

Effects of low-level laser therapy on osteoblastic bone formation and relapse in an experimental rapid maxillary expansion model

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Abstract

Aims and Objectives: The aim of this study was to investigate the effects of low-level laser therapy (LLLT) on osteoblastic bone formation and relapse during expansion of rat palatal sutures.

Materials and Methods: Thirty-two Wistar rats were randomly allocated into two groups of 16 rats each. In the first group, LLLT was applied 4 days after expansion commenced. Seven days after expansion, retainers were applied for 10 days. The second group was similarly treated, with the exception of laser therapy. All rats were sacrificed on day 7 ($n = 1$) (the end of the expansion period; laser group (LG) 1 [LLLT 1] and control group (CG) 1 [control 1]) and day 17 ($n = 8$) (the end of the retention period; LG 2 [LLLT 2] and CG 2 [control 2]) for histological assessment.

Results: The LLLT 1 group had significantly higher numbers of osteoclasts than did the control 1 group ($P = 0.036$). No significant between-group difference in osteoblast cell or capillary numbers was evident when day 7 and 17 data were compared.

Conclusion: Histologically, LLLT stimulated bone formation, as revealed by analysis after the retention period. LLLT during expansion may accelerate bone healing.

Key words: Bio-stimulation, laser, rapid maxillary expansion

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Introduction

Rapid maxillary expansion (RME) and surgically assisted RME are used to expand the transverse dimensions of the palate and maxillary dental arch. These options are widely used well-established techniques in orthodontics and maxillofacial surgery,^[1] and are similar to distraction osteogenesis, a surgical process employed to reconstruct skeletal deformities and lengthen or expand bones.^[2] For successful treatment, induction of new bone formation within the expanded area, reduction of the currently observed relapse rate of 20–25%, and stabilization of expanded tissue, are required.^[3]

Low-level laser therapy (LLLT) has been used to accelerate regeneration of bone tissue.^[4] Several authors have suggested that laser beam delivery directly to bony lesions bio-stimulates bone remodeling.^[5,6] As osteoblasts are responsible for bone formation, LLLT may exert a stimulatory effect on osteoblastic activity.^[7] To explore this question, we evaluated the effects of LLLT on bone regeneration in expanded premaxillary sutures of rats. We thus investigated the effects of LLLT on osteoblastic bone formation and relapsed during expansion of rat palatal sutures.

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Materials and Methods

Sample

We used thirty-two 12-week-old female Wistar albino rats, which were separately housed in plastic cages under artificial fluorescent lighting (a 12–12 h light-dark cycle). The cage temperature was 25°C and food and water were provided *ad libitum*. The Animal Ethics Committee of Gaziantep University approved the study, which was performed in accordance with guidelines for the use of laboratory animals.

Experimental design

All animals were anesthetized via intraperitoneal administration of xylazine and ketamine. Subsequently, expansion appliances (helical springs fabricated from 0.012-inch stainless steel wires) were affixed to the maxillary incisors of all animals to expand the premaxillary sutures [Figure 1]. The springs were placed on a grid and activated using pliers. The initial expansion force was measured with a gauge and adjusted to 30 g. A stainless steel disk was used to prepare a groove at the level of the gingival papilla on the distal sides of the incisors, to ensure retention. Next, a 0.009-inch stainless steel ligature wire was used to fix each spring. Seven days after expansion commenced, retainers were installed, and the rats maintained for a further 10 days (to make a total of 17 days). The animals were monitored for infection and appliance failure throughout the study.

The rats were randomly divided into two groups ($n = 16$ each): The laser group (LG) and the control group (CG). In the first group, LLLT was administered 4 days after expansion began. In the second group, all procedures were the same as for the first group, except for laser therapy. All rats in both groups were sacrificed on day 7 ($n = 8$) (the end of the expansion period; LG 1 [LLLT 1] and CG 1 [control 1]) and day 17 ($n = 8$) (the end of the retention period; LG 2 [LLLT 2] and CG 2 [control 2]) for histological assessment.

Laser therapy

In the LG, premaxillary sutures were irradiated daily on days 1–4 with a Ga-Al-As diode laser (Fotona XD-2 diode laser; Fotona, Ljubljana, Slovenia). A diode laser device with a continuous wavelength of 808 nm was used, and laser therapy was applied with the aid of with a 320 μm -diameter fiber handpiece operated in a sliding mode. Each rat was exposed to laser energy of 250 mW (0.25 W) for 20 s ($0.25 \text{ W} \times 20 \text{ s} = 5 \text{ J}$). The premaxillary regions (approximately 1 cm^2) of the test group were intraorally exposed to 5 J (5 J/cm^2) low-level laser irradiation delivered 1 cm from the target tissue [Figure 2]. The controls received no laser treatment.

