The Anti-inflammatory Effect of Low-Level Laser Therapy on Experimentally Induced Inflammation of Rabbit Temporomandibular Joint Retrodiscal Tissues

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Aims: *To investigate the effect of low-level laser therapy (LLLT) on experimentally induced inflammation in retrodiscal tissues of the rabbit temporomandibular joint (TMJ) using scintigraphic imaging.* ***Methods:*** *Eleven male New Zealand rabbits were included in this study. Six randomly selected rabbits were imaged to provide normal joint images (normal group) before the initiation of the experiment. A 5% formalin solution was locally injected into both right and left TMJs of all rabbits. Subsequently, Ga-Al-As laser (wavelength: 815 nm; energy density: 12 J/cm*^{2}*; output power: 250 mW) was applied for 48 seconds. The treatment was performed six times for 2 weeks to the left TMJ of all rabbits. The right TMJs of the rabbits were used as the control (nontreated) TMJ group, while left TMJs were used as the treated TMJ group. Static images of TMJ were taken at 24 hours, 7 days, and 14 days after the beginning of the treatment. The images of all TMJs were taken in the posteroanterior direction with the rabbit under sedation and its mouth open. The Mann-Whitney* U *test was used to compare group differences, and intragroup differences were determined by the Friedman test and Wilcoxon sign test.* ***Results:*** *Significant differences were found between normal and both the control and treated TMJ groups. A reduction of inflammation in both treated and control TMJ groups was obtained, but there was no statistically significant difference between the groups.* ***Conclusion:*** *Under the conditions used in this study, quantitative scintigraphic measurements of TMJ inflammation of the treated TMJ group decreased but did not differ significantly from those of the control TMJ group.* J OROFAC PAIN 2010;24:293–297

Key words: aseptic inflammation, experimental study, low-level laser therapy, scintigraphy, temporomandibular joint

Inflammatory disorders of the temporomandibular joint (TMJ) are defined as a group of disorders in which internal and related structures of the TMJ become inflamed.[1,2] The aim of treatment of inflammatory joint disorders related to trauma is to control intrinsic micro- and macrotrauma and to allow tissue regeneration by controlling inflammation and internal joint pressure. A multidisciplinary approach consisting of behavioral therapy, soft diet, anti-inflammatory drugs, occlusal splint, and physical treatment has been described for the treatment of inflammatory temporomandibular disorders (TMD).[2] Low-level laser therapy (LLLT) has been proposed as an alternative treatment modality for treating inflammatory TMD.[3–5]

The exact mechanism underlying the effects of LLLT is still unknown, but it has been suggested that LLLT reduces inflammation and pain.[6] The effect of different types, durations, and doses of LLLT in intracapsular diseases of the TMJ has been shown in various clinical studies.[4,7] Hansson[3] applied infrared laser to five patients with arthrogenous pain. He reported that intra-articular inflammation of the TMJ was removed. Mazzetto et al[4] concluded from their double-blind study that LLLT is effective for pain control of subjects with capsulitis, synovitis, retrodiscitis, and painful disc displacement with reduction. Fikácková et al[7] reported that LLLT reduced pain and inflammation in a patient diagnosed as having arthralgia of the TMJ caused by disc displacement with reduction.

Although there are studies showing successful results of LLLT, the findings are open to debate due to utilization of different wavelengths, frequencies, exposure times, and durations of lasers in different TMD conditions.[8] Furthermore, there is a lack of details of the methodology used in many clinical trials, such as direction, diameter, and location of the laser tip.[8] Also, the evaluation of the anti-inflammatory effect of LLLT in clinical studies is subjectively based on the induction of pain rather than the objective measurement of inflammation.

Because of these different opinions and the subjective findings, there is a need for further objective and controlled studies to identify if LLLT is indeed effective on inflammation of TMJ structures. Therefore, the purpose of this study was to use scintigraphic imaging to investigate the anti-inflammatory effect of LLLT on experimentally induced inflammation in retrodiscal tissues of the rabbit TMJ. It was hypothesized that LLLT had an anti-inflammatory effect on retrodiscitis.

Materials and Methods

Animals

Eleven adult male New Zealand white rabbits (2.5 to 3.25 kg) were included in the study. The rabbits were housed in individual cages and fed ad libitum a pelleted feed mixture. They were kept on a 12-hour light/dark cycle. After 2 weeks of acclimatization, the study was initiated.

Before the initiation of the experiment, six randomly selected rabbits out of the 11 were imaged to provide normal joint images (the normal group). Later, inflammation was created in both TMJs of all 11 rabbits by formalin. The right TMJ of all rabbits constituted the control (nontreated) TMJ group; the left TMJ of all rabbits was the treated group. Sedation and anesthesia was induced by intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (10 mg/kg) before all scintigraphic imaging procedures and induction of inflammation. None of the rabbits showed any sign of vital changes after sedation. The same veterinarian applied the anesthesia each time before scintigraphic imaging and remained throughout the imaging procedure until the rabbit recovered from sedation. The study was approved by the Ethical Committee of Istanbul University Faculty of Medicine for Animal Research.

