

The Effect of Blue-Light Emitting Diode Irradiation to Alkaline Phosphatase Levels of Orthodontic Tooth Movement

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Abstract

The acceleration of orthodontic tooth movement can be conducted by light-emitting diodes (LEDs) irradiation. The blue-LED provides a photobiomodulation effect by the absorption of photons by cytochrome c oxidase to increase the production of ATP for the metabolic processes of cells involved in bone remodeling. Increased osteoblast during bone formation will be accompanied by increased of alkaline phosphatase (ALP).

Twenty-four *Cavia cobayas* were divided into 4 groups (group I non-irradiated, group II 25 seconds irradiation, group III 30 seconds, and group IV 35 seconds). The orthodontic force was applied to the inter incisor using open coil. The irradiated group was given blue-LED irradiation every day for 7 days. Gingival crevicular fluid was obtained using paper point to evaluate ALP levels on days 0, 3, and 7 using a spectrophotometer (405 nm). The data were statistically analyzed using Anova $p < 0.05$.

The results showed that ALP levels in the irradiated group was significantly different with the control. Group III has the highest ALP levels. The ALP levels of all groups increase from days 0, 3, and 7. The conclusion is 25, 30, and 35 seconds irradiation of blue-LED increase ALP levels of the GCF. Thirty seconds irradiation is the optimal time to increase ALP levels. The duration of irradiation from days 0, 3, and 7 increase ALP levels.

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Introduction

The duration of fixed orthodontic treatment generally takes two years. Longer duration can increase the risk of periodontal disease, caries, and changes in patient motivation¹. It is necessary to accelerate orthodontic tooth movement (OTM) to reduce these risks, such as by administering chemicals, physical stimulation, and surgery². Some of these methods are quite invasive and require injection procedures that cause discomfort and pain, or sophisticated equipment that requires a long-term application to achieve a therapeutic effect³.

One method of accelerating OTM is using photobiomodulation because it does not have

negative effects either locally or systemically⁴. Photobiomodulation is a therapy that involves photochemical interactions between light and body tissue. The photobiomodulation mechanism is activation of cytochrome C oxidase and production of ATP⁵. The high availability of ATP helps cells to turn over more efficiently in bone remodeling and accelerate OTM⁶.

Photobiomodulation can be originated from the light-emitting diode (LED)⁷, a light source that generates electromagnetic waves at ultraviolet, visible, and infrared wavelengths with narrow or monochromatic frequencies⁸. A light-emitting diode has advantages including emitting a non-coherent or diffuse light so that it is safe to apply to a wider body surface area, does not deliver large amounts of energy to damage tissue, low cost, and easy application^{3,9,10}.

Photobiomodulation can stimulate bone remodeling through accelerating cell proliferation and differentiation¹¹. Tooth movement occurs in the same direction as the bone remodeling response, namely bone resorption in pressure area by osteoclasts and bone apposition in

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tension area by osteoblasts¹². The increase in osteoblasts during bone formation is accompanied by an increase in alkaline phosphatase (ALP)¹³. Alkaline phosphatase activity in the gingival crevicular fluid (GCF) during OTM can be related to the time and force exerted on the periodontal tissue¹⁴. Pagin *et al.*¹⁰ measured ALP at days 3, 4, 7, and 14 on the theoretical basis that ALP peaks occur at the end of the proliferation stage and before matrix maturation. Photobiomodulation accelerates bone remodeling not only influenced by dose but also duration of irradiation. Light irradiation with multiple doses may be more effective than single dose to accelerate bone remodeling¹⁵. This study aims to analyze the effect of length and duration of blue-LED irradiation on ALP levels of the GCF in the tension side of the OTM in marmot (*Cavia cobaya*).

Materials and methods

Animals and group preparation

This research was approved by the Health and Medical Research Ethics Committee of Faculty of Dentistry, Universitas Gadjah Mada (approval no. 00316/KKEP/FKG-UGM/EC/2019). A total of 24 male *Cavia cobayas*, 3-4 months old, and body weight 300-500 g, were grouped randomly into group I non irradiated, group II 25 second irradiation, group III 30 second irradiation, and group IV 35 second irradiation.

