

Role of endogenous opioids in 820 nm low power laser analgesia in the knees of Wistar rats*

Avaliação do papel de opioides endógenos na analgesia do laser de baixa potência, 820 nm, em joelho de ratos Wistar

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SUMMARY

BACKGROUND AND OBJECTIVES: Pain may result in incapacity when it is associated to structural injury. Low power laser is useful in therapies aiming at decreasing joint pain and at tissue repair, but it is still somewhat controversial. This study aimed at evaluating whether analgesia induced by 820 nm low power laser is affected by the application of an endogenous opioids inhibitor.

METHOD: Twenty-four Wistar rats submitted to hyperalgesia were divided into four groups. G1: untreated; G2: treated with 820 nm laser; G3: naloxone injection before injury and untreated; G4: naloxone and treated with 820 nm laser. To induce hyperalgesia, 100 µL of 5% formalin were injected in the right tibiofemoral joint space. Nociception was evaluated by the time for flinching (TFF) in five moments: AV1 (pre-injury), AV2 (15 min/after), AV3 (30 min/after), AV4 (1 hour after) and AV5 (2 hours after).

RESULTS: All groups showed significant difference

between AV1 and AV2, but only G2 showed no difference between AV1 and AV3. There have been no differences for remaining moments as compared to AV1.

CONCLUSION: Low power 820 nm laser analgesia is affected by naloxone.

Keywords: Analgesia, Laser beams, Naloxone, Rats.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A sensação de dor pode resultar em incapacidade, sendo associada à lesão estrutural. O laser de baixa potência mostra-se útil em terapias que objetivam a redução da dor articular e o reparo tecidual, mas ainda com algumas controvérsias. O objetivo deste estudo foi avaliar se a analgesia produzida pelo laser de baixa potência, 820 nm, sofre interferência pela aplicação de um inibidor de opioides endógenos.

MÉTODO: Foram utilizados 24 ratos Wistar submetidos à hiperalgesia e divididos em quatro grupos: G1: não tratados; G2: tratados com laser 820 nm; G3: prévio a lesão, injeção de naloxona e não tratados; G4: naloxona e tratados com laser 820 nm. Para a produção de hiperalgesia foi administrado 100 µL de formalina a 5% no espaço articular tíbio-femoral direito. A avaliação da nocicepção ocorreu pelo tempo de elevação da pata (TEP), em cinco momentos, sendo AV1 (pré-lesão), AV2 (15 min/pós), AV3 (30 min/pós), AV4 (1 hora pós) e AV5 (2 horas pós).

RESULTADOS: Todos os grupos apresentaram diferença significativa entre AV1 e AV2, mas, apenas G2 não apresentou diferença entre AV1 e AV3. Para os momentos seguintes não houve diferenças com relação à AV1.

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CONCLUSÃO: A analgesia oriunda do laser de baixa potência, 820 nm, sofre interferências com aplicação de naloxona.

Descritores: Analgesia, Naloxona, Raios laser, Ratos.

INTRODUCTION

Pain may be associated to real or potential tissue injury and may cause incapacity by decreasing injured tissue function due to structural injury and tissue edema^{1,2}.

Low power laser is widely used in therapies especially aiming at decreasing pain¹ and repairing tissues. This technique also decreases fibrinogen, edema and the content of inflammatory cells, suggesting analgesia by decreasing the inflammatory process³. One may stress biomodulation, ability to stimulate cell division, vasodilation, increased protein and cortisol synthesis. However, there are still controversies with regard to the effects and the way it promotes analgesia and about treatment parameters⁴⁻⁶.

With regard to attenuation of pain caused by some mechanism unpleasant to the body or by tissue injury, it may be mediated by endogenous opioid system interactions. This is responsible for modulating pain by the action of enkephalins and β -endorphins in charge of inducing analgesic effects and influencing nociceptive stimulus perception due to their action on opioid receptors⁷.

A possibility for the analgesic effect of low power laser is peripheral opioids release from immune system cells, with significant release of local β -endorphins^{8,9}, in addition to the independent effect on opioid receptors¹⁰. In this context, the action of opioid antagonist substances and drugs to oppose their effects is studied and one example would be naloxone. Such drug is characterized as a non-specific opioid receptor antagonist³.

Based on some discrepancies in the literature with regard to low power laser parameters and its analgesic action caused by peripheral β -endorphins, this study aimed at evaluating whether low power 820 nm laser analgesia is affected by the application of an endogenous opioid inhibitor such as naloxone.

METHOD

Study with 24 Wistar rats kept in polypropylene cages with free access to water and feed, with controlled dark/light cycle of 12 hours and controlled room temperature ($24 \pm 1^\circ\text{C}$). The study followed the international ethical standards for animal experiments.

