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Effect of Simvastatin and Low-Level Laser Therapy on Sutural Bone Formation After Expansion in Rats: Biomechanical, Computed Tomography and Immunohistochemical Assessment

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Abstract

Introduction: The application of low-level laser therapy (LLLT) and some medications have been shown to accelerate bone formation in rapid palatal expansion (RPE). A combination of these two therapeutic modalities may reduce the time required for the retention period. This study sought to assess the effects of simvastatin and LLLT, alone and combined, on sutural bone formation in rats.

Methods: Sixty male Wistar rats averagely weighing 150 g were divided into five groups (n=12) of control (group 1), 5 mg simvastatin (group 2), 10 mg simvastatin (group 3), LLLT (group 4), and LLLT plus 10 mg simvastatin (group 5). The expansion appliance was placed in the parietal bone in all groups. One week after placing the appliance, the spring was fixed with Duralay acrylic resin to serve as a retainer during the rest of the experiment. The rats were sacrificed after 30 (for biomechanical and computed tomography [CT] assessments) or 60 days (for biomechanical, CT and immunohistochemical [IHC] assessments).

Results: Groups 3 and 4 showed a significant improvement in osteogenesis (confirmed by CT findings, histological analysis and biomechanical test) compared to the control group. Group 5 was significantly superior to all other groups in terms of all parameters ($P<0.001$). Group 2 and the control group were not significantly different ($P>0.05$).

Conclusion: Although LLLT, simvastatin treatment and the combination of both significantly improved sutural bone formation in rats compared to the control group, the combined treatment showed significantly superior clinical results compared to other interventions.

Keywords: Bone biology; Rapid palatal expansion; Laser; Stability.

Introduction

Rapid palatal expansion (RPE) is among the most commonly performed orthodontic procedures. Despite the optimal success rate of this treatment, it requires a relatively long retention period (usually 8 to 9 months), which can be problematic for patients and clinicians.[1] Decreasing the retention period by finding methods to stabilize the results of the active phase of treatment in a shorter time would be highly beneficial. The commonly practiced method includes a mechanical retention period, which involves the placement of an inactive appliance at the site following an active period of expansion to lower the possibility of relapse. Mechanical retention must be maintained for a relatively long period of time in order to efficiently decrease the possibility of postoperative relapse. This often decreases the patients' motivation, compromises patient cooperation and prolongs the course of orthodontic treatment.[2]

The positive efficacy of bisphosphonates for decreasing the possibility of relapse and enhancing osteogenesis at the site of midsagittal sutures has been previously documented in rats.[3,4]

However, to the best of the present authors' knowledge, bisphosphonates have not been used for this purpose in human clinical trials, probably due to their potential side effects.

Statins, including simvastatin, are orally prescribed to lower blood cholesterol. The enhancement of osteogenesis is a side effect of this medication. Simvastatin is hydrophobic and is metabolized in the liver. Following

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consumption, it becomes activated by enzymatic hydrolysis.[5] Evidence shows that simvastatin has high potential for stimulating bone growth.[6-9] Mundy et al[10] were among the first to highlight the positive effects of statins on the formation of mineralized bone tissue. Statins are believed to up-regulate the bone morphogenetic protein-2 (BMP2) and vascular endothelial growth factor in osteoblasts and enhance new bone formation as such.[10,11] Moreover, statins decrease bone resorption by down-regulation of RANKL and cathepsin K pathways. Statins are believed to inhibit the apoptosis of osteoblasts and suppress the activity of osteoclasts.[5] This may be the basic mechanism for alveolar bone regeneration induced by statins.[12] Moshiri et al[13] reported the regeneration of the cortical bone in bone defects 60 days after implanting a simvastatin scaffold in the defects in rabbits. In another study, simvastatin was shown to significantly decrease relapse following orthodontic tooth movement in rats.[7]

Low-level laser therapy (LLLT) with a wavelength of 630 to 1300 nm is commonly used to enhance wound healing, prevent tissue necrosis, decrease inflammation in chronic diseases and traumas, cause pain relief, and resolve edema.[14,15] LLLT also increases the proliferation of osteoblasts.[16-18] Khadra et al[19] showed that LLLT enhanced the attachment, proliferation and differentiation of pre-osteoblasts in a culture medium around dental implants and indicated increased production of tumor growth factor-beta around cells. The same authors in another study reported that LLLT enhanced new bone formation in calvarial bone defects in rats.[20] LLLT of the sutural bone following RPE has shown positive effects on new bone formation at the midpalatal suture and can reportedly accelerate bone regeneration.[21]

Considering the positive effects of simvastatin and LLLT on bone formation, separately reported in previous studies, a combination of them may also be effective for this purpose. This study sought to assess the effects of LLLT and simvastatin, alone and combined, on sutural bone formation in rats using biomechanical, computed tomography (CT) and immunohistochemical (IHC) assessments.

