The Mechanistic Basis for Photobiomodulation Therapy of Neuropathic Pain by Near Infrared Laser Light

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Background and Objective: Various irradiances have been reported to be beneficial for the treatment of neuropathic pain with near infrared light. However, the mechanistic basis for the beneficial outcomes may vary based on the level of irradiance or fluence rate used. Using *in vivo* and *in vitro* experimental models, this study determined the mechanistic basis of photobiomodulation therapy (PBMT) for the treatment of neuropathic pain using a high irradiance.

Study Design/Materials and Methods: In vitro experiments: Cultured, rat DRG were randomly assigned to control or laser treatment (LT) groups with different irradiation times (2, 5, 30, 60, or 120 seconds). The laser parameters were: output power = 960 mW, irradiance = 300 mW/cm^2, 808 nm wavelength, and spot size = 3 cm diameter/area = 7.07cm^2, with different fluences according to irradiation times. Mitochondrial metabolic activity was measured with the MTS assay. The DRG neurons were immunostained using a primary antibody to β-Tubulin III. In vivo experiments: spared nerve injury surgery (SNI), an animal model of persistent peripheral neuropathic pain, was used. The injured rats were randomly divided into three groups ($n = 5$). (i) Control: SNI without LT; (ii) Short term: SNI with LT on day 7 and euthanized on day 7; (iii) Long term: SNI with LT on day 7 and euthanized on day 22. An 808 nm wavelength laser was used for all treatment groups. Treatment was performed once on day 7 post-surgery. The transcutaneous treatment parameters were: output power: 10 W, fluence rate: 270 mW/cm^2, treatment time: 120 seconds. The laser probe was moved along the course of the sciatic/sural nerve during the treatment. Within 1 hour of irradiation, behavior tests were performed to assess its immediate effect on sensory allodynia and hyperalgesia caused by SNI.

Results: In vitro experiments: Mitochondrial metabolism was significantly lower compared to controls for all LT groups. Varicosities and undulations formed in neurites of DRG neurons with a cell body diameter 30 μm or less. In neurites of DRG neurons with a cell body diameter of greater than 30 μm, varicosities formed only in the 120 seconds group. In vivo experiments: For heat hyperalgesia, there was a statistically significant reduction in sensitivity to the heat stimulus compared to the measurements done on day 7 prior to LT. A decrease in the sensitivity to the heat stimulus was found in the LT groups compared to the control group on days 15 and 21. For cold allodynia and mechanical hyperalgesia, a significant decrease in sensitivity to cold and pin prick was found within 1 hour after LT. Sensitivity to these stimuli returned to the control levels after 5 days post-LT. No significant difference was found in mechanical allodynia between control and LT groups for all time points examined.

Conclusion: These *in vitro* and *in vivo* studies indicate that treatment with an irradiance/fluence rate at 270 mW/cm^2 or higher at the level of the nerve can rapidly block pain transmission. A combination therapy is proposed to treat neuropathic pain with initial high irradiance/fluence rates for fast pain relief, followed by low irradiance/fluence rates for prolonged pain relief by altering chronic inflammation. Lasers Surg. Med.

Key words: dorsal root ganglion; fluence rate; laser irradiation; photoneuromodulation; transient neuronal injury

INTRODUCTION

Neuropathic pain is a common, debilitating disorder with a complex etiology [1]. Although a number of pharmacologic agents have been used to treat neuropathic

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pain, reported outcomes have been poor with less than half of the patients reporting satisfactory relief of their symptoms [2]. Photobiomodulation therapy (PBMT), previously referred to as low level laser therapy, has been used to decrease various types of neuropathic pain in preclinical animal models and in randomized controlled clinical trials. PBMT has been reported to reduce pain and improve function in compressive neuropathies such as carpal tunnel syndrome [3]. Also, systematic reviews and meta-analysis of randomized controlled trials found that PBMT reduced acute and chronic neck pain [4,5].

Since PBMT can act in part by causing an anti-inflammatory effect in the target tissue [6–9], it has promise as an effective treatment for neuropathic pain associated with inflammation. Considering the involvement of the central nervous system in pain, it is important to note that photobiomodulation (PBM) can alter microglial phenotypes from pro- to anti-inflammatory across the M1/M2 spectrum in a dose-dependent manner [10]. A number of pre-clinical animal studies investigated the effect of PBMT on inflammatory markers in neuropathic pain models. Hsieh et al. [11] reported a decrease in pro-inflammatory markers (tumor necrosis factor [TNF], interleukin 1 beta [IL-1β], and hypoxia-inducible factor 1-alpha [HIF-1α]) at a chronic constriction injury site in the rat sciatic nerve in PBMT treated animals compared to non-treated-injured animals. Cidral-Filho et al. [12], using a mouse sciatic nerve crush model, reported that PBMT reduced mechanical hypersensitivity and decrease spinal cord and sciatic nerve levels of TNFα. Recently, our laboratory reported that PBMT effectively reduced mechanical hypersensitivity in a spared nerve injury preclinical model of neuropathic pain and modulated macrophage/microglial activation to an anti-inflammatory phenotype [13]. Both studies provide evidence that PBMT is effective for treating neuropathic pain by altering the inflammatory response. In these studies, the fluences (energy densities) were 9 J/cm^2 [11], 2.5 J/cm^2 [12], and 8 J/cm^2 [13] and the irradiances (power densities] were 150 mW/cm^2 at the skin surface [11], 80 mW/cm^2 at the skin surface [12], and 43.25 mW/cm^2 at the target tissue [13].

