Effect of high power dual wavelenght NIR laser emission in a rat model of compressive pain.

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ABSTRACT

Chronic pain condition, such as neuropathic pain, is one of the most important health problems worldwide and, due to its uncertain etiology and poor response to therapy, it represents an important challenge for medicine. Although, nowadays, there are many drugs for the treatment of chronic pain, their use is limited by the frequent side effects and, in some cases, ineffectiveness. Thus, the search for new therapeutic strategies which minimize this important problem is actually growing.

Since many years, laser therapy has been used as a physical therapy for pain relief and has become increasingly popular because it is non-invasive and no side effects have been reported after treatment. However, its true effectiveness is still controversial because of the counteracting results reported in literature.

In the present study we investigated the effectiveness of a high power, dual wavelenght NIR laser source in producing fast analgesic effect in a rat model of neuropathic pain, induced through loose ligation of the sciatic nerve. Twelve animals were included in the study and two treatment protocols were tested, one performed daily, the other every 48 hours, with a total of 5 applications. Both protocols used were able to statistically increase the pain threshold of the ipsilateral paw (the one with loose ligation of the sciatic nerve), starting 5 min after their first applications. The anti-hyperalgesic effect of laser treatment terminated 60 min after application. It started again, showing the same anti-hypersensitivity profile, during the subsequent applications.

The treatment protocol tested in this in vivo study in animal model might be applied to give the patient immediate pain relief and combined with treatments aimed to reduce inflammation, thus leading to analgesic effects that lasts over time.

INTRODUCTION

Pain is a serious health and social problem worldwide. Statistics is alarming and in many countries the difficulty of the patient to access to care is added. Chronic pain causes personal afflictions, social costs and, if not treated, leads to depression, reduced performance and inability to work. Depending on its origin, chronic pain can be classified as inflammatory or neuropathic. Neuropathic pain can be initiated or caused by a primary lesion or dysfunction in the central nervous system (CNS) or the peripheral nervous system (PNS). Millions of people worldwide are suffering from this chronic condition that, as mentioned above, can compromise their engaging in daily activities [1,2].

The etiology of neuropathic pain is very heterogeneous and the underlying pathophysiology is very complex, so it is a very difficult condition to treat [2,4]. Often, the available drugs have limited therapeutic potential in the management of the chronic pain and can induce risks for the patients due to side effects.

Because universally effective therapy for neuropathic pain does not exists and the interest for studies aimed at exploring new therapeutical strategies is growing, neuropathic pain research has been explored with different animal models where intentional damage is inflicted to the sciatic nerve, branches of spinal nerves or in the spinal cord [3,5]. Although these models are not completely representative of the neuropathic pain condition in humans, the development of such experimental models is essential, not only for the detection of new analgesic drugs or therapy, but also for a better understanding of pain syndromes that are difficult to manage clinically [6]. Chronic constriction injury (CCI) of the sciatic nerve with loose ligatures is the most widely used model of neuropathic pain [7,8]. The model simulates the clinical condition of chronic nerve compression in nerve entrapment neuropathy [3].

Conservative treatments, which consist in modifying the pain-precipitating activity, biomechanical correction with physiotherapy, the use of antidepressants, analgesics and/or steroids, are common forms of therapy in the case of neuropathic pain [9,10]. However, treatments with drugs

have undesirable side effects. Therefore, physical therapies which minimize the risk for side effects have been advancing [11]. Among the resources available for treating neuropathic pain within the field of physical therapies, laser therapy has become increasingly popular, due to a large body of evidence that supports its anti-inflammatory [12,15], analgesic [11, 16, 17, repairing and restoring effects [18,21]. Clinical studies on the effects of laser therapy on injured nerves have revealed an increase in nerve function and improved capacity for myelin production [22]. Laser therapy has also been shown to be effective in promoting axonal growth in injured nerves in animal models [23,26].

However, there is controversy about the effectiveness of laser therapy for producing analgesia in cases of neuropathic pain, which may be related, in most cases, to a lack of reproducibility and the doubtful quality of certain studies.

Although many studies using laser therapy reported improvements in symptoms and pain relief, the mechanisms that underlie the analgesic effect are still unclear.

