Vol. 51, Special Number : pp. 225-230, December 2008
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY

A N I N T E R N A T I O N A L J O U R N A L

Comparative Study of the Effects of the Ga-As (904 nm, 150mW) Laser and the Pulsed Ultrasound of 1 MHz in Inflammation of Tibialis Muscle of *Wistar* Rats

Marcus Vinícius de Mello Pinto[1*], Daniel Almeida da Costa[2], Lamara Laguardia Valente Rocha[1], Hélio Ricardo dos Santos[1], André Luis dos Santos Silva[1], Luis Guilherme Barbosa[1], João Batista Alves dos Reis[1] and Mario Bernardo-Filho[3]

[1]Centro Universitário de Caratinga; Av. Moacyr de Mattos, 87; Centro, 35300047; orofacial_1@hotmail.com; Caratinga - MG – Brasil. [2]Faculdade de Minas; Av. Cristiano Machado, 12001; 31760000; Belo Horizonte –MG – Brasil. [3]Departamento de Biofísica e Biometria; Instituto de Biologia Roberto Alcantara Gomes; Universidade do Estado do Rio de Janeiro; Av. 28 de Setembro, 87; 20551030; Rio de Janeiro - RJ - Brasil

ABSTRACT

This paper aims to compare the therapeutic effect of the laser As-Ga of 904nm and pulsed Ultrasound of 1 MHz applied in striated skeletal muscle of inflamed rats. The animals received an intramuscular injection of bupivacaine hydrochloride in tibialis muscle in order to induce the inflammatory process, and after 24 hours, the time was considered 0 for the initiation of therapy, using a laser and ultrasound. Samples collected the muscles of the animals were stained with Hematoxylin-Eosin and histological sections of the groups used for the analysis of the muscle tissue in relation to reducing the inflammatory process, comparing the results of the two therapies used. In this study it is suggested that both treatment with laser as with ultrasound can act as anti-inflammatory. However, the laser seems to have anti-inflammatory effect for all periods observed, while the ultrasound was only able to induce declining inflammatory response to seven days.

Keywords: lasers, ultrasound, inflammation, rats

INTRODUCTION

Inflammation is a complex reaction of vascularized living tissue, which can be caused both by endogenous stimuli as by infectious agents or even by injury (Brito et al., 2004).

Several studies affirm the ability of anesthetics to cause irritation at the site of application even, in fact, miotoxic, causing pain, swelling and inflammation. Carvalho and Okamoto (1987) demonstrated the tissue irritation caused by anesthetics, Pogrel et al. (1995) and McCaughey (1992), evaluated the necrosis of the mucous and neurological disorders caused by local anesthetics, and the group of bupivacaine as causer of inflammation that it presents a inflammatory reaction more intense when compared with lidocain, articaine and mepivacaine (Ribeiro et al., 2003).

The signs and symptoms of inflammatory muscle disorders have been the subject of research in many parts of the world, with the therapy actions

[*]Author for correspondence

of anti-inflammatory drugs, which have undesirable side-effects and get lost in the axis of action of the process of muscle repair after injures (Basford, 1986; Kryuk et al., 1986).

The laser consists of electromagnetic waves, visible or not, that can be classified by the wavelength and by means which were used to produce it (Ortiz et al., 2001a), the effects produced by the laser will depend on the type of fabric and the type of laser used (Nascimento, 2001).

In low-power laser therapy dominates some therapeutic effects observed clinically, such as local analgesia, and antiinflammatory action and increased phagocytosis (Agne, 2005; Ortiz et al., 2001b).

Pugliese et al. (2003) in its study on collagen fibers took standardized skin wounds on the back of seventy-two rats and then punctual implementation of low power laser beam of the type of Arsenate-Gallium Aluminum with different densities of energy. It was observed that the groups submitted to laser therapy, there was further reduction of swelling and inflammatory infiltrate.

Pinto et al. (2007) in a case study based on the treatment of venous ulcer of the medial malleolus suggested that the Low-intensity laser therapy is able to promote healing.

