

Research Article

The Effects of Low-Level Laser Therapy on Orthodontic Tooth Movement: Metrical and Immunological Investigation

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Abstract

The aim of this study was to evaluate the effects of low-level laser therapy on orthodontic tooth movement via metrical measurements and assessment of expressions of PGE₂ and IL-1 in gingival crevicular fluid.

Materials and Methods: The study was consisted of fifteen patients had undergone two or four first premolar extraction treatment. After aligning of dental arches, canine distalization was performed. The right canines of the patients were included in lased experimental group and the left canines were included in control group. An 820 nm Ga-Al-As diode laser with an output power of 100 mW was used for laser treatment (focal spot: 0.2826 cm²; exposure duration: 15s; energy density: 5.3 J/cm²). For evaluation of quantities of signified mediators, fluid was collected from the distobuccal and distopalatal gingival crevice of teeth before, one hour and 48 hour after orthodontic force load.

Results: The statistical analysis of the metrical findings of tooth movement did not represent any significant difference between groups. The levels of PGE₂ and IL-1 did not show any difference between experimental and control groups at any time of observation.

Conclusion: Low-level laser therapy with the irradiation parameters and protocol used in this study was not found effective in accelerating orthodontic tooth movement.

INTRODUCTION

Laser beams are able to create different effects on tissues, ranging from biostimulation to microexplosion. Researchers have been studying the biostimulatory effects of low-level laser radiation since 1966 [1]. In orthodontics, it can be used for the reduction of post-adjustment pain [2], bone regeneration in the midpalatal suture area after rapid maxillary expansion [3] and acceleration of tooth movement [4].

Orthodontic tooth movement occurs as a response of the connective tissue in which several inflammatory mediators, cytokines, and cells, participate. Low-level Laser Therapy (LLLT) is known as a stimulator of the on-going biological process in tissue and has been found to be effective in modulating cell activity and production of endogenous molecules, which are also involved in orthodontic tooth movement [5-7]. In this regard, several researchers have evaluated the effects of LLLT on orthodontic tooth movement. However, the experiments

performed on human beings are inadequate and the results are controversial [8-10].

It has been reported that chemical analysis of Gingival Crevicular Fluid (GCF) is a promising technique to investigate the response of dental and paradental tissues to orthodontic force load in a biochemical manner [11]. Cytokines have been investigated in many clinical studies to provide a non-invasive way to show their involvement in bone remodelling. Prostaglandin E (PGE) has been known as a potent stimulator of bone resorption, and interleukin-1 (IL-1) takes part in its production [12,13]. In addition, IL-1 β is involved in bone metabolism by triggering bone resorption and inhibiting bone formation [14]. PGE₂ and IL-1 β levels in GCF during orthodontic tooth movement have been investigated in several studies [15-17].

In consideration of the previous studies, the aim of this study was to evaluate the effects of low-level laser therapy on orthodontic tooth movement rate. In addition to metrical

investigation, immunological assessments of PGE₂ and IL-1 β levels in GCF were planned to elucidate the effects of the therapy on inflammatory mediators.

MATERIALS AND METHODS

The study consisted of 15 patients (10 girls, 5 boys), between the ages of 13 and 20 yrs, referred to University, Faculty of Dentistry, Department of Orthodontics. Ethical approval was obtained from the Research Ethical Committee of University, Faculty of Dentistry. The patients and their custodians were informed about the procedures, and they gave informed consent to participate in the study. Inclusion criteria were:

- Should not have any systemic illness
- Should not be under medical treatment that could interfere with orthodontic tooth movement (such as analgesics, anti-inflammatory medicine, or antibiotics)
- Should have a clinical indication for the extraction of maxillary first premolars or four first premolars
- Should not have undergone any orthodontic treatment previously
- Should have healthy periodontal tissues (gingival index=0, plaque index \leq 1, probing depths<3mm, no periodontal attachment loss, and no radiographic evidence of periodontal bone loss)

The maxillary first premolars of fifteen patients were extracted. After the extraction of the teeth, dental arches were aligned up to 0.017"×0.025" ss arch wires. Then, canine distalization was performed by a 12-mm closed coil spring on a 0.016"×0.022" ss arch wire with a force of 150 g.

