ORIGINAL ARTICLE

Effect of Blue-light-emitting Diode Exposure on Osteoprotegerin Level During Orthodontic Relapse in Rats

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ABSTRACT

Introduction: The post-orthodontic stabilization is a very important phase because teeth alignment tends to relapse. Osteoprotegerin (OPG) is a glycoprotein produced by osteoblasts and inhibits osteoclastogenesis, which reduces bone resorption. Blue–light emitting diode (LED) provides a photobiomodulation effect, thereby increasing adenosine triphosphate production. This study aimed to determine the effect of blue–LED exposure on the OPG on the pressure sides level in gingival crevicular fluid (GCF) during the relapse phase. **Methods:** Ten Wistar rats were divided into 2 groups (n = 10), control groups and blue–LED groups. An open coil spring was used to apply a 35-gram orthodontic force to the mandibular inter-incisor reciprocally. After seven days, the open coil was maintained without activation as retention by a GIC stopper. Blue-LED was exposed for 30 seconds in the stabilization phase for 7 days. GCF was obtained during the relapse phase on days 0, 3, 7, and 14. ELISA test was used to analyze the OPG level. The ANOVA and Post Hoc LSD tests were used to evaluate the data. **Results:** OPG levels in the blue–LED group were higher than in the control group. OPG levels in the blue–LED group have increased significantly, starting on days 3, 7, and 14, during the relapse phase ($p < 0.05$). **Conclusion:** Blue–LED exposure intensity of 1000 mW/cm2 and a wavelength of 490 nm increased the level of OPG in GCF on the pressure sides during orthodontic relapse in rats.

Keywords: Blue–LED, Osteoprotegerin, Orthodontic, Relapse

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INTRODUCTION

Relapse is the phase where teeth usually return to their original malposition after teeth are orthodontically corrected (1). Relapse is caused by various factors, such as the failure to achieve the six keys to occlusion according to Andrew, failure to prevent malocclusion, inappropriate diagnosis and treatment plans, disharmony of tooth sizes, and poor habits (2). A long and unstable bone remodeling process may also affect the stability of orthodontic treatment results (3).

The interaction of the RANKL, RANK, and osteoprotegerin (OPG) is linked to the remodeling process (4). OPG is a glycoprotein produced by osteoblasts and plays a role in the inhibition of osteoclastogenesis (5). OPG inhibits RANK that binds to RANKL, thus inhibiting osteoclast proliferation and lowering the bone resorption process (6). Relapse in the post–orthodontic stabilization phase occurs due to increased RANKL levels and decreased OPG levels (7). Relapse can be prevented by local OPG gene transfer by reducing osteoclastogenesis and enhancing bone remodeling. An increased OPG level will boost the formation of osteoblasts which help the formation of bone in the remodeling process (8).

Photobiomodulation on orthodontic treatment can accelerate tooth movement, inhibit root resorption, and accelerate bone remodeling (9). Photobiomodulation treatment is a painless therapy that involves irradiation via a low light laser (LLL) or a light emitting diode (LED). It is noninvasive, affordable, simple to use, and has no local or systemic adverse effects (9,10). Photobiomodulation is a non–invasive stimulus to the dentoalveolar complex that potentially increases and boosts adenosine triphosphate (ATP) production by mitochondria (11). The cell's response to the photobiomodulation mechanism involves a photobiological reaction, i.e., the light of a certain wavelength is absorbed by photoreceptor molecules. The mitochondrial photoreceptor known as

cytochrome c oxidase absorbs light wavelengths. Electron excitation will occur, increasing the electron transport rate to increase the capacity of mitochondria to produce ATP (12).

The same cellular pattern is seen in both tooth relapse and orthodontic tooth movement (OTM) (13). The side of pressure in OTM becomes the side of tension in relapse, and there is an increase in osteoblast differentiation, thus causing new bone formation. The side of tension in OTM becomes the side of pressure in relapse, and there is an increase in osteoclasts, thus resulting in bone resorption (13). OPG levels were observed on days 0, 3, 7, and 14 during the relapse phase. OPG levels can be identified through enzymatic changes that occur during the initial phase to the start of the post-lag phase. The initial phase lasts 24 to 48 hours, while the lag phase occurs on days 7 to 14, characterized by the formation of hyaline tissue and undermining resorption (14). .

MATERIALS AND METHODS

Animals and group preparation

This research is a part of thesis research approved by the Research Ethics Commission of the Faculty of Dentistry, UGM, with permit number No. 00751/ KKEP/FKG–UGM/EC/2021. Ten male Wistar rats, 2,5–3 months old, with 200–250 g body weight. Wistar rats were randomly selected and divided into two groups, namely, group C (without exposure to blue–LED/control) and group LED (with exposure to blue–LED) (15). This research used experimental animals according to the 3R principles: Replacement, Reduction, and Refinement. Reporting of the results of studies involving experimental animals refers to the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines released by the National enter for the Replacement, Refinement, and Reduction.