Induction of Aseptic Inflammation of TMJ

The right and left TMJs of all rabbits were shaved and cleansed with a Betadine solution. The rabbits received 0.5 mL of 5% buffered formalin solution injected from a 30-gauge needle inserted beneath the zygomatic process of the temporal bone, just posterior of the condyle; the needle tip was moved medioanteriorly towards the retrodiscal tissue, where the formalin was deposited.

Laser Irradiation

LLLT was performed by a continuous semiconductive Ga-Al-As (Gallium-Aluminium-Arsenide) laser (MC Laser) with an output power of 250 mW and an energy density of 12 J/cm^2, operating at a wavelength of 815 nm. The laser delivers a spot of approximately 10-mm diameter.

Treatment Protocol

The treatment was performed three times a week on alternate days, for two consecutive weeks, for a total of six sessions. The left TMJs of all rabbits were irradiated for 48 seconds at 24 hours and 3, 5, 7, 9, and 11 days after the induction of inflammation. The laser probe was placed in slight contact with the skin behind the condyle near the retrodiscal region, while the rabbit's mouth was open. During the irradiation, the angle of the laser probe was changed without moving the probe in order to irradiate the retrodiscal tissues properly.

Scintigraphic Imaging

Scintigraphic imaging of each rabbit was performed at 24 hours, 1 week, and 2 weeks after formalin injection. A 24-hour evaluation was conducted just

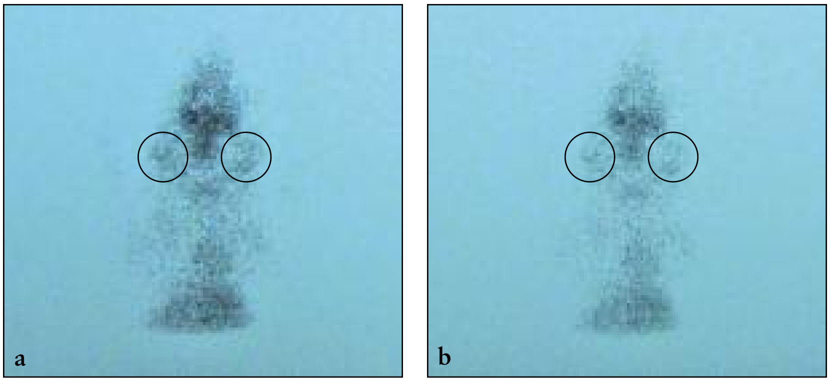

Fig 1 Posteroanterior static images following ^{99m}Tc-HIG administration in a rabbit. *(a)* before treatment; *(b)* after second week of treatment.

before the laser treatment. At 2 hours after intravenous administration of radiopharmaceutical 3–5 mCi ^{99m}Tc-human immunoglobulin G (^{99m}Tc-HIG), static acquisitions of TMJ images were performed for 5 minutes. The images were taken at posteroanterior position on a digital gamma-camera (Elscint Apex-S), equipped with a pinhole collimator. Each image included both TMJs (Fig 1).

Quantitative Analysis

Quantitative analysis using system software was performed by two nuclear medicine specialists and a physics engineer. A region of interest (ROI) was traced around the greatest activity in the TMJ area. Total counts in the ROI were then recorded. To correct injected activity differences between rabbits, an ROI of similar size and shape was drawn on the adjacent site of the TMJ on each image as background. Then all the ROI counts were normalized to their background counts.

Normal group counts were compared with both treated TMJ group and control TMJ group counts. To determine success of the treatment, counts in the treated TMJ group were compared with control TMJ group counts (Table 1).

Statistical Analysis

Statistical analysis was performed using NCSS 2007 and PASS 2008 Statistical Software. The results are shown as mean ± standard deviation. For comparison of the normal group with both treated and control TMJ groups, the Mann-Whitney *U* test was used. Intragroup differences of values before treatment, first week, and second week of treatment were determined by the Friedman test and the Wilcoxon sign test. A $P < .05$ was considered significant.

Table 1 The *P* Values from Comparison of Normal Group with Both Treated and Control TMJ Groups Using the Mann-Whitney *U* Test

	Normal-treated comparison	Normal-control comparison
Before treatment	.002*	.002*
After 2 weeks of treatment	.247	.880

*Significant ($P < .05$)

Table 2 Evaluation of Treated and Control TMJ Groups*

	Treated TMJ	Control TMJ	MW	*P*
Before treatment	2.32 ± 0.44	2.22 ± 0.60	51.0	.533
After 1 week of treatment	1.82 ± 0.53	1.84 ± 0.54	57.5	.844
After 2 weeks of treatment	1.61 ± 0.34	1.49 ± 0.43	46.0	.341
Friedman test	14.364	18.182		
P	.001†	.001†		

*Count ratios of both groups normalized to background counts given in the Table. Mann-Whitney *U* (MW) test and Friedman test data shown for comparison.
†Significant ($P < .05$)

Results

For the normal TMJ group, the average of right and left TMJ counts normalized to background counts (1.41 ± 0.19) was compared to treated and control TMJ group counts (Table 1). Before LLLT, significant differences were found between normal and both control and treated TMJ groups. However, no significant differences were found between normal and both control and treated TMJ groups after 2 weeks.