Orthodontic appliance installation

All subject were carried out under general anesthesia by intramuscular injection of the mixture of ketamine (35 mg/kg body weight) and xylazine (0.5 mg/kg body weight). The lower incisors of all marmots were subjected to orthodontic force and moved distally for a period of one week using a nickel-titanium open coil spring 0.010"x0.030" (American Orthodontics®, USA), applying 35 g of force between the edgewise bracket 0.022-inch slot (American Orthodontics®, USA) with a 0.016" stainless steel wire (Fig. 1).

Blue-Light Emitting Diode Irradiation

Irradiation was performed using Blue-LED 1000mw/cm² (Demi Plus, Ormco, Kerr®, Swiss) for 25 seconds (group II), 30 seconds (group III), and 35 seconds (group IV) once a day for 7 days. Irradiation was performed under general anesthesia to ensure the immobility of the experimental animal. The irradiation was applied

to the gingiva area on the center of resistance incisor marmot from the labial side. The distance between the tip and gingiva was 5 mm with angle of irradiation 90° (Fig. 1).

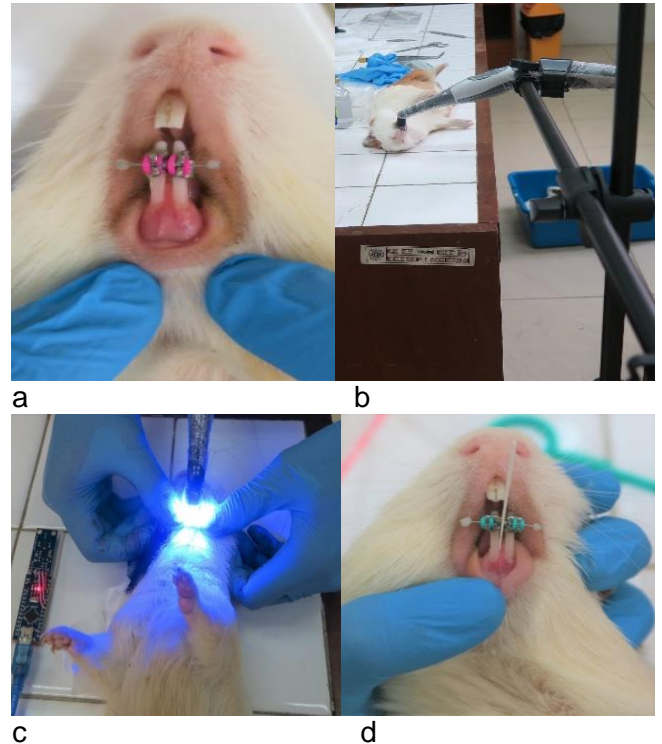


Figure 1. Images of the stages of experiment in marmot (a) bracket placement schematics (b) blue-LED irradiation schematics to the marmot (c) blue-LED irradiation (d) gingival crevicular fluid collection in marmot.

Gingival crevicular fluid collection

Gingival crevicular fluid of each sample in each group was taken on days 0, 3, and 7, its collected using paper point size 15 (Ultradent®, USA). The lower incisors were cleaning with cotton to remove supragingival plaque, and then those teeth were isolated with cotton roll and dried using airway syringe. Paper points were inserted approximately 1 mm into the gingival sulcus of the tension side of teeth (mesial sites) for 30 seconds of each paper point with an interval of 90 seconds to increase the volume of GCF fluid taken each side. A total of three dipped paper points was then placed into a 1.5 mL eppendorf tube containing 350 µL of phosphate buffer saline. Afterwards, those eppendorf tubes were then centrifuged for 5 minutes at 2000 rpm in 4° C using a microcentrifuge refrigerator (Eppendorf Centrifuge 5417R, Germany) to elute the GCF component from the paper points

completely. The paper points were taken and the supernatants were kept frozen and stored at -80°C until assayed. Examination of ALP activities was conducted at Laboratory of Molecular Biology, Faculty of Medicine, Universitas Gadjah Mada (Fig. 1).

