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Anti-inflammatory effects of low-level laser in burn wound models in rats

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Objective: The anti-inflammatory effects of low-level laser in burn wound model in rats were investigated.
Design: Randomized controlled trial.
Methods: The rats were assigned to three experimental groups. Group I received second-degree burn wounds; Group II received dressing film and low-level laser (1.2 J/cm^2) treatment after a burn wound; Group III received dressing film and low-level laser (2.3 J/cm^2) treatment after a burn wound. After inducing a deep second-degree burn wound, the wound was observed every day and the burn area diameter and retraction quantification at 1, 7, and 14 days were evaluated. Low-level laser was investigated on hematological parameters after 14 days. Effects of low-level laser on the inflammatory cytokines (tumor necrosis factor-α [TNF-α] and interleukin-6 [IL-6]) concentrations in the serum were evaluated using immunosorbent assay kits.
Results: Group III showed a significant difference in wound size on days 7 and 14 compared to Group I (p<0.05). Group II showed a significant difference in wound size on day 14 compared to Group I (p<0.05). For wound contraction percentage, both laser therapy treatment groups showed a significant difference compared with Group I (p<0.05). There was also a significant difference in wound contraction percentage in Group III compared to Group II (p<0.05). Compared with the model control group, decreased TNF-α and IL-6 levels in the serum was observed at 14 days after burn wound induction.
Conclusions: The results of this study suggest that low-level laser therapy can assist in burn wound healing, which might be associated with decreased concentrations of TNF-α and IL-6 related proinflammatory cytokines.

Key Words: Inflammation, Low-level lasers, Wounds

Introduction

Thermal burn injury has a high mortality rate and morbidity consequences [1]. Burn is caused by a wide variety of causes such as fire, scald burns, electrical burns, radiation and chemical burns [2]. The healing process of burn wound induces the thermal injury tissue inflammation, edema, hypertrophic, and scar formation [3]. Burn patient management entails significant period of hospitalization, multiple cure procedures, long term rehabilitation and expensive medication. Burn care is important to shorten the duration of treatment for burn patients and to help regenerate tissues [4,5]. Therefore, maintaining skin health is just as crucial as obtaining an early diagnosis and treatment [1].

Burns may be considered as one of the most severe types of physical trauma that has both local and systemic effects. Thermal burn injuries to the skin may respond with inflammation, cellular protection mechanisms, immune suppression, hypermetabolic dysfunction or organs [6,7]. Pain may be due to skin inflammation, along with the appearance of exudate and cytokine production such as tumor necrosis factor (TNF)-α and interleukins (IL) such as IL-6, IL-8 and IL-10 [8,9]. In particular, TNF-α and IL-6 are known to have a complex anti-inflammatory role in burn wound pathogenesis by commencing inflammatory responses [10,11]. Hence, these pro-inflammatory cytokines may also

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serve important roles in the pathogenesis of burn wound.

Some benefits of low intensity laser therapy include microcirculation enhancement, activation of angiogenesis, vasodilatation, enhanced tissue metabolism and analgesic effects histologically [12-14]. Deep penetration of low level laser photons into the body through the skin, nerves, tendons, muscles, ligaments, and even bones can occur to address the painful and injured areas [15]. Thus, the use of low intensity laser therapy is a frequently recommended physical therapy method used for treating burn wounds [16,17]. Although the beneficial effects of laser therapeutic intervention on the burn wound healing process has been reported, information about the interaction of therapeutic laser on burn wounds are insufficient. Thus, the purpose of this study was to determine the low-level laser healing effects in burn wound animal models.

Methods

Animal model

Eighteen male 6-week-old Sprague-Dawley rats with a weight of 160.0±5.0 g were included in the study after an acclimatization period of 1 week. The rats were placed in individually ventilated cages at temperatures of 25.0±1.0°C humidity levels of 55±2% with 12/12-hour light/dark cycles. No restraints were put on for the rats' access to food and water.

