted in some points as the inflammatory processes acceleration, pain and edema reduction, even so the literature presents conflicting results regarding these effects. The aim of this study was analyze the pulsed therapeutic ultrasound effects, in the pain and immediate edema, in rats submitted to the gastrocnemius lateral muscle experimental lesion. Twelve rats Wistar were used, randomly distributed in two groups, being: CG - animals just injured; TUG - injured and treated with pulsed therapeutic ultrasound at 5% with 0,5 W/cm^2 (SATP). For the experimental lesion production, 0,1 mL of formalin 5% was introduced, in the right lateral gastrocnemius muscle belly. The pain evaluation occurred for the Paw Elevation Time (PET), and the edema was evaluated with a metallic caliper, both evaluations occurred before lesion, post-injury, post-treatment, 2, 8 and 24 hours post-injury. The results showed significant PET increase and of the caliper evaluation, after the lesion induction, and for the control group the pain it didn't decrease in any

subsequent moment and the edema just returned to the initial levels 24 hours after the lesion. For the treated group the pain had significant increase after the treatment, and the edema reduction already occurred in the evaluation accomplished with 8 hours after the lesion. It is concluded that the ultrasound in the used parameters, produced edema decrease, even so it produced larger pain increase, in animals submitted to the muscular lesion with formalin 5% injection.

KEYWORDS: ultrasonic therapy, analgesia, edema.

ULTRA-SONS TERAPÊUTICO PULSADO BLESSURE DANS LE MUSCLE DANS RATOS WISTAR

RÉSUMÉ:

La thérapie par ultrasons est largement utilisé dans le domaine physiothérapie, votre action est mise en évidence dans certains points de l'accélération des processus inflammatoires, la réduction de la douleur et l'enflure, mais la littérature présente des résultats concernant ces effets. Cette étude examine les effets des ultrasons thérapeutiques impulsion dans l'immédiat la douleur et l'enflure chez les rats soumis à la gastrocnemius muscle préjudice expérimental. 12 rats ont été utilisées, distribuées au hasard en deux groupes, où: GC - que les animaux blessés; GUT - et en a blessé animaux traités avec l'échographie pulsée de traitement à 5% avec 0,5 W/cm2 (SATP). Pour la production expérimentale de blessure, a fait jusqu'à 0,1 mL de 5% de formol, dans le ventre du droit gastrocnemius muscle latéral. L'évaluation de la douleur a eu lieu au moment de l'élévation de Pata (tep), et l'enflure a été évaluée à l'aide de métal étrier, les deux évaluations ont été pré-blessure, post-blessure, après traitement, 2, 8 et 24 heures après la blessure . Les résultats ont montré augmentation significative sur le bord et d'évaluation avec étrier, après l'induction de blessures, et pour le groupe témoin la douleur n'a pas diminué à tout moment ultérieur que l'enflure et retourné à des niveaux initiaux 24 heures après une blessure. Pour le groupe traité la douleur a augmenté de manière significative après le traitement, et de réduire l'enflure déjà eu lieu dans l'évaluation de 8 heures après une blessure. Il s'ensuit que l'échographie les paramètres utilisés, diminution des produits gonflement, mais produit une plus grande augmentation de la douleur chez les animaux traités avec des dommages musculaires avec injection de formol 5%.

MOTS CLES: la thérapie par ultrasons, l'analgésie, de l'œdème.

ULTRA-SONIDO TERAPÉUTICO PULSADO LESIÓN EN EL MÚSCULO EN RATONES WISTAR

RESUMEN:

El ultrasonido se utiliza ampliamente en el campo fisioterapéuticos, su acción se pone de relieve en algunos puntos como la aceleración de los procesos inflamatorios, lo que reduce el dolor y la inflamación, pero la literatura se presentan resultados contradictorios en relación con estos efectos. Este estudio examina los efectos del ultrasonido pulsado terapéutico en el dolor y la inflamación inmediata en ratones sometidos a las lesiones experimental del músculo gastrocnemio. Se utilizaron ratones 12, distribuidos al azar en dos grupos, siempre y cuando: GC sólo animales heridos; GUT y causó heridas a los animales tratados con pulsos del 5% de ultrasonido para el tratamiento con 0,5 W/cm^2 (SATP). Para la producción experimental de lesión, formado por 0,1 mL de formalina al 5%, en el vientre de la cara lateral derecha del músculo gastrocnemio. La evaluación del dolor producido por el tiempo de elevación de Pata (TEP), y la hinchazón se evaluó con la ayuda de la pinza de metal, ambas evaluaciones se pre-lesión, después de la lesión, después del tratamiento, 2, 8 y 24 horas después de la lesión. Los resultados mostraron aumento significativo en el TEP y la evaluación con la pinza de metal, después de la inducción de las lesiones, así como para el grupo control el dolor no ha disminuido en cualquier momento posterior hinchazón y sólo regresó a niveles iniciales 24 horas después de la lesión. Para el grupo tratado el dolor había aumentado de forma significativa después del tratamiento, y reducir la hinchazón ya se han producido en la evaluación con 8 horas después de la lesión. De ello se deduce que el ultrasonido pulsado en los parámetros utilizados, producen disminución de la hinchazón, pero produce mayor aumento de dolor en los animales tratados con daño muscular con la inyección de formalina al 5%.

PALABRAS CLAVE: terapia de ultrasonidos, analgesia, edema.

ULTRA-SOM TERAPÊUTICO PULSADO EM LESÃO MUSCULAR EM RATOS WISTAR

RESUMO:

O ultra-som terapêutico é amplamente utilizado no campo fisioterapêutico, sua ação é destacada em alguns pontos como a aceleração de processos inflamatórios, redução da dor e edema, porém a literatura apresenta resultados conflitantes a respeito destes efeitos. O objetivo deste estudo foi analisar os efeitos do ultra-som terapêutico pulsado, na dor e edema imediatos, em ratos submetidos à lesão experimental do músculo gastrocnêmio lateral. Foram utilizados 12 ratos Wistar, distribuídos aleatoriamente em dois grupos, sendo: GC animais apenas lesionados; GUT animais lesionados e tratados com ultra-som terapêutico pulsado a 5% com 0,5 W/cm^2 (SATP). Para a produção da lesão experimental, introduziu-se 0,1 mL de formalina 5 %, no ventre do músculo gastrocnêmio lateral direito. A avaliação da dor ocorreu pelo 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, após a indução da lesão, sendo que para o grupo controle a dor não diminuiu em nenhum momento subseqüente e o edema apenas voltou aos níveis iniciais 24 horas após a lesão. Para o grupo tratado a dor teve aumento significativo após o tratamento, e a redução do edema ocorreu já na avaliação realizada com 8 horas após a lesão. Conclui-se que o ultra-som nos parâmetros utilizados, produziu diminuição no edema, porém produziu maior aumento da dor, em animais submetidos à lesão muscular com injeção de formalina 5%.

PALAVRAS-CHAVE: terapia ultra-sônica, analgesia, edema.