Histomorphometric Evaluation of the Effects of Mandibular Advancement Appliance and Low Level Laser Therapy (LLLT) with Different Doses on Condylar Cartilage and Subchondral Bone in Rats

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SUMMARY: The aim of this study was to evaluate the effects of mandibular advancement appliance and low level laser therapy (LLLT) with different doses on cellular hypertrophic changes in the mandibular condyle of rats. Forty-eight 8-week-old male Wistar albino rats weighing between 260 and 280 g were randomly divided into four experimental and control groups. Group I was the control group; group II was the mandibular advancement appliance group; group III was the 8 J/cm² (0.25 W, 20 s) laser irradiation with mandibular advancement appliance group; and group IV was the 10 J/cm² (0.25 W, 25 s) laser irradiation with mandibular advancement appliance group. Mandibular condyle cartilage and subchondral bone changes with different LLLT dose and mandibular advancement appliance were evaluated by histomorphometrical analysis. Subchondral bone fraction results showed that there were no significant differences between groups (p<0.05). The statistically significant differences found between control group and experimental groups in anterior and posterior cartilage layers thickness (p<0.05) and (p<0.01). Posterior and anterior condylar cartilage layers of rats react differentially to LLLT and mandibular advancement application. Maximum changes in condylar cartilage layers were found in 8 J/cm² laser irradiation with mandibular appliance group.

KEY WORDS: Low level laser therapy; Mandibular advancement; Rat; Histomorphometric.

INTRODUCTION

Mandibular deficiency-induced skeletal class II malocclusion has been a common problem in orthodontic practice. Patients with skeletal class II malocclusion and mandibular retrognathia have severe esthetic, psychological, airway and occlusal problems (El-Bialy et al., 2003, 2015). Orthopedic therapy in growing patients has the power to treat underdevelop mandibula by enhancing condylar growth. The functional orthopedic treatment of skeletal Class II malocclusion stimulates the upward and backward growth of mandibular condyle, and this process plays an important role in the forward movement of the mandible (Oksayan et al., 2015). As a result, adaptive mandibular condylar cartilage remodelling occurs with the forward movement of mandible by functional appliances (Owtad et al., 2011). According to patients’ age, sex, severity of skeletal problem and compliance, functional appliance therapy is to achieve the condylar cellular activity and bone remodelling in a shorter treatment time.

Many studies have shown that biomechanical stimulus are important for development and growth process of secondary cartilages like mandibular condyle cartilage (Tingey & Shapiro, 1982; Peltomäki et al., 1997). Through the development of new methods and technologies some researchers studied to increase the chondroblastic and osteoblastic activity in mandibular condylar cartilage with laser and ultrasound for mandibular condyle growth stimulation and acceleration with, or without functional appliances in experimental animals (Oyonarte et al., 2009; Seifi et al., 2010; El-Bialy et al., 2015). Low level laser...
therapy (LLLT) is gaining popularity in medical and dental fields in last years. In recent experimental animal studies, LLLT has become a foremost way to accelerate and stimulate the condylar cartilage growth and mandibular advancement with or without functional appliance (Seifi et al., 2012; Abtahi et al., 2012; El-Bialy et al., 2015).

In this study we aimed to evaluate the effects of mandibular advancement appliance and LLLT with different doses on condylar cartilage layer thickness, total cartilage thickness and subchondral bone changes in anterior and posterior region of mandibular condyle of rats.

MATERIAL AND METHOD

Forty-eight male 8-week-old Wistar Albino rats weighing between 260 and 280 g, divided randomly into four groups. Group I (n=12) was the control group; group II (n=12) was the mandibular advancement appliance group; group III (n=12) was the 8 J laser irradiation with mandibular advancement appliance group; and group IV (n=12) was the 10 J laser irradiation with mandibular advancement appliance group (Table I). The study was approved by İnönü University Experimental Animal Ethical Committee with 2013/A-38 code. The rats were caged in a 23 °C temperature, 12 h night /12 h day environment and regulated humidity conditions. Animals were fed ad libitum with a standard soft diet to prevent any damage to the mandibular advancement appliances. Experimental rats were stimulated with a low-level laser in the temporomandibular joint region bilaterally 15 times over 30 days for symmetrical growth of mandibular condyle. Histomorphometrical changes in the mandibular condyle cartilage and subchondral bone were evaluated on day 30. The animals were anesthetized with an intramuscular injection of 20 % Ketamine hydrochlorur (Ketalar-Eczacıbaşı/Türkiye) and 80 % Xylazine (Rompun-Bayer/Germany) before the mandibular advancement appliance applications.

