Comparison of Low-Level Laser versus Intra-Articular Corticosteroid Therapy for Temporomandibular Joint Osteoarthritis in Rats

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ORIGINAL ARTICLE

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ABSTRACT

Osteoarthritis (OA) is an increasingly common deteriorating disorder of the temporomandibular joint (TMJ) for which there is an urgent need to improve the prevention and cure. Objectives: We aimed to compare low-level laser (LLL) against corticosteroids as an alternative treatment for TMJ-OA. Methods: Sixty rats with TMJ-OA were divided into OA (untreated), corticosteroid-treated, and LLL-treated groups. Animals were sacrificed at 1 and 4 weeks after treatment, and their TMJs were dissected for evaluation by histological analysis, histochemical analysis, and quantitative real-time polymerase chain reaction. Statistical comparison was conducted using one-way analysis of variance. Results: Histopathological examination revealed degenerative changes and loss of normal architecture in the untreated OA group and that these changes were decreased in both treatment groups. In histochemical analysis, collagen formation was higher in both treated groups than in the untreated group. Finally, tumor necrosis factor-α level was the highest in the OA group, followed by the corticosteroid- and LLL-treated groups. Conclusion: LLL may improve joint OA in the TMJ to a similar extent to corticosteroids and appears to have superior anti-inflammatory effects in the short-term.

Key words: dexamethasone, low-level laser therapy, osteoarthritis, temporomandibular joint, tumor necrosis factor-alpha

INTRODUCTION

Osteoarthritis (OA) is an increasingly common deteriorating joint disease characterized by progressive articular cartilage loss, osteophyte formation, synovial inflammation, and subchondral bone changes, including increased thickness that alter the shape and volume. Despite the common perception, it is not simply a disease of increased wear and tear but is a disorder of abnormal remodeling and joint failure.¹ Corticosteroids (CS) are potent anti-inflammatory medications, and intra-articular (IA) injections have widely been used to treat the symptoms of OA since approximately 5 decades. However, there have been alarming reports of post-injection complications, including the inhibition of proteoglycans synthesis, cartilage disintegration, and bone necrosis, that have precluded their use for temporomandibular joint (TMJ) pain. In addition, high doses of corticosteroids increase the possibility of aseptic bone necrosis.² More recently, a credible non-invasive treatment for TMJ disorders has been developed. This is low-level laser (LLL) therapy, which is a conservative and supportive physical therapy that has anti-inflammatory effects and can treat OA of the TMJ (i.e., TMJ-OA).³

To date, assessment of the anti-inflammatory effects of LLL therapy in clinical studies has only been subjective and based on the induction of pain rather than on the objective evaluation of inflammation.⁴ Consequently, there is a need for more objective and controlled studies to determine the effects of LLL therapy on inflammation in TMJ structures.

In this study, we compared the possible anti-inflammatory effects of LLL therapy with those of corticosteroids in albino rats with induced TMJ-OA.
METHODS

All experiments were conducted in the animal house at the Faculty of Medicine, Cairo University, Egypt, after receiving approval from the Ethics Committee on animal experimentation of the Faculty of Dentistry (Approval no.16/9/15).

Animals
Sixty adult healthy female albino rats, approximately 3–6 months old and weighing approximately 180–200 g were used. The animals were housed in a sterile controlled environment (temperature 23°C ± 5°C with a 12 h dark/light cycles) and fed with a standard pellet diet and allowed tap water ad libitum. They were individually kept in stainless steel cages and then randomly distributed into three groups of 20 animals each, as detailed in Table 1.

OA induction and confirmation
A single 2 mg/kg IA injection of monosodium iodoacetate (provided by Sigma-Aldrich Chemical Co. St. Louis, MO 63103 USA) dissolved in sterile 0.9% saline was injected into the right TMJ of rats. After 7 days, blood samples were collected from all animals for biochemical estimation of C-reactive protein.

