The effects of diode laser (660 nm) on the rate of tooth movements: an animal study

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Abstract Low-level laser has been indicated to have the capability to facilitate the differentiation of the osteoclastic and osteoblastic cells which are responsible for the bone remodeling process. The aim of this study was to evaluate the effects of InGaAlP laser with a wavelength of 660 nm on the rate of tooth movement and histological status. Thirty male Wistar rats of 7 weeks old were selected for this study. The rats were randomly divided into two groups of 15 each to form the experimental (laser-irradiated) and control (non-irradiated) groups. The control group received unilateral orthodontic appliance design (one quadrant), but the laser-irradiated group received split-mouth design, with orthodontic appliance on both sides and laser irradiation on one side only (group b) and on the contralateral side (group c). The orthodontic appliance consisted of a NiTi closed coil spring with a length of 5 mm which was ligated to maxillary molar and incisor. A total of 60 g of force was applied to the rat molar. The diode laser (660 nm) was irradiated with an output power of 25 mW in continuous mode for a total time of 5 min in the laser-irradiated group. After 14 days of orthodontic tooth movement, the amount of tooth movements was measured. In the laser-irradiated group, the amount of tooth movement was significantly greater than that of the non-irradiated group (2.3-fold), but there was no significant difference between the non-irradiated and indirectly irradiated groups. Histopathological studies revealed that the number of osteoclasts in the laser-irradiated group was significantly greater than that of the non-irradiated group (1.5-fold) while this number was almost the same in the non-irradiated and indirectly irradiated groups. The results suggested that low-level laser can accelerate the rate of bone remodeling. However, in order to utilize the low-level laser as an adjunct in orthodontic practice on patients, further research studies are needed for finding the appropriate dosage for the human tissues.

Keywords Low-level laser · Orthodontic tooth movement · Alveolar bone remodeling · Histopathology

Introduction

Providing appropriate results of orthodontic treatments that are achieved by tooth movement through force application needs a long period. Long-lasting orthodontic treatments can cause discomfort and unease to many patients and also, in some cases, can be considered as a source of trauma to the oral tissues. Therefore, finding a method to abbreviate the treatment period remains one of the big challenges lying ahead of orthodontists.

Orthodontic tooth movement and bone remodeling are based upon the inflammatory response of tooth periodontium to the applied pressure. Once the sustained orthodontic force is applied to the tooth, the balance of blood flow in the compression side and tension side alters within the periodontal ligament (PDL). The blood flow is decreased in the compressed PDL region while it is increased on the other side where the PDL stretches. This changes the chemical
environment which ultimately leads to the inflammatory response that induces the proliferation, differentiation, and activation of the osteoclastic and osteoblastic cells which carry out the remodeling process via resorption of bone at the pressure side and apposition of bone at the tension side [1, 2]. Any intervention that affects this set of events could alter the rate of tooth movement. As previously reported, various types of drugs, hormones, electric currents, etc. have been tested before but their simulative effects were accompanied by undesirable side effects such as pain, root resorption, bone resorption, etc. [3–5].

Recently, the application of low-level laser (LLL) has been shown to affect many biological and biochemical processes in body tissues, as many beneficial biostimulation effects of LLLT have been demonstrated, including effects on fibroblasts, chondral proliferation, collagen synthesis, nerve regeneration [6]. In regard to bone remodeling, recent studies showed that LLLT can stimulate proliferation and differentiation of osteoblast lineage nodule-forming cells, which led to an increase in the number of osteoblasts [7, 8]. Nicola et al. suggested that diode laser with a wavelength of “660 nm” with proper settings can increase the superficial osteoblast numbers and activity and thickness of the osteoid cells in the area of irradiation [9]. Furthermore, Saito and Shimizu reported that low-energy laser stimulated the process of bone regeneration during suture expansion in rats [10].

In a clinical study, Limpanchikul et al. assessed the effect of low-level laser therapy on retraction of canine. The results of the study did not show a significant difference between laser and control groups [11]. In contrast, Cruz et al. conducted a similar clinical trial in which they applied infrared laser on canines which resulted in significant acceleration of tooth movement in laser-irradiated groups [12]. Mir et al. reported that LLL accelerated the tooth movement (canine retraction) and also lowered the degree of pain felt by the patients as a result of activation of coil springs [13].