In contrast, the pioneering work of Dr. Chow et al. on the clinical efficacy of PBMT for neck pain [14] and the mechanistic basis of pain suppression [15] used irradiances of 670 mW/cm^2 at the skin surface for the clinical study [14] and 300 mW/cm^2 at the cell surface for the *in vitro* experiments [15] at a wavelength of 830 nm. A systematic review on the inhibitory effects of laser irradiation on peripheral mammalian nerves and analgesic effects examined 44 studies: 18 human studies and 26 animal studies [16]. Although inconsistently reported, irradiances that suppressed conduction velocity and/or reduced the amplitude of the action potentials ranged from 300 mW/cm^2 to 1.73 W/cm^2 in the human studies. One important conclusion of this systematic review was that the inhibition of nerve conduction requires comparatively high therapeutic doses [16].

Recently, we completed a pilot, clinical study for treatment of low back pain that compared three treatment modalities: lidocaine injection, radiofrequency, or PBMT with 808 nm wavelength light with high irradiance and fluence (measured at the tip of the fiber optic). The data showed that PBMT applied bilaterally to the dorsal root ganglia (DRG) of the second lumbar spinal nerves decreased low back pain within 5 minutes which was comparable to lidocaine injection [17]. These results lead to the development of this combined *in vitro* and pre-clinical animal study to better understand the mechanistic basis underlying the photoneuromodulation of the neuropathic low back pain at high irradiances. The data from this study and our previous experiments on nerve regeneration and pain suppression serve as the basis for discussion of mechanisms of action for pain suppression based on irradiance.

METHODS

In Vitro Experiments

Cell culture. Primary rat dorsal root ganglion neurons (Lonza Walkersville, Inc. Walkersville, MD) were seeded in Poly-D-Lysine/Laminin (30 μg/ml poly-D-lysine and 2 μg/ml laminin) coated 4-well chamber slides with a seeding density of 2×10^4 cell/well in primary neural growth medium (PNGM) according to manufacturer's instructions. The cells were incubated at 37°C, 5% CO_2 for 48 hours.

Laser irradiation. The laser device used for irradiation of the cells was a CW, 808 nm wavelength diode laser with adjustable output power up to 2 W. Based on our previous clinical pilot study on low back pain in which a high irradiance was delivered to the DRG [17] and a previous *in vitro* study on DRG neurons, in which 300 mW/cm^2 at different treatment times caused varicosity formation and slowing of nerve conduction in the small DRG neurons [15], an irradiance of 300 mW/cm^2 was used in this study. Measurements were made to determine the output power that was needed to deliver 300 mW/cm^2 to the cells (LabMaster Ultima power meter with LM-3 HTD sensor, Coherent, Inc., Santa Clara, CA). After incubating for 48 hours, the cultures were randomly assigned to either control or laser treatment (LT) groups (irradiation times = 2, 5, 30, 60, or 120 seconds). The laser parameters were: output power = 960 mW, irradiance = 300 mW/cm^2, 808 nm wavelength, and spot size: 3 cm diameter/area = 7.07 cm^2.

MTS assay. MTS assay (Promega, Madison, WI) was used to measure the metabolic activity in the living cells. This assay is based on the reduction of tetrazolium salts into formazan, which can be measured colorimetrically. The conversion is presumably accomplished by reductase enzymes in mitochondria. Forty minutes after laser irradiation, MTS solution was added to each well. Forty minutes were chosen based on reports in the literature and previous work in our laboratory that identified that the peak time post-irradiation for change in mitochondrial metabolism was 40 minutes [18]. After incubation for 1.5 hour at 37°C, the supernatant was read for absorbance at 485 nm using FLUOstar OPTIMA plate reader (BMG Labtech, Inc., Cary, NC). Blank controls were medium

alone with MTS solution. Each light parameter setting was measured in triplicate. This experiment has been repeated twice and the data were combined.