Specimen preparation

If any complication, such as an infection, a rapid decrease

in body weight, or appliance failure, was encountered, that animal was excluded from the study. After fixation, springs were removed, and specimens decalcified in aqueous 10% (v/v) formic acid, after which they were dehydrated and embedded in paraffin. The maxillary incisor served as the primary guide for orienting the sections, which were cut perpendicular to the sagittal plane defined by two points, one at the alveolar crest and the other 4 mm apical to the crest. This plane passes through the center of the incisor crown in the gingival region. The paraffin blocks were sliced into 5- μm -thick sections and prepared for hematoxylin and eosin staining prior to optical microscopy. Bone histomorphometry was also performed, centered on the premaxillary suture and a point 175–250 μm (sections 35–50) below the surface of the osseous palate facing the oral cavity. This procedure was used because bone formation in surface regions was sometimes irregular, and quantitative measurements were not possible.

Histological and the histomorphometric assessment

Two examiners blinded to the sources of sections performed all histomorphometric evaluations, and the averages of their counts were calculated. Three histological sections from each animal were analyzed. The study and CGs were compared in terms of the numbers of osteoclasts, osteoblasts, capillaries, and inflammatory cells; and the extent of new bone formation. The sections were rated as mild (+: 0–25 cells), moderate (++: 25–50 cells), strong (+++: 50–75 cells), or very strong (++++: +75 cells) in terms of osteoblastic cells.

Statistical analysis

All data were analyzed using commercially available software SPSS (version 20.0, IBM statistics, USA). Between-group differences in the numbers of osteoclasts, osteoblasts, and capillaries were compared using the Mann–Whitney U-test. A difference was considered statistically significant at a $P < 0.05$.

Results

No animal showed any obvious sign of systemic illness throughout the study. No deep mucosal infection, dehiscence, or other adverse effect was noted in any rat. The midpalatal suture was successfully expanded by the activated helical spring. One rat in the control 1 group and one in the control 2 group were excluded because of appliance failure. In addition, one rat in the control 1 group, one in the LLLT 1 group, and one in the LLLT 2 group died.

Histomorphometric findings

Table 1 shows the numbers of osteoblasts, osteoclasts, and capillaries in the two groups.

Number of osteoblasts

No significant between-group difference was evident in osteoblast numbers.

Number of osteoclasts

All groups except the control 1 group contained osteoclasts. The LLLT 1 group had a significantly higher number of osteoclasts than did the control 1 group ($P = 0.036$). No significant difference between the LLLT 2 and control 2 groups was evident in terms of the numbers of osteoclastic cells.

Number of capillaries

No significant between-group difference was evident in capillary numbers.

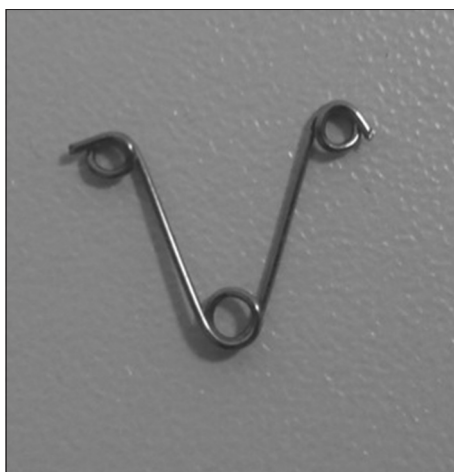


Figure 1: Expansion appliances; helical springs fabricated from 0.012-inch stainless steel wires

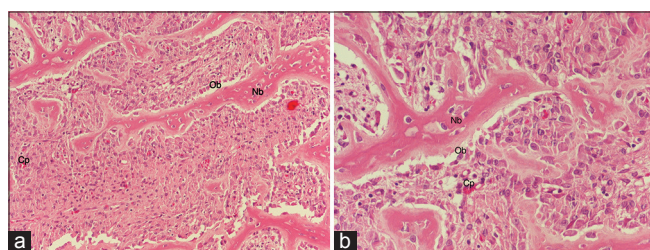


Figure 3: (a and b) Control group (CG); day 7: Representative histological sections showing thin irregular bone development, without osteoclast formation, in the CG on day 7. Ob, Osteoblast; Oc, osteoclast; Nb, new bone; Cp, capillary; Left and right: H and E, $\times 200$ and $\times 400$ (original magnifications)