Woodruff et al. (2004) conducted a meta-analysis of the effectiveness of laser therapy in the healing of tissues and statistical results of this study concluded that laser therapy is an effective treatment modality for treatment of injury tissues and that this varies depending on the parameters used like (power, power density, wavelength, energy, energy density, number and frequency of treatment and duration of treatment), thus confirming the previous studies by other authors.

Before the advent of laser, therapy for Ultrasound was the alternative in the treatment of trauma muscle that has the feature the development of the inflammatory process. However the possibility of therapeutic Ultrasound in relation to a laser for injuries is very small, that because of the depth of reach lesional (Agne, 2005).

Faganello (2003) to evaluate the effects of ultrasound in the process of muscle regeneration, noted that the phagocytosis of the remains of necrotic fibers injured was more efficient in animals treated with U.S., regardless of the intensity used, as well as the emergence of fibroblasts and mio-blasts (for the activation of satellite cells) was stimulated mainly by angiogenesis in animals submitted to the U.S., and the formation of mio-tubes occurred earlier in animals stimulated with U.S.

While comparing the laser with the ultrasound, Demir et al. (2004) made a comparative study of the effects of the laser, ultrasound, and a combination of laser and ultrasound treatment in the trial of the healing of tendon. In this study, although laser, ultrasound and a combination of laser and ultrasound improve the healing of the tendon biochemically more than the control group, there was no statistical difference between the procedures.

Thus, this paper aims to compare the therapeutic effect of laser-As-Ga and pulsed ultrasound applied in striated skeletal muscle of rats (Rattus novergicus albinus) with inflammation induced by bupivacaine.

MATERIALS AND METHODS

Thirty-five rats were used, weighing between 180-220 g. The feeding, balanced diet for the species, and access to water were allowed freely *ad libidum* in terms of maintenance in accordance with the criteria of the Brazilian College of Animal Experiments.

The animals were divided into 7 groups: Control Group (CG, n = 5), control of inflammation of 2 days (CI2, n = 5), control of inflammation of 7 days (CI7, n = 5) Laser Treatment of 2 days (LT2, n = 5) Treatment with Ultrasound of 2 days (TUS2, n = 5) Laser Treatment of 7 days (LT7, n = 5) Treatment with Ultrasound, 7 days (TUS7, n = 5).

The procedures were the same for all animals in the first stage, in order to induce inflammatory process in tibialis muscle, except for the control group which did not receive any procedure.

The animals received an intramuscular injection (99 mg / kg) of bupivacaine hydrochloride (Marcaína ® Heavy 0.5%), in his right tibialis muscle, with sterile equipment, and after 24 hours, the time was considered 0 for the beginning of therapy.

A saline solution in these animals was used to verify a possible tecidual irritation caused by the trauma of the needle, what it did not occur.

The animals of the group CI2, 48 hours after application of bupivacaine, had a sample of the

tibialis muscle collected, and the animals of the CG group also had a sample collected.

The animals of the group CI7, 7 days after application of bupivacaine, had a sample collected, and the animals of the group LT2, from the time 0 received therapy through the Laser, As-Ga, with a wavelength of 904 nm, for 150mW dose of 60 J/cm², by applying spot, in an area of 1cm square. The animals of that group received the therapy on a daily session, with an interval between sessions of 24 hours, for 2 consecutive days with lasers with fiber optics of diamond not to injure the animals. During the period of 24 hours after the last session, was collected a sample.

The animals of the group TUS2, from the time 0, received treatment through the pulsed ultrasound, at 1 MHz frequency, with 0.3 W/cm² that was applied in an area of 1cm square, one session daily, with intervals between sessions of 24 hours for 2 consecutive days, using gel of carbol.

During the period of 24 hours after the last session, was collected a sample.

The animals of the group LT7, from the time 0 received therapy through the Laser in a daily session, with an interval of 24 hours between sessions for 7 consecutive days. During the period of 24 hours after the last session, was collected a sample. The operating procedures of this group were the same as LT2.

The animals of the group TUS7, from the time 0, received treatment through the pulsed Ultrasound in a daily session, with intervals between sessions of 24 hours over 7 consecutive days and the period of 24 hours after the last session, was collected a sample. The operating procedures of this group were the same as LT2.