Therapeutic interventions
Seven days after induction of OA, animals in Group II were sedated and given a single IA injection of 1.2 mg/kg dexamethasone (provided by AMRIYA for pharmaceutical industries, Alexandria, Egypt). An Indium Gallium Arsenide Phosphide (InGaAsP) system model Epic™ Biolase (USA) was used for the LLL therapy. Rats in Group III received mild chloroform sedation, and the tissue over TMJ region 5–10 mm posterior to the lateral eye canthus was irradiated. Seven 60 s sessions of LLL therapy were performed every other day for 2 weeks in accordance with the research by Khozeimeh et al., The selected physical parameters are summarized in Table 2.

Animal sacrifice
Animals were sacrificed by intra-peritoneal injection of 500 mg/kg sodium phenobarbital, as previously described by Al-Saffar et al., and the TMJ was then dissected. Tissues were preserved in 10% neutral buffered formalin for 24 h, dehydrated in ascending grades of ethyl alcohol, immersed in xylene, and embedded in paraffin wax. The sections measured 3–4 μm in thickness.

Histological, histochemical, and polymerase chain reaction analyses
Routine histological examination was performed by light microscopy after sections were stained with hematoxylin and eosin. Next, for histochemical analysis, sections stained with Masson trichrome were examined using a Leica Quin 500 analyzer computer system. Staining was measured as the area and the area percent stained in a standard measuring frame in ten fields per group under magnified (~400) light microscopy transferred to a screen. Areas showing green color staining were chosen for evaluation. These areas were masked by a blue binary color to be measured by the computer system. The means and standard deviations were obtained for each specimen. The mRNA expression level of tumor necrosis factor-alpha (TNF-α) was quantified by real-time quantitative PCR (qRT-PCR). We used 1000 ng of the total RNA from each sample for cDNA synthesis by reverse transcription with a high-capacity cDNA reverse transcriptase kit (Applied Biosystem, USA). The cDNA was subsequently amplified with the Syber Green I

Table 1: Experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (OA group, untreated)</th>
<th>Group II (CS group, treated)</th>
<th>Group III (LLL group, treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>OA induction</td>
<td>IA injection of 2 mg of mono-</td>
<td>IA injection of 2 mg of mono-</td>
<td>IA injection of 2 mg of mono-</td>
</tr>
<tr>
<td></td>
<td>iodoacetate</td>
<td>iodoacetate</td>
<td>iodoacetate</td>
</tr>
<tr>
<td>Treatment</td>
<td>No treatment</td>
<td>IA injection of dexamethasone</td>
<td>LLL therapy</td>
</tr>
<tr>
<td>Date of sacrifice</td>
<td>1 and 4 weeks after OA</td>
<td>1 and 4 weeks after</td>
<td>1 and 4 weeks after</td>
</tr>
<tr>
<td></td>
<td>confirmation</td>
<td>dexamethasone administration</td>
<td>LLLT</td>
</tr>
</tbody>
</table>

Abbreviations: CS, corticosteroids; IA, intra-articular; LLL, low-level laser; OA, osteoarthritis

Table 2. Parameters of the laser device

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser type</td>
<td>InGaAsP diode laser</td>
</tr>
<tr>
<td>Brand</td>
<td>Biolase</td>
</tr>
<tr>
<td>Model number</td>
<td>Epic 10</td>
</tr>
<tr>
<td>Wave length</td>
<td>940 nm</td>
</tr>
<tr>
<td>Operation mode</td>
<td>Continuous wave</td>
</tr>
<tr>
<td>Power output</td>
<td>0.2 W</td>
</tr>
<tr>
<td>Spot size</td>
<td>300 μm</td>
</tr>
<tr>
<td>Energy</td>
<td>4 J</td>
</tr>
<tr>
<td>Irradiation time</td>
<td>20 s</td>
</tr>
</tbody>
</table>
Table 3. Primers sequences for each gene

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence: 5′–3′</th>
<th>Gene bank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Forward: AACTCGAGTGACAAGCCCGTAG</td>
<td>XM00877275.1</td>
</tr>
<tr>
<td></td>
<td>Reverse: GTACCACCAGTTGGTTGTCTTTGA</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward: CACCCTGTTGCTGTAGCCATATTC</td>
<td>XR598347.1</td>
</tr>
<tr>
<td></td>
<td>Reverse: GACATCAAGAAGGTGGTGAAGCAG</td>
<td></td>
</tr>
</tbody>
</table>