Laser Irradiation

The right canines of the patients were included in the laser application group and the left canines were included in the control group. Irradiations were performed on five points of the distal side of the tooth root (2 buccal, 1 occlusal, 2 palatal points). Each part was irradiated for 15 s using an 820-nm Ga-Al-As diode laser (Doris, CTL-1106MX) with an output power of 100 mW. The irradiation was performed with continuous waves by a fiber applicator 6 mm in diameter (CTL-2218) on the first, second, third, and seventh days of the experiment. The tip was held perpendicular and in contact with the mucosa during irradiation (Table 1).

GCF Collection

For evaluation of quantities of signified cytokines in GCF, fluid was collected from the distobuccal and distopalatal gingival crevices of each experimental and control tooth before (T0), 1 hour after (T1), and 48 hours after (T2) orthodontic force load using the method of Offenbacher et al. [18]. At the time of collection, oral hygiene was evaluated with the gingival index and bacterial plaque was assessed using the Silness and Loe index. Afterwards, the teeth were washed gently with water, isolated with cotton rolls, and gently air-dried. Sterile paper strips (Periopaper; ProFlow Inc, Amityville, NY, USA) were inserted 1 mm into each gingival crevice for 30 s and then removed. After 1 min, a second strip was inserted into the same site in the same manner. The four paper strips were sealed in an Eppendorf tube

Table 1: Treatment parameters.

Parameter (unit)	Value
Beam spot size at target (cm ²)	0.2826
Irradiance at target (mW/cm ²)	350
Exposure duration (sec)	15
Radiant exposure (J/cm ²)	5.3
Radiant energy (J)	1.5
Number of points irradiated	5
Area irradiated (cm ²)	1.413
Application technique	contact
Number of sessions	4
Total radiant energy (J)	7.5

and immediately frozen at -80°C until the day of analysis. If there was visible contamination with blood, the strip was discarded.

MEASUREMENT OF TOOTH MOVEMENT

The distalization amounts were defined on dental casts taken just before and at the end of the experimental period (one month) as measuring the distance between canine and second premolar at gingival level.

Determination of PGE₂ and IL-1 β Levels by ELISA Kits

On the day of analysis, 400 μ l of Hank's buffered salt solution containing 1% bovine serum albumin (*Sigma, St Louis, MO, USA*) was added to the tubes containing the sample strips. The tubes were gently shaken for 1 min and then centrifuged at 2000 X g for 5 min. After removal of the strips, the supernatants were divided into aliquots for the determination of each mediator/cytokine. The amounts of PGE₂ and IL-1 β were determined using ELISA assays (*Cayman, Ann Arbor, MI, USA; Invitrogen, Camarillo, CA, USA*) in accordance with the manufacturers' instructions. After the colour development was stopped, the optical density was measured using a microtitre plate-computerized reader (*Bio-kinetics Micro Plate reader EL-312, Bio Tek Instruments, Inc., Winooski, VT, USA*) set to a wavelength of 450 nm. The GCF cytokine levels were calculated from the standard curves and defined as picograms/site for total cytokine levels. Sites with cytokine levels below the limits of the kit's detectability were scored as 0.

Statistical analysis

The data were processed and analyzed using SPSS version 16.0 for Windows. Means and standard deviations at each time interval were calculated as the descriptive statistics for the amount of orthodontic tooth movement and cytokine levels (PGE₂ and IL-1 β). Mann Whitney-U test was used to compare the differences in tooth movement and PGE₂ and IL-1 β levels between the groups. The intra-group comparisons for different time intervals were analysed by using Wilcoxon test. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Amount of tooth movement

The mean orthodontic tooth movement amounts are

presented in Table 2. The statistical analysis of the metrical findings of tooth movement did not reveal any significant difference between the groups.

GCF Analysis of PGE₂ and IL-1β

PGE₂ and IL-1β levels at the experimental and control sites at baseline and 1 and 48 hours after force load are presented in Table 2, and intra-group comparisons are presented in Table 3. PGE₂ expression increased after 1 hour in both groups and started to decrease to baseline levels after 48 hours. IL-1β expression insignificantly decreased at 1 hour in the control group. No significant difference was found between the groups at any observation periods.

