Lasers Med Sci
DOI 10.1007/s10103-014-1552-1

ORIGINAL ARTICLE

Effects of 660- and 980-nm low-level laser therapy on neuropathic pain relief following chronic constriction injury in rat sciatic nerve

M. Masoumipoor · S. B. Jameie · A. Janzadeh · F. Nasirinezhad · M. Soleimani · M. Kerdary

Received: 9 March 2013 / Accepted: 18 February 2014

Abstract Neuropathic pain (NP) is one of the most suffered conditions in medical disciplines. The role of reactive oxygen species (ROS) and oxidative stress in the induction of NP was studied by many researchers. Neuropathies lead to medical, social, and economic isolation of the patient, so various therapies were used to treat or reduce it. During the recent years, low-level laser therapy (LLLT) has been used in certain areas of medicine and rehabilitation. Chronic constriction injury (CCI) is a well-known model for neuropathic pain studies. In order to find the effects of different wavelengths of LLLT on the injured sciatic nerve, the present research was done. Thirty Wistar adult male rats (230–320 g) were used in this study. The animals were randomly divided into three groups (n=10). To induce neuropathic pain for the sciatic nerve, the CCI technique was used. Low-level laser of 660 and 980 nm was used for two consecutive weeks. Thermal and mechanical hyperalgesia was done before and after surgery on days 7 and 14, respectively. Paw withdrawal thresholds were also evaluated. CCI decreased the pain threshold, whereas both wavelengths of LLLT for 2 weeks increased mechanical and thermal threshold significantly. A comparison of the mechanical and thermal threshold showed a significant difference between the therapeutic effects of the two groups that received LLLT. Based on our findings, the laser with a 660-nm wavelength had better therapeutic effects than the laser with a 980-nm wavelength, so the former one may be used for clinical application in neuropathic cases; however, it needs more future studies.

Keywords Low-level laser therapy · CCI · Neuropathic pain

Introduction

Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Two clinical types of pain including acute and chronic were reported; the former one is a protective mechanism that alerts the individual to a certain condition that is immediately harmful to the body, whereas the chronic pain is persistent or intermittent. Acute pain rarely needs medical attention; when it does, nonsteroidal anti-inflammatory drugs (NSAIDs), powerful opioid analgesics, or local anesthetics can adequately control the pain. Chronic pain differs from acute pain not only in its onset and duration, but more importantly in the underlying mechanisms. Chronic pain may not have identifiable ongoing injury or inflammation and often responds poorly to NSAIDs and opioids [2]. Neuropathic pain (NP) refers to chronic pain as a result of damage (due to injury or disease) to the nervous system including nerves, the spinal cord, and other certain CNS regions [3, 4]. Patients with NP often suffer from spontaneous pain, allodynia (pain response to normally innocuous stimuli), and hyperalgesia (aggravated pain evoked by noxious stimuli). NP may have delayed onset after initial nerve injury; therefore, pain may be present in the absence of evident lesion or injury, making proper diagnosis and early treatment difficult [5, 6].

M. Masoumipoor · S. B. Jameie
Department of Nuclear Engineering, Sciences and Researches Branch, Islamic Azad University, Tehran, Iran

S. B. Jameie (✉) · A. Janzadeh · M. Kerdary
Department of Medical Basic Sciences, Faculty of Allied Medicine, IUMS, Tehran, Islamic Republic of Iran
e-mail: behjam@yahoo.com

S. B. Jameie · M. Soleimani
Department of Anatomy, Faculty of Medicine, IUMS, Tehran, Iran

A. Janzadeh · F. Nasirinezhad
Research Center of Physiology, Faculty of Medicine, IUMS, Tehran, Iran

Published online: 16 March 2014

The chronic constriction injury (CCI) model, developed by Bennett and Xie, is a model of mononeuropathy that produces signs of neuropathic pain [7]. The CCI is one of the most common models for peripheral nerve injury and carries a lot of the inflammatory characterization related to the condition/disease. It is reported that the inflammatory component of NP in the case of CCI is present mainly in the first phase of the disease (up to day 14) [8]. In order to relieve or treat chronic pain, it is needed to evaluate its presence in an individual.

