Original Article

Effect of Low-Level Laser on Healing of Temporomandibular Joint Osteoarthritis in Rats

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Abstract

Objective: Temporomandibular disorders (TMD) are clinical conditions characterized by pain and sounds of the temporomandibular joint (TMJ). This study was designed to assess the effect of low-level laser therapy (LLLT) on healing of osteoarthritis in rats with TMD.

Materials and Methods: Thirty-two male Wistar rats (250–200 g) were housed in standard plastic cages. After injection of Complete Freund's adjuvant into the TMJ, rats were randomly divided into two groups of 16 (case and control) and anesthetized; then osteoarthritis was induced via intraarticular injection of 50 µl of Complete Freund's adjuvant; into the bilateral TMJs. In the case group, LLLT was done transcutaneously for 10 minutes daily, starting the day after the confirmation of osteoarthritis. Exposure was performed for 10 minutes at the right side of the TMJ with 880 nm low-level laser with 100 mW power and a probe diameter of 0.8 mm. Control rats were not treated with laser.

Results: After three days of treatment the grade of cartilage defects, number of inflammatory cells, angiogenesis, number of cell layers and arthritis in rats in the case group were not significantly different compared with controls ($P>0.05$). After seven days, the grade of cartilage defects, number of inflammatory cells, number of cell layers, and arthritis in the case group improved compared to controls ($P<0.05$); angiogenesis in both groups was similar.

Conclusion: Treatment of TMD with LLLT after 7 days of irradiation with a wavelength of 880 nm was associated with a greater improvement compared to the control group.

Key Words: TMJ disorders; laser therapy; osteoarthritis

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INTRODUCTION

Temporomandibular disorders are a varied set of clinical conditions characterized by pain in the temporomandibular joint (TMJ) and/or the masticatory muscles. In the body, the TMJ is a synovial, bilateral joint with unique morphology and function, and a stress-sensitive cartilage that is subject to extensive tissue remodeling [1-3]. Associations between developments of osteoarthritis-like, degenerative changes of

articular cartilage and common dysfunction of TMJ have been reported previously. Commonly, progressive and more degenerative processes, with unknown cause in most cases, occur after osteoarthritic changes in the TMJ during life [4-8].

Pain relief and functional recovery can be achieved by inhibiting the factors causing cartilage deformity as much as possible, since they result in cartilage loss and articular deformity. Use of non-steroidal anti-inflammatory drugs (NSAIDs), synovectomy, steroids and immunosuppressants as treatment methods for artificially induced osteoarthritis have been studied [9-13]. Development of drugs or treatment methods that are not harmful seems necessary because of the complications and side effects of most of the existing methods [14]. Low-level laser therapy (LLLT) is a treatment approach with a wide range of applications and with biomodulative and analgesic purposes. In several studies, LLLT was used for the treatment of soft tissue injuries, rheumatoid arthritis, musculoskeletal pain and dental problems. Though controversy was observed in its efficacy, positive clinical results have been reported [14-17]. Some studies evaluated the use of LLLT for treatment of atherosclerosis, non-healing ulcers, and various degenerative conditions [18-20]. Also, augmentation of heat shock proteins and pathophysiological improvement of arthritic cartilage resulted in an osteoarthritis model for treatment with LLL [21]. Dental and periodontal treatment applications of LLLT have been the subject of many in vivo and in vitro studies; and due to its ability to expedite the healing process, it has been used after gingivoplasty and gingivectomy [22-23]. LLLT for temporomandibular disorders, in spite of the common treatment modes available, has proven capable of relieving pain in minutes after administration, bringing about a significant improvement for the patient [24]. Analgesic effects reported by most authors in the literature were the main reason for the use of LLLT for TMD [25-27].

Nonetheless, it has been shown that often laser therapy can be used in lieu of anti-inflammatory medication; thus, preventing side effects [26]. However, LLLT is not the definitive treatment for temporomandibular disorders, regardless of all benefits of laser treatment.

Because of ethical reasons much of the research cannot be done on humans and studies in animal models are used for this purpose. Rats may be used as convenient animals for experimental studies for treatment of TMD, due to the similarity of the TMJ of rats and humans. Therefore, our study was designed to assess the effect of LLLT on healing of osteoarthritis in rats with TMD.