Animals were randomly distributed in four groups:

- Group 1 (G1, n = 6) – submitted to hyperalgesia induction in right knee and untreated;
- Group 2 (G2, n = 6) – right knee hyperalgesia and treated with 820 nm laser, with energy density of 8 J/cm^2 ;
- Group 3 (G3, n = 6) – right knee hyperalgesia with naloxone injection previous to the injury;
- Group 4 (G4, n = 6) – right knee hyperalgesia and naloxone, treated with laser (8 J/cm^2).

Animals were manually contained and received 100 μL of 5% formalin in the tibiofemoral joint space to induce hyperalgesia.

G3 and G4 animals received 1 μg naloxone in the right tibiofemoral joint space 15 minutes before hyperalgesia induction. G1 and G2 animals received 9% saline solution.

Evaluation of nociception

The functional incapacity test was used to evaluate nociception during gait, which is made by a cylinder and a computer program connected to a metal boot adapted to animal's paw.

The experiment started with 5 days of training. Animals walked on a 30 cm diameter cylinder covered by a stainless steel mesh which, through an electric engine, has reached 3 rpm. A metal boot was coupled to animal's hind paws, which has sent information through a wire connecting the boot to the computer with a program showing time for finching values while walking on the cylinder for one minute (time for finching – TFF). Left hind paw was also connected to a boot, however without sending information to the computer, so both paws had the sensation of wearing a boot.

The day after the last training day, normal gait time values were collected (at pre-injury moment (AV1), 15 (AV2) and 30 (AV3) minutes after hyperalgesia induction as well as 1 hour (AV4) and 2 hours (AV5) later.

Treatment protocol

Treatment was started after AV2, that is, 15 minutes after hyperalgesia induction. G1 and G3 suffered no therapeutic intervention, only simulations. G2 and G4 were treated with low power laser (Ibramed®), with 820 nm wavelength, power of 30 mW, energy density of 8 J/cm^2 , with punctual and continuous output area of 0.1160 cm^2 on the medial aspect of the knee joint. Soon after AV3 (30 minutes after injury), animals received new treatment or simulation. After the last evaluation (AV5) all animals were euthanized by guillotine decapitation.

Statistical analysis

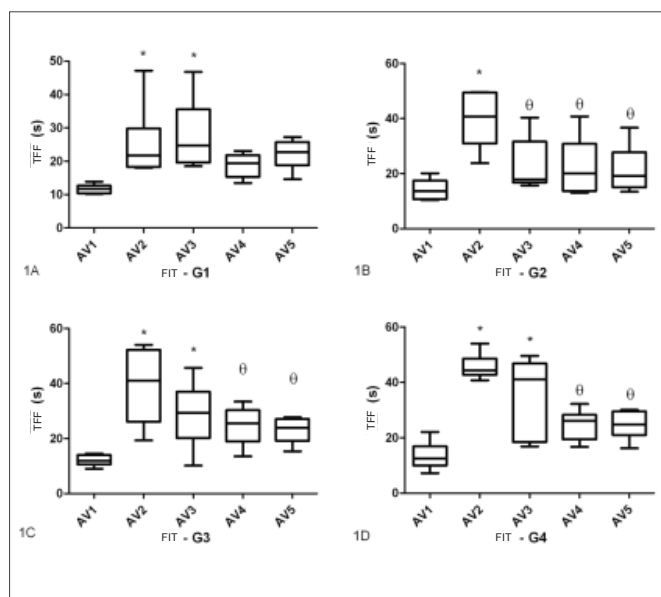
ANOVA test for repeated measures was used to compare within groups, and unidirectional ANOVA was used to compare intra-groups with Tukey's post-test. For all cases, significance level was 5%.

This study was approved by the Ethics Committee for Animal Experiments and Practical Classes, State University of Western Paraná (UNIOESTE), under process 02911/2011.

RESULTS

There has been increased TFF for G1, G3 and G4 when comparing AV1 to AV2 and AV3 ($p < 0.005$). For G2, there has been difference only between AV1 and AV2 ($p < 0.005$). There has been significant TFF decrease for G2, G3 and G4 when comparing AV2 to AV4 and AV5. For G2, there has been also decrease with regard to AV3 ($p < 0.05$) (Graph 1).

When comparing among groups, there has been significant difference only in AV2 when G1 was compared to G4 ($p < 0.05$).



Graph 1 – Functional Incapacity Test (FIT), with time for flinching (TFF) values – 1A:G1, 1B:G2, 1C:G3, 1D:G4. * Significant difference as compared to AV1.

θ Significant difference as compared to AV2.