Mechanical test of paw withdrawal latencies and observations of guarding behavior to certain mechanical stimuli and thermal stimulation including the tail flick test [9], the hindlimb withdrawal plantar test [10], and the hotplate test [11, 12] has been extensively used in assessing pain behavior in animals [13, 14]. In order to decrease the regenerative process and also reach to early functional recovery, certain types of therapies including prescription of analgesic drugs, electrical stimulation, ultrasound, and laser therapy were developed during the recent years [15–17].

Low-level laser therapy (LLLT) is a special type of laser therapy in which the irradiation used is red or near-infrared beams with a wavelength of 600–1100 nm and an output power of 1–500 mW. This type of radiation is a continuous wave or pulsed light that consists of a constant beam of relatively low energy density (0.04–50 J/cm^2) [18, 19]. Since the 1970s, LLLT has been used in several clinical and experimental research studies on peripheral nerve injuries. Irradiation parameters and properties of LLLT, such as dose, intensity, time, and application methods are notably varied among different clinical reports.

The clinical effects of LLLT including cell apoptosis; improved cell proliferation, migration, and cell adhesion, enhancing the cells' mitotic activity; increased blood flow; and local microcirculation were reported in many studies [20–24]. Reis et al. reported on the effectiveness of laser at 660 nm for recovery of the sciatic nerve in rat model following neurotmesis [25]. Belchior et al. also reported on the clinical and functional recovery of an injured sciatic nerve by using gallium-aluminum-arsenide (GaAlAs) laser at a wavelength of 660 nm and density of 4 J/cm^2 for 21 days consecutively. Regarding these studies, the use of low-level laser (660 nm) significantly promotes neural regeneration [26]. Barbosa et al. used laser at 660 and 830 nm for the recovery of sciatic nerve regeneration following crushing injuries, and they reported that 660 nm provided early functional nerve recovery in comparison with 830 nm [27]. Hsieh et al. demonstrated that 660-nm GaAlAs laser at a dose of 9 J/cm^2 significantly reduced neuropathic allodynia in rats with CCI and significantly promoted functional recovery [28]; their result is similar to the findings of Bertolini et al. [29] in 2011.

To our knowledge, there is a controversy on energy densities and wavelengths of LLLT for peripheral neuropathies among different studies, in addition to the lack of specific pain evaluation, i.e., hyperalgesia, leading us to design this study.

Materials and methods

Animals

All the steps of this study were done in a single-blind pattern. Thirty adult male Wistar rats (250–320 g) were used in this study, given with food and water ad libitum. The animals were divided into three groups (n=10) as follows:

- CCI group: subjected to surgical procedure, without undergoing irradiation
- Laser therapy group (660 nm): subjected to laser irradiation with energy density of 4 J/cm^2 and intensity of 0.354 W/cm^2
- Laser therapy group (980 nm): subjected to laser irradiation with energy density of 4 J/cm^2 and intensity of 0.248 W/cm^2

All the animals were subjected to the functional evaluation before surgery. To induce neuropathic pain, the sciatic nerve injury model described by Bennett and Xie (explained elsewhere [7]) was used.

Laser therapy

A couple of CW diode laser emitter with the following specification was used in this study. One is a laser with a wavelength of 660 nm, power of 100 mW (Heltschl, model ME-TL10000-SK), energy density of 4 J/cm^2, and power density of 0.354 W/cm^2; and the other one was a laser with a wavelength of 980 nm, power of 70 mW (Aixiz, model AH980-6015 AC), energy density of 4 J/cm^2, and power density of 0.248 W/cm^2. The beam area on the samples was ~0.238 cm^2. The irradiation time was 11.3 s for visible wavelength and 16.13 s for NIR one.