Materials and Methods

A total of 60 male Wistar rats with a mean weight of 150 g were randomly divided into five groups (n = 12): control group, 5 mg simvastatin, 10 mg simvastatin, LLLT, and LLLT plus 10 mg simvastatin.

Expansion of the Midsagittal Suture

The expansion of the midsagittal suture was performed in all rats for 7 days using a custom-made appliance made of 0.5 mm stainless steel wire (Dentaurum, Germany) with two helixes.[3] All appliances were autoclave-sterilized before use.

General anesthesia was induced with an intraperitoneal injection of 10% ketamine (75 mg/kg; Alfasan, Netherlands) and 2% xylazine (10 mg/kg; Alfasan, the Netherlands) using an insulin syringe. Under aseptic conditions, the scalp was shaved and a midsagittal incision was made on the scalp of rats anteroposteriorly to expose the sagittal suture. Two holes were symmetrically created in the two lobes of the parietal bone at a 6-mm distance from each other using a sterile needle (Soha, Tehran, Iran). The aforementioned appliance was then placed in the holes using approximately 60 g force. The incision line was sutured with three 3-0 silk stitches (Supa, Tehran, Iran) (Figure 1, a-d). Tetracycline ointment (Razak, Tehran, Iran) was applied over the incision site to prevent infection.

Retention Period

All rats underwent another surgical procedure 7 days after the first surgery. Following suture removal, the incision site was opened again and the appliance springs were fixed with Duralay acrylic resin (Reliance, USA) so that the appliance would serve as a retainer during the remaining period (1 to 2 months) (Figure 1e). The incision site was closed with three stitches.

Low-Level Laser Therapy

Groups 4 and 5 were subjected to LLLT (Elexxion, Germany) in direct contact and continuous wave mode. Laser parameters included an 810 nm wavelength, 1 W power, 4 s irradiation time and 5 J energy. The cross-sectional area of the probe (and thus the irradiated surface area) was 0.78 cm^2. LLLT was performed every other day for 30 and 60 days in groups 4 and 5 respectively.

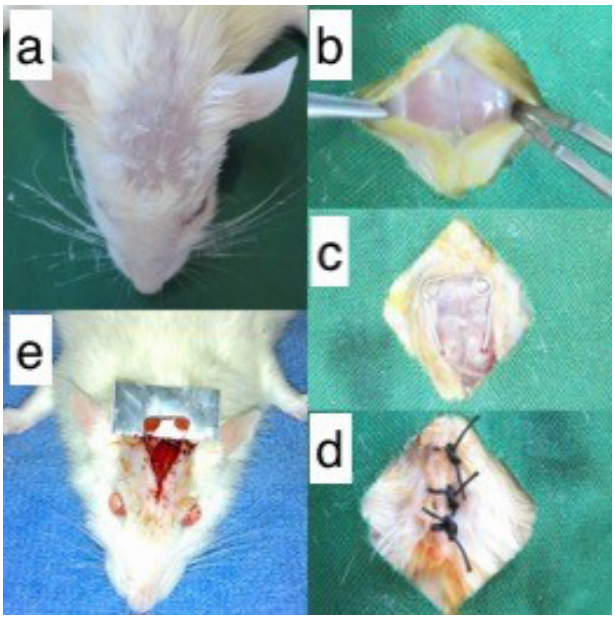

Figure 1. Rat Calvarial Sutural Expansion; (a) preparation of the parietal bone region, (b) exposure of parietal bones, (c) activation of the expansion appliance, (d) closure of incision by silk sutures and (e) deactivation of the expansion appliance by fast set acryl.