In the case of neuropathic pain, the analgesic effects of laser therapy may be due to the local release of neurotransmitters such as serotonin [27], increased mitochondrial ATP production [28], increased release of endorphins [29], or anti-inflammatory effects [30].

The present study was aimed to evaluate the effectiveness of a high power, dual wavelenght NIR laser source in inducing fast analgesic effect in a model of neuropathic pain induced through loose ligation of the sciatic nerve.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, Varese, Italy), weighing 200–250g at the beginning of the experimental procedure, were used for all the experiments. Animals were housed in the Centro Stabulazione Animali da Laboratorio, University of Florence and used no earlier than 1 week after their arrival. Four rats were housed per cage (size 26×41cm); animals were fed with standard laboratory diet and tap water ad libitum, and kept at 23±1°C with a 12-hour light/dark cycle, light at 7am. All animal manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive of 24 November 1986; 86/609/EEC). The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the described experiments was obtained from the Animal Subjects Review Board of the University of Florence. All efforts were made to minimize animal suffering and to reduce the number of animals used

Induction of peripheral mononeuropathy by CCI

Neuropathy was induced according to the procedure described in [31]. Briefly, rats were anaesthetized with 2% isoflurane. Under aseptic conditions, the right (ipsilateral) common sciatic nerve was exposed at the level of the middle thigh by blunt dissection. Proximal to the trifurcation, the nerve was carefully freed from the surrounding connective tissue, and 4 chromic catgut ligatures (4-0, Ethicon, Norderstedt, Germany) were tied loosely around the nerve with about 1-mm spacing between ligatures. After hemostasis was confirmed, the incision was closed in layers. The animals were allowed to recover from surgery and then housed one per cage with free access to water and standard laboratory chow. Another group of rats were subjected to sham surgery in which the sciatic nerve was only exposed but not ligated.

Laser treatments

Treatments have been performed with a Multiwave Locked System laser (MLS laser, ASA Srl, Vicenza, Italy). It is a commercially available laser source built in compliance with EC/EU rules, which received FDA clearance and is widely used in clinics. MLS laser is a class IV, NIR laser with two synchronized sources (laser diodes). The two modules have different wavelengths, peak power and emission mode. The first one is a pulsed laser diode, emitting at 905 nm, with 25 W peak optical power; each pulse is composed of a pulse train (100 ns single pulse width, 90kHz maximum frequency). The frequency of the pulse trains may be varied in the range 1-2000 Hz, thus varying the average power delivered to the tissue. The second laser diode (808 nm) may operate in continuous (power 1 W) or frequenced (repetition rate 1-2000 Hz) mode, 500 mW mean optical power output, duty ratio 50% independently of the repetition rate. The two laser beams work simultaneously, synchronously and the propagation axes are coincident.

14 days after the sciatic nerve ligation, animals were randomly distributed into three groups containing 4 animals each:

- group 1 (control), animals not exposed to laser treatment;
- group 2 (laser treated group I), animals exposed to laser radiation. The treatment was performed daily, every 24 h, for 5 consecutive days and it consisted in the irradiation of two points, one located in the region where the ligature was performed and the other at level of the paw joint. Each point was treated for 23s with the following laser parameters: 10 Hz, 25% intensity, 1 J/cm^2, 3.171 J/tot.
- group 3 (laser treated group II), the treatment was very similar to that administered to the group I, but the animals of the group II were treated every 48 h. Also in this case the treatment was repeated 5 times.

Paw pressure test was performed before laser treatment and 5 min, 30 min, 1 h, 3 h from treatment, after. All the treatments have been performed by a single experienced operator.

All control animals were submitted to the same procedures and handling used for laser-exposed animals.

Paw pressure test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by [32]. Briefly, a constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical mechanical probe. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while rats were lightly restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. Rats scoring below 40 g or over 75 g during the test before starting the experiment (25%) were rejected. An arbitrary cut-off value of 100 g was adopted. The data were collected by an observer who was blinded to the protocol.

Statistical analyses

Behavioral measurements were performed on 5 rats for each treatment carried out in 2 different experimental sets. Measurements were taken in duplicate at least 1 min apart, the responses of both left and right paws were measured. For behavioural experiments One-Way analysis of variance (ANOVA) followed by Fisher's protected least significant difference procedure were used.