MATERIALS AND METHODS

Thirty-two male Wistar rats (200–250 g) were housed in standard plastic cages with food and water available ad libitum. Ethical review of the animal procedures was obtained from the Institutional Animal Care and Use Committee of Rafsanjan University of Medicine, and all experiments were designed to minimize animal suffering and to use the minimum number of animals required to achieve a valid statistical evaluation. The rats were anesthetized intraperitoneally with a ketamine and xylazine mixture (Figure 1-a). Osteoarthritis was induced with an intraarticular injection of 50 µl of Complete Freund's adjuvant (CFA), (oil/saline at a ratio of 1:1) into the bilateral TMJs using a 30-gauge needle and 1-mL syringe (Figure 1-b). After injection of CFA into the TMJ, using random-maker software "Random Allocation", the rats were randomly divided into two groups of 16 (case and control). In the case group, LLLT was done transcutaneously for 10 minutes every day, using a LLL AZOR-2k (Azor Medical Equipment, Moscow, Russia), starting the day after the confirmation of osteoarthritis.

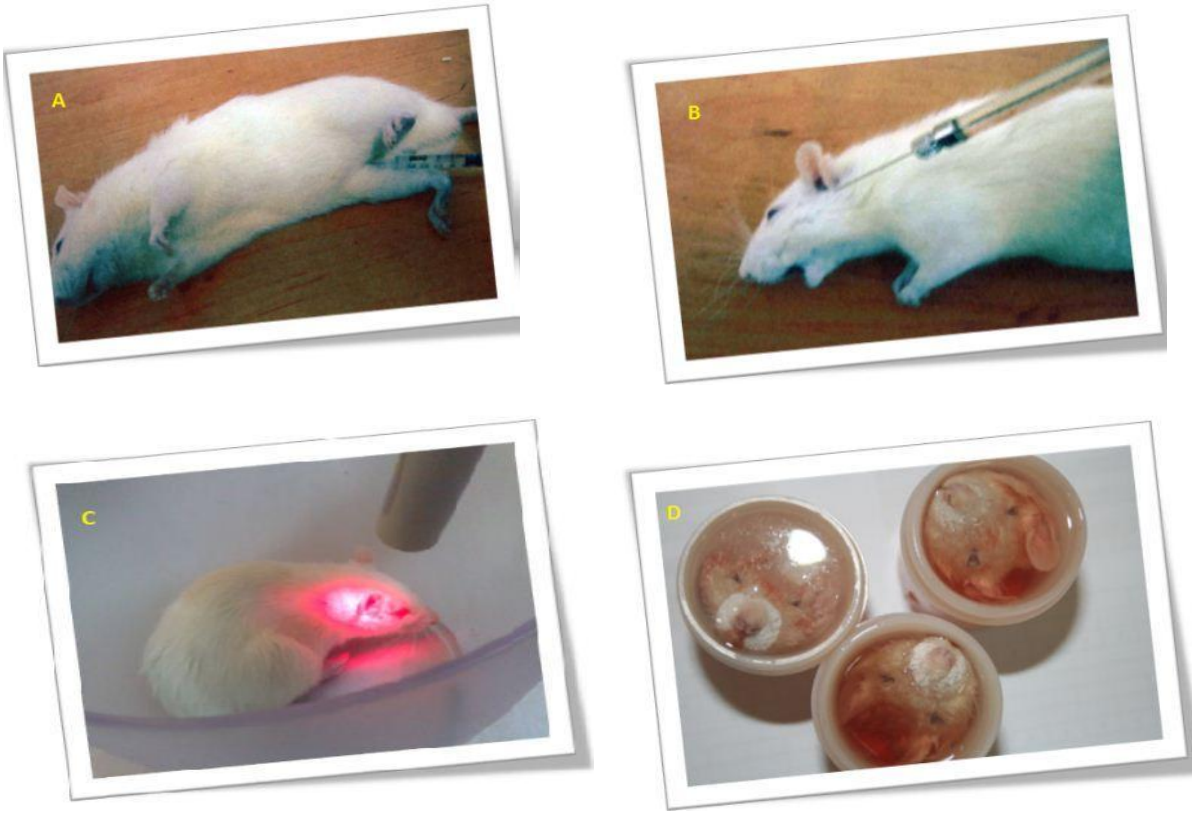

Fig 1. Osteoarthritis induced by an intraarticular injection of 50 µl of complete Freund's adjuvant, into the bilateral TMJs in rats

Exposure was performed for 10 minutes at the right side of TMJ with 880 nm low-level laser with 100 mW power and a probe diameter of 0.8 mm (Figure 1-c). In the control group, rats were not treated with laser, and the same procedure was performed, but the probe was turned off. Each rat was kept in one cage and was able to rotate 360 degrees to obtain food and water freely.