Orthodontic appliance installation

Wistar rats were given the anesthetics ketamine and xylazine (dose ketamine HCL 10% 0.5 mL/kg BW and xylazine 2% 0.5 mL/kg BW) by intramuscular injection in the right upper thigh muscle before being treated. The bracket used in this study was the standard Edgewise 0.022–inch slot (Natural, USA). Each bracket was welded on a matrix band measuring 1 cm long and 0.4 cm wide, which was then shaped like a ring (15).

The welded bracket was inserted on the matrix band using GIC type 1 (GC, USA) (Fig. 1). The bracket was installed with the incisal edge of the bracket at a distance of 2 mm from the mandibular incisors. Using a 0.010”x0.030” nickel–titanium open coil spring (Dynaflex, USA) with a stainless steel wire sized 0.016” (Dynaflex, USA), the mandibular incisors were forced to move distally for seven days. The force generated by the open coil spring was 35 grams. After seven days, the open coil is maintained without activation as retention by a GIC stopper during the stabilization phase (15).

Fig. 1 : Bracket placement in the Wistar rats. The welded bracket was installed with the incisal edge of the bracket at a distance of 2 mm from the mandibular incisors.

Blue–Light Emitting Diode Exposure

Blue–LED with the intensity of 1000 mW/cm^2 and a wavelength of 490 nm (Guilin Woodpecker

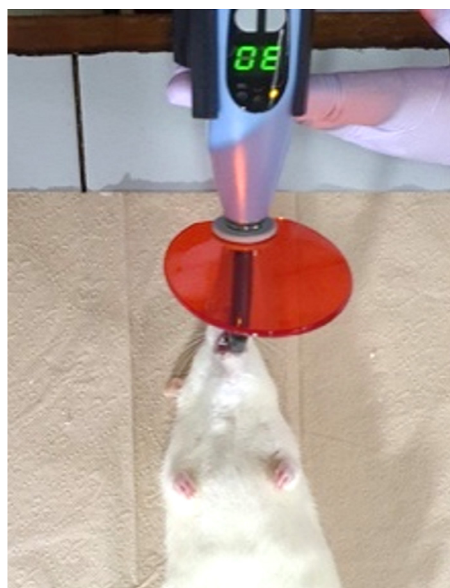

Fig. 2 : Blue–LED exposure to Wistar rats. The exposure of blue–LED with the intensity of 1000 mW/cm^2 and a wavelength of 490 nm for 30 seconds for 7 days in the stabilization phase. Exposure was performed on the labial gingiva, 5 mm from the edge of the gingiva between the right mandibular central incisors, by placing the tip of the optical fiber at a 1 mm distance and an angle of exposure to light of 90°.

Medical Instrument®) was used for the exposure for 30 seconds for 7 days in the stabilization phase. Exposure was performed on the labial gingiva, 5 mm from the edge of the gingiva between the right mandibular central incisors by placing the tip of the optical fiber at 1 mm distance and an angle of exposure to light of 90o (Fig. 2) (15).

Gingival crevicular fluid collection

The gingival crevicular fluid samples were collected on days 0, 3, 7, and 14 of the relapse phase. The GCF on day 0 was performed immediately after the stabilization phase had finished (group C0 and LED0), then on day 3 (group C3 and LED3), day 7 (group C7 and LED7), and day 14 (group C14 and LED14). The GCF was taken by cleaning the right mandibular central incisor of the Wistar rats using cotton pellets to remove the supragingival plaque, followed by isolation using cotton and drying. The gingival sulcus' mesial was inserted with three paper points size 15 each (the side of pressure on relapse) of each rat's incisor with a 1 mm depth for 30 seconds with 90 seconds intervals (Fig. 3). The paper points were then placed in a 1.5 mL Eppendorf tube that was filled with 350 µl of physiological saline solution. The components of the GCF were eluted by centrifuging the Eppendorf tube at 2500 rpm for three minutes (15).

Measurement of OPG Levels

The OPG levels in the samples were analyzed using the ELISA kit. All the components in the ELISA kit and the samples were conditioned to room temperature (18–25°C). A microplate spectrophotometer with a wavelength of 450 nm was used to compare the optical densities of the samples and the standard to determine the OPG levels. The OPG levels were presented in ng/mL (15).

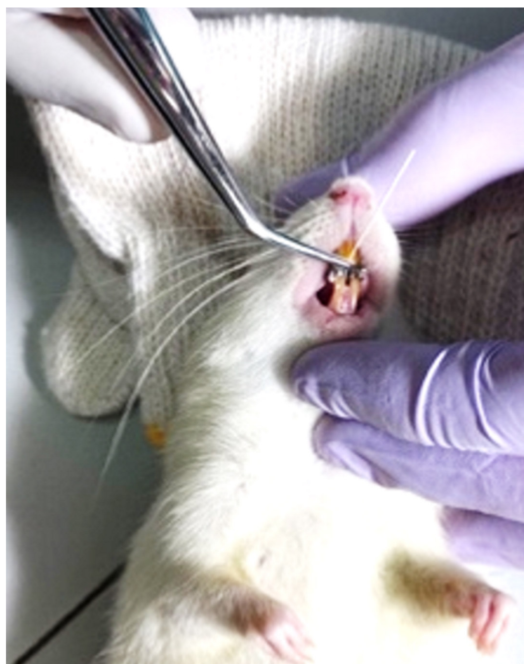

Fig. 3 : Gingival crevicular fluid collection in Wistar rats. The GCF samples were collected on days 0, 3, 7, and 14 of the relapse phase on the side of pressure on relapse.

