Functional Assessment of Knee Nociception of Rats Treated with Low-Level Laser Therapy and Swimming

CIÊNCIAS DO EXERCÍCIO E DO ESPORTE

Original Article

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ABSTRACT

This study aimed at evaluating and comparing the effects of low-level laser therapy (LLLT) and a forced swimming training in a joint nociception model on Wistar rats, in order to functionally register pain by observation of paw elevation time (PET) during gait on a metal cylinder. Thirty-two Wistar rats were divided into four groups: CG – untreated animals submitted to nociception induction on right knee; LG – nociception and treated with 670 nm and 8 J/cm2 LLLT; SG – nociception and swimming for 10 minutes in water at 30-32° C; SLG – nociception and treated with swimming and laser. To achieve the nociception, 50 µL of 5% formalin were injected in the medial tibiofemoral space of each animal. Pain was assessed according to a functional incapacity test, which registered paw elevation time (PET) as well as their gait for one minute on a metallic cylinder. The evaluations occurred before nociception induction (EV1), after 15 minutes (EV2) and after 30 minutes (EV3) of it, with treatment protocols being administered after EV2. The analyses showed that LLLT was the only group to present the restoration values in the EV3 when compared to EV1. SG was the only one which did not show any reduction when compared to EV2 and EV3. Hence, it can be inferred that, by functional evaluation, LLLT had some analgesic effects, while the swimming treatment produced pain increase, which was partially reversed by the use of LLLT.

Keywords: low-level laser therapy, swimming, exercise, pain measurement, physical therapy modalities.

INTRODUCTION

Pain is characterized by a sensory and emotional experience, of unpleasant character, associated to a tissue injury, being it also a protection mechanism. Pain perception and body response to painful stimuli is termed nociception[1].

Submaximal physical exercise can be used as a stressor agent in the form of forced swimming, and even with water temperatures ranging from very low to body one, and in short durations, it can produce decrease of algic stimulus[2-4], such analgesic action is given to the β-endorphin release[5], which is a peptide released during painful and stressing events[6].

Analgesia after physical activity has been reported both in humans and animals. The most tested hypothesis is that activation of the endogenous opioid system may be responsible for the analgesic response. However, especially in chronic cases, it is possible that exercise can exacerbate a pre-existing painful condition[7,8].

Low-level laser is a resource much used aiming tissue repair and reduction of articular pain[9,10]. Research has shown effects of the therapy in reduction of the TNF-α[11], COX-2[12], PGE_2[13], fibrinogen levels[14], edema reduction[15], and content of inflammatory cells[16]; consequently, analgesic effects would also occur, by the reduction of the inflammatory process. However, there is controverse concerning the analgesic action in studies using laser to decrease the muscle pain scenario of late onset[17-20], alteration of sensitive threshold[21-23], release of endogenous opioids [24,25] and even variations on the anti-inflammatory and analgesic effects, due to the time of radiation application[13], or used dose[26,27], still remain.

OBJECTIVE

Due to the controverse mentioned here, as well as a gap in the comparative studies of laser therapies with exercises for nociception reduction, the aim of the present study was to evaluate and compare the low-level laser and forced swimming effects in an articular nociception model of Wistar rats, assessing pain in a functional way, that is to say, by the paw elevation time (PET) during gait on a metallic cylinder.

MATERIALS AND METHODS

Sample and experimental groups

32 male Wistar rats, with 10 ± 2 weeks, kept in polypropylene cages, with free access to water and food *ad libitum*, with controlled 12-hour light/dark cycle and controlled room temperature (24 ± 1ºC) were used. The study was conducted according to the international guidelines of ethics in animal experimentation[28], having been approved by the Ethics in Animal Experimentation and Practical Classes Committee of the Western State University of Paraná (Unioeste).

The animals were randomly divided in four groups:

- Control (CG, n = 8) – composed of untreated animals submitted to nociception induction on right knee;
- Laser (LG, n = 8) – composed of animals submitted to nociception induction on right knee and treated with LLLT;
- Swimming (SG, n = 8) – composed of animals submitted to nociception and which performed swimming as aerobic exercise;
- Laser + Swimming (SLG, n = 8) – composed of animals submitted to nociception induction and treated with swimming and laser.

Experimental model of nociception induction

The animals were manually restrained, at supine position and 50µL of formalin 5% solution was quickly injected in the medial tibiofemoral articular space of right knee, aiming to induce nociception[29].