Data were analyzed using the "Origin 9.0" software (OriginLab, Northampton, MA, USA). Differences were considered significant at a P<0.05.

RESULTS

CCI induces a mononeuropathy characterized by hyperalgesia that appears about 3 days after nerve injury and reaches a plateau from 7 up to 30 days. Laser treatment started 14 days after surgery and, in the first protocol adopted, its application was performed once a day for 5 consecutive days. The nociceptive threshold was measured by Paw Pressure test before and after (5 min, 30 min, 1 h, 3 h) laser application. In Figure 1, the effect of laser treatment 30 min after its application was shown. On day 1, before laser treatment, rats underwent CCI showed a nociceptive threshold significantly reduced, tolerating 43.1 ± 1.1 g on the ipsilateral paw in comparison to the contralateral (nonoperated) paw (68.0 ± 1.8 g). Laser treatment significantly reduced hypersensitivity starting 5 min after application (data not shown) reaching a peak at 30 min (Figure 1; day 1, 57.5 ± 2.4 g). The effect terminated at 60 min. The anti-hyperalgesic effect was no longer recorded after 24 h (day 2, ipsilateral paw before treatment) but a new administration was able to induce an anti-hypersensitive effect comparable to that obtained the previous day. Similar effects were observed also on days 3, 4 and 5 of laser treatment (Fig. 1). The nociceptive threshold of the contralateral paw did not changed during the entire duration of the experiment (not shown).

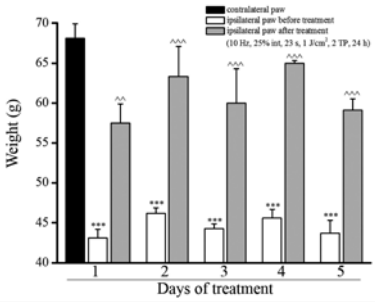

Figure 1. Pain: noxious stimulus, Paw pressure test. Repeated applications of laser (10 Hz, 23s, 25% int, 1 J/cm², 3.171 Jtot, 2 treated points, 24 h) inhibit pain behaviours induced by Chronic Constriction Injury (CCI) in the rat. Peripheral mononeuropathy was induced by CCI of the right sciatic nerve (ipsilateral). Laser treatment was performed daily for 5 consecutive times starting 14 days after surgery. Behavioural measurements were conducted before and after (5 min, 30 min, 1 h and 3 h) laser treatment. The values of the ipsilateral paw before and 30 min after laser application were shown. Each value represents the mean ± sem of 5 rats per group. ***P<0.001 vs the contralateral paw; ^^P<0.01 and ^^^P<0.001 vs the ipsilateral paw before treatments.

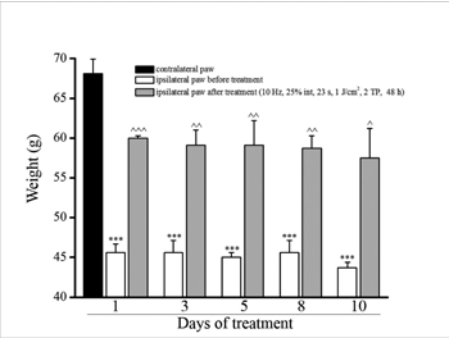

Figure 2. Pain: noxious stimulus, Paw pressure test. Repeated applications of laser (10 Hz, 23s, 25% int, 1 J/cm², 3.171 Jtot, 2 treated points, 48 h) inhibit pain behaviours induced by Chronic Constriction Injury (CCI) in the rat. Peripheral mononeuropathy was induced by CCI of the right sciatic nerve (ipsilateral). Laser treatment was performed every 48h for 5 times starting 14 days after surgery. Behavioural measurements were conducted before and after (5 min, 30 min, 1h and 3h) laser treatment. The values of the ipsilateral paw before and 30 min after laser application were shown. Each value represents the mean ± sem of 5 rats per group. ***P<0.001 vs the contralateral paw; ^P<0.05, ^^P<0.01 and ^^^P<0.001 vs the ipsilateral paw before treatments.