Immunocytochemistry of β-tubulin III. Cells were fixed with 4% paraformaldehyde and then blocked in PBS with 10% goat serum and 0.1% Triton for 30 min at room temperature (RT). Cells were then incubated with Mouse anti-β-Tubulin III antibody (1:75 in PBS with 1% goat serum, Sigma, St. Louis, MO) for 1 hour at RT, followed by incubation with secondary antibody (AlexaFluor488 Goat anti-mouse IgG, 2.5 μg/ml in PBS with 1% goat serum, Life Technologies, Grand Island, NY) for 30 min at RT. After washing with PBS, samples were coverslipped with Vectashield mounting medium with DAPI (Vectors Laboratories, Inc. Burlingame, CA) and sealed with nail polish. The cells were photographed digitally using an Olympus BX43 fluorescence microscope equipped with an Olympus DP72 microscope digital camera (Olympus Imaging America, Inc. Center Valley, PA). The diameters of cell body were measured using Olympus CellSens software (Olympus Imaging America, Inc.). A minimum of 100 cells from each treatment and control groups was measured.

In Vivo Experiments

Animals. The animal use protocol (APG-14-808) was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Use Committee. Sixteen male Sprague–Dawley (SD) rats (201–225 gram, Charles River Laboratories International, Inc., Wilmington, MA) were used in this study. One rat was used for power penetration measurements and the other 15 rats were divided into three groups ($n = 5$) as follows: (i) Control: surgery without LT; (ii) Short term: surgery with LT on day 7 and euthanized on the same day after behavior test; (iii) Long term: surgery with LT on day 7 and euthanized on day 22. Animals were housed two per cage, under a 12-hour light/dark cycle, with access to food and water *ad libitum*.

Power penetration measurement. One male SD rat was anesthetized for light penetration measurement. The fluence rate was measured by a near infrared detector which was designed and built by B&W Tek, Inc. (Newark, DE). A small photo sensor ($2.0 \times 2.5\,mm^2$) was sealed in a glass tube. The output voltage of this sensor was calibrated such that a reading of 1 mV represented $1\,mW/cm^2$. The sensor was placed below the lumbar 4 and 5 DRGs. An 808 nm wavelength laser (Model BWF5-808-20, B&W Tek, Inc.), connected with a probe that had a rolling ball with an irradiation diameter of 4 cm, was placed in direct contact with the skin surface. Light penetration was measured for output powers of 3, 5, and 10 W (which was the maximum output power of this laser). The probe was moved until the highest reading was identified and recorded.

Surgery. Spared nerve injury (SNI) surgery, an animal model for peripheral neuropathic pain, was performed on all rats [19]. Briefly, rats were anesthetized with isoflurane (5% for induction and 0.5–3% for maintenance). An incision was made on the lateral left thigh and the bicep femoris was separated to expose the sciatic nerve and its branches. The common peroneal and tibial nerves were tight-ligated and 3–4 mm of each nerve (distal to the ligation) were cut and removed. Great care was taken to avoid any contact with the intact sural nerve. The muscle and skin were then sutured in two layers.

Laser irradiation. An 808 nm wavelength laser (Model BWF5-808-20, B&W Tek, Inc.) was used for both the long and short term treatment groups. Transcutaneous laser treatment was performed once on day 7 post-surgery with parameters: output power: 10 W, treatment time: 120 seconds. The probe had a small massage ball (circular area with a diameter of 4 cm) and was scanned along the nerve track during the 2 minutes of treatment from the thoracic 13 and lumbar 1 (T13/L1) spinal cord level to the lumbar 4 and 5 (L4/L5) DRG, the sciatic nerve, the sural nerve, and the involved dermatomes on the lateral plantar surface of the hind paw (Fig. 1). The irradiance rate was $270\,mW/cm^2$ at the DRG L4/L5 region according to the power penetration measurements. The rats were lightly anesthetized with isoflurane for the LT. The rats in the control group were handled in exactly the same manner as the irradiated rats but the laser was off. Within 1 hour of treatment, behavior tests were performed to assess the immediate effects of the laser treatment on sensory allodynia and hyperalgesia caused by the SNI model [20].

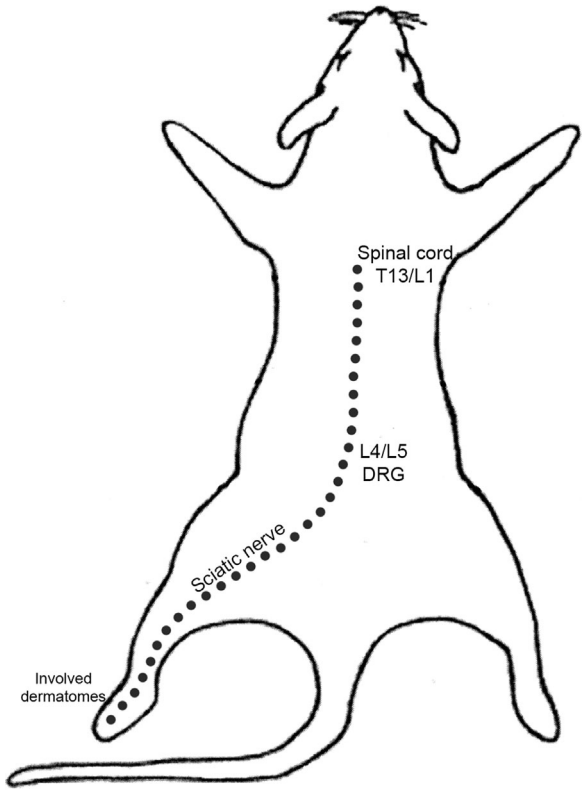

Fig. 1. Illustration of *in vivo* laser scanning pathway and primary targets.