Simvastatin Therapy

Simvastatin (Sina Darou, Tehran, Iran) was purchased in 20 mg tablets. The required dosage (5 mg and 10 mg for groups 2 and 3 respectively)[10] was prepared and administered to the rats by gavage using a rat gavage needle (Ara Teb Fan, Tehran, Iran) and 1cc insulin syringe. For this purpose, the rats were held with one hand in such a way that their gastrointestinal tract was positioned straight. The gavage needle was introduced and the content was injected. This was repeated daily for 30 or 60 days in groups 2, 3 and 5.

CT Scan

On day 40, four rats were randomly chosen from each group to assess the degree of closure of the sagittal suture by three-dimensional and sectional CT scans. CT images were taken after intraperitoneal injection of 10% ketamine (75 mg/kg; Alfasan, the Netherlands) and 2% xylazine (10 mg/kg; Alfasan, the Netherlands) using an insulin syringe. To calculate the percentage of mineralization of the suture, CT scan sections were transferred to Adobe Photoshop CS6 software (Adobe Systems, USA). A standard size of bone tissue (1.08×0.32 cm^2) was selected, and the percentage of suture closure was calculated and reported as a percentage.

Biomechanical Assessment

After 30 and 60 days, four rats from each group were sacrificed to undergo the micro-tension test. After dissection of the calvarial bone, the samples were fixed in 10% formalin for one month. The parietal bone was first dissected using a curved scissor. Next, it was sectioned by a saw (Isomet, USA) with 5 mm thickness in such a way that the sectioned slice contained the holes and part of the sagittal suture. The specimens were placed in the micro-tension device (Bisco, Schaumburg, USA), and after being fixed, the tensile load was applied to the specimen (in Newtons) (Figure 2).

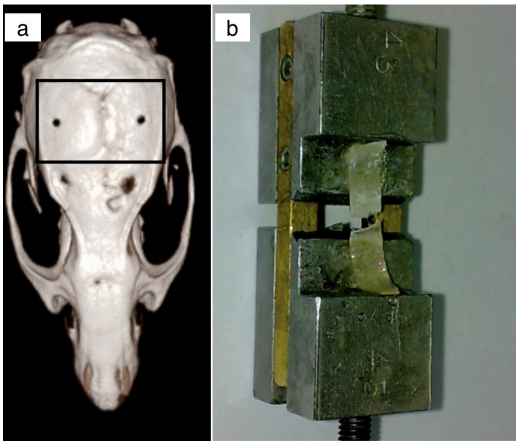

Figure 2. Parietal Bone Specimen Harvestment for the Biomechanical Test; (a) the harvested region marked on three-dimensional radiography and (b) the specimen fixed in the micro-tension device.

Histological Assessment

After sacrificing the rats by toxic inhalation, they underwent aseptic surgery, and the target bones were dissected from the skull. The specimens were fixed in 10% formalin for one month, immersed in 10 % EDTA for decalcification, rinsed under running water, and immersed in 5% sodium sulfate for 12 hours. The specimens were then histologically processed, embedded in paraffin blocks, and transverse sections with 3-5 µm thickness were made at the suture site.[22] After sacrificing the rats after 60 days, four rats from each group were chosen and their bone tissues were subjected to hematoxylin and eosin (H & E) staining and IHC analysis to evaluate osteogenesis.

H & E Staining

The following parameters were evaluated on H & E stained slides under a light microscope at ×40 magnification after 60 days: the distance between the two inner borders of the suture in millimeters in the middle of the suture, attachments along the suture line, percentage of fibrotic tissue, percentage of osteogenesis, number of capillaries, number of fibroblasts, and number of osteocytes.

IHC Analysis

Antibodies used for IHC analysis were purchased from Abcam (USA), which included the mouse specific HRP/DAB (ABC) primary antibody Detection IHC Kit (catalog number: ab64259) and two major antibodies namely anti-BMP2 antibody 65529.111 and anti-osteocalcin antibody OC4-30. The color base of antibodies used in this study was yellow to brown. The target bone tissue became brown in color due to higher expression of antigens. The background color, indicative of the tissue that did not express the respective antigen, was blue. The light microscope was equipped with a digital camera (Dino-Lite, Taiwan). The images were transferred to Adobe Photoshop CS6 software (USA) to calculate the percentage of yellow or brown-stained areas compared to blue-stained areas.