The heads of the rats were dissected from euthanized rats on day three (8 rats in the case and 8 rats in the control groups), and on day seven (8 rats in the case and 8 rats in the control groups). The separated heads were fixed in 10% formalin and were then carefully oriented in the paraffin blocks (Figure 1-d). The TMJ was removed and fixed in 4% paraformaldehyde and demineralized in 15% EDTA.

The specimens were dehydrated in graded concentrations of alcohol and xylene, embedded in paraffin, and cut serially into 4 µm sagittal sections.

Next, they were stained with hematoxylin-eosin. An observer, blinded to the experimental design, in consultation with two pathologists, evaluated histopathological alterations of the joints after standardization of the measurements.

The presence of angiogenesis, grade of cartilage defects, the number of cell layers, the number of inflammatory cells, and arthritis were assessed (Figure 3). Arthritis in rats was confirmed using clinical signs and based on swelling and redness.

All statistical analyses were performed using SPSS software (version 20; SPSS Inc., Chicago, IL).

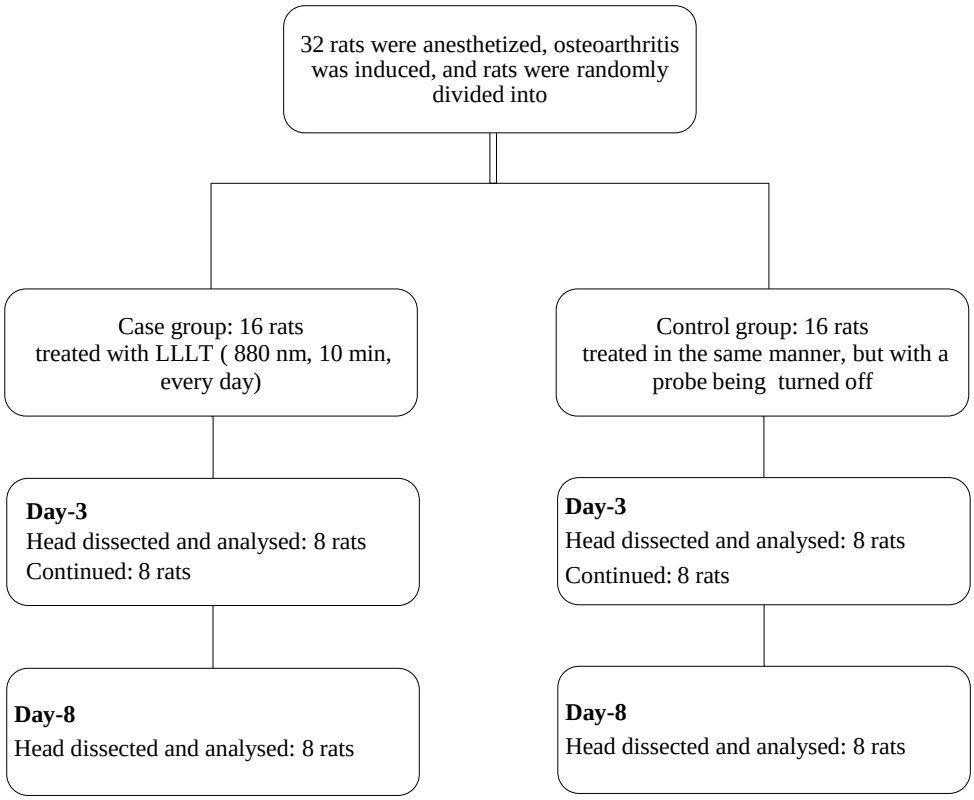

Fig 2. Flowchart of the study

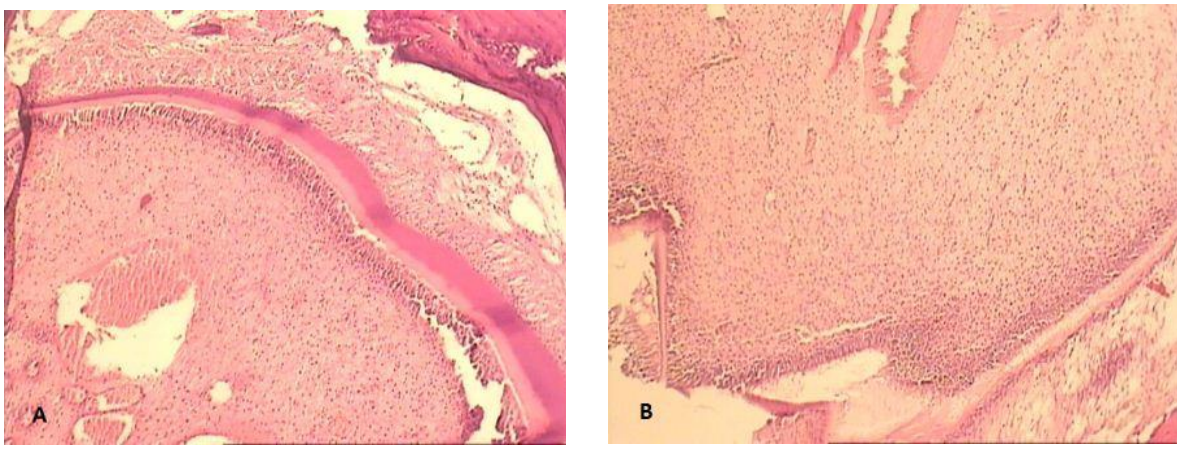

Fig 3. Photomicrographs of the histopathological analysis of TMJ. A: control group, B: case group (H&E staining 100×)

The data were presented and statistically significant differences among the groups were compared using the Mann-Whitney U test. P-values less than 0.05 were considered to indicate statistical significance.

RESULTS

Figure 2 shows the algorithm of the study, number of rats, treatment, follow-up and analyses.

Results of the comparison of the frequencies, grade of cartilage defects, number of inflammatory cells, number of cell layers, arthritis and angiogenesis between the case and the control groups after three days of treatment are shown in Table 1.

As shown, the grade of cartilage defect in all rats in the case group was irregular and superficial erosion, and deep defects in the cartilage were more than in the control group, but differences were not statistically significant (P>0.05).

Inflammatory cells and angiogenesis between the groups were similar (P>0.05).