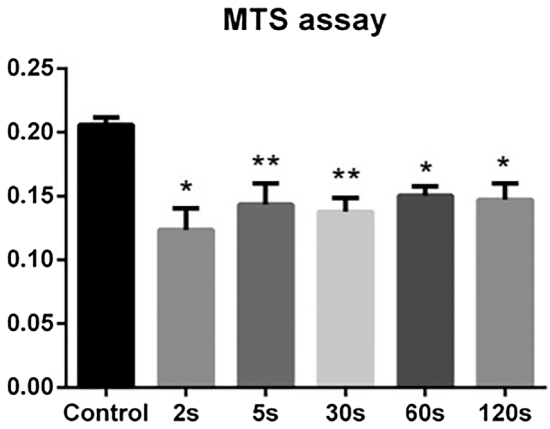

Fig. 2. Statistically significant reduction of mitochondrial metabolism as measured by MTS assay. $^{*}P < 0.005$, $^{**}P < 0.01$ compared to control group.

Behavior tests. Behavior tests included Heat Hyperalgesia, Cold Allodynia, Mechanical Hyperalgesia (Pin Prick), and Mechanical Allodynia (Electronic Von Frey). The animals were placed in an inverted plastic box on an elevated metal grid to allow for stimulation on the lateral plantar surface of the hind paw. Tests were performed before surgery (baseline), day 7 before LT and 1 hour after LT and on days 10, 12, 15, 18, and 21. The behavior tests done on day 7 post-irradiation were done at 1 hour after PBMT because the animals had been anesthetized and needed this amount of time to completely wake up. The behavior data from day 7 before and after LT represents the immediate effects from the laser irradiation. For long term effects, data from long term group were compared with the control group.

Heat hyperalgesia. The test used to measure heat hyperalgesia was modified from Hargreaves et al. [20]. The thermal stimulation was generated by a beam of radiant heat using an 808 nm wavelength laser with a 2 W output power and 3 mm diameter. Animals were placed on the elevated grid and acclimated for 5 min. The laser beam was positioned on the lateral plantar surface of the hind paw which is the region innervated by the intact sural nerve. The time when the hind paw was briskly withdrawn was recorded with a maximum cut-off time of 10 seconds. Both left (injured) and right (uninjured) sides were tested. The ratio was calculated as response time of left side divided by the response time of the right site. Rats were

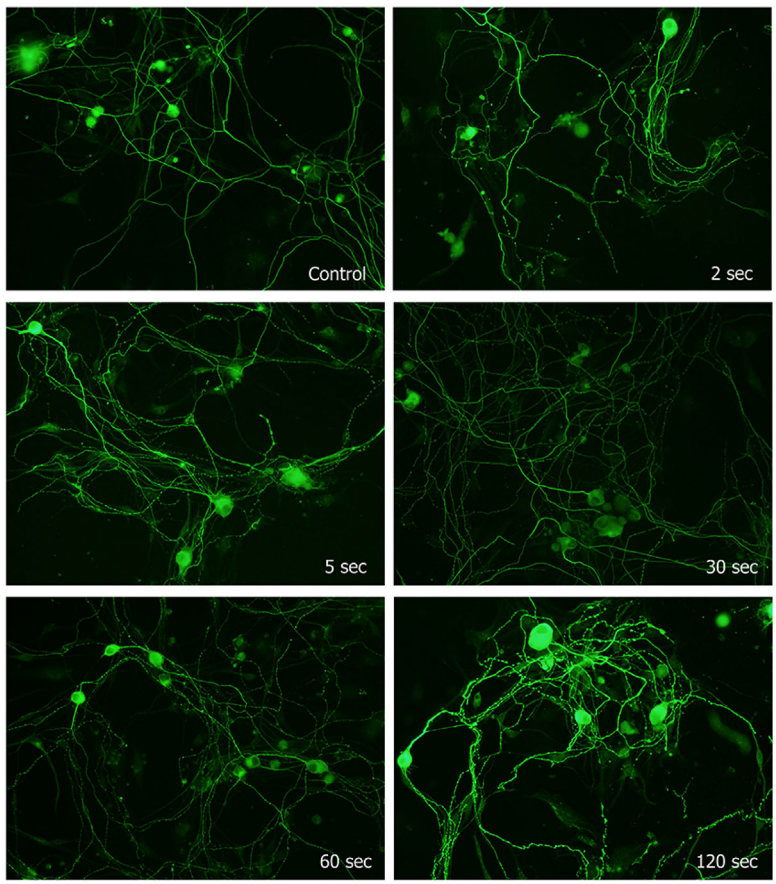

Fig. 3. Photomicrographs of control and LT DRG neurons immune-labeled with β-Tubulin III. Varicosities and undulations were present in LT groups.