Statistical Analysis

Data were analyzed using SPSS version 24 (SPSS Inc., IL, USA). Descriptive data were reported as mean ± standard deviation. The Kolmogorov-Smirnov test was applied to assess the normal distribution of data, which showed that all biomechanical test data after 30 days, CT scan data and IHC assessment data were normally distributed ($P > 0.05$). Other data were not normally distributed ($P < 0.05$). The five groups were compared using ANOVA. In case of the presence of a significant difference, pairwise comparisons were carried out using post hoc Tukey's test.

Results

In general, group 5 had a significant difference with the

control group in all tested variables ($P<0.001$). Group 2 had no significant difference with the control group in any variable. Groups 3 and 4 had significant differences with the control group in some parameters.

Biomechanical Test Results

Table 1 shows the results of the biomechanical test in the five groups after 30 and 60 days. As shown, the five groups were significantly different in terms of quantitative values, and group 5 (LLLT plus simvastatin) showed the best results after both 30 ($P<0.001$) and 60 ($P=0.007$) days. Group 5 was significantly superior to all other groups at both time points. Post hoc Tukey's test showed significant differences between all groups ($P<0.05$) except for groups 1 and 2 ($P=0.43$) after 30 days. Post hoc Tukey's test revealed significant differences between groups 1 and 5, 2 and 5, 3 and 5, and 4 and 5 ($P<0.001$) after 60 days. No other significant differences were noted ($P>0.05$).

CT Scan Results

Table 2 presents the quantitative mean values of mineralization obtained from CT scans. As shown, the five groups were significantly different in this respect and group 5 showed superior results (ANOVA, $P<0.001$). On cross-sectional CT scans, group 5 also showed significantly higher mineralization ($P<0.001$, Figures 3 and 4). Tukey's test revealed significant differences between groups 1 and 3 ($P=0.03$), 1 and 4 ($P<0.001$), 1 and 5 ($P<0.001$), 2 and 5 ($P<0.001$), 3 and 5 ($P<0.001$), and 4 and 5 ($P=0.01$). No other significant differences were noted ($P>0.05$).

Table 1. Results of the Biomechanical Test in the Five Groups After 30 and 60 Days

Time Point	Group	Mean ± SD (Newton)	*P* Value
30 days	1 (control)	17.07 ± 1.10	<0.001
	2 (5 mg simvastatin)	19.5 ± 0.90	
	3 (10 mg simvastatin)	23.90 ± 2.25	
	4 (LLLT)	28.08 ± 1.95	
	5 (LLLT plus simvastatin)	36.04 ± 1.16	
60 days	1 (control)	28.30 ± 1.80	0.007
	2 (5 mg simvastatin)	32.90 ± 2.40	
	3 (10 mg simvastatin)	31.85 ± 2.70	
	4 (LLLT)	35.70 ± 3.85	
	5 (LLLT plus simvastatin)	59.95 ± 5.60	

SD: standard deviation; LLLT: low-level laser therapy.

Table 2. Mean Values of Mineralization Obtained From CT Scans

Group	Mean ± SD (Percent)	*P* Value
1 (Control)	15.00 ± 0.00	<0.001
2 (5 mg simvastatin)	30.00 ± 0.00	
3 (10 mg simvastatin)	33.33 ± 5.77	
4 (LLLT)	42.50 ± 5.00	
5 (LLLT plus simvastatin)	60.00 ± 10.00	

SD: standard deviation; LLLT: low-level laser therapy.

Results of Histological Assessment (H & E Staining)

Table 3 shows the results of the histological assessment in terms of distance between the two suture borders, attachment along the suture line, percentage of fibrotic tissue in the suture, percentage of bone tissue in the suture, number of capillaries, number of fibroblasts, and number of osteocytes in the five groups.

Regarding the distance between the two suture borders, a significant difference was noted among the five groups (ANOVA, $P=0.012$). Tukey's test revealed significant differences between groups 1 and 5 ($P<0.001$), 2 and 5 ($P<0.001$), 3 and 5 ($P=0.001$), and 4 and 5 ($P=0.003$). No other significant differences were found between the