Before use, laser calibration was done routinely. Three points of the surgical incision were irradiated transcutaneously with no direct skin contact as follows: two points on two ends of surgical incision and another at their midpoint. The laser therapy was started on the first day after the surgery and was continued for 2 weeks daily at the same time between 10 and 12 a.m.

Functional analysis

Thermal withdrawal threshold

By using a plantar test apparatus (Ugo Basile, Italy), thermal hyperalgesia, the latency to withdrawal of the hind paws from a focused beam of radiant heat applied to the plantar surface,

was studied [10]. The animals were placed in an acrylic box with glass floor, and the plantar surface of their hind paw was exposed to a beam of infrared radiant heat. The paw withdrawal latencies were recorded at infrared intensity 50; three trials for the right hind paws were performed, and for each reading, the apparatus was set at a cutoff time of 25 s. Each trial was separated by an interval time of 5 min.

Mechanical withdrawal threshold

Mechanical paw withdrawal thresholds were assessed with the Randall–Selitto method by using an Analgesy-meter apparatus (Ugo Basile, Italy; [30]). This instrument exerts a force that increases at a constant rate. The force was applied to the hind paw of threat, which was placed on a small plinth under a cone-shaped pusher with a rounded tip (1.5 mm in diameter). The rat was held upright with the head and limb to be tested free, but most of the rest of the body cradled in the hands of the experimenter. The paw was then put under the pusher until the rat withdrew its hind paw. Each hind paw was tested twice, with a 10-min interval between the measurements, and mechanical paw withdrawal thresholds were calculated as the average of two consecutive measurements.

Statistical analysis

By using SPSS 19.0, statistical analysis was done, and the results were presented as means±SD. P_V less than 0.05 was considered to be significant.

Results

For functional evaluation of gait, we used the plantar test and the Randall–Selitto method recorded preoperatively and those recorded for 7 and 14 days. The results were as follows:

Plantar test

The thermal withdrawal threshold of the control group was, on average, 18.91±4.08 s from the data collected prior to the injury. For the CCI group, it was 12.42±4.82 and 10.70±5.02 s on the 7th and 14th days after surgery, respectively. For the LLLT 660-nm group, the mean value was 18.34±4.29 and 19.88±3.13 s on the 7th and 14th days after surgery, respectively. For the LLLT 980-nm group, the mean value was 16.13±4.11 and 14.18±3.35 s on the 7th and 14th days after surgery, respectively. There was a significance in the statistical comparison of results among the groups. There were no significant difference between the 7th and 14th postsurgery days in the LLLT 660-nm group and the control group, but there was a significant difference between the 7th and 14th postsurgery days in the LLLT 980-nm group and the control group (P<0.01, P<0.001), respectively. Also, there was significant difference between the two laser therapy groups on the 14th postsurgery day (P<0.001, Fig. 1).

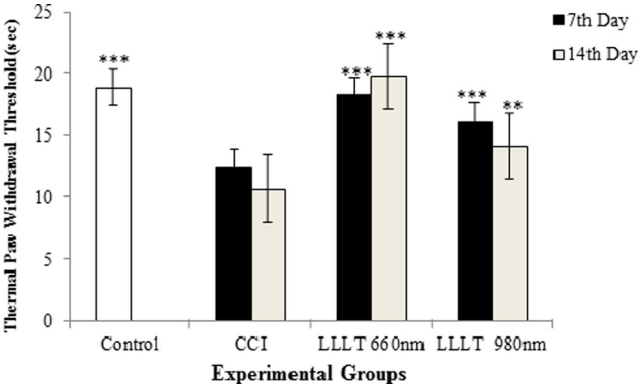

Fig. 1 Mean values of the thermal withdrawal threshold obtained from the groups during the study period (before surgery (*control*), the 7th and 14th days after surgery). *Asterisks* represent significant differences from the CCI group (***P<0.001, **P<0.01)

Randall–Selitto method

The mean of the mechanical withdrawal threshold of the control group was 19.18±4.66 g before surgery. For the CCI group, it was 10.17±4.18 and 9.15±4.20 g on the 7th and 14th days after surgery, respectively. For the LLLT 660-nm group, the mean value was 15.54±4.50 and 14.36±5.43 g on the 7th and 14th days after surgery. For the LLLT 980-nm group, the mean value was 14.15±4.25 and 12.35±5.28 g on the 7th and 14th days after surgery.