Functional mandibular advancement appliance application. The functional mandibular advancement appliance applied in this research was similar to that developed for growing rats in previous studies (Xiong et al., 2004; Owtad et al.). Silicon impressions were obtained from mandibular incisors and plaster models were made. Mandibular advancement appliances were constructed from hard Essix material for the mandibular incisors and identical inclination planes were fitted to the lower incisors of rats in the experimental groups. And mandibular advancement appliances repositioned the rats mouths vertically open and cross bite position. These appliances bonded to lower incisors with the self etch bonding system (Transbond Plus, 3M, Monrovia, USA) and advancement appliances provided 2.5–3 mm continuous forward mandibular movement in experimental groups (Fig. 1). The functional mandibular advancement appliance was not applied to rats in the control group.

Table I. Design of experimental study protocol.

| Groups | N | Appliance | LLLT energy density | Experimental period time |
|--------|---|-----------|---------------------|-------------------------|
| Grup I | 12 | - | - | 30 days |
| Grup II | 12 | + | - | 30 days |
| Grup III | 12 | + | 8 J/cm² | 30 days |
| Grup IV | 12 | + | 10 J/cm² | 30 days |

Fig. 1. Experimental mandibular advancement appliance for rat mandibular incisors.

Laser application. Laser applications were performed in temporomandibular joint region of rats in group III and IV with a 810 nm GaAs diode laser (Cheese, Wuhan, China) with a probe diameter of 0.625 cm². Application was applied bilaterally and in direct contact to the skin 15 times in 30 days. In group III received 8 J/cm² (0.25 W, 20 sec) energy density on each side per session. And group IV received 10 J/cm² (0.25 W, 25 sec) energy density on each side per session.

Histomorphometrical preparations and analysis. All experimental and control group rats were sacrificed with injection of high amount of ansthesia. Left condyles of rats were dissected and suspended in 10 % formalin solution (Merck, Darmstad, Germany) in 48 hours. After this protocol, condyle specimens were decalcified in 5 % formic acid solution (Tekkim, Bursa, Türkiye) for 2 weeks at 4 °C.
Samples washed with distilled water at the end of the 2 week and tissue was placed in the trays. Rapid biopsy program was started with the help of automatic tissue processing machine (Thermoscientific, ExcelsiorES, USA) to follow tissue. Then the samples were embedded in parafin blocks (Gurr, Leuven, Belgium). For examination of cartilage and subchondral structures clearly, outer surface of the condyles were trimmed 200-250 µm. Parasagittal serial sections (5 µm) were cut and stored at 37 °C in the oven for approximately 24 h. Hematoxylin eosin (Surgipath, Peterborough/USA) staining procedure were applied to each sample for histomorphometrical evaluations. The samples obtained from subjects were examined under light microscope (Leica DM5000 B, Wetzlar/Germany) and Image acquisition was performed Leica Qwin.plus image analysis system (Leica Qwin.Plus, Cambridge, United Kingdom).

The condylar cartilage was equally divided into six regions (three of them anteriorly and three of them posteriorly) and the condylar cartilage was typically organized into the fibrous, proliferative, mature, and hypertrophic layers (Jiao et al., 2010; Li et al., 2013) (Fig. 2). Thickness of condylar cartilage layers and total layers were measured at the six regions (three of them anteriorly and three of them posteriorly) of the condylar cartilage. The three measurements in anterior and posterior parts were averaged. In the middle of the anterior and posterior parts of the condyle two square (0.5 mm × 0.5 mm) bone area fraction (bony area / total area) measurement was performed under the interface of hypertrophic cartilage layer (Jiao et al.) (Fig. 3).

Statistical analysis. Statistical analysis was performed using SPSS software (Statistical Package for Social Science, SPSS Inc. Chicago, Illinois, America, Windows version 20.0). To evaluate the normality Kolmogorov-Smirnov test was used. The nonparametrical statistical Kruskal–Wallis and Mann–Whitney U tests were applied to analyze the intragroup and intergroup significant differences on histomorphometric datas.

RESULTS

Body weight. Body weight of the animals did not change in 30-day experimental process in any group. The increase of the weight in the group I (control) was more than that of other experimental groups (Table II).

Subchondral bone. According to subchondral bone area fraction (bony area / total area) results, anterior and posterior subchondral bone measurements demonstrated no significant differences between the groups (p<0.05) (Table III).

Anterior condylar cartilage layers. The results of this research showed that anterior hypertrophic layer thickness in condylar cartilage was not significantly changed, with LLLT and a mandibular advancement device during the 30 day experimental period (p<0.05).

