## The Journal of Physical Therapy Science

SPTS

Original Article

# Effects of low-level laser therapy on inflammatory symptoms in an arthritis rat model

Seima Okita, RPT, BS[1, 2], Ryo Sasaki, RPT, PhD[2, 3], Yasutaka Kondo, RPT, MS[1, 2],
Junya Sakamoto, RPT, PhD[2, 4], Yuichiro Honda, RPT, PhD[2, 4],
Minoru Okita, RPT, PhD[2, 4]*

[1] Department of Rehabilitation, The Japanese Red Cross Nagasaki Genbaku Hospital, Japan
[2] Laboratory of Locomotive Rehabilitation Science, Nagasaki University Graduate School of
  Biomedical Sciences: 1-7-1 Sakamoto, Nagasaki-shi, Nagasaki 852-8520, Japan
[3] Department of Rehabilitation, Juzenkai Hospital, Japan
[4] Institute of Biomedical Sciences, Health Sciences, Nagasaki University, Japan

**Abstract.** [Purpose] This study evaluated the effect of low-level laser therapy on inflammatory signs in an arthritis rat model as a foundation for elucidating the mechanism of the anti-inflammatory effect. [Materials and Methods] Eighteen Wistar rats were divided into three groups: group I (arthritis without low-level laser therapy), group II (arthritis with low-level laser therapy), and the control group (sham arthritis control). Arthritis was induced in the right knee by injecting a mixture of kaolin and carrageenan. Low-level laser therapy was continued for seven days after the onset of arthritis by 60 times of repeated irradiation for 10 seconds in the right knee joint area. The joint transverse diameter, pressure pain threshold in the affected knee joint, and mechanical paw withdrawal threshold at the distant site were evaluated the day before the injection and one, three, and seven days after the injection. Pathological changes were observed. [Results] Group II showed better improvement in swelling and pain in the affected knee joint and secondary hyperalgesia at the distance site when compared to group I. In group II, there was only mild infiltration of synovial cells, and the progression of arthritis was suppressed compared with that of group I. [Conclusion] Low-level laser therapy can mitigate swelling and inflammatory pain in the affected knee joint and prevent secondary hyperalgesia.
Key words: Acute pain, Low-level laser therapy, Pain management

## INTRODUCTION

Acute pain correlated with inflammation evoked by burn injury, trauma, arthritis and surgical procedures is a protective system that begins the healing process[1]. Inflammation is characterized by redness, swelling, heat, pain, and dysfunction of the body at the peripheral tissue level. Acute pain is time dependent once ordinary physiological responses occur, including permeability changes, leukocyte recruitment and accumulation, and release of inflammatory mediators.

Once acute pain is a general body response system, outlasting severity pain enhanced more greater sensitivity to general noxious stimulation. These changes are featured in peripheral or central processes called primary and secondary hyperalgesia, which is a risk factor for developing chronic pain[2, 3]. The mechanisms by which increasing responsiveness of nociceptive neurons in the spinal dorsal horn with long-pronged stimulation are referred to as central sensitization[4].

Therefore, accurate inflammation management is essential for medical handling during the acute phase. Gold standard anti-inflammatory treatments are widely used as pharmacological therapies; for instance, non-steroidal anti-inflammatory

---

drugs (NSAIDs) are recommended in most knee osteoarthritis clinical treatment guidelines and in the acute phase of muscle injury[5, 6]. The use of NSAIDs has been a potentially safer alternative; perhaps the occurrence of systemic side effects has been argued since the drug penetrates slowly and in small amounts into the blood circulation[7]. However, a recent study focused on alternative agents for non-invasive treatment and reported that low-level laser therapy (LLLT) has anti-inflammatory effects in clinical physiotherapy setting[8]. Low-level laser therapy involves the application of light for therapeutic purposes that promotes tissue regeneration, reduces inflammation, and relieves pain[9, 10]. In a systematic review and meta-analysis, LLLT has proven to be effective in reducing pain caused by lateral elbow tendinopathy (tennis elbow), fracture, and plantar fasciitis[11–13]. Thus, LLLT is an effective electrophysiological agent for the management of acute pain.