Measurement of alkaline phosphatase level

Alkaline phosphatase level was determined using a spectrophotometer (Jenway 6330, UK). Approximately 50 mL of 40 mM carbonate buffer (pH 9.8) mixed with 3 mM magnesium dichloride (MgCl_2) was put into Eppendorf tubes using pipettes. A total 50 μL of GCF samples and 50 μL of 3mM p-nitrophenyl phosphate were added to the same tubes. The tubes were then incubated for 30 min at 37°C . The enzymatic reaction was stopped by adding 50 mL of 0.6 M sodium hydroxide (NaOH), and then the absorbance was measured immediately at 405 nm wavelength, referred to a standard curve prepared from phosphatase substrates (Sigma 104, Sigma-Aldrich, St. Louis, USA). Alkaline phosphatase activity was presented in the form of enzyme unit (U) as μmol of p-nitrophenol released per minute at 37°C .

Statistic analysis

Two-way Anova was used to analyse the differences between the groups and determine its significance. P-values $<.05$ were considered statistically significant, and least significant difference (LSD) post-hoc test was used to detect pairwise disagreements between the groups.

Results

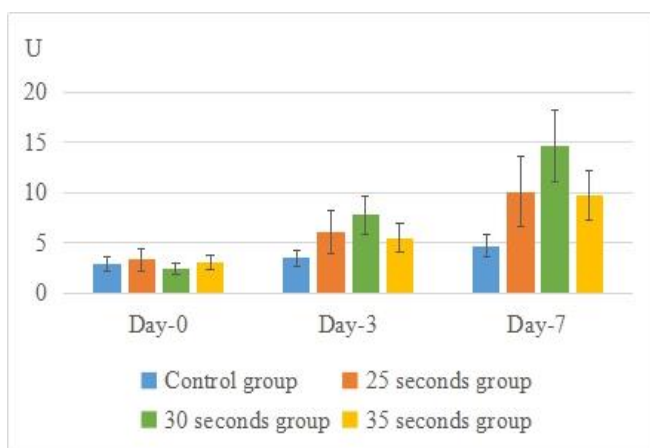


Figure 2. Mean (standard deviation) of ALP levels (U) from 4 groups tested.

The results of measurement of the ALP levels is shown in Figure 2. The results of the study showed that the lowest ALP levels were in the control group, followed by group IV (35 seconds), group II (25 seconds), and highest in the group III (30 seconds). Alkaline phosphatase levels in all groups changes from day 0, 3, to 7. Changes in ALP levels in the group III (30 seconds) from day 0 to day 3 increased sharply 3 times and from day 3 to 7 increased 2 times compared to the previous level. Changes in ALP levels in the group II (25 seconds) and IV (35 seconds) from day 0 to day 3 increased 2 times and day 3 to 7 increased 1.5 times compared to the previous level. Changes in ALP levels in the control group from day 0 to day 3 and day 3 to day 7 each only increased 1 time.

Group	Group I	Group II	Group III	Group IV
Group I Control	-	-	-	-
Group II (25 seconds)	2,692*	-	-	-
Group III (30 seconds)	4,616*	1,924*	-	2,625*
Group IV (35 seconds)	1,991*	-	-	-

*) significant difference $p <.05$

Table 1. Two-way Anova test on ALP levels.

Group	Group I	Group II	Group III	Group IV
Control	-	-	-	-
Group I (25 seconds)	2,692*	-	-	-
Group II (30 seconds)	4,616*	1,924*	-	2,625*
Group III (35 seconds)	1,991*	-	-	-

*) significant difference ($p <.05$)

Table 2. Post Hoc LSD test for differences between the length of irradiation groups.

