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Original Article

Combinative Effect of Red and Far-Infrared Laser Therapy on Histological Changes of Injured Muscle in Rats

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ABSTRACT

Background and Aim: The effects of red or far-infrared laser therapy are well known, but combinative effect of laser on muscle injury has not yet been investigated in experimental studies. Here, we aim to examine the combinative effect of red and far-infrared laser therapy in a rat muscle injury model. **Methods:** A total of male Wistar rats were randomly distributed into 3 groups: healthy Control Group (CG), untreated Muscle injury Group (MG) and combination of Red and Far-infrared laser therapy Group (RFG). Gastrocnemius injury in rat was induced by a single blunt-impact trauma based on previous studies. Laser therapy was started within 24 hr after muscle injury and applied every day for 21 days (1 min/day). **Results:** After combinative laser therapy (RFG), the area of damaged fibers, percentage in the area of collagen and leukocyte infiltration decreased significantly compared to MG. **Conclusion:** These results showed that the combination of Red and Far-infrared laser therapy was effective in muscle recovery and could modulate collagen and inflammatory process in injured muscle.

Keywords: Gastrocnemius Injury, Low-level laser therapy, Far-infrared laser therapy.

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INTRODUCTION

Injuries to muscle tissue frequently affect people in every group. They arise from workplace demands, sports activities, or mishaps, and their commonality underscores the demand for superior treatment strategies. Thus, exploring alternative strategies becomes essential to safeguard tissues from the devastating effects of muscular harm. Skeletal muscle healing after injury follows a relatively consistent sequence comprising three main stages. The destruction phase involves rupture and subsequent necrosis of muscle fibers, with hematoma formation and an associated inflammatory cell response. This is followed by the repair phase, characterized by phagocytic removal of necrotic tissue, regeneration of myofibers, and development of a fibrous connective tissue scar. Finally, during the remodeling phase, the regenerated myofibers mature, the scar tissue retracts and reorganizes, and the muscle progressively regains its functional capacity.[1,2] Although muscle tissue is a dynamic tissue with an excellent capacity for repair after the injury, such a process is considered slow and some injuries might even affect muscle functioning, leading to atrophy, contracture, pain and increased likelihood of re-injury.[3] Low-Level Laser Therapy (LLLT) has emerged as a promising approach, as it promotes muscle cell proliferation and supports skeletal muscle regeneration after injury. These effects are largely attributed to improved modulation of the inflammatory response and enhanced activation of satellite cells.[4] Low-Level Laser Therapy (LLLT) is commonly used to alleviate muscle pain, yet the underlying biological mechanisms driving the positive outcomes seen in clinical trials are still not well understood. Intense and repeated skeletal muscle contractions can trigger an inflammatory response, which is typically associated with muscle damage.[5,6] Far-Infrared Radiation (FIR) refers to electromagnetic waves with wavelengths ranging from 5.6 to 1000 μm. Owing to its advantageous effects, FIR has been widely utilized in diverse fields, particularly in food preservation, health enhancement, and improvement of cardiovascular function.[7,8] Some studies indicated that FIR therapy has potentially beneficial effects in the treatment of wound healing,[9] diabetes,[10] tumor thermal therapy,[11] chronic fatigue syndrome,[12] and knee osteoarthritis.[13] These studies revealed that the potential effects of FIR therapy played a significant role in the protective effect on vascular function. However, only a limited number of studies have investigated the effects of combining conventional recovery strategies with Low-Level Laser Therapy (LLLT) or infrared

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laser therapy for enhancing muscle injury recovery. Although these modalities are known to support tissue regeneration, the underlying mechanisms through which LLLT or infrared laser therapy act on injured muscle are not yet fully elucidated, making their clinical application a subject of ongoing debate. In this study, we revealed the combinative effects of Red and Far-infrared laser therapy on muscle healing after muscle injury in rats through histopathological findings.

MATERIALS AND METHODS

Animals and Groups

Male Wistar rats (260 g) were provided by Laboratory Animal Centre of Pyongyang University of Medical Sciences and adapted in a lab environment before experiments for a week. 45 rats are randomly chosen and during the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2℃ and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences.

Rats were randomly divided into 3 groups (n=15/group): Control Group (CG), Model Group (MG) and Combination of Red and Far-infrared laser therapy Group (RFG).