The anti-inflammatory action of LLLT may inhibit and/or attenuate the release of inflammatory mediators and pain markers, especially tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and prostaglandin E2 (PGE2)[14, 15]. Another study showed that pharmacological interventions, such as NSAIDs and LLLT, reduced the levels of inflammatory markers in cells[8]. However, the biological mechanisms of LLLT in acute pain remain unclear. Therefore, it is necessary to re-examine how LLLT affects acute pain in clinical settings. With respect to these perspectives, this study aimed to evaluate the effect of LLLT on inflammatory signs of swelling and pain threshold in carrageenan-induced arthritis in rats and to outline its potential in inflammatory pain management.

## MATERIALS AND METHODS

The experiment animals comprised male Wistar rats (n=18; 7-weeks-old), which were housed two per cage and maintained at 22–24 °C with a 12:12 h light:dark cycle. Food and water were provided *ad libitum* throughout the experiments. All the rats were subjected to a pre-experimental period to acclimatize them to experimental environment and evaluation process for seven days.

To investigate the therapeutic effects of LLLT on acute inflammation, 18 rats were randomly divided into the following three groups: arthritis without LLLT (AR; n=6), arthritis with LLLT (LLLT, n=6), and sham arthritis control (CON; n=6). In the AR and LLLT groups, all the rats were anesthetized with combination anesthetic agents (5 mL/kg, i.p.), which consisted of 0.375 mg/kg medetomidine hydrochloride (Dorbene, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan), 4.0 mg/kg midazolam (Midazolam, Sandoz K.K., Tokyo, Japan), and 5.0 mg/kg butorphanol (Vetorphale, Meiji Seika Pharma Co., Ltd, Tokyo, Japan). Subsequently, the rats were injected with 300 μL of a mixture of 3% kaolin (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 3% carrageenan (Sigma Chemical Co., St. Louis, MO, USA) in the right knee joint anteriorly[16]. Rats in the CON group were injected with 300 μL saline as a sham treatment.

All experimental procedures were approved by the Ethics Review Committee for Animal Experimentation at Nagasaki University (approval no. 1510051250).

In this study, a low-level gallium-aluminum-arsenide (Ga-Al-As) laser (MLD-2001, Mochida) was used. The laser device had a wavelength of 830 nm, output intensity of 60 mW, and spot size was 3 cm$^2$. The LLLT was performed continuously for seven days, starting one day after the carrageenan and kaolin injection. Specifically, LLLT group were anesthetized, placed in the supine position, and their extremities were fived to the experimental table with taping tape. Owing to not maximum extended position under anesthesia, the knee joint of rat was held as slightly flexed position. The irradiation time of the laser beam was 10 s per time, and this was repeated 60 times (total 10 min) while shifting slightly around the right knee joint through the skin.

During the experiment, the swelling and pressure pain thresholds (PPT) of the right knee joint and mechanical hyperalgesia of the right hind paw were evaluated. These assessments were first performed prior to injection and served as baseline. Evaluations during the experimental period were performed 1, 3, and 7 days after injection, and before intervention to prevent the immediate acute effects of LLLT irradiation from affecting evaluation results. The evaluation methods were as follows:

To track the changes in joint swelling, we measured the transverse diameter of the inflamed knee joint under anesthesia using Vernier calipers. The transverse diameter was measured in the supine position, and the knee joint was held in the maximum extended position[17].

The PPT of the inflamed knee joint was assessed using a Randall–Selitto apparatus (Ugo Basile, Varese, Italy). The rats were lightly restrained by hand. The rounded tip of the transducer probe (base diameter=9 mm) was applied to the lateral side of the knee joint with a linearly increasing pressure (48 g/s). The threshold was defined as the force required to elicit hind limb flexion reflex or vocalization. Seven measurements were taken at intervals of at least 3 min, and the average of five measurements (excluding the maximum and minimum) was recorded as the PPT[17].

To evaluate the mechanical paw withdrawal threshold (MPWT) of the right hind limb, an electronic von Frey anesthesiometer (IITC-2391, IITC Life Science Instruments, Los Angeles, CA, USA), adapted with a 0.5 mm$^2$ contact area polypropylene tip, was used. The rats were individually housed in a homemade plastic container with a mesh on the floor. The investigator was trained to apply the tip perpendicular to the central area of the hind paw with a gradual increase in pressure, which was characterized by the removal of the paw (the animal actively lifted the whole paw upon the tip of the anesthesiometer, bit or licked the paw, or shook the paw with high-amplitude movements in response to the stimulus). The measurement was performed seven times, and the average of five measurements (excluding the maximum and minimum) was considered the MPWT[18].