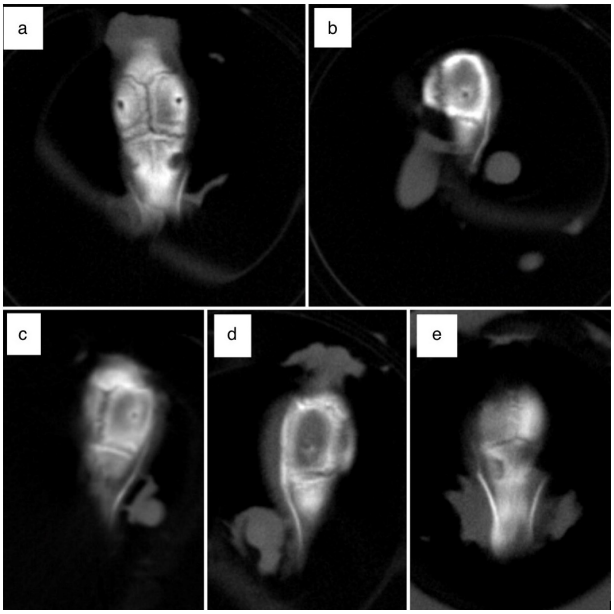

Figure 4. Sectional CT Scans after 30 days (from left to right: control, 5 mg simvastatin, 10 mg simvastatin, LLLT and LLLT plus simvastatin groups).

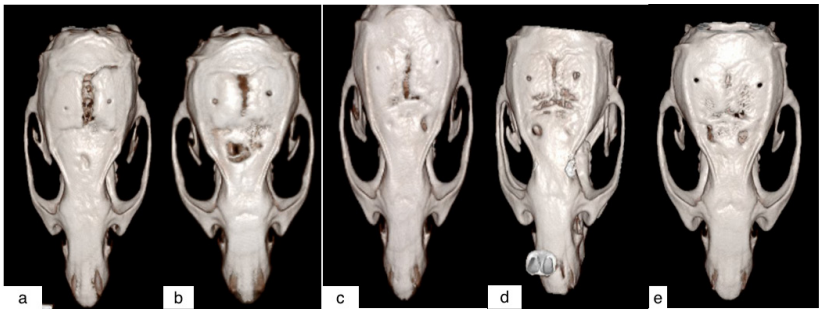

Figure 3. Three-dimensional CT Scans After 30 days (from left to right: control, 5 mg simvastatin, 10 mg simvastatin, LLLT and LLLT plus simvastatin groups).

Table 3. Results of the Histological Assessment in the Five Groups (in 40x Magnification)

Group	Mean Percentage of Free Space Between the Two Suture Borders	Mean Percentage of Attachment Along the Suture Line	Mean Percentage of Fibrotic Tissue in the Suture	Mean Percentage of Bone Tissue in the Suture	Mean Number of Capillaries	Mean Number of Fibroblasts	Mean Number of Osteocytes
1 (Control)	28.20±4.40	22.50±2.90	41.25±8.55	25.00±4.10	0.25±0.50	47.50±9.60	35.00±5.80
2 (5 mg simvastatin)	30.35±4.65	23.35±2.90	43.35±5.80	35.00±5.00	1.00±0.00	50.00±10.00	40.00±10.00
3 (10 mg simvastatin)	27.70±4.65	28.70±7.50	33.35±5.80	36.70±5.80	0.35±0.60	43.35±5.80	46.70±5.80
4 (LLLT)	22.00±2.75	31.20±6.35	34.00±5.50	44.00±5.50	1.40±0.90	40.00±10.00	54.00±5.50
5 (LLLT plus simvastatin)	5.00±8.70	56.70±5.80	16.70±5.80	73.35±5.80	2.70±1.20	13.35±5.80	100.00±10.00
P value	0.012	0.024	0.0026	0.006	0.015	0.059	0.008

LLLT: low-level laser therapy.

groups ($P>0.05$).

Regarding attachment along the suture line, the five groups showed a significant difference ($P=0.024$). Tukey's test showed significant differences between group 5 and all other groups (all P values <0.001). No other significant differences were found between the groups ($P>0.05$).

The five groups were also significantly different regarding the percentage of fibrotic tissue in the suture ($P=0.026$). Tukey's test revealed significant differences between groups 1 and 5 ($P=0.002$), 2 and 5 ($P=0.002$), 3 and 5 ($P=0.048$), and 4 and 5 ($P=0.019$).

The five groups were also significantly different regarding the percentage of bone tissue in the suture ($P=0.006$), and pairwise comparisons revealed significant differences between groups 1, 2, 3 and 4 with group 5 ($P<0.001$) and also between groups 1 and 4 ($P<0.001$).

The five groups were significantly different in terms of the number of capillaries ($P=0.015$). Groups 1 and 5 ($P=0.007$) and 3 and 5 ($P=0.015$) had significant differences in this regard.