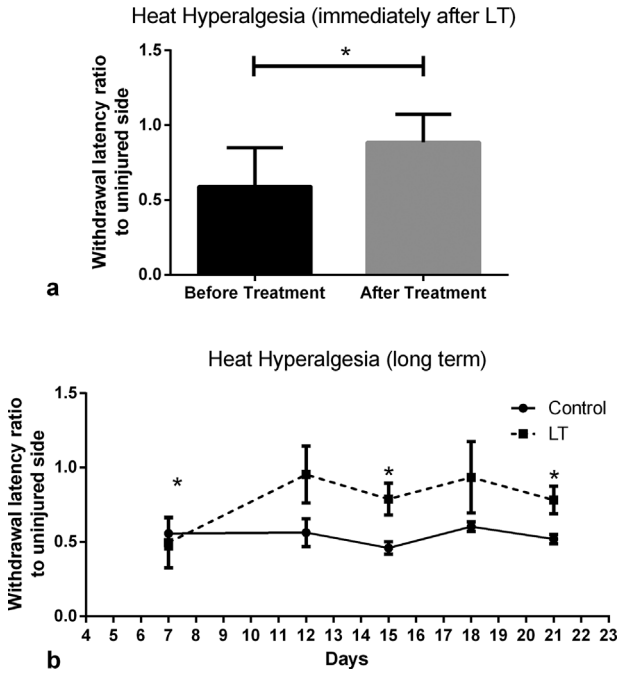

Fig. 4. Heat hyperalgesia. There was a decrease in sensitivity to the heat stimulus ($P < 0.05$) within 1 hour after irradiation (**a**). For the long term, a decrease was found in the LT group ($P < 0.05$) on days 5 and 21 (**b**).

tested three times with a 5-minute interval between each test.

Cold allodynia. After 5 min of acclimation on the elevated grid, cold acetone (20 μl, −20°C) was sprayed on the lateral plantar surface of the hind paw. The duration of the withdrawal response to the cold stimulation was recorded and graded in five levels: 0, no visible response; 1, startle response without paw withdrawal; 2, clear withdrawal of the paw; 3, prolonged withdrawal (2–30 seconds) often combined with flinching and licking of the paw; and 4, prolonged, repetitive withdrawal (30 seconds) and/or vocalization.

Mechanical hyperalgesia (pin prick). Pin prick test was performed using a safety pin after 5 min acclimation of the elevated grid [13]. A brief stimulation was applied to the lateral part of the plantar surface of the hind paw. The duration of paw withdrawal was recorded. An arbitrary minimal time of 0.5 second was used as normal response time. The maximum cut-off time was 15 seconds.

Mechanical allodynia (electronic von frey). SNI animals developed hypersensitivity to mechanical stimulation, which was measured using an electronic von Frey (Bioseb, Chaville, France) device [21,22]. The hand-held force transducer of the device can generate force from 0 to 500 g in 0.1 g intervals. After an acclimation period of 15 min, the plastic tip of the transducer was applied perpendicularly to the lateral plantar surface of the hind paw. The lowest force at which a brisk withdraw of the hind paw was recorded. The test was repeated three times with a 5-minute interval between each measurement, and the mean of the three measurements was computed for analysis.

Statistical Analysis for *In Vivo* and *In Vitro* Experiments

For the MTS assays, one way ANOVA with Tukey's multiple comparisons test were used to compare the effects between groups. For behavior data, results were presented as mean + standard error of the mean (SEM). Unpaired two-tailed t-test was used to compare the immediate effects between before and after irradiation. For long-term behavior data, Two-way ANOVA with Sidak's multiple comparisons test was used to compare control and long-term LT groups. Family-wise significance and confidence levels were set at 0.05 (95% confidence interval).

RESULTS

In Vitro Experiments

All the LT groups with treatment times of 2, 5, 30, 60, and 120 seconds had statistically significant lower mitochondrial metabolism compared to controls (Fig. 2). There was no difference in the metabolic inhibition for all irradiation times tested. This finding is due to the fact that 40 minutes post irradiation was previously identified as the peak time post-irradiation for change in mitochondrial metabolism [18].