Muscle injury model

Gastrocnemius injury was induced by a single blunt-impact trauma.[14] Rats were anesthetized intraperitoneally with ketamine (95 mg/kg) and xylazine (12 mg/kg) and placed in the prone position, with the right hind limb secured in knee extension and the ankle maintained at 90° dorsiflexion. A standardized injury was induced in the right gastrocnemius muscle by dropping a 0.459 kg metal mass from a height of 18 cm through a guiding apparatus, generating a kinetic energy of 0.811 J. Control animals underwent anesthesia alone without induction of muscle injury.

Red and Far-Infrared Laser Therapy Device and Irradiation

The therapy device named as Red and Far-Infrared laser was purchased from Pyongyang Medical Instruments Company, DPR Korea and certified by the National Board of Medical Instruments Certification. This device consists of 2 main parts: Red laser irradiation part (λ=670 nm; 40 J/cm^2; 50 mW/cm^2) and Far-Infrared laser irradiated wooden boards. Red laser irradiation part was surrounded with 4 of Far-Infrared laser irradiated wooden boards and posited in the center of this device. The height from Red laser irradiation part to the ground was 40cm. The wooden boards (45×20×1.2cm; length×width×thickness) were coated with Nano-paste (ZnO). The spectrums of the wooden board exhibited high emissivity (>0.8) between 8~14 μm wavelengths. Red laser irradiation was targeted on the medial belly of right gastrocnemius muscle and at the same time Far-infrared laser was irradiated around fixed rats. Laser therapy was started within 24 hr after muscle injury and applied every day for 21 days (1 min/day).

Muscular Sample Collection

On day 22 post-injury, rats from all groups were euthanized via anesthetic overdose (ketamine and xylazine administered intraperitoneally), followed by excision of the right gastrocnemius muscles using scissors and forceps.

Histopathological Analysis

The excised muscles were fixed in 4% paraformaldehyde for 24 hr and subsequently embedded in paraffin for histological analysis. Longitudinal sections of 5 μm thickness were prepared and stained with Hematoxylin and Eosin (H&E), followed by examination under a light microscope. For each animal, ten randomly selected images were captured at 50× magnification using a Moticam system and analyzed with Motic Image Plus 2.0 software. Each image was divided into 100 fields (100 μm^2 per field), yielding a total of 1,000 fields per animal, which were evaluated to quantify the area of damaged muscle fibers, collagen deposition, and inflammatory cell infiltration.

Statistical Analysis of Data

Quantitative data are reported as mean±standard error of the mean. Statistical differences in basal characteristics between the groups were calculated by one-way analysis of variance and t-test for continuous variables. $P<0.01$ was considered statistically significant. All statistical analyses were performed using the SPSS 16.0 software.

RESULTS

During the study period, there was no loss of animals.

Change of the Area of Damaged Muscle Fibers

The area of damaged muscle fibers in each group at the 22th days after muscle injury was shown in Figure 1. At the 22th days after muscle injury, the mean area of damaged muscle fibers in MG group was significantly elevated by 2347.8 (μm^2) compared to CG (120.4 μm^2). But combination of lasers showed this index was significantly decreased compared to MG.

Change of Collagen Percentage

After combination with Red and far-infrared laser therapy, the change of collagen percentage in each group was measured using histopathologic findings (Table 1). Table 2 shows the effects of combinative laser on collagen presence in injured muscle.

At the 22th days after muscle injury, the percentage in the area of collagen increased by approximately 28% in MG. Combination of laser inhibited this increase significantly compared with MG.

Change of Leukocyte Infiltration

Table 2 shows reduced leukocyte infiltration after therapy of combinative laser for 21 days. After treatment of laser, leukocyte cells of injured muscle in MG increased significantly compared to CG, but RFG inhibited this increase significantly.