The number of fibroblasts was significantly different among the five groups ($P=0.056$). Groups 1 and 5 ($P=0.002$), 2 and 5 ($P=0.002$), 3 and 5 ($P=0.008$), and 4 and 5 ($P=0.008$) were significantly different in this respect.

The number of osteocytes was significantly different among the five groups ($P=0.008$). Group 5 had significant differences with all other groups ($P<0.001$). Also, groups 1 and 4 were significantly different in this respect ($P=0.013$). Figure 5 shows some histological findings in the five groups.

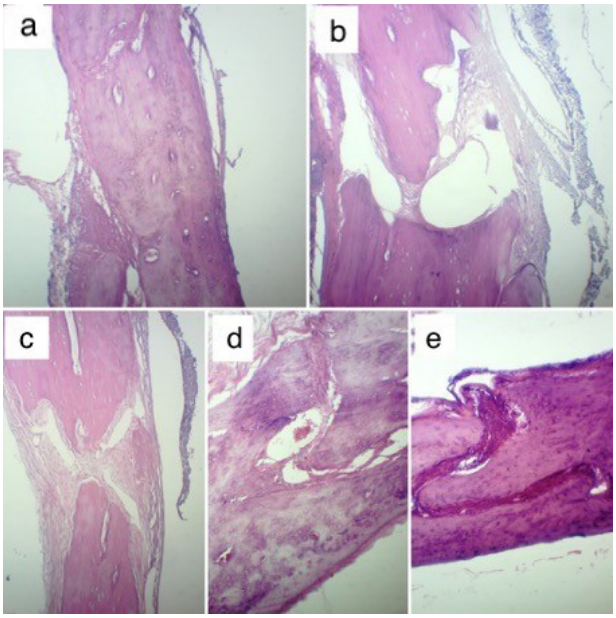

Figure 5. Some Histological Findings (Number of Osteocytes and Degree of Osteogenesis) in the Parietal Bone of Rats in the Five Groups at×40 Magnification (1: control, 2: 5 mg simvastatin, 3: 10 mg simvastatin, 4: LLLT and 5: LLLT plus simvastatin group).

Results of IHC Analysis

The results showed a higher percentage of positivity of the experimental group compared to the control group for the expression of BMP2 and osteocalcin (Figures 6 and 7). Table 4 shows the results of IHC analysis for the expression of BMP2 and osteocalcin in the five groups. As shown, the five groups were significantly different regarding the expression of BMP2 ($P=0.002$). Tukey's test showed significant differences between groups 1 and 4, 1 and 5, 2 and 5, 3 and 5, and 4 and 5 ($P<0.001$) and also between groups 1 and 3, and 2 and 4 ($P=0.01$) in this respect. The five groups were also significantly different regarding the expression of osteocalcin ($P<0.001$). Pairwise comparisons revealed significant differences between groups 1 and 4, 1 and 5, 2 and 5, and 3 and 5 ($P<0.001$) and also between groups 2 and 4, and 5 and 4 ($P=0.003$).

Discussion

Maxillary constriction is a common orthodontic problem. Transverse palatal deficiency can impair normal growth, and RPE is required to correct this problem and prevent later compilations.[23] The relapse of the expanded suture is a great concern after RPE, and several methods have been used to improve expansion results and decrease the retention period. Our study assessed the quality and quantity of sutural osteogenesis in the parietal bone of rats following treatment with 5 and 10 mg doses of simvastatin