PCR Master Kit (Fermentas) in a 48-well plate using a Step One instrument (Applied Biosytem, USA), as follows: 10 min at 95°C for enzyme activation followed by 40 cycles of 15 s at 95°C, 20 s at 55°C, and 30 s at 72°C for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold values of the GAPDH housekeeping gene by the ΔΔCt method. We used 1 μM of primers specific for each target gene. Primers sequences are shown in Table 3.

Statistical analysis
Data are presented as means and standard deviations (SD). Given that analysis using the Kolmogorov–Smirnov test for normality indicated that most data were normally distributed, one-way analysis of variance (ANOVA) was used for comparisons between groups. This was followed by Tukey’s post-hoc test when the difference was found to be significant. Unpaired t-tests were used to compare the same treatment group between the two observation dates. The significance level was set at p < 0.05. Statistical analysis was performed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Histological results at 1 week
Histopathological examination of the OA group showed mild degenerative changes in the condyle (Figure 1A). In the LLL group, similar to that of CS group, the condyle appeared more or less normal but retained some degenerative changes. However, in the CS group, the central region of the disc was ankylosed to the condyle. At the anterior region along the ligaments’ insertion, osteoclasts were observed in the Howship lacunae (Figures 1B and 1C). As for the synovial membrane, there were mild signs of inflammation in the OA group (Figure 2A), but this appeared normal in the CS and LLL groups. Large amounts of extravasated, ruptured, and congested blood vessels were noticed in the LLL group (Figures 2B and 2C).

Histological results at 4 weeks
Histopathological examination of the OA group showed moderate degenerative changes in the condyle at 4 weeks (Figure 3A). In the CS and LLL groups, most of the inflammatory signs had subsided, but with some evidence of degeneration persisted (Figures 3B and 3C). The synovial membrane appeared thickened and inflamed in the OA group and there was fatty degeneration in the sub-lining layer (Figure 4A). This appeared normal in the treated groups, with few mononuclear cell infiltrates (Figures 4B and 4C).
Figure 3. Sagittal section in condyle (4 weeks)
A: The OA group shows abnormal thickness (asterisk), calcified cartilage (cc), osteoclasts (OCL), many reversal lines (arrows), and sclerosis (circle). B: The CS group shows roughness (arrows), calcified cartilage (cc), atrophied chondrocytes (asterisks), and marrow cavities filled with chronic inflammatory cells (m). C: The LLL group shows calcified cartilage (cc), sclerosis (asterisks), and many reversal lines (arrows). Also shown: fibrous layer (F), proliferative layer (P), hypertrophic layer (H), subchondral bone (SB), and disc (D). (hematoxylin and eosin, ×400.)

Figure 4. Photomicrograph of the synovial membrane (4 weeks)
A: The OA group shows thickened synovial lining (L) with few cellular contents in the sub-lining (SL) layer and fatty degeneration (asterisks). B: The CS group has many villi projecting into the upper joint cavity (arrow heads) and few mononuclear cells (arrows) C: Normal L and SL layers are shown. (hematoxylin and eosin, ×400.)

Histochemical results (Masson trichrome stain) at 1 and 4 weeks
The highest mean values for the Masson trichrome stain at 1 week were recorded in the LLL group, followed by the CS group, and then the OA group. ANOVA revealed that the difference between all groups was statistically significant (P < 0.0001), with Tukey’s post-hoc test indicating significant differences between each compared pair (Table 4). The highest mean results for the Masson trichrome stain at 4 weeks were recorded in the CS group, followed by the LLL group, and then the OA group. ANOVA revealed that the difference was statistically significant between all groups (P < 0.0001), with Tukey’s post-hoc test indicating significant differences between each compared pair (Table 4).