For anterior mature layer thickness parameter, a statistically significant difference was
detected between the groups (p<0.01). Group III (p<0.01) and IV (p<0.05) showed statistically significant low thickness values than group I (control).

A statistically significant difference was found between the groups for anterior proliferative layer thickness in condylar cartilage (p<0.01). Experimental groups III (p<0.01) and IV (p<0.05) showed statistically significant low thickness values according to group I (control).

According results of this experimental study anterior fibrous layer thickness in condylar cartilage was not significantly changed with LLLT and mandibular advancement during the experimental period (p<0.05) (Table IV).

**Posterior condylar cartilage layers.** Histomorphometrical results for posterior hypertrophic layer thickness in condylar cartilage showed statistically significant differences between the groups (p<0.05). Group II (p<0.01), group III (p<0.05) and IV (p<0.01) showed statistically significant low thickness values than group I (control).

Statistically significant differences were found between groups in posterior mature layer thickness of condylar cartilage variable (p<0.05). Group II (p<0.05), group III (p<0.01) and IV (p<0.01) showed statistically significant low thickness values than group I (control).

For posterior proliferative layer thickness parameter, a statistically significant difference was detected between the groups (p<0.05). Group II (p<0.05), group III (p<0.05) and IV (p<0.01) showed statistically significant low thickness values than group I (control).

According results of this experimental study posterior fibrous layer thickness in condylar cartilage did not showed statistically significant differences between groups with LLLT and mandibular advancement during the experimental period (p<0.05) (Table V).

### Table II. Body weight of rats before and after the experimental study period.

| Variables                          | N | Groups | Mean   | Standard | P   |
|-----------------------------------|---|--------|--------|----------|-----|
| Anterior subchondral bone area fraction (bony area / total area) (%) | 1 | I      | 0.55   | 0.13     |     |
|                                   | 2 | II     | 0.44   | 0.13     |     |
|                                   | 1 | III    | 0.45   | 0.12     | 0.115 |
|                                   | 1 | IV     | 0.41   | 0.11     |     |
| Posterior subchondral bone area fraction (bony area / total area) (%) | 1 | I      | 0.36   | 0.16     |     |
|                                   | 2 | II     | 0.42   | 0.17     |     |
|                                   | 1 | III    | 0.43   | 0.15     | 0.369 |
|                                   | 1 | IV     | 0.34   | 0.16     |     |

### Table III. Comparisons of subchondral bone area fraction (bony area / total area)(%) measurements between groups. (p<0.05)

| Groups | T (g)       |
|--------|-------------|
| Group I | 262.75 ± 48.69 |
| Group II | 268.7 ± 22.54  |
| Group III | 273.81 ± 25.51  |
| Group IV | 270.75 ± 30.98  |

### Table IV. Statistical results of anterior layer thickness in condylar cartilage according to Kruskall-Wallis and Mann Whitney U tests.

| Variables                          | P   |
|-----------------------------------|-----|
| Anterior hypertrophic layer (µm)    |     |
| Anterior mature layer (µm)         |     |
| Anterior proliferative layer (µm)  |     |
| Anterior fibrous layer (µm)        |     |

### Table V. Comparisons of subchondral bone area fraction (bony area / total area)(%) measurements between groups. (p<0.05)

| Groups | T (g)       |
|--------|-------------|
| Group I | 0.55 ± 0.13  |
| Group II | 0.44 ± 0.13  |
| Group III | 0.45 ± 0.12  |
| Group IV | 0.41 ± 0.11  |

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Total condylar cartilage thickness. Histomorphometrical results showed statistically significant difference for anterior total cartilage layer thickness between groups (p<0.05). Group III showed statistically significant low anterior thickness values than group I (control) (p<0.01).

A statistically significant difference was found between the groups for posterior total cartilage layer thickness in condylar cartilage (p<0.01). Experimental groups II (p<0.01), III (p<0.01) and IV (p<0.01) showed statistically significant low thickness values according to group I (control) (Table VI).

DISCUSSION

Our study researched the histomorphometrical effects of different doses LLLT with mandibular advancement appliance on mandibular condyle cartilage of growing rats. The experimental rat model was chosen for this research because the condyle morphology and cartilage layer structures are similar to humans (Oksayan et al., 2015). To investigate experimentally the condylar growth, young and adolescent rats were studied. Similar to our study, Khan et al. (2013) studied on 8-week rat condyles for detecting the effects of growth hormone and ultrasound application. Previous experimental rat studies have shown that functional advancement appliances can stimulate and improve mandibular forward position (Rabie et al. 2003; Xiong et al.; Oksayan et al., 2014). When determining the experimental period we benefited from many other rat mandibular advancement studies, according to these researches the experimental process lasted approximately 4 weeks (Liu et al., 2007; Li et al.).