Samples collected of the muscles of the 35 animals followed the procedures for histological routine and were stained with Hematoxylin-Eosin (HE). The histological sections of the groups used for the analysis of the muscle tissue in relation to reducing the inflammatory process, comparing the results of the two therapies used.

They were produced six histological slides with three sections in each individual animal and subsequently submitted to the cuts by hematoxylin and eosin staining, for histological analysis. For morphometry, each of the three pieces of each of the six blades was chosen at random and this photographed a field, which led to the final 18 camps photographed by animals. The morphometry was done by counting these 18 files/ animal as the following parameters: the muscle tissue with normal aspect, with inflammatory infiltrate connective tissue, muscle fibers normal in appearance, muscle fibers with inflammation, areas of degeneration / regeneration and artifacts. Statistical analysis of the various parameters measured in different groups was performed by SigmaStat software for Windows version 1.0, using variance test for multiple comparisons of means of treatments taken hand in hand using non-parametric test Kruskal-Wallis and Dunnys with significance at $p < 0.05$. All measures were presented as mean and standard deviation.

RESULTS

In the counting of normal tissue the control group had higher values for this parameter when compared to all other groups used in this experiment, which is consistent with the lack of change in the fabric produced by inflammation. Thus, there was a significantly higher ratio of conjunctive normal in appearance and lack of inflammatory infiltrate in the control group (19.23 ± 8.62), compared with two (3.73 ± 4.34) and seven days of inflammation (0.2 ± 0.88). In calculating the presence of conjunctive with inflammatory infiltrate, animals with inflammation and treated with laser, both the two on the seven days, showed similar to that controlled. Only registered are significant differences with increase in this parameter, the comparison made between the control group (1.12 ± 1.80) and those with seven days of inflammation (10.2 ± 18.9) for seven days or treated with ultrasound (5.18 ± 9.01), when there is increased inflammatory response which affects connective tissue. These results suggest that inflammation induced by bupivacaine is more evident for seven days and that while the laser seems to have antiinflammatory effect for all periods observed, the ultrasound was only able to induce decrease inflammatory response to seven days. However, when compared to animals with inflammation and untreated and those undergoing treatment and injection of bupivacaine were significant differences for conjunctive with signs of inflammation only for two days (3.16 ± 9.80), this how the animals undergo two days of laser had less quantity of this parameter (0.75 ± 2.17) than animals with inflammation and without treatment and the group submitted to ultrasound (3.19 ± 10.83), whose value for this parameter for two

days of inflammation was higher than the other two cited above. These figures suggest that the inflammatory infiltrate two days to reach the connective tissue in different ways depending on the treatment used, so the laser acts of anti way in contrast to the pro-inflammatory action of ultrasound. Although there are no significant differences in the presence of inflammatory infiltrate in the muscle tissue to seven days, there is tendency to decrease the inflammation in the groups treated for untreated, and still less the difference in the group treated with laser.

The induction of inflammation, both the two on the seven day, generated an increase of myones inflamed compared to controlled (14.27 ± 4.77) and untreated groups (inflammation two days: (27.01 ± 7.56) and inflammation seven days: (55.04 ± 13.95) and between controls and those who received treatment with ultrasound two days: (25.72 ± 9.89) and ultrasound seven days (47.61 ± 13.9). Meanwhile, the laser treatment with only the group treated with seven days (35.03 ± 17.67) showed a significant difference of control, suggesting increased inflammatory response in the muscle fibers only in this period.

Furthermore, the inflammatory response which reached the muscle fibers showed significantly reduced in the groups treated with laser: laser two days: (11.68 ± 4.90) and laser seven days: (35.03 ± 17.67) compared those with inflammation and not treated the same periods inflammation two days: (27.01 ± 7.56) and inflammation seven days: (55.04 ± 13.95).

The groups treated with laser to seven days also had significantly lower values when compared to those treated with ultrasound (47.61 ± 13.9) in the same period.