J. Phys. Ther. Sci. Vol. 35, No. 1, 2023

56

All results are expressed as the mean ± standard deviation. All data were statistically evaluated using SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA). Statistically significant differences among groups at each evaluation day were assessed using one-way analysis of variance, followed by the Bonferroni post-hoc test. The level of significance was set at a p-value of <0.05.

After the final behavioral testing on day seven, the rats were deeply anesthetized, and transcardial perfusion of saline and 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB; pH 7.4) was performed. The right knee joint was subsequently excised. The right knee joint was fixed with 4% paraformaldehyde and then decalcified with 10% ethylenediaminetetraacetic acid (EDTA) in 0.1 M PB (pH 7.4). The knee joint was dehydrated using a graded series of ethanol solutions and embedded in paraffin.

Longitudinal 6-μm serial sections were cut with a microtome, and the sections from each right knee sample were stained with hematoxylin-eosin to identify pathological changes, such as degeneration of articular cartilage and cell infiltration in the joint capsule.

## RESULTS

One day after injection, the transverse diameter of the knee joint was significantly higher in the AR and LLLT groups than in the CON group, but there were no significant differences between the two groups. The AR and LLLT groups showed significantly higher scores three days after the injection. The AR group was still significantly higher at seven days after the injection; however, in the LLLT group, the width of the knee joint significantly recovered compared to the AR group at seven days after induction of inflammation (Table 1).

For the AR and LLLT groups, the PPTs of knee joints at one day after the induction of arthritis were significantly lower than those in the CON group, and there were no significant differences between the two groups. These results persisted at day three after the injection, but an increase in PPTs of LLLT groups was observed at seven days after the induction compared to that in the AR groups (Table 1).

The MPWT of the hind limbs in the AR and LLLT groups scored significantly lower on the first day after injection than at baseline. For the LLLT groups, a significant increase in PRB was observed seven days after the injection, and the AR group was markedly lowered throughout the experimental period compared to the CON group (Table 1).

In the AR group, marked cell infiltration was observed in the synovium, and the occurrence of arthritis was remarkable. In contrast, in the LLLT group, synovial cell infiltration was mild and the progression of arthritis was suppressed (Fig. 1).

## DISCUSSION

In the present study, we found that LLLT intervention after arthritis induction resulted in reduction of knee swelling, improvement of PPT of inflamed knee joints, and secondary hyperalgesia of the ipsilateral hind paws.

The results of knee joint swelling and PPT in the AR and LLLT groups one day after the injection indicated that injection of carrageenan produced acute inflammation and primary hyperalgesia with the same level of inflammation in the affected knee joint. Additionally, the AR group showed persistent acute inflammation and primary hyperalgesia for up to seven days after the injection. This phenomenon is also supported by the pathological changes in the knee joints of the AR group, which were examined seven days after the injection, such as marked cell infiltration of the synovium.

**Table 1.** Changes in the swelling, the pressure pain thresholds (PPT), and the mechanical paw withdrawal threshold (MPWT) of the right knee joint over time

|  | Group | Baseline | 1 day | 3 days | 7 days |
|---|---|---|---|---|---|
| Swelling; transverse diameter (mm) | CON | 10.3 ± 0.5 | 10.3 ± 0.8 | 10.5 ± 0.6 | 10.5 ± 0.6 |
|  | AR | 9.8 ± 0.4 | 15.7 ± 1.2* | 15.7 ± 0.8* | 14.8 ± 1.7* |
|  | LLLT | 10.2 ± 0.4 | 16.2 ± 1.7* | 14.3 ± 1.0* | 12.5 ± 1.0*, # |
| PPT (g) | CON | 215.4 ± 14.0 | 216.8 ± 12.0 | 206.1 ± 10.3 | 197.4 ± 25.5 |
|  | AR | 220.1 ± 20.1 | 133.5 ± 12.8* | 127.7 ± 7.0* | 129.1 ± 7.9* |
|  | LLLT | 213.3 ± 13.7 | 129.1 ± 9.5* | 145.5 ± 19.2* | 166.3 ± 3.9*, # |
| MPWT (g) | CON | 46.8 ± 9.8 | 48.8 ± 8.3 | 50.9 ± 7.5 | 50.4 ± 6.2 |
|  | AR | 52.3 ± 10.3 | 25.1 ± 3.5* | 22.7 ± 3.0* | 25.2 ± 2.4* |
|  | LLLT | 46.1 ± 9.8 | 26.3 ± 4.1* | 27.3 ± 1.7* | 43.0 ± 2.6# |

