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Original Article

The effect of low-intensity laser therapy (LILT) on cutaneous wound healing and pain relief in rats

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Abstract. [Purpose] This study examined the impact of low-intensity laser therapy on wound healing and pain control using a rat cutaneous wound model. [Subjects and Methods] Twenty-four adult male Sprague-Dawley rats (between 220−240 g, 7 weeks) were used in this study. The rats were anesthetized and a circular fragment of skin was removed from the dorsal region of the back by a punch with an 8-mm diameter. The animals were randomly divided into 6 groups, Groups C 1, C 3, and C 5, control groups, received no laser treatment. Groups T 1, T 3, and T 5 received laser treatment for 20 min per day for 1, 3 and 5 days, respectively. Lumbar spine and dorsal skin were extracted and processed using western blot analysis. [Results] Periodical observation showed increases in NGF expression on the skin, and decreases in c-*fos* expression by the spinal cord in the treatment groups compared to the control group. [Conclusion] The present findings suggest that low-intensity laser therapy could be used as an effective therapy for wound healing and pain relief, and could be further used as a clinical approach for treating cutaneous wounds.

Key words: Low-intensity laser therapy, Wound healing, Pain relief

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INTRODUCTION

A wound is known as any loss of continuity in the skin that must be healed or repaired to provide protection and prevent contamination of the body[1)]. Wound healing is a complex biological and biochemical process that involves the removal of invading pathogens from the damaged tissues and the remodeling of injured tissues. During the process of wound healing, a variety of growth factors that promote cell proliferation and differentiation are released into the wound space. The function of these growth factors, which include epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF) and nerve growth factor (NGF) have been identified by previous studies[2–4)]. Specifically, NGF is known to be an important growth factor for neural regeneration and it also induces tissue repair in the remodeling stage during wound healing[5)].

There is much evidence that growth factors play an important role during wound healing, but few studies have investigated the role played by pain. In the healing phases of an injury, pain signaling should be considered when assessing clinical strategies. To confirm pain, c-*fos* can be used as a marker of painful sensations in the spinal cord[6)]. A study of c-*fos* demonstrated that it is a useful marker of nociceptive neuron stimuli in the dorsal horn (DH) of the spinal cord[7)].

Low-intensity laser therapy (LILT) is a therapeutic modality that has been used in a variety of clinical applications, including wound healing. The effects of laser therapy on pathological conditions such as wound healing, qualified scar formation, and relief of pain have been reported by many studies[8–12)]. In cutaneous wounds, LILT has been shown to accelerate the wound healing process via growth factors[13–15)]. Although studies have demonstrated the positive effects of LILT on wound healing, the correlation between LILT and pain control has not yet been reported. It is necessary to confirm pain control (via c-*fos*) during wound healing in conjunction with LILT. Therefore, the purpose of this study was to investigate wound healing and pain control using LILT and a rat model.

SUBJECTS AND METHODS

Experimental procedures were performed according to the protocols established by the Institution of Animal Care and Use Committee (IACUC) of Daegu University, which are based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH, 1996).

Twenty-four adult male Sprague-Dawley rats (between 220−240 g, 7 weeks old) were used in this study. The animals were kept under a 12 light/12 h dark schedule at 22°C, and were freely fed during the experimental period. They were anesthetized with 2 mL/kg 50% Zoletil and 50% xyla-

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Table 1. The comparison of NGF expressions in skin tissue across the six groups (Unit: pixels)

Expressions of NGF (Mean±SD)						
Group	Control (n=12)			Treatment (n=12)		
Day	1	3	5	1	3	5
	3,011.2±89.4	2,838.6±289.9	6,643.0±498.2	3,400±489.9	6,182.8±225.1*	13,203.4±416.4*

Table 2. The comparison of c-*fos* expressions by the spinal cord across the six groups (Unit: pixels)

Expressions of c-*fos* (Mean±SD)						
Group	Control (n=12)			Treatment (n=12)		
Day	1	3	5	1	3	5
	9,711.2±771.4	9,387.4±160.9	9,272.6±423.1	5,985.6±131.9*	5,411.2±131.9*	874±217.4*

*significant difference from matched control day. $p<0.05$
Mean±SD: mean±standard deviation, Control: No low-intensity laser therapy, Treatment: Low-intensity laser therapy for 20 min per day for 1, 3, and 5 days, respectively

zine hydrochloride mixture via intraperitoneal (IP) injection, and a circular fragment of skin was removed from region of the back by a punch with an 8-mm diameter[16]. The animals were randomly divided into 6 groups, Control groups C1, C3, and C5 received no laser treatment, and acted as matched controls for the treatment groups: T 1, which received laser treatment for 20 min for 1 day; T 3, which received laser treatment for 20 min per day for 3 days; and T 5, which received laser treatment for 20 min per day for 5 days.