These data suggest again that the treatments used to act differently in the control of inflammation of the model used to reach the muscle fibers, with the laser antiinflammatory agent acting as both the two on the seven days of inflammatory response, since the ultrasound only acts antiinflammatory agent as the period of seven days. Moreover, our findings suggest the increased efficiency of laser in combating inflammation induced by bupivacaine in relation to ultrasound.

Regarding the area of regeneration in the model used, there was no statistically significant differences between groups, however, these areas were observed only in the blades of animals with inflammation which are seven days treated or not. In all comparisons between pairs of experimental groups there was no significant difference (P> 0.05).

DISCUSSION

The action of laser therapy has been tested for the repair of other tissues and other models of inflammation. Rocha et al. (2007) concluded in their studies that in the induction of experimental granulomatous inflammation in mice, the laser is inflammatory action in the first weeks and from twenty-first day the answer is known as antiinflammatory.

Our findings suggest presence of areas of regeneration from seven days in all groups treated with inflammation or not, which is consistent with several authors who describe the occurrence of areas of repair of skeletal muscle after seven days and in periods of greater inflammation as Sadeh (1985) that when performing their study with bupivacaine in tibialis muscle of rats, noted the process of tissue repair between seven and fourteen days and Milburn (1976) observed that the same process in the third week.

Moreover, could not be established in this experimental model of the stimulation effect of laser on muscle regeneration, unlike Weiss et al. (1992) observed that in periods of 8 and 11 days the presence of more muscle fibers in young rats treated in the injured area when compared to control, indicating a faster process of regeneration in mice irradiated laser. It is believed that this fact can not be confirmed in our work because of the kinetics of the processes of degeneration and regeneration involved in tissue repair, perhaps they were considered times greater inflammatory response as ten, fourteen and twenty days of this action and even the laser ultrasound could have been better understood.

CONCLUSION

Our results suggest that the injection of 0.9 mL of bupivacainee is efficient in the induction of inflammation in tibialis muscle of rats, which becomes more intense for seven days, when they can be observed also areas of regeneration. Both treatment with laser like the ultrasound can act as antiinflamatory, however, this action is more

effectively fulfilled by the laser compared with the ultrasound after seven days of treatment.

ACNLOWLEDGEMENTS

Prof. Carlos Brown Scavarda on its review in the English language.

RESUMO

O presente trabalho tem como objetivo comparar o efeito terapêutico do Laser As-Ga de 904nm e do Ultra-som pulsado de 1 MHz aplicado em músculo estriado esquelético inflamado de ratos Wistar. Os animais receberam uma injeção intramuscular de cloridrato de bupivacaína no músculo tibial, a fim de induzir o processo inflamatório, e após 24 horas, foi considerado o tempo 0 para o início da terapia, utilizando-se o laser e o ultra-som. As amostras coletadas dos músculos dos 35 animais foram coradas com Hematoxilina-Eosina e as secções histológicas dos grupos serviram para as análises do tecido muscular em relação à redução do processo inflamatório, comparando os resultados das duas terapias utilizadas. Neste trabalho sugere-se que tanto os tratamentos com laser quanto com ultra-som podem agir como antiinflamatórios, no entanto, o laser parece ter efeito antiinflamatório durante todos os períodos observados, enquanto o ultra-som somente foi capaz de induzir diminuição da resposta inflamatória aos sete dias.

Palavras-chave: lasers, ultra-som, inflamação, ratos

REFERENCES

Agne, J. E. (2005), *Eletrotermoterapia Teoria e Prática***.** Orium, Santa Maria, pp. 24-32.

Basford, J. K. (1961), Low-energy laser treatment of pain and wounds: Hype,hope or hokum. *Mayo Clinic Proc*., **61**, 671-675.

Brito, M. V. H. et al. (2004), Modelo experimental para indução de miosite em ratos: Avaliação microscópica. *Rev Para Med***.**, **18**, 6-10.

Demir, H.; Yaray, S.; Kirnap, M.; Yaray, K. (2004), *Comparison of the effects of laser and ultrasound treatments on experimental wound healing in rats.* J Rehabil Res Dev., **41**, 721-728.