LLLT is known as a stimulator of the current biological process in tissue. Several studies have reported on the effects of LLLT on orthodontic tooth movement [4,7-10]. To our knowledge, this study is the first to report both clinical data and immunological response in terms of several inflammatory cytokines in gingival crevicular fluid.

According to Karu et al., the mitochondrial cytochromes absorb the photon energy, leading to an increase in ATP synthesis and improvement of the potential activity of the cells [19]. Because osteoclasts are multinuclear cells with high-activity mitochondria, they are readily affected by low-level laser irradiation. Furthermore, Hentunen et al. reported that the bone matrix liberates a protein that stimulates osteoclast formation, which is light-dose dependent [20]. It is known

Table 2: Mean values for Orthodontic Tooth Movement (OTM), PGE₂ and IL-1β, and statistical comparison of the control and laser groups (p<0.05, Mann Whitney-U test).

		Mean	SD	SEM	P value	Sign.
OTM (mm)	Control	1.21	0.61	0.15	0.52	NS
	Laser	1.34	0.60	0.15		
T0 PGE ₂	Control	392.6	146.5	37.83	0.72	NS
	Laser	367.6	184.1	47.55		
T1 PGE ₂	Control	500.0	179.4	46.32	0.67	NS
	Laser	495.0	209.2	54.03		
T2 PGE ₂	Control	435.8	227.0	58.63	0.85	NS
	Laser	425.4	248.9	64.26		
T0 IL-1β	Control	12.93	9.48	2.53	0.22	NS
	Laser	10.04	8.26	2.20		
T1 IL-1β	Control	7.0	5.28	1.46	0.05	NS
	Laser	12.40	7.75	2.33		
T2 IL-1β	Control	9.80	9.22	2.66	0.37	NS
	Laser	11.8	6.29	1.81		

NS: Non-significant

Table 3: Intra-group comparisons of PGE₂ and IL-1β levels at different time intervals.

	PGE ₂		IL-1β	
	T0-T1	T1-T2	T0-T1	T1-T2
Control	0.03*	0.29	0.10	0.26
Laser	0.02*	0.19	0.20	0.65

(*P<0.05, **P<0.01, Wilcoxon test)

that osteoclastic activity may influence posterior osteoblastic activity, and vice versa. Similarly, Zaidi et al. observed that both osteoblasts and osteoclasts have hormonal interaction [21]. Consequently, stimulation of orthodontic tooth movement by LLLT is conceivable, as it consists of concurrent bone resorption and formation.

No significant difference in tooth movement rate was seen between the groups in this study, although a dose (5.3 J/cm²) which has been attested to be appropriate was used [4,8,22-24]. This finding is in accordance with some of the studies [9,25-27] but in disagreement with the findings of the researchers who have claimed that orthodontic tooth movement could be stimulated with LLLT [4,8,10,24]. Although it has been established that LLLT is dose-dependent [28] the effective dose for tooth movement stimulation has not been defined yet. There is a great variety of applied doses between 5.25 J/cm² and 6000 J/cm² [23-27]. When an important factor, scattering, is considered, to keep the energy dose higher seems advisable. Luger et al. have suggested that the energy amount at the target area was 3-6% of the total energy because of scattering of light while transmitting through the tissue [29]. Yamagishi et al. [30] claimed that only 50% of the light of a diode laser could reach 1 mm depth in bovine mandibular cortical bone [31]. Similarly, Esnouf et al. reported a significant reduction in intensity in the first mm of penetration [32]. The negative results of this study may depend on the fact that the energy density's being remained low. On the other hand, its revealed that the therapy produces better results when delivered at low doses when compared to high doses [33]; as already implied by the name of the therapy (*low-level* laser therapy).

In the present study, some of the subjects presented a significant increase in the amount of tooth movement while the movement was not notable at that much in some of them. Depending on this, it could be suggested that the capacity of the effects of the LLLT differs among individuals. It is already known that molecular absorption of laser light is a prerequisite for any cellular effect [34]. The differences in the thicknesses or densities of the soft and the hard tissues, the inflammatory responsiveness and the healing potential of individuals may affect the amount of the effects of the therapy. Cells with a pH lower than the normal, where the redox state is shifted toward reduction, are considered to be more sensitive to the stimulative action of light than those in which the respective parameters are optimal or near optimal. The proposed redox-regulation mechanism may be a fundamental explanation of some clinical effects of irradiation [35]. Light action on the redox state of a cell via the respiratory chain also explains the diversity of low-power laser effects [36].

