Anti-inflammatory Effects of Low Intensity Laser Therapy on Adjuvant-induced Rheumatoid Arthritis in Rat

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Abstract. [Purpose] The purpose of this study was to evaluate the anti-inflammatory and analgesic efficacy of low-intensity laser therapy (LILT) on adjuvant-induced arthritis in a rat model. [Subjects] Thirty Sprague-Dawley rats were randomly divided into 3 groups: normal, placebo, and LILT groups. The arthritis group was treated with low intensity laser therapy using a gallium-aluminum-arsenide diode laser device for 3 weeks. Each group had 10 rats. All treatments were applied once a day, 5 days a week, for a total experimental period of 21 days. The hind paw volume and articular index of the knee joint along with TNF-α and IL-6 levels in the serum were used as outcome measures. [Results] Hind paw volume and the arthritis index of rats in the placebo group were significantly higher than those in the normal group (p<0.05). In the laser treatment group, the hind paw volume, the arthritis index, and TNF-α and IL-6 concentrations were significantly decreased than those in the placebo group. [Conclusion] We concluded that LILT is effective at alleviating RA symptoms such as edema, arthritic index, and the expression profiles of inflammatory cytokines in adjuvant-induced arthritic rats.

Key words: Inflammation, Low intensity laser, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease presenting with a wide range of articular and periarticular symptoms. The inflammation predominantly affects the synovial joint, resulting in the hyperplasia of synovial cells, excess synovial fluid, and pannus formation. This leads to joint pain, edema, stiffness, deformity, and disability. As most joint damage is largely irreversible, persistent damage will inevitably result in greater disability.

It has been reported that the inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin (IL)-1β, and IL-6, play key roles in mediating inflammation and joint damage during the development of RA. These inflammatory factors lead to the development of extra-articular features, such as alveolitis, glomerulonephritis, vasculitis, nerve compression, and neuropathy. Anti-inflammatory and analgesic drugs, such as steroids, anti-rheumatic drugs (DMARDs), anti-TNF-α therapy, and abatacept are often required to inhibit or halt the underlying immune process. However, these medications are associated with numerous adverse effects.

Treatment modalities of RA include analgesic drugs, non-steroidal anti-inflammatory drugs, physical therapy, electrotherapy, steroid injections, ultrasound, iontophoresis, and low intensity laser therapy. Low intensity laser therapy has many benefits, such as microcirculation enhancement, vasodilatation, tissue metabolism improvement, activation of angiogenesis, nerve regeneration, and analgesic effects at the tissue level. Low level laser photons can penetrate deep into the body through the skin, muscles, tendons, ligaments, nerves, and even bones in order to pinpoint and target painful and damaged areas. Therefore, low intensity laser therapy belongs to commonly recommended physical therapy methods used to treat arthritis. However, the effect of low intensity laser therapy on the therapeutic modulation for RA remains unclear. Particularly, no report has investigated the effect of low intensity laser therapy (LILT) on pain related to inflammatory cytokines.

Therefore, the present study was designed to investigate the effect of LILT on the evaluation of anti-arthritic activity and how it alters the response of inflammatory cytokines in adjuvant-induced arthritis in rats.

SUBJECTS AND METHODS

All experimental procedures in this study were reviewed and approved by the Ethical Review Committee of Dongshin University. In this study, we used 30 male Sprague-Dawley rats (Orient, Seoul, Korea), each weighing 150–160 g (age, 5 weeks). After rats were acclimatized for 1 week, chicken collagen II (Sigma, St. Louis, MO, USA) was dissolved in 0.1 mM acetic acid to a final concentration of 2 mg/mL. Rats were immunized by subcutaneous injection at the base of the tail with 100 µg collagen II emulsified in an equal
of the joints for the signs of arthritis was performed daily.

First immunization. These rats were regularly weighed and during the laser treatment for this safety. For the placebo arthritis induction. The researcher used protective eyeglasses.

A total 30 rats (10 normal rats, 20 arthritic rats) were used. These rats in the normal group received an injection of an equal volume of saline at the same location. In our study, a total 30 rats (10 normal rats, 20 arthritic rats) were used. These rats were randomly divided into 3 groups of 10 rats after adjuvant-induced arthritis (Table 1).