In all the investigations which evaluated the effect of low-level laser therapy on tooth movement, the study methods are variable and so are the reported results. Since the true cellular mechanism through which low-level laser alters the remodeling process is still unknown and there is no documented protocol for laser application on orthodontic tooth movement, the aim of this study was to evaluate the effects of InGaAlP laser with a wavelength of 660 nm on the rate of tooth movement and histological status.

Materials and methods

A total of 30 male Wistar rats of 7 weeks old with an average weight of 200 g were obtained (Razi Institute, Iran) and kept at the animal center of the Pharmacology Department of Tehran University of Medical Sciences in separate cages in a 12:12-h light/dark environment. Their nutrition consisted of soft substances and water in order to avoid any displacement of the orthodontic appliance (the animal experimental protocol was approved by the “ethics committee for animal experiments” of Tehran University of Medical Sciences). The rats were randomly divided into two groups of 15 each to form the experimental (laser-irradiated) and control (non-irradiated) groups.

The rats were under anesthesia during the operations. The anesthetic drug (mixed ketamine hydrochloride (Budapest, Hungary) and xylazine hydrochloride (Rompoun, Bayer, Leverkusen, Germany) was injected intraperitoneally. The control group received unilateral orthodontic appliance design (one quadrant), but the laser-irradiated group received split-mouth design, with orthodontic appliance on both sides and laser irradiation on one side only (group b) and on the contralateral side (group c, this group was specifically designed in order to test any possible effect of LLLT on the tooth movement on the contralateral side). The orthodontic appliance consisted of a NiTi closed coil spring that was placed between the maxillary first molar and incisor. The spring was ligated to the maxillary first molar and incisors via 0.010-in. steel ligature wires (Dentaurum, Newton). In order to keep the appliance in place, a cervical groove was prepared on the incisor to seat the ligature wire on and also the ligature wire was covered with a composite resin (Fig. 1). The exerted force by this appliance was about 40 cN. The tooth movement was performed for a period of 14 days.

Laser irradiation

In this study, InGaAlP diode laser with a wavelength of 660 nm (Azor, Russia) was used. The laser was irradiated with an output power of 25 mW in continuous mode for a total time of 5 min. Under general anesthesia, the laser was applied by placing the end of the optical fiber tip, with a spot area of 1 cm² and a total energy of 7.5 J, in contact with the buccal side of the gingiva around the maxillary first molar. Irradiation was performed once a day, with 48-h intervals for six sessions.

Measurement of tooth movement

Forty-eight hours after the last irradiation session (day 14), the rats were sacrificed (with ether overdose) and their upper jaws were removed. Orthodontic tooth movement was measured using a filler gauge directly in the mouth to reveal the distance between the first and second molars. The rats had tight contacts between the molars at the beginning of the experiment. An additional silicone impression (President, Liechtenstein) was taken and poured with ultra strength dental stone (Gildard, Germany) before removing the appliance, to prevent any relapse of tooth movement. The final measurement was performed using a filler gauge on the plaster replica. The same operator performed all the measurements.
Histological study

In order to evaluate the histological changes of bone and tissue around the tooth, the posterior hemi maxilla containing three molar teeth, bone, and soft tissue was dissected and immersed in 10% formalin solution for 10 days. The samples were washed with water and placed in 5% formic acid for 1 week in order to make the bone soft enough to cut. Samples were seated in paraffin, and 5-mm-thick mesiodistal sections were cut and every fifth section was stained with hematoxylin and eosin. The osteoclast count, PDL width, and bone resorption were the criteria for comparison between the samples (Figs. 2 and 3).

Statistical analysis

The orthodontic tooth movement data are shown as mean ± standard deviation. Because two sets of data were from one group of rats, the obtained data were essentially correlated. So in order to evaluate tooth movements among the study groups, considering the data correlations (as the direct and indirect data are gathered from one group of rats), the generalized estimating equation method was applied using linear model and unstructured correlation matrix.

To compare the three histopathological variables (osteoclast count, alveolar bone resorption, and PDL width) among the groups, Kruskal-Wallis test (with Dunn's procedure for pairwise comparison) was performed due to small sample size. A p value <0.05 was considered statistically significant.