The mode of the laser device is a controversial topic and could affect the results of the therapy. In the present study, the irradiations were performed with continuous mode. While Bradley et al. [37] and Takeda [38] have supported the use of continuous mode, Kim et al. [39] and Yoshida et al. [40] have preferred pulsed mode. Yoshida et al. claimed that laser units functioning in pulsed mode show more bio-stimulatory response [40].

As Cruz et al. have stated [8], the acute effect of LLLT may be another explanation of the lack of difference in rate of orthodontic tooth movement in this study. The researchers have

suggested that LLLT has a positive effect in the early phases of tooth movement. However, as the lased tooth moves more than the control tooth, the force on the lased tooth decreases. Then the control tooth, which becomes receiving more force, catches up the lased one in one month.

Glinkowski and Pokora indicated that biostimulating laser application to bone increased phagocytosis and cytokine (IL-1, TGF- β) synthesis by increasing macrophage migration [22]. Moreover, Fujita et al. found that increased amount of cytokines accelerated tooth movement via increasing RANKL release while depression of them decelerated the movement [41]. This is why the (non-null) hypothesis of this study was to observe an increase in cytokine levels in GCF with accelerated tooth movement.

In several studies, PGE₂ and IL-1 β levels in GCF have been shown to increase due to orthodontic forces [16,17]. It was reported that PGE₂ and IL-1 β reached a peak at 24 hours after force load, and both decreased to baseline levels in 168 hours [16]. However, we could not observe an expression curve (up-regulation) similar to these studies as the curve's being so transient in comparison with the literature. PGE₂ expression was found to be increased at one hour in both groups and started to decrease to baseline levels after 48 hours. The amount of force or it's being the first phase of tooth movement may cause such a flying effect. Interestingly, IL-1 β expression decreased lower than baseline levels at all observation periods in the control group. Contrarily, it represented a slight increase at 1 hour followed by a decrease at 48 hours in the lased group. This may depend on the merely stimulation of IL-1 β syntheses via LLLT while PGE production might not been affected by the therapy as it is known that cellular responses and activities could be affected diversely by LLLT.

Evaluation of the PGE₂ levels revealed that there was no difference between the control and lased group at any observation periods. IL-1 β levels in GCF were also found to be indifferent between groups. Similar to the metrical findings, the immunological assessment revealed no positive effect of LLLT (with the dose of 5.3 J/cm²) on the orthodontic force induced inflammatory response of the periodontal ligament. In this regard, the authors concluded that the dosage might be remained insufficient. In any case, the crucial point of LLLT is to transmit the effective dose to the required depth of the target tissue.

The reason for the inconceivable IL-1 β levels at 1 hour measurements of the control group may be resulted from a problem with the GCF sampling method, due to repeated collections of fluid at the same time. Repeated sampling is often necessary to collect a sufficient amount of GCF from healthy tissue [42]. Very small amounts of GCF could be collected from healthy sample sites in this study due to the fact that GCF volume has been shown to be correlated with an inflammatory state of periodontium, and the patients with inflammatory conditions had not been included in the study in order to prevent misleadingly increased levels of cytokines. Besides, the pocket depths are shallow in healthy patients and inserting the strips can lead to physical irritation, causing an increase in volume of GCF. These two handicaps of this method may have generated changes in cytokine levels via dilution of GCF.

Some studies have demonstrated reduced quantities or absence of PGE₂ and IL-1 β in GCF at non-inflamed sites using ELISA method [18,43]. On the other hand, Grieve et al. have found that although PGE₂ and IL-1 β levels were consistently low in healthy sites, a sensitive RIA (radioimmunoassay) detected measurable levels of PGE₂ and IL-1 β in 98% and 44% of sites, respectively [15]. Thus, the sensitivity and detection limits of ELISA kits should be considered also.

Further studies investigating the molecular events and mechanisms underlying the differential effects of varying doses and intensities are necessary for a comprehensive understanding of LLLT.

CONCLUSION

Low-level laser therapy with the irradiation parameters and protocol used in this study was not found effective in accelerating orthodontic tooth movement. Further studies are required to optimize treatment parameters and explain the action mechanisms of the therapy.

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