According to the Friedman test, there were significant differences within each group, but according to the Mann-Whitney *U* test, there was no statistically significant difference between control and treated TMJ groups (Table 2).

Table 3 Evaluation of Intragroup Significance

	Treated TMJ	Control TMJ
Before treatment vs 1 week	.003*	.004*
Before treatment vs 2 weeks	.004*	.003*
1 week vs 2 weeks	.100	.007*

*Significant ($P < .05$); Wilcoxon sign test.

For the treated TMJ group, there were significantly reduced scintigraphic values at the first and second week of LLLT, compared to scintigraphic values before treatment. There was no significant difference in scintigraphic values after the second week of LLLT, compared to the first week of treatment (Table 3).

For the control TMJ group, there were significantly lower scintigraphic values at the first and second week of LLLT, compared to scintigraphic values before treatment. Values after the second week of LLLT were significantly reduced, compared to those after the first week of treatment (Table 3).

Discussion

There are studies showing the anti-inflammatory effect of LLLT on other tissues,[9–11] but no studies could be found that evaluated the anti-inflammatory effect of LLLT on retrodiscal tissues of the TMJ. Studies using laser treatment for TMD were reviewed, and it was difficult to compare them with the present study since their methodologies were not clear as far as application point and direction of laser probe position, different laser parameters, etc. Hansson[3] evaluated the anti-inflammatory effect of LLLT on five patients with arthrogenous pain by measuring maximum mouth opening and subjective pain parameters, and concluded that intra-articular inflammation was controlled in all patients after infrared laser application. Mazetto et al[4] reported that capsulitis, synovitis, retrodiscitis, and painful disc displacement with reduction treated with LLLT resulted in pain control. Fikácková et al[7] found similar results and reported that an 80% reduction in pain was experienced in a patient with disc displacement caused by chronic overload.

Although improvement in pain and inflammation has been shown in these studies, the lack of a control group plus the small number of subjects and diversity of the techniques employed are problematic.[12] Also, the success of LLLT in the treatment of myogenic and arthrogenic TMD cases has been based on observational results in clinical trials.[4,7,8,12–15] Measurements used in these studies were also subjective since it was assumed that the pain was the result of inflammation. So, pain relief by LLLT has been assumed to reflect a reduction of inflammation. However, inflammation cannot be measured clinically. Since the reviewed LLLT studies showed major differences in methodology such as diagnosis of TMD, frequency and duration of laser application, and the measurement of success definitions, no comparison could be made between the present data and earlier published results.

In the present study, LLLT was carried out with a Ga-Al-As laser (output power of 250 mW, wavelength of 815 nm, and energy density of 12 J/cm^2) in six sessions. There is no agreement on the laser parameters, and the optimal dose in treating TMD has a wide range of energy density (2-100 J/cm^2).[7] Although the effects of LLLT on inflammation at the cellular level have not been explained thoroughly, it is known that the absorbed light energy within the tissue is converted into adenosine triphosphate (ATP). The cellular activity needed for tissue healing is increased with the stimulation of ATP production.[16]

In the present study, inflammation in retrodiscal tissues was induced by formalin injection. Different inflammatory agents, such as Complete Freund's adjuvant, carrageenan, and formalin, have been used to induce inflammation in the TMJ.[17–19] Despite the similarity between the inflammatory reactions caused by these agents, formalin has been shown to be the most persistent inflammatory agent.[19] Goulart et al[19] observed histologically the presence of a mild inflammatory process 15 days after formalin injection in the rat TMJ. Since the LLLT was applied for 2 weeks, formalin injection was considered the most acceptable inflammatory model for the present study.

The present study used scintigraphy to test the anti-inflammatory effect of LLLT. Scintigraphic imaging is widely used for detecting infection, as well as inflammation, by determining the location and number of inflammatory foci throughout the body.[20] Scintigraphic evaluation with various radiopharmaceuticals has been shown to detect and measure inflammation with specificity and sensitivity.[21–24] ^{99m}Tc-HIG is one of the radiopharmaceuticals used to detect inflammation. Quantitative analysis can be performed more objectively to measure the severity of inflammation with ^{99m}Tc-HIG. In the present study, the effects of LLLT in inflamed retrodiscal tissues were objectively investigated using scintigraphic imaging.

Conclusions

The present study on the effect of LLLT on experimentally induced inflammation in retrodiscal tissues of the rabbit TMJ revealed a statistically significant decrease in the intensity of inflammation at different time intervals for both treated and control (nontreated) groups. On the other hand, when treated and control (nontreated) groups were compared, no significant effect of LLLT was shown. Thus, within the limits of this objective study, the effect of LLLT on inflammation of TMJ retrodiscal tissues was not demonstrated. Since it has been proposed that LLLT has no adverse effects, further objective animal research is needed to define the optimal treatment of LLLT parameters, such as energy doses, frequency, and duration.

Acknowledgments

The authors are grateful to Prof Dr Kutlan Özker for his contributions and to Tolga Yontucu for technical veterinarian support.

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