In all LT groups, varicosities, and undulations were present in neurites of DRG neurons with a cell body diameter of 30 μm

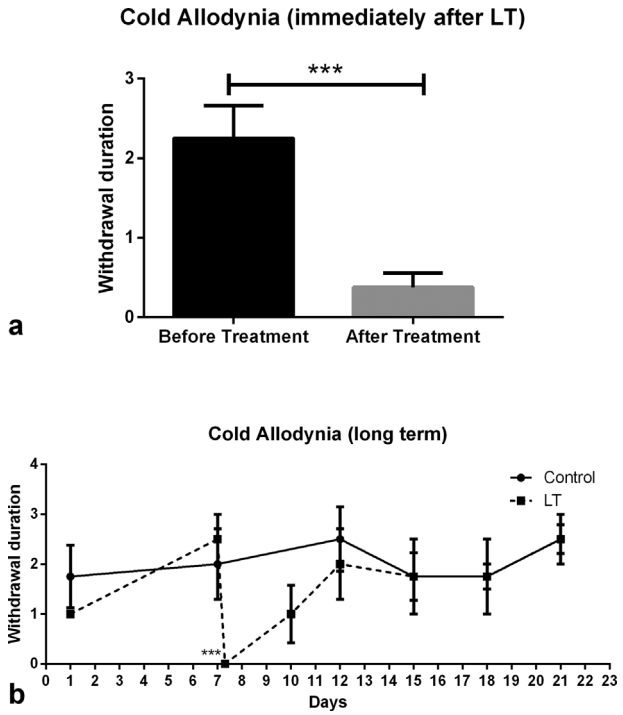

Fig. 5. Cold allodynia. A decrease in sensitivity to the cold stimulus was found within 1 hour post-LT ($P < 0.001$, **a**). The sensitivity to the cold stimulus returned to the control levels after 5 days post-LT (day 12) (**b**).

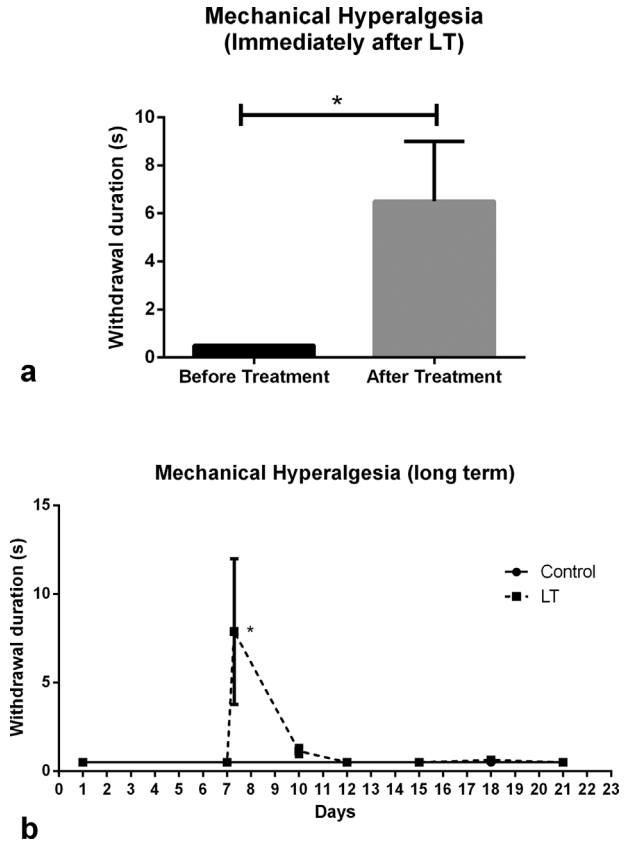

Fig. 6. Mechanical hyperalgesia. A significant decrease in sensitivity to the pin prick stimulus ($P < 0.05$) occurred within 1 hour after LT (**a**) and returned to the control level after 5 days post-LT on day 12(**b**).

or less compared to the control group (Fig. 3). These neurons are associated with C and Aδ fiber types which *in vivo* are the unmyelinated and lightly myelinated axons conveying pain and temperature sensory information. The neurites of DRG neurons with a cell body diameter $\geq 30\,\mu m$ began to form varicosities only in the 120 seconds group (Figure 3). Neurons of this size are associated with Aα and Aβ fiber types, which *in vivo* are myelinated and convey proprioceptive, two-point tactile and vibration sensory information. It is critical to remember that in our culture model there are no Schwann cells and therefore no myelination of the neurites. Therefore, the response of the neurons to the laser irradiation is not related to the degree of myelination.

In Vivo Experiments

Power measurement. For an output power of 3, 5, and 10 W, the fluence rate was measured as 80, 165, and 270 mW/cm^2, respectively. The fluence rate of 270 mW/cm^2 was the closest power density to the target 300 mW/cm^2 which was used in the *in vitro* experiments. Therefore, an output power of 10 W was chosen for *in vivo* laser irradiation experiments.