CON group: sham arthritis control; AR group: arthritis without low-level laser therapy (LLLT); Low-level laser therapy (LLLT) group: arthritis with low-level laser therapy (LLLT).
Statistically significant differences among groups at each evaluation day were assessed using one-way analysis of variance, followed by the Bonferroni post-hoc test. *p<0.05, significantly different from CON group; #p<0.05, significantly different from AR group.
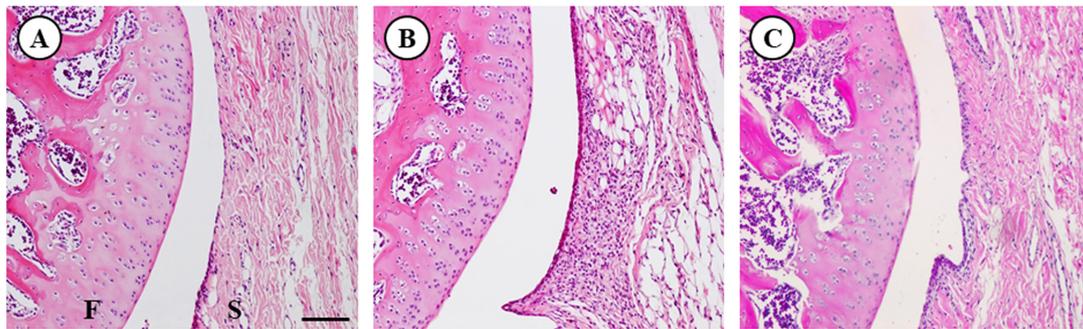
**Fig. 1.** Pathological changes in the knee.
Representative photographs of cell infiltration in the synovium of the sagittal slice of the posterior knee joint capsule in the CON (A), AR (B), and LLLT (C) groups. Marked cell infiltration was observed in the synovium of the AR group. In contrast, in the LLLT group, synovial cell infiltration is mild; scale bar, 100 μm.
CON group: sham arthritis control; AR group: arthritis without LLLT; LLLT group: arthritis with LLLT; LLLT: low-level laser therapy; F: femor; S: synovium.

Furthermore, the results of MPWT in the AR and LLLT groups one day after the injection indicated that carrageenan induction caused secondary hyperalgesia at distant sites. Secondary hyperalgesia persisted for seven days after injection in the AR group. In this study, we examined the pain sensitivity of the hind paw using von Frey filaments. Extreme sensitivity to pain condition is known as hyperalgesia, which involves elevating excitability of neurons within the peripheral and central nervous system[19]. Exposed severe pain caused exceed noxious stimulus into the spinal cord that leads to central sensitization or secondary hyperalgesia at distant sites, increasing the responsiveness of spinal nociceptive neurons. Once acute inflammation occurs in the affected joint, inflammatory agents contribute to nociceptive sensitivity, which is delivered to multiple segments of the spinal cord, activating a sensible responsive state of afferent sensory neurons in the central nervous system. Therefore, central sensitization and secondary hyperalgesia may occur in the affected knee joint. The reduction in MPWT in our animal models indicated the inhibition of secondary hyperalgesia, in other words, inhibition of central sensitization in the spinal cord. One of the central sensitization mechanisms as the windup phenomenon by Mendell and Wall[20]. Windup indicated that repeated low-frequency electrical stimulation of C-fibers progressively increased the response of dorsal horn neurons[4].

In contrast, the transverse diameter in the LLLT group decreased to a level similar to that at baseline seven days after injection. Additionally, the PPT in the LLLT group was significantly higher than that in the AR group seven days after inflammatory induction. These results are also consistent with the pathological changes in the synovial membrane of the LLLT group, in which the cellular infiltration was mild. Therefore, LLLT may be effective in the recovery of inflammatory pain and swelling during the acute phase of a rat arthritis pain model.