The low-intensity laser used for the irradiation procedures had a wavelength, of 660 nm and a power output of 60 mW and 1–4 J/cm^2. During irradiation, the laser probe was held with the tip just in contact with the dorsal surface of the wound. Treatment was given for 20 min per day.

The spinal cord and dorsal skin tissues extracted from the rats were homogenized in lysis buffer (50 mM Tris, 120 mM Nacl, pH 7.4) with added protease inhibitors (Complete, Roche, Mannheim, Germany). Total proteins were collected and the protein concentrations were determined by the Bradford method (Bio-Rad, Richmond, CA, USA). To validate NGF and c-*fos* protein expression, western blot analysis was performed. The protein extracts from spinal cord (20 μg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After protein separation, the samples were transferred to nitrocellulose and blots were probed with anti-NGF 1:1000 (cat# sc-365944, Santa Cruz, CA, USA), anti-C-*fos* 1:1000 (cat# sc-8047, Santa Cruz, CA, USA). Horseradish peroxidase conjugated anti-mouse 1:5000 (cat# sc-2005, Santa Cruz, CA, USA) was used as a secondary antibody. The thickness of the bands was photographically measured using Scion Image software Beta 4.0.3 (Scion Corp., Frederick, MD, USA).

The data are expressed as the mean ± standard deviation (SD), and the statistical analysis was performed using one-way analysis of variance (ANOVA) and SPSS 18.0 software. A post-hoc analysis was performed using the LSD method. Significance was accepted for values of $p<0.05$.

RESULTS

Low-intensity laser treatment is a well-established and widely-used clinical model of wound healing. In this study, differences were found in NGF expression between the groups after the laser treatment. Periodical observation of wound healing for 5 days revealed that the treatment groups showed increases in NGF expression on the skin compared to their respective control groups (Table 1). Over the 5 days, the treatment groups, with the exception of group T1, showed higher expression of NGF than their respective control groups, and the differences were significant. There was no significant difference between the T 1 group and the C1 control group.

This study also confirmed the effect of low-intensity laser treatment on pain control via c-*fos* expression by the spinal cord. A significant decrease in c-*fos* expression after LILT was observed in all the treatment groups. However, the control groups (C 1, 3, 5) showed no significant change in c-*fos* expression at any of the time points (Table 2). There was a trend of greater decrease in c-*fos* in the T5 group compared to the T1, and T3 groups.

DISCUSSION

The aim of the present study was to verify the effect of low-intensity laser therapy on the rate of wound healing. Low-intensity laser therapy is one of the clinical therapeutic modalities that has been used for wound healing. Previous studies of laser treatments have demonstrated that low-intensity laser therapy accelerates and facilitates wound healing[17–19].

During the wound healing process the secretion of biological substances, such as growth factors, plays an important role in healing or repairing wounded skin[20]. A variety of growth factors have previously been found to assist wound healing, and a study that incorporated the application of NGF showed that it enhanced the rate of healing[21]. The results of the present study also show that low-intensity laser therapy had a positive effect on growth factor via NGF expression

in the healing of wounded skin. Our present study showed that NGF expression, especially in T 3 and T 5, accelerated wound healing in the treatment groups as compared to their control groups.

Pain control is an important parameter in wound healing because it influences the application of treatment. The positive results observed with pain control may also contribute to its clinical application in therapy[22]. Many studies have focused on the effects of low-intensity laser therapy on the mechanisms of wound healing or the mechanical laser therapy approach, but few studies have shown a correlation with pain relief[9, 20, 23–25]. This study found that LILT suppressed c-*fos* expression, which is known as a marker of pain, by the spinal cord. The c-*fos* protein has been used for 25 years as a marker of pain control in many studies[26]. It has been used as a marker of nociceptive neuron stimuli in the dorsal horn (DH) of the spinal cord, and increased levels of c-*fos* protein have been shown to be correlated with spinal sensitization in the DH[6]. C-*fos* expression is a parameter of pain control and our results show that LILT decreased c-*fos* expression by the spinal cord in the treatment groups. Moreover, our results also show that the level of c-*fos* decreased over the course of the experimental time period.

In conclusion, low-intensity laser therapy not only helps accelerate cutaneous wound healing via NGF expression in the skin, it is also useful for pain control due to the decrease in the amount of c-*fos* released by the spinal cord. Therefore, our results suggest that LILT could be used as an effective therapy for wound healing and pain relief, and could be further used as a clinical approach for treating cutaneous wounds.