A gallium-aluminum-arsenide (Ga-Al-As, infrared laser) diode laser device (Chattanooga group, USA) with a wavelength of 850 nm, power output of 200 mV, continuous wave, and 0.07 cm² spot-area laser were used for the laser therapy. The therapeutic point was positioned medially to the patellar ligament, and treatment was applied towards the joint surface of the femur. The laser therapy group was treated with a dosage of 1.8 J/cm² for 1 minute in the peri-articular region of both hind paw. The laser therapy was applied 5 times per week during the 21 days following arthritis induction. The researcher used protective eyeglasses during the laser treatment for his safety. For the placebo laser group, the diode laser was applied in the same way, but the device was turned off during therapy sessions.

The paw volume of all the animal groups was measured by plethysmograph at 0, 7, 14, and 21 days after low intensity laser therapy. Paw volume measurements were performed using a water displacement plethysmometer, UGOBASIL (Biological Research Apparatus Co., Comerio-Varese, Italy), and the researcher was blinded to the treatment groups. The mean values were calculated and plotted at each time point. The clinical symptoms of arthritis in the hind limbs were evaluated by 2 blinded observers according to the visual appearance. The scores were rated on a scale of zero (no swelling or erythema) to 4 (excess edema with joint rigidity) for each knee joint. The mean articular index score was given for each foot of the adjuvant-induced arthritic rat. The total score for each animal was then calculated and used as the articular index with a maximum value of 16 points.

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 and IL-6, from the serum of the rats were measured by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers’ instructions (R&D System, Minneapolis, MN, USA). The concentrations of TNF-α and IL-6 were calculated from a standard curve. The serum biochemistry examination was assayed by microplate readers (Bio-Rad, USA).

Data analysis was performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). All the data are expressed as mean ± standard deviation (S.D.) of 3 replications. Differences between two groups were tested by one-way ANOVA, followed by the Student–Newman–Keuls multiple comparisons test when difference were detected. A p-value of less than 0.05 at the 95% confidence level was considered significant.

RESULTS

As shown in Table 2, rat paw edema volume increased significantly in the adjuvant-induced arthritic group compared to the normal group (p < 0.05). The laser therapy group showed a significant reduction in rat paw edema volume as compared with that of the placebo group (P < 0.05). The effects of laser therapy on the arthritis index of adjuvant-induced arthritic rats are shown in Table 3. The laser therapy group had a significantly lower arthritis index than that of the placebo group, assessed at days 14 and 21 days after the adjuvant-induced arthritis began (p < 0.05). The changes in the inflammatory cytokines (TNF-α, IL-6) in the adjuvant-induced arthritic rats are shown in Table 4. The level of TNF-α and IL-6 in the placebo group was significantly higher than that observed in the normal group. However, the TNF-α and IL-6 levels after treatment with the low intensity laser were significantly lower than that of the placebo group (p<0.05).

DISCUSSION

In the present study, we investigated the therapeutic effects of low intensity laser therapy on the edema, the articular index and inflammation in adjuvant-induced arthritis rat model. In this study, we have found that low intensity laser therapy may offer therapeutic benefits for the treatment of adjuvant-induced arthritis, at least partially through the reduction of edema, articular index, and inflammatory cytokines (TNF-α and IL-6).

Generally, the symptoms of RA such as synovitis, progressive pannus formation, marginal erosion of bone and cartilage destruction closely resemble those observed in the experimental model of type II collagen-induced arthritis (CIA)23, 24. Therefore, CIA in rats or mice is the most commonly use animal model to evaluate approved and pending therapies or to investigate the pathogenesis of RA. Paw edema is the main feature of RA induced by adjuvant collagen, and it is commonly used as a parameter for evaluating the presence of RA16, 25.