Behavior tests. For all hyperalgesia and allodynia behavior tests done, there was no significant difference found between control and experimental groups on day 7 post-surgery prior to the laser treatments. On day 7 within 1 hour after irradiation, the involved area of the lateral plantar surface of the hind paw was tested.

For heat hyperalgesia, there was a statistically significant decrease in sensitivity to the heat stimulus ($P < 0.05$) compared to the measurements done on day 7 prior to the laser treatment (Fig. 4a) within 1 hour after irradiation. For the long term measurements, a decrease in the sensitivity to the heat stimulus was found in the LT group compared to the control group ($P < 0.05$) on days 15 and 21 (Fig. 4b). The lack of a significant difference on days 12 and 18 was due to the greater variability in the standard error of the means. A greater number of animals in each group would likely result in decreased heat hyperalgesia for all time points. For cold allodynia, a significant decrease in sensitivity to the cold stimulus was found within 1 hour post-LT ($P < 0.001$, Fig. 5a). The sensitivity to the cold stimulus returned to the control levels after 5 days post-LT (day 12) (Fig. 5b). For mechanical hyperalgesia, a significant decrease in sensitivity to the pin prick stimulus ($P < 0.05$) occurred within 1 hour after LT (Fig. 6a) and also returned to the control level after 5 days post-LT on day 12 (Fig. 6b). For mechanical allodynia using electronic Von Frey test, no significant difference was found (Fig. 7a and b) between control and LT groups for all time points examined post-laser treatment.

DISCUSSION

In the present study, *in vitro* and pre-clinical animal experiments explored the mechanistic basis underlying

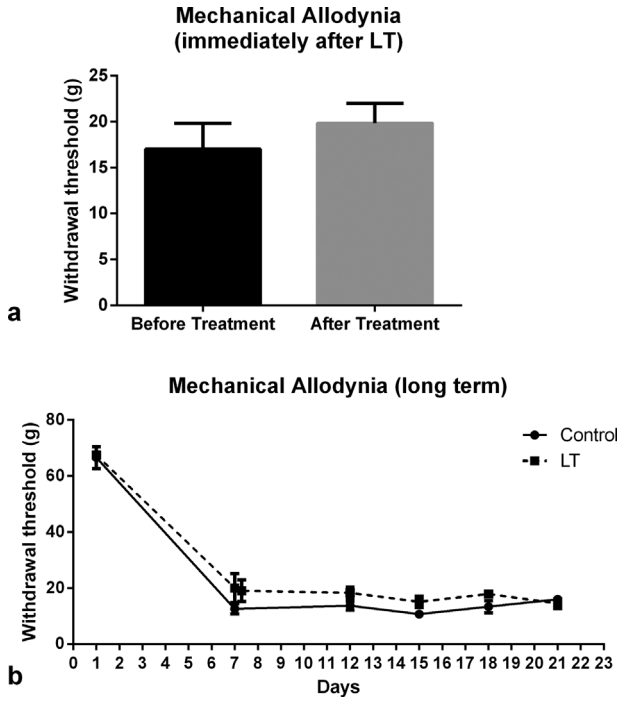

Fig. 7. Mechanical allodynia. No significant difference was found (**a** and **b**).

photoneuromodulation of neuropathic pain at an irradiance of 300 and 270 mW/cm^2. Chow et al. [15] reported that laser irradiation using a CW 830 nm wavelength laser with an irradiance of 300 mW/cm^2 induced axonal varicosities in DRG small and medium neurons which represent the type C and Aδ fibers related to pain, temperature, and light touch perception. Chow et al. [11] hypothesized that the laser irradiation blocks nociceptor-specific neurons by microtubule disruption with varicosity formation as the key morphological feature. With our treatment parameters, varicosities, and undulations formed in *in vitro* neurites of DRG neurons with a diameter of 30 μm or less at all irradiation times examined and in neurites of DRG neurons with a diameter greater than 30 μm in the 120 seconds irradiation group in contrast to Chow's study. These large neurons represent the Aα and Aβ large myelinated neurons related to the perception of proprioception, vibration, and two point tactile discrimination.