Statistical analysis indicated that the difference between the control group and CCI group on the 7th and 14th postsurgery days was significant (P<0.001); also, the difference between the LLLT 660-nm group and the CCI group on the 7th and 14th postsurgery days was significant (P<0.001); and the difference between the LLLT 980-nm group and the CCI group on the 7th and 14th postsurgery days was significant

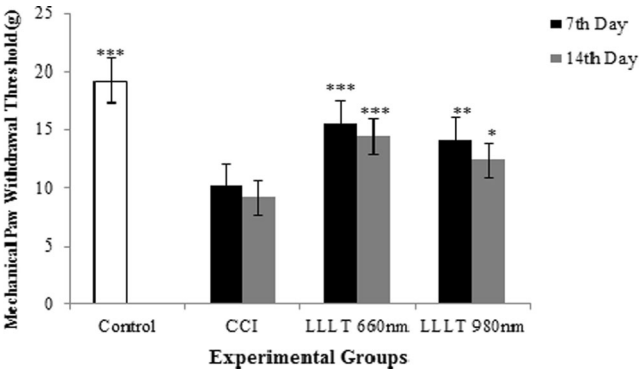

Fig. 2 Mean values of the mechanical withdrawal threshold obtained from the groups during the study period (before surgery (*control*), the 7th and 14th days after surgery). *Asterisks* represent significant differences from the CCI group (***P<0.001, **P<0.01, *P<0.05)

($P<0.01$, $P<0.05$), respectively. There was a significant difference between the 7th and 14th postsurgery days in the LLLT 660-nm group and the control group ($P<0.01$, $P<0.001$), respectively; also, there was significant difference between the 7th and 14th postsurgery days in the LLLT 980-nm group and the control group ($P<0.001$). There was no significant difference between the two laser therapy groups on the 7th and 14th postsurgery days (Fig. 2).

Discussion

Our data significantly confirmed the therapeutic effects of LLLT on pain reduction in CCI model, in which the wavelength of 660 nm was more effective than 980 nm. In discussion, first we discuss on the mechanisms of LLLT effectiveness on pain reduction and then on the possible reasons of the differences between the wavelength of 660 and 980 nm.

It is known that CCI as a model of neuropathic pain induces an inflammatory condition that activates inflammatory cascade marked by the increase of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α that play important role in the etiology and continuation of neuropathic pain [31–34]. Also, prostaglandin E_2 (PGE_2) or prostaglandin I_2 (PGI_2) administration induces hyperalgesia and enhances the sensitivity of primary afferents to either mechanical or chemical stimulation [35–38]. During the pre-inflammatory phase, the production of reactive oxygen species (ROS) increases which in turn activates NF-κB [39, 40]. The activated NF-κB increases the expression of the iNOS and subsequent synthesis of NO [41, 42]. NO and its reactive nitrogen intermediates may destruct cells and tissues and play significant role in the pathology of certain inflammatory conditions [43]. Regarding the LLLT effectiveness on pain reduction, various mechanisms were postulated.