Pain evaluation

The functional incapacity test (*Rat Knee-Joint Incapacitation Test*), originally described by Tonussi and Ferreira was used for pain evaluation[30]. This test has the aim to evaluate pain during the animal's gait, that is, in a functional way. It basically characterizes by a metallic cylinder in movement and a computer program connected to a metallic boot adapted to the animal's paw. The animal walks during one minute over the cylinder and the time at which it keeps its paw on the air is evaluated. The animal, with no pain, normally keeps the paw on the air during 10s, while when algenic substances are injected in the knee, this time increases. The evaluations occurred before nociception induction (EV1), after 15 (EV2) and 30 (EV3) minutes of induction.

Treatment protocols

The treatment protocols occurred after assessment of EV2 moment. Control group did not undergo any therapeutic intervention. LG treatment consisted of laser use (Ibramed®) with wave length of 670nm, 30mW power, 8J/cm^2, fluency, in a punctual and continuous way, on the knee medial articular interline. SG was submitted to 10 minutes of swimming in a 200-liter water oval container, manufactured in plastic, 60cm deep and with water temperature kept between 30-32ºC. SLG performed laser therapy preceded by swimming, identically to the procedure mentioned above.

Statistical analysis

Data normality was verified by the Kolmogorov-Smirnov test, with subsequent analysis within groups by one-way ANOVA with repeated measurements for comparison between groups, with Tukey post-test. In all cases significance level accepted was 5%.

RESULTS

The results demonstrated significant differences between EV1 when compared to EV2 for all groups ($p < 0.05$). When time decrease was observed, from EV2 to EV3, it was observed that only the Swimming group did not obtain significant decrease in data. When EV1 values were compared to EV3 values, it was observed that only the group treated with laser did not produce significant difference, indicating only to this group, restoration of values (figure 1).

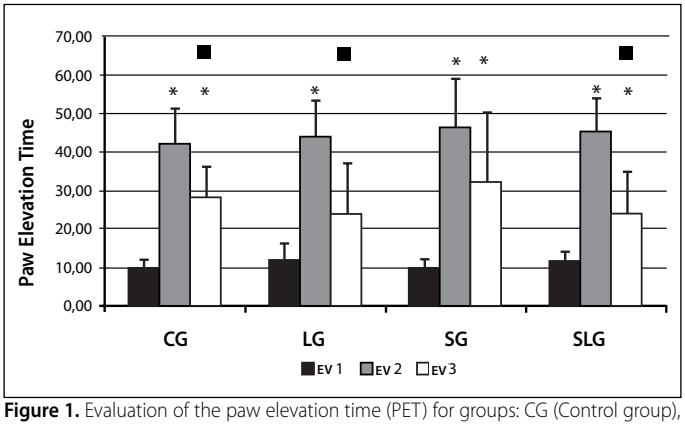

Figure 1. Evaluation of the paw elevation time (PET) for groups: CG (Control group), LG (Laser group), SG (Swimming group) and SLG (Swimming + Laser group). At the different moments EV1 (before nociception induction), EV2 (after 15 minutes) and EV3 (after 30 minutes). *Statistically difference when comparing to EV1. ■Statistically significant difference when comparing to EV2.

DISCUSSION

The pain experimental model produced by formalin injection, is used both in subcutaneous application models[31], and in intra-articular injections, as the one used in the present study, with the aim to assess pain and procedures for its reduction[29].

Pain evaluation was performed by the functional incapacity test, which assesses the paw elevation time of the animal, walking over a metallic cylinder, during one minute, where the animal with pain absence presents PET at around 10s while animals with knee joint pain increase this value, even with intra-articular injection of formalin 5%[29,30]. It should be highlighted that such test, despite being a forced task, assesses pain in a functional way.

Since nociception induced by formalin is characterized by two distinct phases, with a quiescence period between them, around the fifth to the 10th minute after induction[29], it was chosen in the present study to compare the pre-injection values with those found 15 and 30 minutes after pain induction., since for the group in which the two techniques were associated (laser and swimming), the animals' manipulation lasted about 15 minutes. Therefore, assessment after formalin injection was related to the nociception second phase and the final assessment still corresponded to this phase.