Low intensity laser therapy can result in pain relief, collagen proliferation, anti-inflammation effects, circulation enhancement, and peripheral nerve stimulation25. However, the mechanism of pain and inflammation reduction by LILT

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**Table 1. Experimental group design**

<table>
<thead>
<tr>
<th>Group (Total n=30)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group (n=10)</td>
<td>Arthritis not induced; no subsequent treatment</td>
</tr>
<tr>
<td>Placebo group (n=10)</td>
<td>Placebo laser therapy for 1 minute at peri-articular each point therapy after adjuvant-induced arthritis</td>
</tr>
<tr>
<td>LILT group (n=10)</td>
<td>LILT 1.8 J/cm² for 1 minute at peri-articular each point therapy after adjuvant-induced arthritis</td>
</tr>
</tbody>
</table>

*Low Intensity Laser Therapy
has not been fully understood yet. Studies rarely report any systematic recording of adverse effects. A clinical study by Meireles et al.\textsuperscript{26} reported that LILT is not beneficial at the wavelength, dosage, and power studied for the treatment of hands among patients with RA. However, in this study inflammation of the interphalangeal joint was significantly reduced. Inflammatory cytokines may play a role in the induction and maintenance of chronic inflammation in synovial tissue. In several experimental models of inflammation, TNF-α and IL-6 are both expressed very early on and play key roles\textsuperscript{1}. Our current study showed that low intensity laser therapy had a potent inhibitory effect on the levels of TNF-α and IL-6, and edema and the articular index were also reduced. Previous studies also reported the anti-inflammatory effect of LILT in RA by both a 780-nm and 830-nm Ga-Al-As diode laser\textsuperscript{16, 27}. Different experimental studies suggest that LILT has analgesic effects\textsuperscript{28}. Moreover, it has been reported that LILT has anti-inflammatory and analgesic effect due to its reductive effect in prostaglandin synthesis\textsuperscript{29}. Our findings are consistent with these reports.

Improvements in hind paw local microvascular circulation leads to the reduction of edema and better absorption of effusion. Thus, it may result in the reduction of the articular index. In conclusion, this study revealed that short-period application of LILT was more effective in reducing edema and inflammation than placebo laser treatment, in the arthritis group. Thus, our data suggest that LILT can be a potentially important aid in the treatment of RA, especially in patients who experience adverse effects to current drug and invasive treatments.

REFERENCES


### Table 2. Effects of low intensity laser therapy on the hind paw volume in adjuvant-induced arthritic rats (ml)

<table>
<thead>
<tr>
<th></th>
<th>0 day</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0.24±0.02</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>Placebo group</td>
<td>2.24±0.22</td>
<td>2.15±0.41</td>
<td>2.15±0.14</td>
<td>2.07±0.12</td>
</tr>
<tr>
<td>LILTa group</td>
<td>2.24±0.16</td>
<td>2.08±0.13</td>
<td>1.66±0.14</td>
<td>1.04±0.17</td>
</tr>
</tbody>
</table>

*Low Intensity Laser Therapy. Data were presented as mean±S.D. *: p<0.05 as compared to normal group. **: p<0.05 as compared to placebo group

### Table 3. Effect of low intensity laser therapy on the articular index in adjuvant-induced arthritic rats (point)

<table>
<thead>
<tr>
<th></th>
<th>0 day</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Placebo group</td>
<td>15.50 ± 0.71</td>
<td>15.50 ± 0.53</td>
<td>15.30 ± 0.68</td>
<td>15.20 ± 0.63</td>
</tr>
<tr>
<td>LILTa group</td>
<td>15.40 ± 0.84</td>
<td>15.20 ± 0.74</td>
<td>12.10 ± 1.29</td>
<td>9.40 ± 2.07</td>
</tr>
</tbody>
</table>

*Low Intensity Laser Therapy. Data were presented as mean±S.D. *: p<0.05 as compared to normal group. **: p<0.05 as compared to placebo group

### Table 4. Effect of low intensity laser therapy on inflammation cytokines in adjuvant-induced arthritic rats (pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>TNF-α</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>26.74±5.12</td>
<td>0.93±0.26</td>
</tr>
<tr>
<td>Placebo group</td>
<td>71.18±7.91</td>
<td>3.12±0.51</td>
</tr>
<tr>
<td>LILTa group</td>
<td>46.30±9.81</td>
<td>1.64±0.51</td>
</tr>
</tbody>
</table>

*Low Intensity Laser Therapy. Data were presented as mean±S.D. *: p<0.05 as compared to normal group. **: p<0.05 as compared to placebo group