REFERENCES

1) Ousey K, McIntosh C: Physiology of wound healing. Lower extremity wounds: a problem-based approach. 2008, 25.
2) Micera A, Vigneti E, Pickholtz D, et al.: Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. Proc Natl Acad Sci USA, 2001, 98: 6162–6167. [Medline] [CrossRef]
3) Kato YP, Silver FH: Formation of continuous collagen fibres: evaluation of biocompatibility and mechanical properties. Biomaterials, 1990, 11: 169–175. [Medline] [CrossRef]
4) Werner S, Grose R: Regulation of wound healing by growth factors and cytokines. Physiol Rev, 2003, 83: 835–870. [Medline]
5) Matsuda H, Koyama H, Sato H, et al.: Role of nerve growth factor in cutaneous wound healing: accelerating effects in normal and healing-impaired diabetic mice. J Exp Med, 1998, 187: 297–306. [Medline] [CrossRef]
6) Hunt SP, Pini A, Evan G: Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature, 1987, 328: 632–634. [Medline] [CrossRef]
7) Coggeshall RE: Fos, nociception and the dorsal horn. Prog Neurobiol, 2005, 77: 299–352. [Medline]
8) Ribeiro MS, Da Silva DF, De Araújo CE, et al.: Effects of low-intensity polarized visible laser radiation on skin burns: a light microscopy study. J Clin Laser Med Surg, 2004, 22: 59–66. [Medline] [CrossRef]
9) Ferreira DM, Zângaro RA, Villaverde AB, et al.: Analgesic effect of He-Ne (632.8 nm) low-level laser therapy on acute inflammatory pain. Photomed Laser Surg, 2005, 23: 177–181. [Medline] [CrossRef]
10) Stadler I, Lanzafame RJ, Evans R, et al.: 830-nm irradiation increases the wound tensile strength in a diabetic murine model. Lasers Surg Med, 2001, 28: 220–226. [Medline] [CrossRef]
11) Simunovic Z, Ivankovich AD, Depolo A: Wound healing of animal and human body sport and traffic accident injuries using low-level laser therapy treatment: a randomized clinical study of seventy-four patients with control group. J Clin Laser Med Surg, 2000, 18: 67–73. [Medline]
12) Demir H, Balay H, Kirnap M: A comparative study of the effects of electrical stimulation and laser treatment on experimental wound healing in rats. J Rehabil Res Dev, 2004, 41: 147–154. [Medline] [CrossRef]
13) Hawkins D, Houreld N, Abrahamse H: Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. Ann N Y Acad Sci, 2005, 1056: 486–493. [Medline] [CrossRef]
14) Almeida-Lopes L, Rigau J, Zângaro RA, et al.: Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. Lasers Surg Med, 2001, 29: 179–184. [Medline] [CrossRef]
15) Schindl A, Merwald H, Schindl L, et al.: Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation. Br J Dermatol, 2003, 148: 334–336. [Medline] [CrossRef]
16) Rezende SB, Ribeiro MS, Núñez SC, et al.: Effects of a single near-infrared laser treatment on cutaneous wound healing: biometrical and histological study in rats. J Photochem Photobiol B, 2007, 87: 145–153. [Medline] [CrossRef]
17) Avci P, Gupta A, Sadasivam M, et al.: Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg, 2013, 32: 41–52. [Medline]
18) Solmaz H, Gülsoy M, Ülgen Y: 635nm diode laser biostimulation on cutaneous wounds. SPIE Photonics Europe, International Society for Optics and Photonics, 2014, pp. 91292S-91292S-91297.
19) Santos NR, de M Sobrinho JB, Almeida PF, et al.: Influence of the combination of infrared and red laser light on the healing of cutaneous wounds infected by Staphylococcus aureus. Photomed Laser Surg, 2011, 29: 177–182. [Medline] [CrossRef]
20) Tamariz-Domínguez E, Castro-Muñozledo F, Kuri-Harcuch W: Growth factors and extracellular matrix proteins during wound healing promoted with frozen cultured sheets of human epidermal keratinocytes. Cell Tissue Res, 2002, 307: 79–89. [Medline] [CrossRef]
21) Nithya M, Suguna L, Rose C: The effect of nerve growth factor on the early responses during the process of wound healing. Biochim. Biophys. Acta (BBA)-. Gen Subjects, 2003, 1620: 25–31. [CrossRef]
22) Suyama T, Takahashi K, Shibuta H, et al.: Pain and rehabilitation in patients with spinal cord injury. J Phys Ther Sci, 2001, 13: 59–64. [CrossRef]
23) do Nascimento PM, Pinheiro AL, Salgado MA, et al.: A preliminary report on the effect of laser therapy on the healing of cutaneous surgical wounds as a consequence of an inversely proportional relationship between wavelength and intensity: histological study in rats. Photomed Laser Surg, 2004, 22: 513–518. [Medline] [CrossRef]
24) Mendez TM, Pinheiro AL, Pacheco MT, et al.: Dose and wavelength of laser light have influence on the repair of cutaneous wounds. J Clin Laser Med Surg, 2004, 22: 19–25. [Medline] [CrossRef]
25) Kim G, Kim E: Analgesic efficacy of low intensity laser therapy in a monosodium iodoacetate-induced osteoarthritic rat model. J Phys Ther Sci, 2013, 25: 309–312. [CrossRef]
26) Gao YJ, Ji RR: c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury? Open Pain J, 2009, 2: 11–17. [Medline] [CrossRef]