The animal experimental protocols were performed according to the Dongshin University Animal Care and Use Committee guidelines. All rats had undergone random assignment into three groups (Table 1). Group I (control group) received no treatment after burn wound induction. Group II was treated with dressing film and low level laser (1.2 J/cm^2) treatment after burn wound. Group III received dressing film and low level laser (2.3 J/cm^2) treatment after burn wound.

Burn injury

Eighteen rats were assigned randomly into three groups and were intramuscularly preanesthetized with atropine sulfate at 0.04 mg/kg. After 10 minutes, a combination of ketamine 90 mg/kg and xylazine 10 mg/kg anesthetics were injected intramuscularly followed by dorsum trichotomy by direct hair tension (area of −4 cm^2) and antisepsis with 1% polyvinylpyrrolidone-iodine. Following anesthesia, the dorsal areas of the rats were shaved and depilated using an electric clipper. An aluminum bar (r=20 mm) that was preheated for 100°C for 15 seconds was directly placed onto the depilated area in order to create symmetrical burns. Following the burn injury, animal awakening, and completion of the procedure, intramuscular dipyrane application (0.01 mg/kg) was used for analgesic purposes. Burn wound injuries were examined during the course of 14 days and 100 μl of hydrogel was applied onto the burn wound [18].

Table 1. Classification of experimental groups (N=18)

Group	Design of each group
Group I	Non-treatment after burn wound injury (n=6)
Group II	Low level laser therapy (1.2 J/cm^2) after burn wound injury (n=6)
Group III	Low level laser therapy (2.3 J/cm^2) after burn wound injury (n=6)

Low laser therapy and biochemical analysis

The laser device used for the study was a gallium-aluminum-arsenide diode laser (Ga-Al-As, infrared laser; Chattanooga Group Inc., Chattanooga, TN, USA) with a wavelength of 850 nm, a power output of 200 mV in pulsed mode, continuous wave, and a 0.07 cm^2 spot-area was used. The laser source probe was in light contact with the burn surface at the center of each square, and was aimed at a 90-degree angle to the injured tissue during the assigned treatment time period. Dosages of 1.2 J/cm^2 and 2.4 J/cm^2 at a maximum of 6 points for 1 minute per point were used in the burn regions.

The laser device was applied within the time limit and dosage at every centimeter along the burn wound edges in non-contact mode, five times a week during the 14 days after the induction of a second-degree burn. For group I (control), the diode laser was applied in an equipvalent manner but the device was shut off during the treatement periods. Protective eyeglasses were worn by the researchers during the laser therapy sessions to ensure safety.

Quantification of wound retraction

Each week, the rats were observed for wound contracture under anesthesia. Wound retraction was assessed on day 7 and day 14 post burn-wound induction. Decreased original wound size percentage was used to express wound contraction: % wound contraction on day-X=[(area on day 0−open area on day X)/area on day 0]×100 [19].

Enzyme-linked immunosorbent assay

Upon completion of the therapy sessions (day 14) the animals were sacrificed and blood of the animals were placed