Rabeloet al. reported on the therapeutic effects of LLLT on the wound healing of a diabetic rat throughout by reducing the inflammatory progress and reducing inflammatory cell density [44]. Same evidences also were reported for the reduction of rat paw edema by red LLLT with a wavelength of 632.8 nm (He-Ne) and 650 nm [45, 46]. It is also shown that 660 and 684 nm from red diode lasers are effective in reducing edema formation [47]. In another study on posttraumatic muscular tissue repair, it is shown that LLLT reduced the inflammatory response, collagenesis, expression of iNOS, and the activation of NF-κB [48, 49]. It is reported that the expression of the pro-inflammatory gene such as IL-1β is suppressed by LLLT in human keratinocytes [50, 51]. Aimbire et al. reported that LLLT (650 nm) reduced the expression of TNF-α, after acute immune complex lung injury, in Wistar rats [52]. It is also shown that LLLT is able to inhibit the production of PGE_2 and decrease the mRNA levels of cyclooxygenase-2 [53]. The role of ROS as a natural cytotoxic production of the normal metabolism of oxygen was also reported. ROS has important roles in cell signaling, regulating nucleic acid synthesis, protein synthesis, enzyme activation, and cell cycle progression [54, 55]. Following the application of LLLT in some cases, a shift in overall cell redox potential in the direction of greater oxidation and increased ROS generation and cell redox activity has been shown [56–62].

Wu et al. only used transcranial LLLT with 36 J/cm^2 of a 665-, 810-, or 980-nm laser 4 h after traumatic brain injury. Both lasers with the wavelength of 665 and 810 nm, not 980 nm, were highly effective in improving the motor performance during the succeeding 4 weeks [63]. They concluded that the different effects among various wavelengths are due to the absorption spectrum of the different chromophores located in the mitochondria and cell membrane [63] of which role is considered to be important for LLLT therapeutic effects [64, 65]. At 980 nm, however, there is a notable absorption band of water, and therefore, 980-nm photons are more likely to produce tissue heating rather than the photochemical effects resulting from absorption by cytochrome c oxidase (complex IV mitochondrion) [63]. Cytochrome c oxidase is the photoreceptor in the red region of the spectrum and is responsible for activating the synthesis of ATP and, consequently, cell metabolism [66]. The ability of the cell to have a greater energy source during the repair process might be the reason for the better response observed in the group treated with laser at 660 nm. LLLT transmits energy at low levels and therefore does not emit heat, sound, or vibrations. Experiments following LLLT exposure have shown that the immediate increase in heat of the target tissue is negligible [67]. In addition to the above mechanism, it is also reported that the wavelength of 660 nm such as with He-Ne laser used for wound healing leads to photo reactivation of cellular superoxide dismutase (SOD). Thus, the laser light acts as a catalase or superoxide dismutase. It would be to suggest that laser radiation reactivated one of those enzymes [68].

Conclusion

The significance of this study is to provide new ways in laser therapy for clinical trials to reduce certain types of pain in patients suffering from peripheral nerve injuries.

Acknowledgements The authors would like to thank the Medical Basic Sciences Laboratory (Faculty of Allied Medicine, TUMS) and Pain Laboratory (Faculty of Medicine, TUMS).

References

1. Merskey H, Bogduk N (1994) Part III: Pain terms, a current list with definitions and notes on usage. In: Merskey H, Bogduk N (eds) Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. IASP, Seattle, pp 209–214