Group	Day-0	Day-3	Day-7
Day-0	-	-	-
Day-3	2,794*	-	-
Day-7	7,024*	4,230*	-

*) significant difference ($p <.05$)

Table 3. Post Hoc LSD test for differences between groups of the duration of irradiation.

Table 1 showed that significant differences in ALP levels between the length of irradiation groups (control, 25 seconds, 30 seconds, and 35 seconds). There was a significant difference in ALP levels between the duration of irradiation groups (day of observation) days 0, 3, and 7. Furthermore, there was an

interaction between the length and duration of irradiation ($p < 0.05$). The differences between groups were calculated through the Post Hoc LSD test.

The post hoc test between the length of irradiation groups (Table 2) showed significant differences in ALP levels between all groups ($p < 0.05$). ALP levels also showed significantly differ from group III to group II and IV ($p < 0.05$). The post hoc test between the duration of irradiation groups (Table 3) showed significant differences in ALP levels between all groups ($p < 0.05$).

Discussion

This study showed irradiated groups had higher ALP levels than control group (Table 1). The increase in ALP levels in the irradiated groups probably occurred due to the penetration of blue-LED into the osteoblasts which play a role in the bone apposition. Osteoblasts will secrete ALP so that the increase in osteoblasts due to blue-LED irradiation will also increase ALP levels. Photobiomodulation can stimulate osteoblast proliferation and differentiation for bone formation in the tension side followed by increased ALP activity¹⁶.

A light that passes through the skin or mucosa will spread causing the light energy level to decrease by 3-6% of its original intensity¹⁷. Photobiomodulation exposure has a low absorption coefficient on hemoglobin and water resulting in high penetration depth of body tissue¹⁸. Light exposure in this study aimed to stimulate osteogenic cells that are located deep in soft tissue (gingiva) so that we selected the LED device that had wavelengths of 420-490 nm, intensity 1000 mW/cm², with variations in irradiation time of 25 seconds, 30 seconds, and 35 seconds.

Thirty seconds irradiation produced the highest ALP levels (Table 1). This occurred due to the right dose of blue-LED. The biostimulation effect is based on the Arndt-Schulz Law which stated that low to moderate energy levels are required for cellular response activation but excess energy actually inhibits cell proliferation, so LED parameters must be precise, such as dose, exposure time, and intensity to achieve the optimal effect on tissue¹⁹. The dosage in this study is based on the conversion of the law of photons (energy is inversely proportional to

wavelength but is directly proportional to intensity and time) from the research of Ekizer *et al.*³ regarding OTM acceleration in Wistar using LED wavelength 618 nm, intensity 20 mW/cm², and 20 minutes irradiation time. This study used blue-LED wavelength 420-490 nm and intensity of 1000 mW/cm², we found that the optimal exposure time was 30 seconds.

Group I (non-irradiated) produced lower ALP levels than group II (25 seconds) ($p < 0.05$). Twenty five seconds irradiation was sufficient to increase osteoblast proliferation which was indicated by increasing ALP levels. The increase in ALP levels in group II was 2 times higher than the control group, but the dose was not as optimal as group III. This is probably because the exposure dose is too low so that it does not have a photobiomodulation effect²⁰.

Group III (30 seconds) produced higher ALP levels than group IV (35 seconds) ($p < 0.05$). This condition occurs possibly due to 35 seconds irradiation exceeds the optimal dose. At the initial 35 seconds irradiation had the effect increasing ALP levels but to a certain point, the effect decreases because it reaches a saturation point caused by the photoinhibitor effect. The dose exceeds the saturation point because the long radiation time can also produce a photoinhibitor effect thereby reducing the photobiomodulation effect⁹. The control group had the lowest ALP levels because no additional stimulus to increase cell metabolism so that OTM occurred only because of the orthodontic force.