In the present study the stress produced by swimming did not produce the expected analgesic effect, since the PET in this group did not return to the initial values; difference in the values observed after 15 and 30 minutes from nociception induction was not observed either.

Such fact is contrary to the what has been mentioned by Mogil *et al.*[4], who reported that physical exercise is able to act over the release of endogenous opioids when the stressor agent is of low intensity

and non-opioid when the stressor agent is of high intensity. As in the present study it was decided to use 30-32º C of temperature and for a short period of time (10 minutes), it was believed that there would be β-endorphin release, which would produce analgesic effects, a fact which probably did not actually occur, since swimming analgesic effects were not proved.

According to Lana *et al.*[1], high-intensity physical exercises could, differently from the low-intensity ones, be a more intense stressor stimulus and hence, they would be able to trigger more evident neuroendocrine responses in the body, with increase in the seric levels of corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and glucocorticoid hormones, and with the CRH increase, β-endorphin release could occur.

Concerning the swimming group, when EV2 and EV3 were compared, the significant reduction observed in the other groups was not observed here, indicating maintenance of the algic scenario. This fact can be explained by Quintero *et al.*[32], Who observed by the formalin test, hyperalgesia in rats trained in swimming for three days. These authors speculate that sub-chronic swimming produces decrease in serotonin release. This episode may have occurred in the present study, since physical activity was trained during three days prior to the experiment.

Swimming ended up producing deleterious effects also to the group in which it was associated to laser, since the observed outcomes for the laser therapy were not seen in the association of techniques. The only fact observed was significant decrease of PET of EV3 compared to EV2, also observed in laser therapy as well as in Control group, demonstrating reduction of the pain levels for the three groups, but not for the Swimming group.

The literature presents contradictory results for the low-level laser use on pain[17-25,33,34]. According to Bjordal *et al.*[26], in acute pain cases, many of the reported adverse results occur due to laser low levels. The dose used in this study was 8J/cm^2, similar to the one used by Campana *et al.*[14], who conducted treatment in rats with osteoarthritis, induced by urate crystals, and observed an anti-inflammatory effect of radiation. Lopes-Martins *et al.*[16] also while assessing the inflammatory process, observed more remarkable reduction of inflammatory cells after pleurisy induction by carrageenan injection, com 5J/cm^2 than with 3 and 15J/cm^2.

The wave length used was 670nm, close to the lengths used by Albertini *et al.*[12] and Bortone *et al.*[15], who used 660 and 684nm in animals exposed to inflammation by carrageenan and observed decrease of the edema and RNAm of COX-2 and of kinin B1 and B2.

The outcomes show that low-level laser was efficient in decreasing nociception during animals' deambulation, since it was the only group which did not present significant difference when EV1 and EV3 were compared. Moreover, the group in which laser was associated with swimming had behavior similar to the Control group, that is to say, presented significant decrease in EV3 when compared to EV2, differently from what occurred to the Swimming group. Thus, it is inferred that although laser had not produced effects of restoration values, as LG did, there was a positive laser effect, since the high nociception level found in the SG did not occur. Such statement is supported in other studies, such as the one by Laakso and Cabot[35], Who observed pain reduction by pressure on the paw of rats submitted to a pain experimental model by Freund's complete adjuvant injection and treated with 780nm laser and 2.5J/cm^2 dose, but with no effect with 1J/cm^2.

Soriano *et al.*[36] observed positive effects of the laser therapy in rats submitted to arthritis by crystals and treated with low-level laser and also in humans with arthritis by rheumatic gout. There was reduction of the fibrinogen PGE_2 and TNF-α levels for the animals, which may have led to pain reduction in the animals, being also corroborated for humans, in which pain reduction was observed.

Again in humans, Mizutani *et al.*[37] used 830nm laser to treat different cases (shoulder adhesive capsulitis, also known as frozen shoulder; cervical spondylosis; knee osteoarthritis; de Quervain's disease; Morton's neuroma; piriformis syndrome, to name some) in 83 patients, and observed in 80.7% of the cases, significant pain reduction, which was related to the decrease in PGE_2 levels in the serum.

In the present study, characteristics of the inflammatory process or endorphins level have not been correlated with the PET data, and these limitations should be suggestions for further studies.

CONCLUSION

It was concluded in the present study that, by the PET functional evaluation, low-level laser presented analgesic effects, while swimming produced increase in pain, which was partially reverted with the associated use of laser.

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