DISCUSSION

Muscle damage is often caused by unexpected injuries. A key issue in recovery is to improve tissue repair following muscle injury. Laser technology, introduced in the 1960s, is characterized by distinctive properties such as monochromaticity, coherence, and minimal beam divergence. Low-Level Laser Therapy (LLLT) utilizes low-power laser light (1-500 mW) in the management of pathological conditions and is proposed to enhance tissue regeneration, suppress inflammation, and relieve pain. In parallel, accumulating evidence suggests that Far-Infrared (FIR) therapy exerts significant protective effects on vascular function. So that, Red or infrared laser therapy has been widely used in promoting health and has been shown to exert beneficial effects in human body. Although photomodulation therapy has been widely used to ameliorate muscle injury, research has produced contradictory results. Furthermore, the combination of Red and Far-infrared laser therapy on muscle injury will become as a newer challenge in sports and clinical field. In particular, the non-thermal effect of FIR has been found to play a significant role in the protective effect on some vascular-related diseases, but its improvement effects and use against muscle injury have not been clearly presented. Present study demonstrated that the combination of red and far-infrared laser therapy has a positive effect on the recovery and treatment of injured muscle in rat. As a result, after treatment of Red and Far-infrared laser irradiation on muscle injury, the mean area of damaged muscle fibers decreased significantly compared to MG. Muscle fibers were considered damaged when they showed features such as acidophilic cytoplasm, disappearance of striations, nuclear loss, fiber rupture, or evidence of phagocytosis by inflammatory cells. Inflammatory cell infiltration-comprising neutrophils, eosinophils, macrophages, and lymphocytes-was evident within the perimysium, where these cells were actively engaged in the clearance of injured muscle fibers.[15] Our results demonstrated that the combination of two laser irradiation can inhibit infiltration and deposition of inflammatory cells into injured muscle at the period evaluated (Figure 1). The effects of photomodulation therapy on collagen metabolism are still unclear.[16] Some studies suggest laser therapy enhances collagen synthesis[17,18] while others report reduced collagen production. Additionally, the wide variation in laser fluences applied by different researchers to injured muscles prevents identification of an optimal treatment protocol. According to our results, the combination of two laser irradiation showed the positive effect against increasing collagen in injured muscle (Table 1). It suggests that decreased collagen in RFG may be a type III -immature collagen. The authors suggested that laser therapy such as LLLT could inhibit the infiltration and deposition of inflammatory cells of skeletal muscle after injury. However, there were no reports about the combination of Red and Far-infrared laser therapy used in this work on inflammatory process after muscle injury. Table 2 exhibited the positive effect of combination of two laser irradiation on inhibition of inflammatory process in injured muscle. Therefore, the combination of Red and far-infrared laser therapy would be possible to be used on the acceleration of muscle healing.

Table 1: Percentage in the area of collagen.

Group	%
CG	17.2±1.5
MG	27.7±2.3△△
RFG	20.9±1.4 *

Each value represents the mean ± SEM of 15 rats per group. CG: Control group. MG: Model group, RFG: Combination of Red and Far-infrared laser therapy group.*P< 0.05 as compared with MG. △△ P<0.01 as compared with CG.

Table 2: Leukocyte infiltration.

Group	Number of cells/100μm²
CG	1.8±0.5
MG	13.6±2.7△△
RFG	5.1±1.3 **

Each value represents the mean ± SEM of 15 rats per group. CG: Control group. MG: Model group, RFG: Combination of Red and Far-infrared laser therapy group.**P<0.01 as compared with MG. △△ P<0.01 as compared with CG.

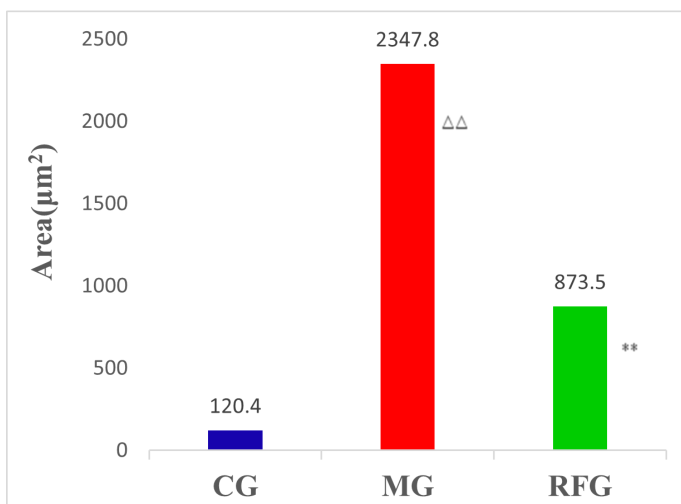

Figure 1: Combinative Effect of laser therapy on the area of damaged muscle fibers CG: Control group. MG: Model group, RFG: Combination of Red and Far-infrared laser therapy group. Each value represents the mean ± SEM of 15 rats per group. **P<0.01 as compared with MG. △△P<0.01 as compared with CG.

CONCLUSION

In conclusion, this study shows that the combination of Red and Far-infrared laser therapy has a healing potency in muscle injury. The results suggest that the combination of Red and far-infrared laser therapy might pave the way for the management of recovery after muscle injury.

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ABBREVIATIONS

CG: Control Group; **MG:** Model Group/Muscle Injury Group; **RFG:** Combination of Red and Far-infrared Laser Therapy Group; **LLLT:** Low-Level Laser Therapy; **FIR:** Far-Infrared; **H&E:** Hematoxylin and Eosin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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