Half the rats in the control group showed one cell layer; while most rats in the case group showed more than one cell layer; also, arthritis in most rats in the case group was average but in the control group was severe. Differences in the number of cell layers and arthritis between the case and control groups were not statistically significant (P>0.05).

Table 1. Comparison of the frequencies of studied variables between the case and control groups three days after the intervention

		Cases	Controls	P-value
Grade of cartilage defect	Normal	0	2 (12.5)	
	Irregularity and superficial erosion	10 (62.5)	7 (43.8)	
	Deep cartilage defects	6 (37.5)	5 (31.2)	
	Calcified cartilage defect spread to the tissues	0	2 (12.5)	
	Median [IQR]	2 [2-3]	2 [2-3]	0.89
Number of inflammatory cells	Low	3 (18.8)	2 (12.5)	
	Medium	6 (37.5)	5 (31.2)	
	High	7 (43.7)	9 (56.3)	
	Median [IQR]	2 [2-3]	2 [2-3]	0.56
Number of cell layers	1	3 (18.7)	8 (50)	
	2-3	9 (56.3)	7 (43.7)	
	4-5	2 (12.5)	1 (6.3)	
	> 5	2 (12.5)	0	
	Median [IQR]	2 [2-2.75]	1.5 [1-2]	0.056
Arthritis	Mild	0	2 (12.5)	
	Average	12 (75)	5 (31.2)	
	Severe	4 (25)	9 (56.3)	
	Median [IQR]	2 [2-2.75]	2 [2-3]	0.31
Angiogenesis	Limited number of vessels	5 (31.2)	4 (25)	
	Slight increase in small blood vessels	2 (12.5)	5 (31.2)	
	A moderate increase in small blood vessels	6 (37.5)	6 (37.5)	
	A large number of small blood vessels	3 (18.8)	1 (6.3)	
	Median [IQR]	3 [1-3]	3 [2-3]	0.62

Data presented as number (percentage), and median [IQR]
P-values calculated by the Mann-Whitney U test

Table 2 shows the the results seven days after the intervention assessing the grade of cartilage defect, number of inflammatory cells, number of cell layers, arthritis and angiogenesis between the case and the control groups. The grade of cartilage, number of inflammatory cells, number of cell layers, and arthritis in the case group improved compared to the controls (P<0.05); but angiogenesis was similar between the case and the control groups after seven days (P=0.05).

Arthritis in all rats in the case group on day 3 was severe or average but on day seven, arthritis in half of these rats improved while in the control group slight arthritis progressed to severe arthritis. These changes between groups were statistically significant (P=0.005). Also, the grade of cartilage defect, number of inflammatory cells and number of cell layers in the case group improved after increasing the time of treatment but in controls exhibited no change after increasing the treatment time.