Early varicosity formation in axons have been associated with injury of the central and peripheral nervous systems [23–25] and many neurodegenerative diseases [26,27]. Interestingly, many reports suggest that varicosity formations in dendrites are neuroprotective (see the recent publication by Liebert for discussion) [28]. Besides laser irradiation, a number of chemical and physical agents including anesthetics have been reported to cause varicosity formation in neurons of various sizes [29–31]. Varicosities form when there is breakage of the microtubules [23]. This cytoskeletal disruption affects axonal flow and mitochondrial function [15,28], impairs nerve conduction [32], and signal transduction [33]. In the present study, mitochondrial metabolism measured using the MTS assay was significantly lower for all irradiated groups compared to controls. These data were based on the addition of the MTS solution at 40 minutes post-irradiation which has been previously identified as the peak time for change in mitochondrial metabolism [18]. Previously, Chow et al. (2007) examined mitochondrial membrane potential in DRG neurons treated with 830 nm wavelength light with an irradiance of 300 mW/cm^2 for 30 seconds. A statistically significant decrease in the mitochondrial membrane potential was found by 5 min post-irradiation and progressed over the total 30 minutes post-irradiation time [15]. In preliminary studies to determine a maximal irradiance that could block pain transmission and not cause permanent damage, the effect of 600 mW/cm^2 at irradiation times of 60 or 120 seconds was examined. This combination of parameters caused a statistically significant decrease in mitochondrial metabolic activities compared to the controls and the cells irradiated with 300 mW/cm^2 at 60 or 120 B indicating that an irradiance higher that 300 mW/cm^2 may be more effective (data not shown).

Different sizes and types of neurons have differential susceptibility to stimuli that cause axonal varicosity formation. Magdesian et al. [34] designed experiments to apply gradual nano-scale forces to compress axons of rat hippocampal or DRG neurons in a microfluidic chamber. They found that the two types of neurons undergo similar morphological changes including varicosity formation but their response differed in intensity and time. The hippocampal axons completely recovered axonal transport when compressed to pressures up to 65 ± 30 Pascal (Pa) for 10 min while the DRG axons resisted pressures up to 540 ± 220 Pa. The authors related the differences in the neuronal response to the composition of the cytoskeletal elements and thus the viscoelastic properties of the axons. They determined that the DRG axons had seven times more neurofilaments than the hippocampal axons [34]. Also, neurofilament/microtubule ratios are three times higher in the peripheral nervous system than in the central nervous system [35]. Furthermore, there are higher numbers of microtubules in unmyelinated axons compared to myelinated axons [36]. It is important to remember that in our culture model there were no Schwann cells and therefore no myelination of the neurites. Therefore, the response of the neurons to the laser irradiation was not related to the degree of myelination but may be related to differences in the neurofilament/microtubule ratios.

The primary target of the near-infrared light within neurons that results in the cytoskeletal disruption and varicosity formation has not been identified. It has been suggested that the light may be directly absorbed by proteins involved in microtubule stability/instability inducing a conformational change [28]. Another possibility is that a calcium influx occurs which is followed by the activation of calcium-dependent proteases, such as calpain, which cleave and degrade cytoplasmic proteins [37].

The present study demonstrates that transcutaneous irradiation with an output power of 10 W delivered 270 mW/cm^2 to the DRG and sciatic nerve and blocked pain and thermal transmission, but did not affect mechanical allodynia which relayed by large myelinated fibers. Laser irradiation of the rat sciatic nerve decreased small and medium sizes fiber somatosensory evoked potentials but did affect fast conducting large fibers [32].

Of clinical relevance is the duration of the effect. As demonstrated, even after 21 days, the laser treated group showed less sensitivity to the heat stimulus compared to control group. For cold allodynia and mechanical hyperalgesia, the duration of the effect was 5 days. In contrast to our results, a study on the assessment of neuropathic pain relief in a chronic constriction injury rat sciatic nerve model reported that laser treatment (980 nm wavelength, irradiance on the surface of the skin = 248 mW/cm^2, irradiation times 16 s at three sites) caused an increase in mechanical allodynia and thermal threshold at 7 and 14 days post-surgery. Unlike the present study in which irradiation was done only once, irradiation was done daily for 14 days [38].

Based on our studies on PBMT and nerve regeneration as well as neuropathic pain and a survey of the literature, we propose two methods that can be used to modulate neuropathic pain based on irradiance levels of near infrared light at the level of the target tissue. The first of these methods uses low irradiances in the range of 10–100 mW/cm^2 and causes a decrease in pain response by altering chronic inflammation [13] and decreasing mechanical

allodynia [13,39]. The mechanisms involved at these irradiance levels are the currently know and accepted mechanisms of PBM [40–42]. The second method utilizes irradiances in a range from 250 mW/cm^2 to 1.73 W/cm^2 which suppress conduction velocity and/or reduce the amplitude of the action potentials [16] and rapidly block pain transmission as demonstrated in the current preclinical animal data and human data [17]. The mechanism involved at these irradiance levels is the alteration of the neuronal microtubules as discussed above. It is important to note that to transcutaneously deliver these irradiance levels at the target tissue much high levels need to be used at the surface of the skin. We further propose that a combination therapy approach may result in the improved clinical outcomes for treating neuropathic pain. This approach would involve initial use of a high irradiance treatment to block the pain transmission followed by a series of low irradiance treatments along the course of the involved nerve to alter the chronic pathology and inflammation.

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