Anti-Inflammation Effects of Low Intensity Laser Therapy on Monosodium Iodoacetate-induced Osteoarthritis in Rats

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Abstract. [Purpose] The purpose of this study was to investigate the effects of low intensity laser therapy (LILT) on inflammatory osteoarthritis in the knee joint of rats. [Subjects] Thirty Sprague–Dawley rats were randomly divided into 3 groups with 10 rats each: the normal, control, and LILT groups. The arthritis group was treated with LILT using a gallium–aluminum–arsenide diode laser over during 3 weeks. All treatments were applied once a day, 5 days per week for 21 days. The inflammation cytokine and the articular index were used to assess the arthritic symptoms. The levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 in the serum were used as outcome measures. [Results] The arthritis index points of rats in the LILT group were lower than that of the control group. Similarly, TNF- α , IL-1 β , and IL-6 concentrations in serum were significantly decreased in the LILT group compared to the control group, which coincided with behavioral studies. [Conclusion] This study shows that LILT is capable of reducing inflammatory cytokine concentrations induced by monosodium iodoacetate osteoarthritis in rats.

Key words: Low intensity laser, Inflammation, Osteoarthritis

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INTRODUCTION

Osteoarthritis (OA) is a degenerative disease and the major cause of disability in weight-bearing joints. Over half of all people older than 65 years of age demonstrate radiographic OA changes in the knees¹⁾. OA usually has a slow and subtle onset. Risk factors for the development and progression of OA include local biomechanical factors like obesity, joint injury, extensive sport participation, and joint deformity, as well as systemic factors, including age, gender, bone density, and estrogen deficiency²⁾.

OA primarily affects weight-bearing joints, for example joints of the hands, feet, knees, and hips, and involves both the degeneration of articular cartilage as well as changes to the subchondral bone. The histological changes in joints affected by OA can be evidenced by gradual development of pain, stiffness and bony crepitus with motion, effusions, and joint movement limitations^{3, 4)}.

Pain in OA patients is induced by local inflammation in knee joints, such as in the synovial membrane, and pathological OA is followed by the production of several cytokines, such as tumor necrosis factor (TNF)- α and interleukins (IL), such as IL-1 β and IL-6^{5–7)}. These inflammatory mediators further contribute to OA pathogenesis by increasing cartilage degradation and inducing hyperalgesia. TNF- α activates sensory neurons directly via its receptors and initiates a cascade of inflammatory reactions through the production of IL (IL-1 β , IL-6, IL-7, IL-17, and IL-18)^{8–10)}.

Specifically, IL-6 is reported to play a complex role in OA pathogenesis by initiating inflammatory responses such as the production of tissue inhibitors of metalloproteinases-1, which may act to limit cartilage damage through a negative feedback loop^{11, 12)}. Therefore, these pro-inflammatory cytokines may also play a significant role in the pathogenesis of human OA.

Inflammatory cytokines activities control may play an important role in the maintenance joint function and pain control. TNF- α , IL-1 β , and IL-6 will key targets for an early intervention in human arthritis. Animal models allow us to evaluate the appearance and the changes in cytokine levels over time, starting from the initiation of joint inflammation. The therapeutic goals of OA include pain and inflammation reduction and improvement of joint function; nonpharmacologic interventions are the universality of OA therapy and include weight control, exercise, physical therapy, patient education, self-help programs and joint unloading in arthritic joints^{13–15)}.

There are biological effects that have been observed with the use of therapeutic lasers and phototherapy in general, which makes laser therapy unique among the various healing methods available today¹⁶⁾. However, the details regarding the anti-inflammation effects of laser in OA joints are unclear. Therefore, the present study was designed to investigate the anti-inflammatory and chondroprotective effects of LILT in a monoiodoacetate (MIA)-induced OA model.

Table 1. Experimental group design

Group (Total n=30)	Treatment
Normal group (n=10)	Non-arthritis group
Control group (n=10)	Placebo laser therapy for 20 second at peri-articular after MIA induced arthritis
LILT ^a group (n=10)	LILT 3.6 J/cm ² for 20 seconds at peri-articular after MIA induced arthritis

^aLow Intensity Laser Therapy.

SUBJECTS AND METHODS

This study used 30 male Sprague–Dawley rats, each weighing 150–160 g. After the rats were acclimatized for 1 week, they were given a single intraarticular injection of monosodium iodoacetate (MIA) through the infra-patella ligament of the left knee. MIA was dissolved in sterile physiological saline and administered in a 60 μ L volume. The rats of the normal group were given a single intraarticular injection of equal volume of sterile saline. Up to 5 days after the MIA injection substantial inflammation of synovial joints was observed. The general health of the animals was monitored and no signs of distress were observed. A total of 30 rats were used in this study, and they were randomly divided into 3 groups of 10 rats after MIA-induced arthritis (Table 1). All experimental procedures in this study were reviewed and approved by the Ethical Review Committee of Dongshin University.

A gallium–aluminum–arsenide diode laser with the following characteristics was used for LILT: a wavelength, 850 nm; power output, 200 mV; wave pulse, 16 Hz; and spot area, 0.07 cm². The therapeutic point was positioned medially to the patellar ligament, and the treatment was applied on towards the joint surface of the femur. The laser group was treated with dosage of 3.6 J/cm² for 20 s in the peri-articular region of both hind paws. The laser device was applied 5 times per week over 21 days following arthritis induction. The researchers wore protective eyeglasses during the laser treatment for safety. For the control group, the diode laser was applied in the same way, but the device was turned off during therapy sessions.