DISCUSSION

In the search for analgesic and anti-inflammatory methods, low power laser is an interesting method for

decreasing pain and the inflammatory process, and for producing few side effects⁸. Laser effects are dose-dependent and the expected response is not obtained with it is used in under or overdoses; however, in adequate doses there is effective analgesic effect^{11,12}, in spite of a huge discrepancy with regard to adequate dose and wavelength¹³, thus raising the need for other studies to evaluate such dosimetric parameters. Our study has observed that the dose used (8 j/cm^2) with 820 nm laser was adequate to induce analgesia as already observed by other study¹. The evaluation tool used is also referenced in the literature, showing that animals with increased nociception have longer time for flinching while ambulating for one minute, as compared to animals without painful stimulation¹⁴. Naloxone was the first opioid receptor antagonist widely used in studies and clinical practice. Our study has observed that low power 820 nm laser analgesic effect has suffered significant interference and was antagonized by naloxone. To justify such effect, it is inferred that the group receiving naloxone and low power laser association has maintained pain in intra-group comparison for the third evaluation moment. It is then believed that laser might have induced endogenous opioids production and release by blood cells¹⁵, thus explaining our results. Such situation is in line with findings of a study using peripheral opioid receptor antagonists, however with 660 nm wavelength, that is within the visible length³. However, as already described with regard to controversies, in another study, laser analgesic effect in a carrageenan-induced nociceptive model, was not antagonized by naloxone¹⁰. On the other hand, other studies show opioid-mediated laser analgesic effects antagonized by systemic naloxone, that is injected by the intraperitoneal route^{8,9}. It is worth reminding that our study has used low naloxone doses so just an intra-articular and not systemic effect was expected.

Our study has used naloxone as the sole endogenous opioid receptor antagonist, suggesting the use of other drugs in association to laser to evaluate their possible effects and efficacy on analgesia and joint edema.

CONCLUSION

Low power 820 nm laser analgesia is affected by the peripheral application of an endogenous opioid antagonist such as naloxone.

REFERENCES

1. Bertolini GRF, Matos CMP, Artifon EL, Ferrari D, Vituri RF. Avaliação funcional da nocicepção do joelho de ratos tratada com laser de baixa potência e natação. *Rev Bras Med Esporte* 2011;17(1):45-8.
2. Cruz M. Lesão estrutural e incapacidade – nem só da artrite, também da artrose. *Acta Reum Port* 2008;33(4):475-6.
3. Serra AP, Ashmawi HA. Influência da naloxona e metisergida sobre o efeito analgésico do laser em baixa intensidade em modelo experimental de dor. *Rev Bras Anestesiol* 2010;60(3):302-10.
4. Kujawa J, Zavodnik L, Zavodnik I, et al. Effect of low-intensity (3.75-25 J/cm²) near-infrared (810 nm) laser radiation on red blood cell ATPase activities and membrane structure. *J Clin Laser Med Surg* 2004;22(2):111-7.
5. Campana VR, Moya M, Gavotto A, et al. Laser therapy on arthritis induced by urate crystals. *Photomed Laser Surg* 2004;22(6):499-503.
5. Bortone F, Santos HA, Albertini R, et al. Low level laser therapy modulates kinin receptors mRNA expression in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation. *Int Immunopharmacol* 2008;8(2):206-10.
6. Lana AC, Paulino CA, Gonçalves ID. Influência dos exercícios físicos de baixa e alta intensidade sobre o limiar de hipernocicepção e outros parâmetros em ratos. *Rev Bras Med Esporte* 2006;12(5):248-54.
7. Honmura A, Ishii A, Yanase M. Analgesic effect of Ga-Al-As diode laser irradiation on hyperalgesia in carrageenin-induced inflammation. *Lasers Surg Med* 1993;13(4):463-9.
8. Hagiwara S, Iwasaka H, Okuda K, et al. GaAlAs (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. *Lasers Surg Med* 2007;39(10):797-802.
9. Ferreira DM, Zângaro RA, Villaverde AB. Analgesic effect of He-Ne (632.8 nm) low-level laser therapy on acute inflammatory pain. *Photomed Laser Surg* 2005;23(2):177-81.
10. Albertini R, Aimbire FS, Correa FI, et al. Effects of different protocol doses of low power gallium-aluminum-arsenate (Ga-Al-As) laser radiation (650 nm) on carrageenan induced rat paw o edema. *J Photochem Photobiol B* 2004;74(2-3):101-7.
11. Schindl A, Schindl M, Pernerstorfer-Schön H, et al. Low-intensity laser therapy: a review. *J Investig Med* 2000;48(5):312-26.
12. Bjordal JM, Couppe C, Chow RT, et al. A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. *Aust J Physiother* 2003;49(2):107-16.
13. Martins MA, Bastos LC, Tonussi CR. Formalin injection into knee joints of rats: pharmacologic characterization of deep somatic nociceptive model. *J Pain* 2006;7(2):100-7.
14. Hagiwara S, Iwasaka H, Hasegawa A, et al. Pre-irradiation of blood by gallium aluminum arsenide (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. *Anesth Analg* 2008;107(3):1058-63.

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