Experimental rat condylar cartilage reaction to biostimulation is now a controversial topic in orthodontic literature. Nowadays, there is scarce research published in orthodontics, regarding the use of LLLT and its impact on biostimulation of condyle cartilage and subchondral bone (Seifi et al.; El-Bialy et al., 2015). Laser biostimulation has many effects on cartilage and bone tissues depending on doses.

In this study we revealed that the biostimulatory effects of different LLLT doses (8 J/cm² and 10 J/cm²) on condylar cartilage and growth changes during mandibular advancement. Seifi et al. revealed a significant difference between 904 nm LLLT with Diode laser group and control group on rat condyle region, and they concluded that laser irradiation can stimulate condylar growth. Abtahi et al. studied the effect of 630 nm LLLT on condylar growth in rabbits and found no increase in cartilage thickness.

The results of our study are in line with Tang & Rabie (2005), they agreed that condylar active growth has a relationship with decreased thickness of cartilage. In addition to this Ghafari & Degroote (1986) found a reduction in total thickness of rat condylar cartilage in mandibular advancement group. Our results referred us that the bite-jumping mandibular advancement appliance 1-2 mm open-bite effect may reduce the TMJ stress and decrease cellular activity in the
proliferative zone and condylar cartilage thickness decreased. Oyonarte et al. focused on the ratio between proliferative zone/mature zone and they concluded that reductions could then be shown as an enhancement in proliferative activity of the cartilage.

Histomorphometrical examination showed that subchondral bone area fraction (bony area / total area) was a greater amount in posterior region of condyle in group III but this increase was not statistically significant. Abtahi et al. analyzed the thickness of new bone in their LLLT study on rabbits and they found improvement in new bone formation. Oyonarte et al. used ultrasound stimulation without mandibular advancement and found increase in subchondral trabecular perimeter by 20 min ultrasound application.

Our results showed that changes are more noticeable in posterior condylar region than anterior part. This situation let us to think on two factors; mesenchymal cell movement and mandibular advancement appliance posterior condylar affect. Rabie et al. (2002) support our findings they conclude their results due to an excess of blood vessels, undifferentiated mesenchymal cells reached posterior cartilage region more easily.

According to histomorphometrical findings group III (8 J/cm²) showed more pronounced changes in condylar cartilage layers than group IV(10 J/cm²). Light of Arndt-Schultz law, excess dosage of LLLT can lead inhibition on tissue instead of activation (Masuyama et al., 1954).

The power of our study was to have a chance to observe different doses of LLLT and use histomorphometrical analysis for condylar cartilage evaluation. Furthermore, there is scarce information in the literature on mandibular condyle biostimulation with LLLT.

Limitations of this study was the absence of only LLLT groups (ethical committee did not give a permission for naked LLLT groups) without any mandibular advancement appliance, for determining the naked LLLT impact on condylar cartilage tissue.

The clear mechanism through which LLLT can improve condylar growth is not exactly known. These findings may be used as a clinical approach in functional orthopedic therapy. LLLT applications in condylar regions might be useful for shortening functional appliance therapy in skeletal class II patients.

Further studies are needed with more subjects for evaluating the effective LLLT dose on condylar cartilage growth and mandibular advancement

CONCLUSIONS

- Posterior and anterior condylar cartilage layers of rats react differently to varying doses of LLLT and mandibular advancement application. Histomorphometrical posterior condylar cartilage layer changes were found more than anterior region.

- Mandibular advancement application with LLLT produces histomorphometrical changes in condylar cartilage. And maximum changes in condylar cartilage layers were found in 8 J/cm² laser irradiation with mandibular appliance group.

- According to the LLLT impact on condylar cartilage layers, if later studies confirm these results, laser application may be useful in functional orthopedic therapies. And further research is needed to evaluate the different LLLT application parameters and different radiological and cellular analyzing methods.

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RESUMEN: El objetivo de este estudio fue evaluar los efectos del aparato de avance mandibular y la terapia con láser de bajo nivel (TLBN) con diferentes dosis en cartílago condilar y hueso subcondral en ratas. Int. J. Morphol., 38(2):252-258, 2020.
nera diferencial a la aplicación de TLBN y avance mandibular. Se encontraron cambios significativos en las capas de cartílago condilar con irradiación láser de 8 J/cm² con el grupo de dispositivos mandibulares.

PALABRAS CLAVE: Terapia con láser de bajo nivel; Avance mandibular; Rata; Histomorfométrica.

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