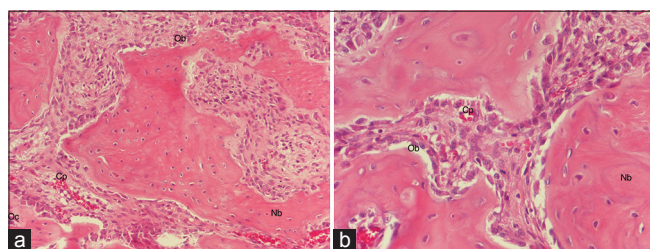


Figure 5: (a and b) Control group; day 17: Representative histological sections showing trabecular bone formation with many osteoblasts and osteoclasts. Ob, Osteoblast; Oc, osteoclast; Nb, new bone; Cp, capillary; Left and right: H and E, $\times 200$ and $\times 400$ (original magnifications)

Histological analysis

On day 7, although thin trabecular bone with numerous osteoblasts was observed in both the LLLT 1 and control 1 groups, the trabecular bone was larger in the LLLT 1 group [Figures 3a, b, 4a and b], indicating that healing was more advanced in the latter group. Inflammation was more obvious in the control 1 than the LLLT 1 group.

On day 17, both the LLLT 2 and control 2 groups exhibited increased trabecular bone formation [Figures 5a, b, 6a and b]. The LLLT 2 group displayed better ossification than the other groups.



Figure 2: Administration of laser therapy with a 320- μ m fiber handpiece operated in a sliding mode

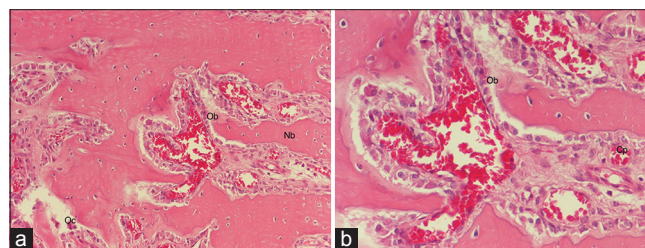


Figure 4: (a and b) Laser group; day 7: Representative histological sections showing thin trabecular bone with many osteoclasts on day 7. Ob, Osteoblast; Oc, osteoclast; Nb, new bone; Cp, capillary; left and right: H and E, $\times 200$ and $\times 400$ (original magnifications)

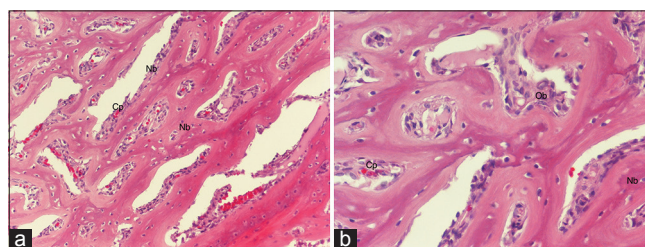


Figure 6: (a and b) Laser group; day 17: Representative histological sections showing good bone formation with osteoblasts. Ob, Osteoblast; Oc, osteoclast; Nb, new bone; Cp, capillary; Left and right: H and E, $\times 200$ and $\times 400$ (original magnifications)

Table 1: Effects of LLLT on the numbers of osteoblasts, osteoclasts, and capillaries at the end of days 7 and 17 of the experimental period (mean±SD)

	Osteoblast score	Number of osteoclasts	Number of capillaries
7 th day			
Control 1	2.67±1.03	0.67±0.34	16.00±9.73
LLLT 1	3.00±0.69	3.57*±4.07	18.00±2.31
17 th day			
Control 2	2.86±0.72	1.14±1.68	18.14±9.79
LLLT 2	2.50±1.04	1.67±2.25	11.17±5.77

*P<0.05 compare with control 1. SD=Standard deviation; LLLT=Low-level laser therapy