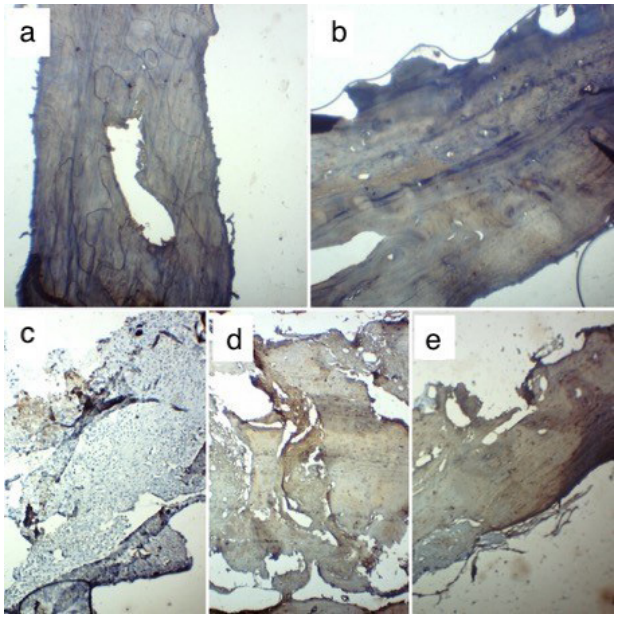

Figure 6. IHC Staining for BMP2 at × 40 Magnification (1: control, 2: 5 mg simvastatin, 3: 10 mg simvastatin, 4: LLLT and 5: LLLT plus simvastatin group).

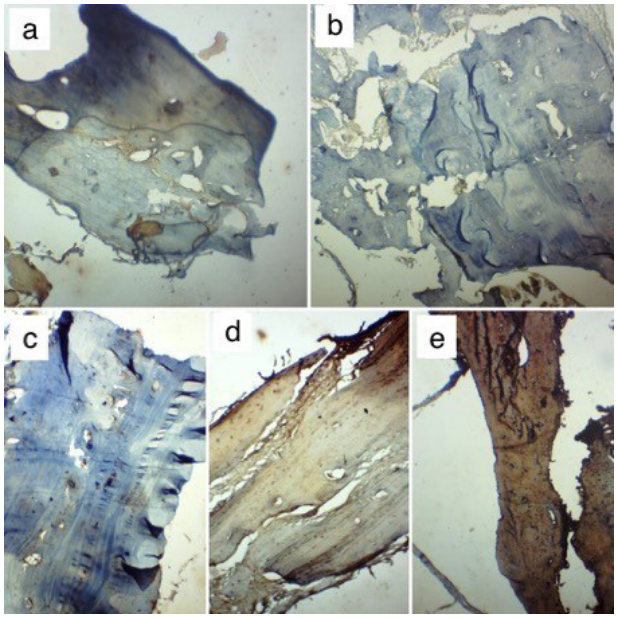

Figure 7. IHC Staining for Osteocalcin at x40 Magnification (1: control, 2: 5 mg simvastatin, 3: 10 mg simvastatin, 4: LLLT and 5: LLLT plus simvastatin group).

Table 4. Results of the IHC Analysis for the Expression of BMP2 and Osteocalcin in the Five Groups

	Mean for BMP2	Mean for Osteocalcin
1 (Control)	22.50 ± 5.00	17.50 ± 9.60
2 (5 mg simvastatin)	30.00 ± 8.22	23.35 ± 5.80
3 (10 mg simvastatin)	37.50 ± 5.00	33.35 ± 5.80
4 (LLLT)	45.00 ± 5.80	50.00 ± 8.20
5 (LLLT plus simvastatin)	82.00 ± 2.75	75.00 ± 5.80
P value	0.002	< 0.001

LLLT: low-level laser therapy.

and LLLT, alone and combined. This assessment was performed using the biomechanical micro-tension test, CT scan and histological (H & E staining) and IHC analyses. In the biomechanical micro-tension test, we applied the tensile load to the suture and found that the load required for the separation of the two parts of the bone increased from group 1 to group 5 in such a way that some samples in group 5 underwent fracture in bone mass rather than the separation of the suture line. CT scan assessment by 3D and sectional methods also showed that the gap at the suture line became smaller from group 1 to group 5. Histological analysis by H & E staining revealed that the majority of evaluated parameters significantly improved from group 1 to group 5. IHC analysis also revealed that the percentage of staining with specific antibodies increased from group 1 to group 5.

Several studies have attempted to enhance osteogenesis following palatal expansion using different medications.

Lee et al[3] evaluated the effects of bisphosphonates on expanded sutures in rats and noticed that bisphosphonates significantly enhanced sutural bone formation and decreased the rate of relapse after palatal expansion. Accordingly, considering our results, simvastatin and LLLT may also be able to decrease the rate of relapse following palatal expansion. This needs to be evaluated in future studies.

Ozturk et al[4] evaluated the effect of zoledronic acid on sutural bone formation in rats following palatal expansion using the CT scan. They showed that the percentage of mineralized tissue relative to the entire volume of the suture increased in the treatment group compared to the control group. This finding is similar to ours.