Table 2. Comparison of the frequencies of studied variables between the case and control groups seven days after the intervention

		Cass	Control group	P-value
Grade of cartilage defect	Normal	6 (37.5)	2 (12.5)	
	Irregularity and superficial erosion	9 (56.3)	4 (25)	
	Deep cartilage defects	1 (6.2)	8 (50)	
	Calcified cartilage defect spread to the tissues	0	2 (12.5)	
	Median [IQR]	2 [1-2]	3 [2-3]	0.003
Number of inflammatory cells	Low	6 (37.5)	2 (12.5)	
	Medium	6 (37.5)	2 (12.5)	
	High	4 (25)	12 (75)	
	Median [IQR]	2 [1-2.75]	3 [2.25-3]	0.015
Number of cell layers	1	2 (12.5)	8 (50)	
	2-3	4 (25)	5 (31.2)	
	4-5	8 (50)	3 (18.8)	
	> 5	2 (12.5)	0	
	Median [IQR]	3 [2-3]	1.5 [1-2]	0.007
Arthritis	Mild	8 (50)	2 (12.5)	
	Average	5 (31.2)	2 (12.5)	
	Severe	3 (18.8)	12 (75)	
	Median [IQR]	1.5 [1-2]	3 [2.2-3]	0.004
Angiogenesis	Limited number of vessels	4 (25)	5 (31.2)	
	Slight increase in small blood vessels	3 (18.8)	3 (18.8)	
	A moderate increase in small blood vessels	1 (6.3)	5 (31.2)	
	Large number of small blood vessels	8 (50)	3 (18.8)	
	Median [IQR]	3.5 [1.2-4]	2.5 [1-3]	0.31

Data presented as number (percentage), and median [IQR]
P-values calculated by the Mann-Whitney U test

DISCUSSION

The data of histological analysis in this study suggest that no improvement was observed in the case group after 3 days of irradiation around the TMJ compared to the controls with regard to the grade of cartilage defect, number of inflammatory cells, number of cell layers, arthritis and angiogenesis. With an increase in the laser irradiation days from 3 days to 7 days, statistically significant improvements in the grade of cartilage defect, number of inflammatory cells and number of cell layers were seen.

Based on our findings, increase in the laser irradiation sessions can be useful for treatment of osteoarthritis in rats with TMD.

The stimulatory effects of 630 nm low level laser irradiation on bone formation in the condylar region during mandibular advancement in rabbits were assessed by Abtahi et al, [28]. They showed that after 3 weeks of irradiation around TMJ, a significant increase in newly formed bone was observed.

Other studies reported an over-arching clinical rationale for use of LLL in conditions such as arthritis. Shen and colleagues assessed the efficacy and safety of 650 nm laser irradiation in 40 patients with knee osteoarthritis which were randomly allocated to an active laser group or to a placebo laser group (20 per group). They showed the advantages of laser treatment in these patients [29]. Also, Ekim et al. [30] evaluated the efficacy of LLLT in patients with rheumatoid arthritis with carpal tunnel syndrome and showed that laser therapy seemed to be effective for pain and hand function and suggested that LLLT may be used as a good alternative for treatment of patients with rheumatoid arthritis and carpal tunnel syndrome [33].

These studies concluded that this treatment is associated with anti-inflammatory effects. In agreement with previous in vitro and in vivo studies, our findings showed that laser irradiation can be useful for treatment of TMD.

Cho et al. [35] reported the induction of osteoarthropathy into both knees of 25 normal rabbits and demonstrated that edema and heat sensation significantly decreased and no inflammatory cells were observed histologically in the groups treated with LLLT, as compared to the control groups. They reported that after 2 weeks, no significant treatment effect was seen, but significant improvement was observed after 4 weeks of treatment. They believed that LLLT should be continued for at least 3 weeks in these patients. Brosseau et al. [36] reported that LLLT is effective for rheumatoid arthritis. Amano et al. [37] reported that LLLT is effective for rheumatoid arthritis due to a direct photochemical effect. Our study differed from the studies mentioned above, as we induced non-inflammatory osteoarthritis. However, unlike our study Brosseau et al. [36] reported that LLLT is not effective in osteoarthritis.

The wavelength and the type of irradiation as well as the time of exposure are key factors for the efficacy of laser therapy, and we think that time of exposure, use of just one wavelength and a narrow spectrum of therapeutic doses should be noted as limitations of this study. Therefore, we suggested further studies in order to examine the efficacy of LLL irradiation in various conditions and time periods.

CONCLUSION

Treatment of TMD with LLLT after 7 days of irradiation with a wavelength of 880 nm was associated with a greater improvement compared to the control group.

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