Results

After 14 days of orthodontic tooth movement, the mesial movement of the maxillary first molar was measured and the following results were obtained: The amount of tooth movement in the control group (group a), group directly irradiated with laser (group b), and group indirectly irradiated with laser (group c) was 0.11 ± 0.04, 0.39 ± 0.07, and 0.12 ± 0.03, respectively. The amount of tooth movement in the laser-irradiated group (group b) was significantly (2.3-fold) greater than that of the control group (group a) (p < 0.001) but there was no any significant difference between group c (the group which received laser indirectly and the non-irradiated group (group a) (p = 0.65). The mean amount of tooth movements can be seen in Fig. 4.

In the histopathological studies, three parameters were evaluated. Out of these three parameters, only osteoclast count at the pressure side was statistically different (p = 0.008). The number of osteoclastic cells on the pressure side in the laser-irradiated group (arrow) showed a multinucleated giant cell in Howship lacunae on the bone surface. Original magnification ×200.
irradiated group was significantly higher than those in the control group. Again, the difference between the group indirectly irradiated with laser and control group was very little and insignificant. The results can be seen in Table 1.

**Discussion**

Long-term orthodontic treatment is one of the main concerns in this field. The aim of this study was to assess the effects of diode laser irradiation on the rate of tooth movement.

In this study, the effects of low-level laser irradiation on bone remodeling and tooth movement were quite prominent. The rate of tooth movement in the laser-irradiated group was nearly 2.3-fold greater than that in the non-irradiated group. This difference was also observed in the rate of bone remodeling, as the number of osteoclasts was remarkably higher at the pressure side in the laser-irradiated group (p<0.001).

However, there was almost no significant difference between the group indirectly irradiated with laser (group c) and the non-irradiated group (group a) in the rate of tooth movement and the osteoclast count.

PDL width and alveolar bone resorption as the other histopathologic parameters were also evaluated. PDL width varies through the process of remodeling. By the exertion of sustained force, PDL decreases in response to the applied pressure while it increases when the alveolar bone resorption begins. PDL width is an important factor in determining the amount of tooth movement in the initial strain stage of tooth movement, which is the first mechanical response of tooth to the sustained force. At this stage, the initial displacement of the tooth depends upon factors like PDL width, root length, and also anatomical configuration. After the initial strain stage, the lag phase begins, which is marked by hyalinization of the PDL tissue [2]. Kim et al. who evaluated the effect of LLL irradiation on collagen turnover reported that LLLT facilitated the rate of collagen turnover in rats [14].

In the present study, the statistical analysis of data from the PDL width and alveolar bone resorption did not indicate any marked difference between the irradiated and non-irradiated groups.

Despite the fact that infrared laser can penetrate more into the soft tissues, it is well proven that every single photon of red laser has 50% higher level of energy than that of infrared laser. Also, it has a higher absorption in cell organelles (nucleus, mitochondria, etc.) where the biostimulation properties of low-level laser begin. It results in the expression of specific proteins that amplify some cellular activity like synthesis of mediators and cytokines which are essential to the formation and activation of both osteoclasts and osteoblasts [6]. Thus, diode laser (660 nm) could be considered like a viable source of biostimulation for bone remodeling. So unlike the several other studies, we chose to apply red laser with a wavelength of 660 nm.

Although the mechanism by which LLLT can affect the bone remodeling process is still unknown, the laser’s ability to

![Graph showing mean (and standard error) of tooth movement in the study groups](image)

**Table 1** The mean of osteoclast count, alveolar bone resorption, and PDL width on the pressure and tension sites

<table>
<thead>
<tr>
<th>Group</th>
<th>Laser-irradiated (direct)</th>
<th>Laser-irradiated (indirect)</th>
<th>No laser</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>PDL_t</td>
<td>775.00</td>
<td>700.00</td>
<td>900.00</td>
</tr>
<tr>
<td>PDL_p</td>
<td>425.00</td>
<td>350.00</td>
<td>500.00</td>
</tr>
<tr>
<td>osteo_t</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>osteo_p</td>
<td>5.00</td>
<td>4.00</td>
<td>7.00</td>
</tr>
<tr>
<td>resorb_t</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>resorb_p</td>
<td>0.00</td>
<td>0.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>
modify cell metabolism has been documented in the literature. It can accelerate the rate of ATP synthesis via the oxidative phosphorylation pathway in inner mitochondrial membranes [15]. Low-level laser chromophore is present in protein complexes (cytochrome oxidase, NADH dehydrogenase) which carry out redox reactions that led to energy release and ATP synthesis [16]. Absorption of special wavelengths of low-level laser stimulates the electron transport chain and accelerates the synthesis of ATP which is the molecular unit of energy for cell functions [17]. As a result, the abundance of ATP molecules facilitates many cell biologic processes [6]. Thus, via acceleration of ATP synthesis, LLLT could demonstrate its biostimulation effects.