In Figure 2 is depicted the effect of laser treatments performed every 48 h. The paw hypersensitivity of CCI animals (ipsilateral, 45.6 ± 1.1 g vs contralateral, 68.1 ± 1.8 g), recorded on day 1 before laser treatment, was significantly increased 30 min after laser application, reaching a value of 60.0 ± 0.3 g in the rat Paw pressure test (Figure 2). The effect onset was 5 min after application and vanished at 60 min (data not shown). Forty-eight h after, the CCI-dependent pain threshold alteration (45.6 ± 1.5 g) was newly improved by the 2nd laser application (59.1 ± 1.9 g, day 3) for 30 min. The efficacy of laser treatments was verified on days 5, 8 and 10.

DISCUSSION

The present data show the anti-hypersensitivity effect of two different laser treatments in a rat model of peripheral neuropathy induced by sciatic nerve ligation (CCI). CCI induces a damage characterized by pain sensation correlated with evident tissue alterations [33,34]. Fourteen days after the unilateral loose ligation of the sciatic nerve, which

is one of the most frequently used model for the study of neuropathic pain and its treatments [35,36], rats showed a high degree of hyperalgesia against mechanical stimulus (Paw Pressure test). Laser treatments were performed daily (first protocol adopted) and every 48 hours (second protocol adopted) with a total of 5 applications for treatments. Both protocols used were able to statistically increase the pain threshold of the ipsilateral paw starting 5 min after their first applications. The anti-hyperalgesic effect of laser treatments terminated 60 min after application and started again, showing the same anti-hypersensitivity profile, during the subsequent applications. Accordingly, a recent work evaluated the effect of 660 and 980 nm low level laser therapy on neuropathic pain relief in the same model of CCI in the rat. Laser application (energy dose 4J/cm^2), started the first day after surgery and continued for 2 weeks, increased significantly thermal and mechanical threshold. In particular, the laser radiation with 660 nm wavelength had better therapeutic effects with respect to the laser radiation with 980 nm wavelength [37]. However, the efficacy of 980 nm laser therapy was also demonstrated in CCI-induced neuropathy by Jameie et al.[38]. Moreover, these authors highlighted a synergistic effect between the simultaneous use of laser and CoQ10 on pain relief [38]. The reduction of oxidative damages has been postulated as one of the main mechanisms following the exposure to laser therapy, which induces an increase in SOD activity, thus leading to decrease tissue damages and promote the healing process [39,41].

The efficacy of 660 nm GaAlAs laser at energy dose of 9 J/cm^2 in significantly reducing CCI-allodynia was demonstrated by Hsieh et al. [42], who found decreased levels of HIF-1α and IL-1β in treated animals and hypothesized an antinflammatory effect of the therapy. It is noteworthy that previous research demonstrated the ability of the laser source used in the present study to increase the expression of NLRP10 [43], a protein which inhibits the inflammasoma decreasing the IL-1β production. The effectiveness of laser therapy in improving neuropatic pain symptoms was further demonstrated by [44], applying a low power laser with 830 nm laser and energy density 4J/cm^2 to an experimental model of sciatica in rats. Clinical studies on carpal tunnel syndrome also showed significant improvement in pain and nerve conduction in patients undergoing low-level laser therapy over the carpal tunnel area [45,46].

CONCLUSION

The results of this study demonstrate that the application of a high power, dual wavelength (808 nm + 905 nm), NIR laser in a model of neuropathic pain induced a statistically significant increase in the pain threshold. The anti-hyperalgesic effect of laser treatment occurred immediately after treatment and terminated 60 min after application. Subsequent applications were able to reproduce the effect, showing the same anti-hypersensitivity profile. The effectiveness of red-NIR low power laser radiation in reducing neuropathic pain was demonstrated by several studies. It is noteworthy that in the present study, where a high power, dual wavelength NIR laser has been used, the pain relief has been obtained with very short exposure time (23 sec) and lower energy density (1J/cm^2) in comparison with that applied in other studies, ranging from 3 to 9 J/cm^2. The treatment protocol tested in this study might be applied to give the patient an immediate pain relief and might be combined with treatments aimed at reducing the inflammatory component to have an analgesic effect that lasts over time.

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