Generally, the synthesis of pro-inflammatory cytokines is regulated by the inflammatory cell accumulation[21]. The reduction of inflammatory cell accumulation in our experiment would promote the typical decrease in pro-inflammatory cytokine release that occurs as an anti-inflammatory response according to LLLT irradiation[14]. A placebo-controlled study performed by Leal Junior et al. found that LLLT decreased the mRNA expression of various inflammatory cytokines, including TNF-a, IL1-b, IL6, and IL10[22]. These cytokines contribute to inflammatory symptoms, especially acute pain, and the suppression of their expression by LLLT may alleviate inflammatory pain and PPT. Although several studies have demonstrated the effectiveness of LLLT on inflammatory cytokines, we did not examine any cytokines in this experimental model. Therefore, we understand that this representation is an experimental limitation.

Additionally, the MPWT in the LLLT group at seven days after injection also significantly recovered to similar level as that in the CON group. This result suggests that LLLT during the acute phase of arthritis alleviated secondary hyperalgesia in the hind paw quickly and easily. Once inflammation was alleviated by LLLT irradiation in this experiment, these windup mechanisms were prevented by decreasing the stimulation input of the spinal dorsal horn. However, we understand that we have not investigated biomarkers to clarify nociceptive transmission, and we only examined the von Frey filament in this experiment. Therefore, it is important to highlight the various factors involved in central sensitization, such as expression of neurotransmitters, substance P, and glutamate in future research.

Over the years, LLLT has been used to reduce acute pain in a variety of inflammatory disorders like knee osteoarthritis[23], plantar fasciitis[13], lateral elbow tendinopathy[11], fracture[12], and acute skeletal muscle injury[6]. Although it has been suggested that the mechanism of the reduction of inflammation and promotion of tissue repair by LLLT may involve the decreased expression of inflammatory cytokines[14, 15] and the kinetics of inflammatory cells such as macrophages[24, 25], the details of the relationship between the reduction of acute pain by LLLT and cytokines, inflammatory cells have not been clarified yet. This study will be a foundation for solving those research questions.

J. Phys. Ther. Sci. Vol. 35, No. 1, 2023

58

In conclusion, LLLT irradiation after the onset of arthritis mitigated the development of swelling and pain in the affected knee joint and prevented the progression of secondary hyperalgesia in the right hind paw. Clinically, LLLT contributes to the management of the acute phase of inflammation. Therefore, we believe that LLLT is a potentially beneficial electrophysiological agent for the management of acute pain and secondary prevention of chronic pain. Further studies are needed to clarify the mechanism of cellular kinetics, especially of macrophages and cytokines, and to establish the effectiveness of LLLT for inflammatory pain in animal experimental models.

## *Funding and Conflict of interest*

# REFERENCES

1) Amaya F, Izumi Y, Matsuda M, et al.: Tissue injury and related mediators of pain exacerbation. Curr Neuropharmacol, 2013, 11: 592–597. [Medline] [CrossRef]

2) Sluka KA, Westlund KN: Behavioral and immunohistochemical changes in an experimental arthritis model in rats. Pain, 1993, 55: 367–377. [Medline] [CrossRef]

3) Radhakrishnan R, Moore SA, Sluka KA: Unilateral carrageenan injection into muscle or joint induces chronic bilateral hyperalgesia in rats. Pain, 2003, 104: 567–577. [Medline] [CrossRef]

4) Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain, 2011, 152: S2–S15. [Medline] [CrossRef]

5) Stausholm MB, Naterstad IF, Joensen J, et al.: Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: systematic review and meta-analysis of randomised placebo-controlled trials. BMJ Open, 2019, 9: e031142. [Medline] [CrossRef]

6) Tomazoni SS, Frigo L, Dos Reis Ferreira TC, et al.: Effects of photobiomodulation therapy and topical non-steroidal anti-inflammatory drug on skeletal muscle injury induced by contusion in rats-part 1: morphological and functional aspects. Lasers Med Sci, 2017, 32: 2111–2120. [Medline] [CrossRef]

7) Moore RA, Derry S, McQuay HJ: Topical agents in the treatment of rheumatic pain. Rheum Dis Clin North Am, 2008, 34: 415–432. [Medline] [CrossRef]

8) Bjordal JM, Lopes-Martins RÁ, Joensen J, et al.: The anti-inflammatory mechanism of low level laser therapy and its relevance for clinical use in physiotherapy. Phys Ther Rev, 2010, 15: 286–293. [CrossRef]