Our total energy corresponding to the exposure time was 7.5 J which is considerably lower than that reported by previous studies which utilized GaAlAs with a wavelength in the range of infrared laser. Another difference in performing the laser irradiation in this study was the 48-h intervals given between the irradiation sessions.

The osteoclastic cells differentiate from their precursors deriving from monocyte–macrophage lineage [18]. At the end of the experiment, the number of multinuclear osteoclasts increased 1.5-fold in the irradiated group (group b) as compared to groups a and c, which indicated that low-level laser therapy contributed to the differentiation of osteoclastic cells from their precursors.

Xing et al. demonstrated the role of the receptor activator of nuclear factor-kB (RANK)-RANKL ligand and OPG system in inducing the differentiation and activation of osteoclasts precursors. RANK is a cytokine from the tumor necrosis factor family which is produced by osteoblasts and bone marrow stromal cells and has a major role in the process of osteoclastogenesis [19].

It is well documented that the rate and type of the orthodontic tooth movement are dependent on bone turnover [20]. Petri et al reported that LLLT stimulated differentiation human osteoblastic cells grown on titanium [21]. These studies as well as the study by Yaakobi et al. suggested that LLLT can induce the proliferation and differentiation of osteoblastic cells, which translates into new bone formation [22].

In 2000, Kawasaki and Shimizu evaluated the effects of LLL on tooth movement in rats. They reported that LLLT had stimulated the tooth movement and also, in histopathological analysis, found that the amount of bone formation, the rate of cellular proliferation, and the number of osteoclasts on the pressure side were all increased in the laser-irradiated group [23]. Kim et al. demonstrated that the application of GaAlAs laser with a wavelength of 808 nm can induce the formation and activation of osteoclasts through expression of locally formed cytokines which are the markers of osteoclastic differentiation and activation (RANK, RANKL, OPG) [24]. Seifi et al. assessed the effects of two different types of low-power laser (850 nm (pulse mode) and 630 nm (continuous mode)) on the orthodontic tooth movement in rabbits and reported that the effects of laser were mostly inhibitory as the amount of tooth movement in the laser group was significantly less than that in the control group [25]. Also, Fujita et al. conducted the same study with GaAlAs (810 nm) laser and reported that low-level laser stimulated both the formation of osteoclasts and the orthodontic tooth movements [26].

Furthermore, Marquezan et al. observed no increase in the rate of tooth movement as a result of LLL therapy in experimental tooth movements in rats; however, the osteoclast numbers were higher in the laser-irradiated group [27]. According to Habib et al., LLLT induced the proliferation of osteoblasts as well as osteoclastic differentiation and also deposition of collagen matrix in both pressure and tension areas [28].

The amount of tooth movement in orthodontic treatments depends on the magnitude of the force that is applied to the tooth. An optimal amount of force is one which induces the least amount of undermining resorption [29]. Ren et al. suggested that 20 cN of force is the optimal force for orthodontic movement of rat molars [30]. In the present study, the coil springs exerted up to 40 cN of force, which explains why the amount of tooth movement in our study was rather small compared to that reported by previous studies.

The alveolar bone of rat is denser than that of humans due to the lack of osteons and bone marrow space. Also, there are some differences in the arrangement of periodontal fibers and supporting structures between rats and humans. Although these differences may affect the extrapolation of results to human condition, rats can be considered as a good option for orthodontic tooth movement studies for they exhibit some advantages like being inexpensive for providing large sample size and simple histological sample preparation [30, 31].

For all the clues like those mentioned above, LLLT seemed a viable choice for testing on bone remodeling and evaluation of the effects. Controversial results were reported from different studies due to the different wavelengths and parameters used and the various circumstances.

In order to parlay this LLL ability into clinical use, we have to consider different circumstances in terms of laser parameters and laser dosage due to differences in bone density and soft tissue thickness in humans compared to animals. Thus, there is a need to do more research studies towards the clinical use of LLLT and obtaining optimal dosage for this purpose.

**Conclusion**

The diode laser (660 nm) used in this study could be considered as an effective tool during orthodontic tooth movement in rats, as the rate of tooth movement raised significantly and the number of osteoclastic cells on the pressure side were increased significantly.
References