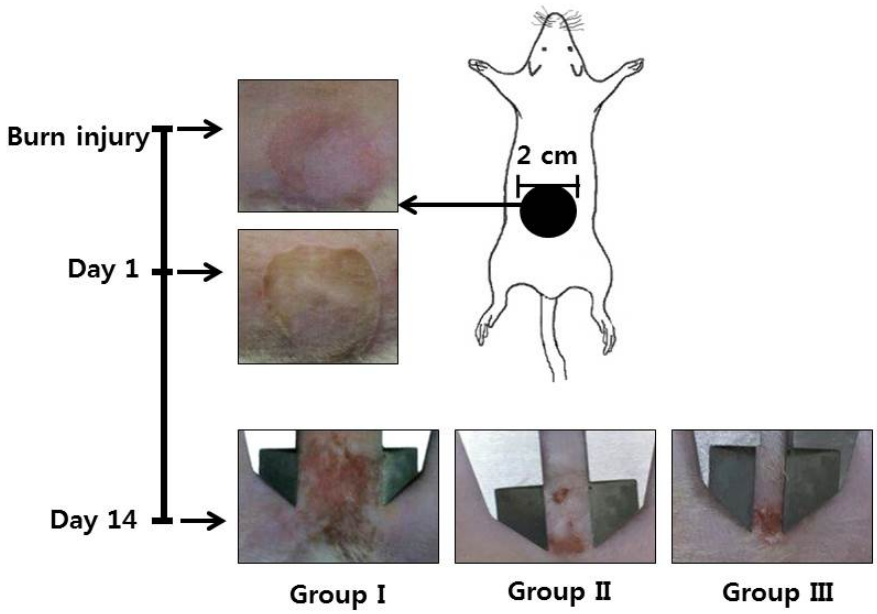

Figure 1. Wound healing effects of low level laser.

into EDTA-containing tubes for the purpose of serum separation. The enzyme-linked immunosorbent assay kits (R&D System, Minneapolis, MN, USA) were used to assess the cytokines TNF-α and IL-6 from the rat serum according to the manufacturers' instructions. Cytokine concentrations calculations were made from a standard curve. The assay of the serum biochemistry examination was performed by microplate readers (Bio-Rad, Hercules, CA, USA). The sensitivity limit of both assays was about 10 pg/ml, and the the linear assay range was between 10 pg/ml and 1,000 pg/ml based on the manufacturer's instructions.

Data analysis

IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used for data analyses. All values are expressed as mean±standard deviation (SD) of 3 replications. The one-way ANOVA was used to compare the differences between groups, and the Student-Newman-Keuls multiple comparisons test was used when differences were detected. The *p*-values of less than 0.05 at a 95% confidence interval level were considered as significant.

Results

Figure 1 show the burn injured area according to time intervals after the second-degree burn. In each group, the repair process after a scalding injury was studied by comparing wound area, wound closure, and decrustation on day 1 and day 14. In the first day, there was no obvious difference among the groups. On the day 14, whereas wounds treated with different laser therapy showed wound reduced or no edema. In Table 2, the body weight changes showed that the growth of the SD rats was normal, and all the experimental groups of rats were comparative and homogenous. The wound size was significantly reduced in the group III on day 7 and day 14 compared with group I ($p<0.05$; Table 3). Compared with group I (control group), low level laser treatment could significantly shorten the contraction percentage ($p<0.05$). The effects of low level laser therapy on the inflammation related biological factors TNF-α and IL-6 concentration are shown in Table 4.

Table 2. Changes in body weight of rats during the experimental period

Group	Starting body weight (g)	Final body weight (g)
Group I[a]	176.83 (4.40)	252.17 (14.27)
Group II[b]	177.33 (3.27)	239.50 (16.54)
Group III[c]	177.83 (3.13)	245.00 (13.29)

Values are presented as mean (SD).
[a]Non-treatment after burn wound injury. [b]Low level laser therapy (1.2 J/cm^2) after burn wound injury. [c]Low level laser therapy (2.3 J/cm^2) after burn wound injury.

Discussion

The wound healing process is composed of three stages:

Table 3. The change of wound size and contraction percentage in each groups

Group	Day 1	Day 7	Day 14	Wound contraction (%)
Group I[a]	18.37 (1.30)	16.50 (2.33)	10.63 (1.41)	58.10 (8.62)
Group II[b]	17.56 (1.72)	13.63 (1.85)	7.38 (1.06)*	42.23 (6.48)*
Group III[c]	18.25 (0.71)	12.75 (0.71)*	5.63 (1.59)**	30.67 (8.22)**

Values are presented as mean (SD).
Tested by one-way ANOVA.
[a]Non-treatment after burn wound injury. [b]Low level laser therapy (1.2 J/cm^2) after burn wound injury. [c]Low level laser therapy (2.3 J/cm^2) after burn wound injury.
*p<0.05 compared with group I, **p<0.05 compared with group II on low level laser therapy after burn wound injury.