Discussion

Fracture healing involves complex aspects of cell and tissue proliferation and differentiation. Many players are involved, including growth factors, inflammatory cytokines, antioxidants, bone breakdown cells (osteoclasts), bone-building cells (osteoblasts), hormones, amino acids, and many nutrients.^[8] Efforts have been made to shorten the periods of fracture healing and distraction osteogenesis protocols.^[9,10] To stimulate bone healing, certain materials (e.g., autologous marrow cells, demineralized bone matrix, Vitamin D analogs, and cultured periosteal cells) have been transplanted into the lengthening area.^[9,10] To stimulate callus formation in the healing region, certain mechanical procedures^[11] have been applied, including electrical stimulation,^[12] electromagnetic stimulation,^[13] and LLLT.^[4-7]

Bone engages in a continuous cycle of resorption and apposition mediated by the balanced activities of osteoblasts and osteoclasts of the normal adult skeleton. RME is characterized by opening of the midpalatal suture, and the resorption/apposition cycle is also in play in the suture during RME. To accelerate healing, several studies have sought to stimulate bone growth via LLLT.^[4-7] Saito and Shimizu^[6] explored the effect of LLLT on bone regeneration in the midpalatal suture of rats. It was suggested that LLLT effectively accelerated bone regeneration, especially during the initial stages of bone formation. The following laser parameters were used: Wavelength 830 nm; spot area 0.6 cm-diameter fiber; energy per point 126 and 420 J; number of points 1; total energy 126 and 420 J; time per point, 420 and 600 s; power, 100 mW; and density 35.3 W/cm.^[2] Although it was suggested that LLLT accelerated bone formation, the laser parameters used are controversial. da Silva et al.^[7] evaluated *in vivo* bone formation in the midpalatal suture after RME, followed by LLLT; the proliferation and expression of osteoblastic activity were enhanced. The following laser parameters were used: Wavelength 830 nm; spot area 0.00785 cm²; energy per point 1.26 J;

number of points 1; total energy 1.26 J; time per point 42 s; power 30 mW; and density 160 J/cm². Although LLLT was claimed to increase osteoblastic activity, we did not find any significant differences in osteoblast numbers between the laser and CGs on either days 7 or 17. Cepera et al.^[14] suggested that LLLT associated with RME afforded efficient opening of the midpalatal suture and influenced bone regeneration, accelerating healing. The laser parameters used were: Wavelength 780 nm; spot area 0.04 cm²; energy per point 0.4 J; number of points 10; total energy 4 J; time per point 10 s; power 40 mW; and density 10 J/cm². Angeletti et al.^[15] suggested that low-level laser irradiation (Ga-Al-As) accelerated bone regeneration in the midpalatal suture after surgically assisted RME. The following laser parameters were used: Wavelength 830 nm, spot area 0.06 cm²; energy per point 8.4 J; number of points 8; total energy 67.2 J; time per point 84 s; power 100 mW; and density 140 J/cm². Santiago et al.^[16] suggested that a soft laser stimulated the repair process, contributing to suture reorganization and the palatal bone osteogenesis both during and after expansion. However, the laser parameters were not reported in detail. We also found that LLLT stimulated bone regeneration of the midpalatal suture after RME. The following laser parameters were used in our study: Wavelength 808 nm, delivery using a 320-μm fiber handpiece operated in the sliding mode; energy per point 8.4 J; many points in the sliding motion (over approximately 1 cm²); total energy 5 J; time per point 20 s; power 250 mW; and density 5 J/cm². We found that only osteoclast numbers were significantly higher in the LLLT than the CG on day 7. Although osteoblast numbers did not increase in the LG on either day 7 or 17, the increase in osteoclast numbers on day 7 suggests that resorption accelerates the healing process.

The stability of maxillary expansion remains controversial.^[14] It is known that 8–9 months of stabilization using an expander appliance is required to guarantee complete ossification of the midpalatal suture.^[17] Cepera et al.^[14] claimed that LLLT can aid in preventing relapses and reducing retention times after RME. Although Angeletti et al.^[15] suggested that LLLT accelerated bone regeneration and increased mineralization of the midpalatal suture, the cited authors also considered that further studies should be conducted to explore whether laser therapy inhibits relapse and reduces the retention period. Although previous studies suggest that LLLT may be helpful to prevent relapse, no such evidence was obtained in our study. We are also of the view that further studies should evaluate relapse rates after RME.

We thus conclude that low-level laser treatment associated with RME aids bone regeneration in sutures. However, we do not yet have any evidence as to whether laser therapy effectively prevents relapse after RME procedures in rats.

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