2. WangLX WZJ (2003) Animal and cellular models of chronic pain. Adv Drug Deliv Rev 55:949–965
3. WoolfCJ MRJ (1999) Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 353:1959–1964
4. Zimmermann M (2001) Pathobiology of neuropathic pain. Eur J Pharmacol 429:23–37
5. Dubner R (1991) Neuronal plasticity and pain following peripheral tissue inflammation or nerve injury. In: BondMR CJE, Woolf CJ (eds) Proceedings of the VIth World Congress on Pain. Elsevier, Amsterdam, pp 263–276
6. Woolf CJ (1996) Windup and central sensitization are not equivalent. Pain 66:105–108
7. Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33: 87–107
8. Gary J, Bennett G, Chung JM, Honore M, Seltzer Z (2003) Models of neuropathic pain in the rat. Current Protocols in Pharmacology. doi: 10.1002/0471141755
9. Amour FE, Smith D (1941) A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79
10. Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88
11. Espejo EF, Mir D (1993) Structure of the rat's behaviour in the hot plate test. Behav Brain Res 56:171–176
12. Woolfe G, Macdonald AD (1944) The evaluation of the anelgesic action of pethidine hydrochloride (Dermol). J Pharmacol Exp Ther 80:300–307
13. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994) Quantitative assessment of tactile allodynia in the rat paw. J NeurosciMethods 53:55–63
14. Dubner R (1989) Methods of assessing pain in animals. In: Textbook of pain Edited by: Wall PD and Melzack R. Edinburgh, Churchville Livingstone: 247-256.
15. Mendonça AC, Barbieri CH, Mazzer N (2003) Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods 129:183–190. doi:10.1016/S0165-0270(03)00207-3
16. Gigo-Benato D, Geuna S, Rochkind S (2005) Phototherapy for enhancing peripheral nerve repair: a review of the literature. Muscle Nerve 31:694–701. doi:10.1002/mus.20305
17. Raso VVM, Barbieri CH, Mazzer N, Fasan VS (2005) Can therapeutic ultrasound influence the regeneration of the peripheral nerves. J Neurosci Methods 142:185–192
18. Ohshiro T, Calderhead RG (1988) Low-level laser therapy: a practical introduction. Wiley, New York, pp 17, 28–30, 33, 34.
19. Huang Y, Chen ACH, Carroll JD, Hamblin MR (2009) Biphasic dose response in low-level light therapy. Dose Response 7:358–383
20. Kitchen SS, Partridge CJ (1991) A review of low level laser therapy, part I: background, physiological effects and hazards. Physiotherapy 77:161–163
21. Karu TI, Pyatibrat L, Kalendo G (1995) Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. J Photochem Photobiol B 27:219–233
22. Khullar SM, Brodin P, Fristad I, Kvinnsland IH (1999) Enhanced sensory reinnervation of dental target tissues in rats following low level laser (LLL) irradiation. Lasers Med Sci 14:177–184
23. Schindl A, Schindl M, Schindl L, Jurecka W, Hönigsmann H, Breier F (1999) Increased dermal angiogenesis after low-intensity laser therapy for a chronic radiation ulcer determined by a video measuring system. J Am AcadDermatol 40:481–484
24. Manteifel V, Bakeeva L, Karu T (1997) Ultrastructural changes in chondriome of human lymphocytes after irradiation with He-Ne laser: appearance of giant mitochondria. J Photochem Photobiol B 38:25–30
25. Reis FA, Belchior ACG, Carvalho PTC, Silva BAK, Pereira DM, Silva IS, Nicolau RA (2009) Effects of laser therapy (660 nm) on recovery of the sciatic nerve in rats after injury through neurotmesis followed by epineural anastomosis. Lasers Med Sci 24:741–747