Table 4. Effect of low level laser on TNF-α and IL-6 in burn wound rat (pg/ml)

Group	TNF-α	IL-6
Group I[a]	24.63 (3.70)	127.88 (8.14)
Group II[b]	12.44 (4.69)*	81.88 (14.78)*
Group III[c]	9.38 (4.27)**	60.63 (18.68)**

Values are presented as mean (SD).
Tested by one-way ANOVA.
TNF-α: tumor necrosis factor-α, IL-6: interleukin-6.
[a]Non-treatment after burn wound injury. [b]Low level laser therapy (1.2 J/cm^2) after burn wound injury. [c]Low level laser therapy (2.3 J/cm^2) after burn wound injury.
*p<0.05 compared with group I, **p<0.05 compared with group II on low level laser therapy after burn wound injury.

inflammation, proliferation and remodeling of the extracellular matrix in the skin. The events that occur during the proliferative phase include angiogenesis, deposition of collagen, epithelialization, and wound contraction [20,21]. Burn healing occurs by secondary intention, a slow process with a high infection risk and produces scar retraction, which may lead to extensive scarring and subsequent high burn-related treatment costs, depending on the injury location [22]. This study demonstrated in vivo clinical effects of low-level laser therapy (LLLT) on second-degree thermal burns in rats. LLLT is an important intervention for wound healing that has been shown to create beneficial effects on the stimulation of the extracellular matrix, myofibroblasts, and granulation tissue activities for the skin healing process [23-25]. The presence of myofibroblasts creates a centripetal contraction from the edges of the burn lesion. Myofibroblast apoptotic cell death may promote lesion contraction more than 50% of the original size [26]. Photomicrographs showed faster wound contraction in the group on day 14 compared to untreated scald wounds.

Inflammatory cytokines serve a significant role in maintaining and inducing inflammation in skin tissue. Based on experimental models of inflammation, early expressions of TNF-α, IL-6, and IL-1β occur and play key roles. The release of pro-inflammatory mediators such as interferon-γ, TNF-α, IL-6, and IL-8 are increased in burn patients with major burn injury [27,28]. This study shows that low intensity laser therapy has a remarkable effect that inhibits TNF-α and IL-6 levels along with the reduction of edema and inflammatory responses. The action of low intensity laser therapy is based on the light absorption in thermally injured skin tissue, which will generate proliferation in cellular metabolism [26,29]. LLLT has been shown to accelerate the acute inflammatory process and tissue repair of the wound [30,31].

The use of low level laser to expedite tissue healing has been examined and the photostimulation has been shown to promote reepithelization, synthesis of collagen, proliferation of fibroblasts, and angiogenesis to reduce inflammatory cytokine levels [32]. Bayat *et al*. [33] examined the effects of low-level helium-neon laser on rats that had received third-degree burns and reported a significant increase in total quantity of mast cells and newly created epidermal cells at the burn lesion site in the group treated with laser therapy compared with the control group. Chiarotto *et al*. [16] evaluated the effects of InGaP (670-nm) and GaAlAs (830-nm) on second-degree burns in rats and reported similar results of decrease in granulocyte numbers, increase in angiogenesis and fibroblast proliferation, which were reported to expedite the healing process of the second-degree burns after laser therapy. We found similar results of a reduced inflammatory period, fibroblast proliferation and increases in granulation tissue resulting in the promotion of third-degree burn wound healing after laser therapy [34].

Our findings are consistent with the previous findings and reports. Improvement in wound areas local microvascular circulation leads to decreased cytokine concentrations of TNF-α and IL-6. This study results showed that low-level

laser as a therapeutic intervention on burn animal wound models is beneficial for the wound and can suppress inflammatory cytokines (TNF-α and IL-6). The low-level laser observed may provide a rational rehabilitation therapeutic strategy to improve burn care. Based on this study, it is suggested that low-level laser treatment is beneficial since it burn wound necrosis was reduced through inflammatory cytokine reduction.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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