The clinical arthritis symptoms in hind limbs were evaluated according to visual appearance by 2 observers, who were blinded to which group the animals belonged. The symptoms were rated on a scale of 0–4 for each knee joint: 0 = no swelling or erythema, 1 = slight swelling and/or erythema, 2 = low to moderate edema, 3 = pronounced edema with limited joint usage, and 4 = excess edema with joint rigidity¹⁷⁾. At the end of the experimental period (day 21) all animals were sacrificed and blood was collected in EDTA containing tubes, respectively for serum separation. The isolated cytokines, including TNF- α , IL-1 β , and IL-6 from the rat serum were measured using enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. The concentrations of these cytokines were calculated from a standard curve. The serum biochemistry examination was assayed using microplate readers.

Data analysis was performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). All the data are expressed as the mean $\pm$ standard deviation (SD) of

3 replicates. Differences between the 2 groups were tested using one-way ANOVA followed by Student–Newman–Keuls multiple comparisons test if a difference was detected. *p* value less than 0.05 at a 95% confidence level were considered significant.

RESULTS

The effects of laser therapy on the arthritis index of MIA-induced arthritic rats are shown in Table 2. The LILT group had a significantly lower arthritis index than that of the control group, assessed 21 days after the LILT began (*p* < 0.05). The changes in the inflammatory cytokines (TNF- α , IL-1 β , and IL-6) in the MIA-induced arthritic rats are shown in Table 3. The levels of TNF- α , IL-1 β and IL-6 in the control group were significantly higher than in the normal group. However, the inflammatory cytokine (TNF- α , IL-1 β , and IL-6) levels after treatment with the low intensity laser were significantly reduced compared to the control group (*p* < 0.05).

DISCUSSION

In the present study, we investigated the anti-inflammation effects of low intensity laser therapy on the arthritic index and on TNF- α , IL-1 β , and IL-6 production in a MIA-induced osteoarthritis rat model. We found that LILT may offer therapeutic benefits for the treatment of MIA-induced osteoarthritis, at least partially through the reduction in the articular index and inflammatory cytokine production (TNF- α , IL-1 β , and IL-6).

Upon evaluation of articular index, our study determined that LILT group improve more than control group. Articular cartilage destruction is a key pathological characteristic of OA. An important step in the pathophysiology of OA is the breakdown of the extracellular matrix of articular cartilage by tissue proteinases, enzymes whose expression is upregulated by inflammatory stimuli, such as primary inflammatory cytokines^{18, 19)}. Therefore, OA is a complex disease with a number of underlying cellular, biochemical, and physical causes.

Pro-inflammatory mediators, including TNF- α , IL-1, IL-6, and prostaglandins are all overproduced in chondrocytes in pathological conditions and contribute to amplifying the intraarticular inflammatory process^{20, 21)}. Among these cytokines, IL-6 was detected in patients with OA as well as in patients with rheumatoid arthritis²²⁾. Pallotta et al.²³⁾ demonstrated that laser irradiation was effective in significantly reducing the total number of leukocytes as well as neutrophils in joint cavities.

Table 2. Effect of low intensity laser therapy on the articular index of rats with monosodium iodoacetate induced osteoarthritis

	Normal group	Control group	LILT group
21 days	0.00 ± 0.00	12.57 ± 1.84*	7.05 ± 1.02**

(point) ^aLow Intensity Laser Therapy. Data were presented as mean ± SD *: p<0.05 as compared to the normal group. **: p<0.05 as compared to the control group. Unit: point

Table 3. Effect of low intensity laser therapy on inflammation cytokine levels of rats with monosodium iodoacetate induced osteoarthritis

	TNF-α	IL-1β	IL-6
Normal group	20.52 ± 4.56	0.35 ± 0.06	0.86 ± 0.18
Control group	56.28 ± 7.91*	1.50 ± 0.27*	2.64 ± 0.32*
LILT group	24.25 ± 6.50*	1.05 ± 0.35**	1.06 ± 0.30**

^aLow Intensity Laser Therapy. Data were presented as mean ± SD *: p<0.05 as compared to the normal group. **: p<0.05 as compared to the control group. Unit: pg/ml

Cartilage extracellular matrix degradation and suppression of extracellular matrix biosynthesis are regulated by a significant increase in the levels of cytokines in the affected synovial joint²²). On the other hand, a critical function of IL-10 is to limit inflammatory responses²⁴). In our study, we could observe that laser irradiation group was able to significantly reduce the concentration of TNF-α, IL-1β, and IL-6 than control group. IL-10 inhibits IL-1β and TNF-α expression and is present in OA chondrocytes, where it may counteract their catabolic effects²⁵).

Interestingly since there are many pro-inflammatory cytokines exerting action on OA initiation and development, it is hard to obtain complete therapeutic effects by blocking the activity select cytokines. Our present results demonstrate that treatment with LILT increased the arthritic index and decreased TNF-α, IL-1β, and IL-6 concentration on day 21. From this point of view, LILT was significantly effective in reducing inflammatory cytokines (TNF-α, IL-1β, and IL-6) in joints of MIA-induced OA model, which can indicate an effective reduction in joint cartilage degradation. The responsiveness of the OA model to low-intensity laser therapy indicates that it may be an effective therapeutic approach for treating joint discomfort associated with OA.

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