Faganello, F. R. (2003), *Ação do ultra-som terapêutico no processo de regeneração do músculo esquelético*. Dissertação (Mestrado em Biodinâmica da Motricidade Humana) - Instituto de Biociências, Universidade Estadual Paulista, Rio Claro.

Kryuk, A. S., Mostovnikov, V. A., Khkhlov, Serdyuchenko, N. S. (1986), *The therapeutic efficiency of low-Intensity Laser Light*. Science and Techn. Publishes, Minnsk, pp. 45-50.

McCaughey, W. (1992), Adverse effects of local anesthetics. *Drug Saf*., **7**, 178-189.

Milburn, A. (1976), The effect of the local anaesthetic bupivacainee on the muscle spindle of rat. *J Neurocytol*., **5**, 425-46.

Nascimento, P. M. (2001), *Efeito da variação da intensidade e do comprimento de onda do laser não cirúrgico em feridas cirúrgicas em dorso de ratos: estudo histológico*. Dissertação (Mestrado em Engenharia Biomédica), Universidade do Vale do Paraíba, Instituto de Pesquisa e Desenvolvimento, São José dos Campos.

Ortiz, M. C. S.; Carrinho, P. M.; Santos, A. A. S.; GonçalveS, R. C.; Parizotto, N. A. (2001), Laser de baixa Intensidade: príncipios e generalidades, Parte 1. *Fisioter Bras.*, **2**, 221-240.

Ortiz, M. C. S.; Carrinho, P. M.; Santos, A. A. S.; Gonçalves, R. C.; Parizotto, N. A. (2001), Laser de baixa Intensidade: efeito sobre os tecidos biológicos – Parte 2. *Fisioter Bras.*, **2**, 337-352.

Pinto, M. V. M.; Costa, D. A.; Aguiar, J. L. N.; Barauna, M. A.; Silva, A. L. S.; Biagini, A. P.; Oliveira, R. G.; Silva, C. M.; Junior, P. C. T. (2007), Análise do Efeito da Terapia Laser de Baixa Intensidade e do Controle Doloroso na cicatrização de Úlcera Venosa. *Dor* (São Paulo), **8**, 1113-1137.

Pogrel, M. A.; Bryan J.; Regezi, J. (1995), Nerve damage associated with inferior alveolar nerve blocks. *J Am Dent Assoc*., **126**, 1150-1155.

Pugliese, L. S.; Medrado, A. P.; Reis, S. R. A.; Andrade, Z. A. (2003), The influence of low-level laser therapy on biomodulation of collagen and elastic fibers. *Pesq Odontol Bras*., **17**, 307-313.

Ribeiro, P. D.; Sanches, M. G.; Okamoto, T. (2003), Comparative analysis of tissue reactions to anesthetic solutions: Histological analysis in subcutaneous tissue of rats. *Anesthet Prog*.; **50**, 169-180.

Rocha, L. L. V.; Pinto, M. V. M.; Maria, J.; Vieira, A. J. D.; Martins, P. R.; Costa, K. F. F. V.; Valerio, L. R. S. (2007), Efeito da Terapia laser sobre o modelo experimental de Inflamação Granulomatosa. *Fisioter Bras*., **8**, 335-341.

Sadeh, M.; Stern, L. Z.; Czyzewski, K. (1985), Changes in end-plate cholinesterase and axons during muscle degeneration and regeneration. *J Anat*., **1401**, 165-176.

Weiss, N.; Oron, V. (1992), Enhancement of muscle regeneration in the Rat gastrocnemius muscle by low energy laser irradiation. *Anat Embriol*., **186**, 497-503.

Woodruff, L. D.; Bounkeo, J. M.; Brannon, W. M.; Dawes, K. S.; Barham, C. D.; Waddell, D. L.; Enwemeka, C. S. (2004), The efficacy of laser therapy in wound repair: a meta-analysis of the literature. *Photomed Laser Surg*., **22**, 241-247.

Received: August 20, 2008;
Revised: September 10, 2008;
Accepted: September 13, 2008.