Polymerase chain reaction for tumor necrosis factor-alpha at 1 and 4 weeks
The highest mean values were recorded in the OA group, followed by the CS group, and then the LLL group. ANOVA revealed that the difference between all groups was statistically significant (P < 0.0001), and Tukey’s post-hoc test revealed a significant difference between each compared pair (Table 4).

DISCUSSION
Our histological results in the OA group at 1 and 4 weeks revealed a general destruction of the condylar cartilage and subchondral bone. This resembled the clinical and experimental observations of Wang et al., who reported that the condyle was active and underwent severe destruction and remodeling. We also found thickening and roughness of the fibrous layer with atrophy of the proliferative layer, consistent with previous findings of chondrocyte hypertrophy-like changes in OA. The hypertrophic layer also showed focal chondrocyte loss with some areas of hyalinization. Similarly, in rats injected with collagenase, it was reported that large areas of chondrocytes in the superficial and middle zones of the cartilage disappeared by weeks 1 and 6 because of cartilage disintegration in the deep and calcified zones. The hypertrophic layer also showed areas of calcified cartilage in our study. Goldring and Marcu also showed that naturally resting chondrocytes in OA were activated and underwent a phenotypic alteration, resulting in chondrocyte proliferation, which was followed by raised catabolic activity and hypertrophy-like maturation and calcification of the cartilage.

The subchondral bone showed vertical and horizontal cracks with widened marrow cavities filled with chronic inflammatory cells and fibroblast-like cells. Goldring also reported increases in microcracks, vascular communication channels, and fissures in joints with OA. Moreover, they demonstrated that vascular invasion of bone marrow tissue into the

Table 4: Masson trichrome mean area percent and TNF-α relative quantitation at 1 and 4 weeks (ANOVA)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Masson trichrome (Mean ± SD)</th>
<th>TNF-α (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA1</td>
<td>*8.63 ± 2.32</td>
<td>***3.49 ± 0.27</td>
</tr>
<tr>
<td>CS1</td>
<td>*22.02 ± 2.53</td>
<td>***2.63 ± 0.07</td>
</tr>
<tr>
<td>LLL1</td>
<td>*27.85 ± 3.42</td>
<td>***1.90 ± 0.34</td>
</tr>
<tr>
<td>OA4</td>
<td>**4.62 ± 2.02</td>
<td>****4.61 ± 0.35</td>
</tr>
<tr>
<td>CS4</td>
<td>**0.60 ± 0.57</td>
<td>*****2.27 ± 0.43</td>
</tr>
<tr>
<td>LLL4</td>
<td>**6.38 ± 2.94</td>
<td>****1.52 ± 0.36</td>
</tr>
</tbody>
</table>

Primary comparison was by ANOVA. Significance level p < 0.05, asterisks indicate that the differences between compared groups at 1 week (OA1, CS1, LLL1) and 4 weeks (OA4, CS4, LLL4) were significant. Tukey’s post-hoc test: means of comparing groups at 1 and 4 weeks with different superscript letters are significantly different.
subchondral bone induced the synthesis of matrix metalloproteinases (MMPs) that caused disintegration of the adjacent cartilage.\textsuperscript{13} The subchondral bone in our OA group also showed areas of sclerosis with large numbers of osteoclasts. Other researchers also reported that early OA was associated with an increase in bone resorption.\textsuperscript{14,15} concluding that subchondral bone sclerosis and increased adjacent bone volume were possible adaptive responses.

Similar to previous findings, we observed a tide mark.\textsuperscript{12} Goldring and Marcu described this as a basophilic line in histological sections. They concluded that it reflected an accumulation of macromolecular debris, including DNA that did not penetrate the barrier between non-calcified and calcified regions. As for the synovial membrane, it has been reported that the synovial fluid obtained from patients with OA can enhance bone resorption by activating the OPG/RANK/RANKL system. Both OPG and RANKL are synthesized by chondrocytes and contribute to bone remodeling by enhancing the maturation and activation of synovial osteoclasts.\textsuperscript{16} The changes we observed in the synovium are consistent with these.