The role of ALP in the mineralization process is to prepare an alkaline condition in the osteoid tissue so that calcium is easily deposited. This enzyme also increases the concentration of phosphate so that calcium-phosphate bonds are formed in the form of hydroxyapatite crystals which will remain in the bones²¹. Alkaline phosphate expression can describe the biochemical changes in the periodontal tissue after orthodontic force application so that this enzyme can be used as a biomarker of osteoblastic activity in alveolar bone formation which shows an increase on days 3 and 7 in tension side and on days 17, 21, and 24 in the pressure side^{22,23}.

Photobiomodulation will increase osteoblast activity that lead to increase new bone formation, and mineralization of the bone matrix by the ALP enzyme will occur²³.

Photobiomodulation can also influence RANKL, an osteogenesis-related transcription factor expressed by osteoblasts. The RANKL/RANK bonds allow preosteoclasts to differentiate into osteoclasts, leading to bone resorption²⁴. Increased new bone formation and bone resorption due to blue-LED irradiation will increase the acceleration of OTM.

The results showed that differences in ALP levels between observation (duration of irradiation) days 0 to 3 and 3 to 7 ($p < 0.05$) (Table 3). The increase in ALP levels from day 0, 3, to 7 in both groups was in accordance with the study of Coombe *et al.*²⁵ which showed that ALP levels increased from day to day in the irradiation and control group.

The initial phase of OTM is characterized by rapid tooth movement and immediately after the orthodontic force was applied²⁶. Tissue that is exposed to orthodontic force will give an acute inflammatory response, namely the migration of cytokine cells that release leukocyte signaling molecules²⁷. On day 3, there was an increase in ALP levels probably due to an increase in osteogenic differentiation of cells involved in bone formation. Alkaline phosphatase activity will be detected in the osteoid area of new bone formation but not in the calcified bone matrix. Cells that show high ALP activity include pre-osteoblasts and osteoblasts²⁶. This is in accordance with the study of Wu *et al.*²⁸ which stated that ALP activity increased on day 3 possibly due to increased osteogenic differentiation in periodontal ligament cells. The increase in ALP levels on the 7th day can occur because of the hyalinization area formed by orthodontic forces. Enzyme activity on the 7th day can show a lag phase which is the hyalinization phase of OTM²⁹.

Alkaline phosphatase is a glycoprotein produced by various types of cells such as leucocytes, osteoblasts, macrophages, and fibroblasts in the periodontal tissue. Bacteria in the gingival sulcus can also trigger ALP production³⁰. The presence of ALP on day 0 even though there was no treatment on marmot was probably due to the biological response to bacteria.

Alkaline phosphatase is a biomarker used to evaluate early-stage osteogenic differentiation²⁸. Saito and Shimizu¹⁵ conducted photobiomodulation exposure to bone regeneration in rats during palatal expansion,

which showed that photobiomodulation would be effective in stimulating bone formation during the initial period, namely the first 3 days of irradiation. Irradiation for up to 7 days still plays an important role in maintaining this bone regeneration activity because if the photobiomodulation is stopped, it may cause regeneration activation also stop. A single dose irradiation only on day 0 did not affect the activation of bone regeneration. Photobiomodulation can accelerate bone regeneration depending not only on the total dose of exposure but also on the duration of exposure.

LED irradiation only induces an acceleration of regional metabolic activity³. Photobiomodulation uses a low power (usually below 500 mW depending on the target tissue) so as not to cause temperature increases and structural changes in the exposed tissue¹⁶. We did not observe changes in tissue temperature. Biological tissue is not a good conductor of heat so the increase in tissue temperature during LED exposure can vary between species.

Conclusions

The 25, 30, and 35 seconds irradiation of blue-LED increase ALP levels of the GCF in the tension side of the OTM in marmot (*Cavia cobaya*). Thirty seconds irradiation is the optimal time to increase ALP levels. The duration of blue-LED irradiation from days 0, 3, and 7 increase ALP levels of the GCF in the tension side of the OTM in marmot (*Cavia cobaya*).

Declaration of Interest

The authors report no conflict of interest.

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