26. Belchior ACG, Reis FA, Nicolau RA, Silva IS, Pereira DM, Carvalho PTC (2009) Influence of laser (660 nm) on functional recovery of the sciatic nerve in rats following crushing lesion. Lasers Med Sci 24: 893–899
27. Barbosa RI, MarcolinoAM GRRJ, MazzerN BCH, Fonseca MCR (2010) Comparative effects of wavelengths of low-power laser in regeneration of sciatic nerve in rats following crushing lesion. Lasers Med Sci 25:423–430
28. HsiehYL CLW, ChangPL YCC, KaoMJ HCZ (2012) Low-level laser therapy alleviates neuropathic pain and promotes function recovery in rats with chronic constriction injury: possible involvements in hypoxia-inducible factor 1a (HIF-1a). The Journal of Comparative Neurology Research in Systems Neuroscience 520:2903–2916
29. Bertolini GR, Artifon EL, Silva TS, Cunha DM, Vigo PR (2011) Low-level laser therapy, at 830 nm, for pain reduction in experimental model of rats with sciatica. Arq Neuro Psiquiatr 69:356–359
30. RandallLO SJJ (1957) A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 111:409–419
31. Cui JG, holmin S, mathiesen T (2000) Possible role of inflammatory mediators in tactile hypersensitivity in rat models of mononeuropathy. Pain 88(3):239–248
32. Martucci C, Trovato AE, Costa B, Borsani E, Franchi S, Magnaghi V, Panerai AE, Rodella LF, Valsecchi AE, Sacerdote P, Colleoni M (2008) The purinergic antagonist PPADS reduces pain related behaviours and interleukin-1 beta, interleukin-6, iNOS and nNOS overproduction in central and peripheral nervous system after peripheral neuropathy in mice. Pain 137:81–95
33. Sommer C, Kress M (2004) Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 361:184–187
34. leung l, Cahill CM (2010) TNF-alpha and neuropathic pain–a review. J Neuroinflammation 7:27
35. Ferreira SH, Nakamura M, Abreu Castro MS (1978) The hyperalgesic effects of prostacyclin and prostaglandin E2. Prostaglandins 16(1):31–37
36. Higgs EA, Moncada S, Vane JR (1978) Inflammatory effects of prostacyclin (PGI_2) and 6-oxo-PGF1[alpha] in the rat paw. Prostaglandins 16(2):153–162
37. Schepelmann K, Linger K, Schaibleh G (1992) Inflammatory mediators and nociception in the joint: excitation and sensitization of slowly conducting afferent fibers of cat's knee by prostaglandin I_2. Neuroscience 50(1):237–247
38. DevorM WDM, Goetzl EJ (1992) Eicosanoids, but not tachykinins, excite C-fiber endings in rat sciatic nerve-end neuromas. Neuroreport 3(1):21–24
39. Cuzzocrea S, Thiemermann C, Salvemini D (2004) Potential therapeutic effect of antioxidant therapy in shock and inflammation. Curr Med Chem 11:1147–1162
40. Torres SH, De Sanctis JB, Briceño ML, Hernandez N, Finol H (2004) Inflammation and nitric oxide production in skeletal muscle of type 2 diabetic patients. J Endocrinol 181:419–427
41. Adams V, Nehrhoff B, Spate U, Linke A, Schulze PC, Baur A, Gielen S, Hambrecht R, Schuler G (2002) Induction of iNOS expression in skeletal muscle by IL-1b and NF-kB activation: An in vitro and in vivo study. Cardiovasc Res 54:95–104
42. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J (2005) Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 15:113–120
43. Gilad E, Wong HR, Zingarelli B, Virag L, O'Connor M, Salzman AL, Szabo C (1998) Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: Role of inhibition of NFkappaB activation. FASEB J 12:685–693