In our study, dexamethasone was used as the gold standard anti-inflammatory treatment for OA. Although we gave the rats a single injection, the condylar head showed many osteoclasts, which indicated resorption. El-Hakim et al. also observed that IA injection of dexamethasone into the condylar heads of rats resulted in massive degradation of the articular fibrocartilage cap.\textsuperscript{7} In this study, the ankylosis observed at the disc in the CS group may have been caused by repeated IA injections (this group received injections of monosodium iodoacetate and dexamethasone). Similar findings were seen on MRIs, with anterior disc dislocation and severe condylar necrosis after a second IA injection of triamcinolone.\textsuperscript{17}

We compared LLL therapy with traditional corticosteroid therapy by IA injection. The laser was applied to three points (capsule, retrodiscal area, and condylar neck), using the physical parameters Khozeimeh et al. had previously applied.\textsuperscript{8} Graciele et al. also selected multiple points to irradiate a wider joint area.\textsuperscript{18} It was notable that most signs of inflammation subsided after LLL and therapy, which is consistent with the findings of Peimani and Sardary. They showed that LLL therapy resulted in marked improvements in the grade of cartilage defects and the number of inflammatory cells in rats with induced TMJ-OA.\textsuperscript{19} Similarly, Barbosa et al. demonstrated that LLL therapy had a positive effect on bone regeneration and the synthesis of cartilage.\textsuperscript{20} In other research, Alves et al. reported that LLL therapy inhibited the inflammatory reaction in the early late stages of RA.\textsuperscript{21} and Sobol et al. reported that LLL therapy accelerated cellular activity, especially in cells responsible for cartilage repair.\textsuperscript{22}

Another noteworthy histological finding in our study was that LLL therapy increased the number of blood vessels. In research by da Rosa et al., neovascularization was shown in laser-treated groups on the seventh day of treatment.\textsuperscript{23} Dantas et al. suggested that the high rates of newly formed capillary blood vessels enhanced the formation of granulation tissue, an initial step in the healing process.\textsuperscript{24} LLL therapy causes vasodilation by enhancing smooth muscle relaxation, which increases oxygenation of the treated cells and facilitates the influx of immune cells, thereby accelerating the healing process.\textsuperscript{25}

Regarding our histochemical results, Masson trichrome staining showed that the amount of collagen formed in both of the treated groups was higher than that in the untreated group. Supporting this, a previous report indicated that low-dose dexamethasone preserved matrix formation, decreased proteoglycan and collagen loss from the ECM, and maintained chondrocyte vitality in OA.\textsuperscript{26} Also, LLL therapy has been shown to accelerate the initial breakdown of cartilage destroyed by collagenase and to stimulate fibroblasts to produce collagen type III.\textsuperscript{27} In this way, LLL therapy may exert its beneficial effects in OA.

Concerning our qRT-PCR results. LLL therapy at the selected dose reduced the TNF-α levels, resulting in an anti-inflammatory effect superior to that of dexamethasone. Similarly, LLL therapy may reduce TNF-α and IL1-β levels, effectively decreasing the inflammatory reaction and oxidative stress in rat tibialis anterior muscles after creating a cryolesion.\textsuperscript{28}

Elsewhere, LLL therapy was reported to decrease the expression of inflammatory mediators such as IL-1α, IL-1β, and TNF-α.\textsuperscript{29}

Further objective animal research is now required to define the optimal parameters for LLL therapy (e.g., energy doses, frequency, and duration) before translation into human trials can be justified. Efforts should also be made to investigate the combined effect of CS and LLL therapy in treating OA. In addition, we recommend that other investigatory techniques be used to clarify the mechanism of the anti-inflammatory effects of LLL in the treatment of OA. Perhaps more importantly, however, we must ensure that studies include long-term follow-up periods so that we understand the implications of this therapeutic option.

**CONCLUSION**

We conclude that LLL therapy using the parameters in this study has anti-inflammatory effects superior to those of dexamethasone in rats. LLL therapy is a safe, effective, and non-invasive tool which has the potential to be applied in a wider scale.
CONFLICT OF INTEREST

The authors declare that there were no financial or non-financial conflicts of interest in this article.

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