Moshiri et al[13] reported successful use of a simvastatin-loaded scaffold for the regeneration of large bone defects. In line with their finding, our study showed that gavage of 10 mg simvastatin significantly enhanced sutural osteogenesis in rats.

Mundy et al[10] showed the positive effects of statins on bone formation and attributed it to the up-regulation of BMP2. Similarly, we evaluated the expression of BMP2 and osteocalcin and revealed their significant up-regulation in group 5.

Stein et al[9] and Özeç et al[24] showed the positive efficacy of simvastatin for the regeneration of bone defects, which was in agreement with our findings in the 10 mg simvastatin group. Wu et al[25] evaluated the effect of simvastatin on bone remodeling after tooth extraction in rats and showed that local simvastatin preserved the residual ridge by the stimulation of osteogenesis. Park et al[26] demonstrated that administration of simvastatin increased the volume of the cancellous bone, enhanced osteogenesis and increased the compressive strength of the cancellous bone. Martiz et al[27] also discussed that statins induced new bone formation. Similarly, our study showed the positive effects of 10 mg simvastatin on

osteogenesis. Ayukawa et al[28] revealed that intraperitoneal injection of simvastatin induced new bone formation around titanium implants. In line with our study, Oliveira et al[12] reported that local simvastatin improved the quality of bone, decreased bone resorption and increased bone density.

A previous study evaluated the effect of LLLT on the sutural bone following RPE and highlighted its positive effects on new bone formation in the midpalatal suture and acceleration of bone healing.[21] Migliario et al[17] used LLLT to increase the proliferation of osteoblasts and concluded that reactive oxygen species along with LLLT enhanced the proliferation of preosteoblasts. In agreement with their findings, LLLT increased the number of osteocytes in our study. Khadra et al[19] evaluated the effect of LLLT on attachment, proliferation and differentiation of osteoblast-like cells in a culture medium around titanium implants and concluded that LLLT increased attachment, proliferation and differentiation of cells, and production of tumor growth factor beta-1. Our study also showed that suture closure was more favorable in the LLLT groups compared to the control group. Ferreira et al[21] evaluated the effect of LLLT on sutural bone formation following RPE and pointed to its positive effects on new bone formation at the midpalatal suture. LLLT also accelerated bone healing in their study. Their findings were in accord with ours. Petrov et al[29] showed that LLLT enhanced the attachment of osteoblasts to the implant surface and angiogenesis and decreased tissue damage. In our study, osteogenesis was greater in LLLT groups compared to the control group. Khadra et al[20] indicated that LLLT enhanced new bone formation in calvarial bone defects in rats. Sella et al[30] evaluated the effect of LLLT on bone fractures in rats and pointed to its significant role in new bone formation at the site of the fracture, which was also in line with our findings. Hübler et al[31] evaluated the effect of LLLT on new bone formation after distraction osteogenesis treatment by assessing the chemical composition of the bone and measuring the calcium and phosphorous contents and found that the laser-irradiated site was more mineralized and had a higher percentage of minerals.

Our findings showed that all interventions effectively improved bone density. This effect was confirmed by biomechanical, CT scan and histological analyses. However, a combination of the laser and simvastatin yielded significantly superior results. This result was not far from expectation since each intervention improves bone quality via a different mechanism. Thus, the application of LLLT and simvastatin synergistically improved bone quality.

To the best of the present authors' knowledge, this study is the first of its kind. However, this study was performed on rats; thus, the results cannot be accurately generalized to the clinical setting. Future clinical trials are required to assess the efficacy of these modalities in humans.

Similar studies are also recommended on patients undergoing SARPE expansion and distraction osteogenesis. We also recommend evaluating local delivery methods for simvastatin administration, which may help compensate for the potential side effects of systemic administration of the drug in humans.

Conclusion

Although LLLT and simvastatin, alone and in combination, enhanced sutural osteogenesis compared to the control group, combined treatment yielded significantly superior results in terms of biomechanics as well as CT scan and histological findings.

Ethical Considerations

The study was conducted in full accordance with the Guidelines for Ethical Conduct in the Care and Use of Animals. Institutional Review Board (IRB) approval by Zanjan University of Medical Sciences (ZUMS.REC.1394.281). All rats were kept at 25°C temperature in the 12 h light/12 h dark cycle. They were fed hard foods, and food and water were available ad libitum.

Conflict of Interests

All authors declare that they have no conflicts of interest.

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