44. RabeloSB VAB, NicolauR SMC, MeloMdaS PMT (2006) Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. Photomed Laser Surg 24(4): 474–479
45. AlbertiniR AFS, CorreaFI RW, CogoJC AE, TeixeiraSA DNGHC, NetoCF ZRA, Lopes-Martins RA (2004) Effects of different protocol doses of low power gallium–aluminum–arsenate (Ga–Al–As) laser radiation (650 nm) on carrageenan induced rat paw oedema. J Photochem Photobiol B 74(2–3):101–107
46. FerreiraDM ZRA, VillaverdeAB CY, FrigoL PG, LongoI BDG (2005) Analgesic effect of He–Ne (632.8 nm) low-level laser therapy on acute inflammatory pain. Photomed Laser Surg 23(2):177–181
47. AlbertiniR VAB, AimbireF SMAC, BjordalJM ALP, MuninE CMS (2007) Anti-inflammatory effects of low-level laser therapy (LLLT) with two different red wavelengths (660 nm and 684 nm) in carrageenan-induced rat paw edema. J Photochem Photobiol B Biol 89:50–55
48. RizziCF, MaurizJL, Freitas CorreaDS, MoreiraAJ, ZettlerCG, FilippinLI, MarroniNP, Gonzalez-GallegoJ (2006) Effects of low-level laser therapy (LLLT) on the nuclear factor (NF)-kappaB signaling pathway in traumatized muscle, Lasers Surg. Med.
49. Moriyama Y, Moriyama EH, Blackmore K, Akens MK, Lilge L (2005) In vivo study of the inflammatory modulating effects of low level laser therapy on iNOS expression using bioluminescence imaging. Photochem Photobiol 81(6):1351–1355
50. Gavish L, Asher Y, Becker Y, Kleinman Y (2004) Low level laser irradiation stimulates mitochondrial membrane potential and disperses subnuclear promyelocytic leukemia protein. Lasers Surg Med 35(5):369–376
51. Gavish L, Perez L, Gertz SD (2006) Low-level laser irradiation modulates matrix metalloproteinase activity and gene expression in porcine aortic smooth muscle cells. Lasers Surg Med 38:779–786
52. Aimbire F, Albertini R, Pacheco MT, Castro-Faria-Neto HC, Leonardo PS, Iversen VV, Lopes-Martins RA, Bjordal JM (2006) Low-level laser therapy induces dose-dependent reduction of TNF alpha levels in acute inflammation. Photomed Laser Surg 24(1):33–37
53. Sakurai Y, Yamaguchi M, Abiko Y (2000) Inhibitory effect of low-level laser irradiation on LPS-stimulated prostaglandin E2 production and cyclooxygenase-2 in human gingival fibroblasts. Eur J Oral Sci 108(1):29–34
54. Storz P (2007) Mitochondrial ROS—radical detoxification, mediated by protein kinase D. Trends Cell Biol 17:13–18
55. Brondon P, Stadler I, Lanzafame RJ (2005) A study of the effects of phototherapy dose interval on photobiomodulation of cell cultures. Lasers Surg Med 36:409–413
56. Karu T (1999) Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B 49:1–17
57. Alexandratou E, Yova D, Handris P, Kletsas D, Loukas S (2002) Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. PhotochemPhotobiolSci 1:547–552
58. Chen AC-H, Arany PR, Huang YY, Tomkinson EM, Saleem T, Yull FE, Blackwell TS, and Hamblin MR (2009) Low level laser therapy activates NF-κB via generation of reactive oxygen species in mouse embryonic fibroblasts. Proc SPIE in press.
59. Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O, Eichler M, Kaplan D, Lubart R (2003) Low energy visible light induces reactive oxygen species generation and stimulates anincrease of intracellular calcium concentration in cardiac cells. J Biol Chem 278:40917–40922
60. Lubart R, Eichler M, Lavi R, Friedman H, Shainberg A (2005) Low-energy laser irradiation promotes cellular redox activity. Photomed Laser Surg 23:3–9
61. Pal G, Dutta A, Mitra K, Grace MS, Romanczyk TB, Wu X, Chakrabarti K, Anders J, Gorman E, Waynant RW, Tata DB (2007) Effect of low intensity laser interaction with human skin fibroblast cells using fiber-optic nano-probes. J Photochem Photobiol B 86: 252–261
62. Zhang J, Xing D, Gao X (2008) Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway. J Cell Physiol 217:518–528
63. Wu Q, Huang YY, Dhital S, Hamblin MR, Anders JJ, Waynant RW (2010) Low level laser therapy for traumatic brain injury. Mechanisms for Low-Light Therapy V. Proc SPIE. 7552 Article No. 755206.
64. Amat A, Rigau J, Waynant RW, Ilev IK, Anders JJ (2006) The electric field induced by light can explain cellular responses to electromagnetic energy: a hypothesis of mechanism. J Photochem Photobiol B 82:152–160
65. Vladimirov Yu A In: Chikin S (1994) Efferent medicine. Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow,pp.51–66.
66. Manteifel VM, Karu TI (2005) Structure of mitochondria and activity of their respiratory chain in successive generations of yeast cells exposed to He-Ne laser light. Izv Akad Nauk Ser Bioll 32:556–566
67. Hrnjak M, Kuljic-Kapulica N, Budisin A, Giser A (1995) Stimulatory effect of low-power density He-Ne laser radiation on human fibroblasts in vitro. Vojnosanit Pregl 52:539–546
68. Romm AR, Sherstnev MP, Volkov VV, Vladimirov Yu A (1986) The action of laser irradiation of peroxide chemiluminescence of wound exudation. Byul EkspBiol Med 102:426–428