9) Bjordal JM, Lopes-Martins RA, Iversen VV: A randomised, placebo controlled trial of low level laser therapy for activated Achilles tendinitis with microdialysis measurement of peritendinous prostaglandin $E_2$ concentrations. Br J Sports Med, 2006, 40: 76–80, discussion 76–80. [Medline] [CrossRef]

10) Cotler HB, Chow RT, Hamblin MR, et al.: The use of low level laser therapy (LLLT) for musculoskeletal pain. MOJ Orthop Rheumatol, 2015, 2: 00068. [Medline]

11) Bjordal JM, Lopes-Martins RA, Joensen J, et al.: A systematic review with procedural assessments and meta-analysis of low level laser therapy in lateral elbow tendinopathy (tennis elbow). BMC Musculoskelet Disord, 2008, 9: 75. [Medline] [CrossRef]

12) Neto FC, Martimbianco AL, de Andrade RP, et al.: Effects of photobiomodulation in the treatment of fractures: a systematic review and meta-analysis of randomized clinical trials. Lasers Med Sci, 2020, 35: 513–522. [Medline] [CrossRef]

13) Dos Santos SA, Sampaio LM, Caires JR, et al.: Parameters and effects of photobiomodulation in plantar fasciitis: a meta-analysis and systematic review. Photobiomodul Photomed Laser Surg, 2019, 37: 327–335. [Medline] [CrossRef]

14) de Almeida P, Tomazoni SS, Frigo L, et al.: What is the best treatment to decrease pro-inflammatory cytokine release in acute skeletal muscle injury induced by trauma in rats: low-level laser therapy, diclofenac, or cryotherapy? Lasers Med Sci, 2014, 29: 653–658. [Medline] [CrossRef]

15) Macias DM, Coughlin MJ, Zang K, et al.: Low-level laser therapy at 635 nm for treatment of chronic plantar fasciitis: a placebo-controlled, randomized study. J Foot Ankle Surg, 2015, 54: 768–772. [Medline] [CrossRef]

16) Sluka KA, Christy MR, Peterson WL, et al.: Reduction of pain-related behaviors with either cold or heat treatment in an animal model of acute arthritis. Arch Phys Med Rehabil, 1999, 80: 313–317. [Medline] [CrossRef]

17) Tong Y, Ishikawa K, Sasaki R, et al.: The effects of wheel-running using the upper limbs following immobilization after inducing arthritis in the knees of rats. Physiol Res, 2021, 70: 79–87. [Medline] [CrossRef]

18) Ishikawa K, Oga S, Goto K, et al.: Voluntary forelimbs exercise reduces immobilization-induced mechanical hyperalgesia in the rat hind paw. Pain Res Manag, 2021, 2021: 5592992. [Medline] [CrossRef]

19) Klyne DM, Moseley GL, Sterling M, et al.: Are signs of central sensitisation in acute low back pain a precursor to poor outcome? J Pain, 2019, 20: 994–1009. [Medline] [CrossRef]

20) Mendell LM, Wall PD: Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. Nature, 1965, 206: 97–99. [Medline] [CrossRef]

21) Bondeson J, Wainwright SD, Lauder S, et al.: The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. Arthritis Res Ther, 2006, 8: R187. [Medline] [CrossRef]

22) Leal-Junior EC, de Almeida P, Tomazoni SS, et al.: Superpulsed low-level laser therapy protects skeletal muscle of mdx mice against damage, inflammation and morphological changes delaying dystrophy progression. PLoS One, 2014, 9: e89453. [Medline] [CrossRef]

23) Hegedus B, Viharos L, Gervain M, et al.: The effect of low-level laser in knee osteoarthritis: a double-blind, randomized, placebo-controlled trial. Photomed Laser Surg, 2009, 27: 577–584. [Medline] [CrossRef]

24) Souza NH, Mesquita-Ferrari RA, Rodrigues MF, et al.: Photobiomodulation and different macrophages phenotypes during muscle tissue repair. J Cell Mol Med, 2018, 22: 4922–4934. [Medline] [CrossRef]

25) da Silva EM, Mesquita-Ferrari RA, Rodrigues MF, et al.: The effects of photobiomodulation on inflammatory infiltrate during muscle repair in advanced-age rats. J Gerontol A Biol Sci Med Sci, 2020, 75: 437–441. [Medline]