Statistic Analysis

Statistical program SPSS was used to analyze the data. Levene's homogeneity test revealed homogenous data, while the Shapiro–Wilk normality test demonstrated that the data were normally distributed. The two–way Analysis of Variance (ANOVA) parametric test was then used to tabulate the data and identify group differences. In addition, Post Hoc Least Significance Difference (LSD) was also performed to determine different groups. The confidence level was 95% (α = 0.05) (15).

RESULTS

The mean values and standard deviation (SD) of the OPG level on the pressure side of the gingival crevicular fluid post–stabilization phase can be seen in Table I. Compared to the blue–LED group, the OPG levels in the control group were lower.

The OPG levels in the control and blue–LED groups increased from days 0, 3, 7, and 14 of observation. The OPG levels increased gradually and were highest significantly ($p<0.05$) in 14 days relapse phase compared to all other groups (Fig. 4). OPG levels on days 0 and 3 were comparable ($p>0.05$), and the level started higher in blue–LED on day 7 ($p<0.05$).

According to the outcomes of the two–way ANOVA test, OPG levels between the control group and the blue–LED group were significantly different; there were significant differences in the observation period on days 0, 3, 7, and 14; there was an interaction

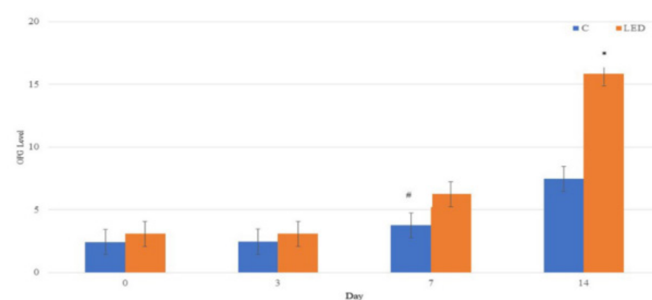

Fig. 4 : Graph of mean OPG levels on the pressure side of the GCF during the relapse phase of Wistar rats. The OPG level was highest significantly ($p<0.05$) in 14 days relapse phase. OPG levels on days 0 and 3 were comparable ($p>0.05$), and the level started higher in blue LED on day 7 ($p<0.05$). # Higher than the other group% *Lower ($p<0.05$) than blue–LED group on day 7.

Table I : The mean values and standard deviation (SD) of the OPG level on the pressure side of the gingival crevicular fluid post-stabilization phase of the Wistar rat

	Mean values ± Standard Deviation (SD)			
Group	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Control	2.428 ± 0.019	2.452 ± 0.023	3.752 ± 0.033	7.474 ± 0.015
***Blue*-LED**	3.072 ± 0.025	3.075 ± 0.033	6.240 ± 0.037	15.868 ± 0.016

The OPG levels in the control group were lower than in the blue-LED group. The OPG levels in the control and blue-LED groups increased from days 0, 3, 7, and 14 of observation. The OPG levels increased gradually, reaching the peak on day 14 in both groups.

Table II : Two-way ANOVA test OPG levels on the pressure side of the gingival crevicular fluid in the relapse phase of Wistar rat

Variable	F	p *value*
Day	276158.951	.000*
Treatment	142527.504	.000*
Day*Treatment	52523.979	.000*

*Significant differences between groups (p < 0.05)
OPG levels between the control group and the blue–LED group were significantly different; there were significant differences in the observation period on days 0, 3, 7, and 14; there was an interaction between the observation period and exposure to blue–LED (p<0.05).

Table III : LSD post hoc test results differences between groups during observation day on OPG levels on the pressure side of the gingival crevicular fluid in the post-orthodontic stabilization phase of Wistar rats

Day	Day 0	Day 3	Day 7	Day 14
Day 0	-	.074	.000*	.000*
Day 3	.074	-	.000*	.000*
Day 7	.000*	.000*	-	.000*
Day 14	.000*	.000*	.000*	-

*Significant differences between groups (p < 0.05)
There was a significant difference in the OPG levels between the two groups on days 0 to 7 and day 14 (p<0.05). There was no significant difference in the OPG levels on day 0 and day 3 (p>0.05).

between observation period and exposure to blue–LED (p<0.05) (Table II). The LSD (Least Significant Difference) Post Hoc test was used to determine how the two groups' observation times differed in the two–way ANOVA test (Table III). The outcomes of the LSD Post Hoc test to compare the mean OPG levels between the two groups based on the observation periods. As a result, there was a significant difference in the OPG levels on days 0 to 7 and day 14 (p<0.05). There was no significant difference in the OPG levels on day 0 and day 3 (p>0.05).

DISCUSSION

According to this study, the blue–LED exposure group had higher OPG levels than the control group (Table I). The increased OPG levels in the blue–LED group might be the result of the ability of the blue–LED to penetrate the periodontal ligament cells, one of which is osteoblasts. An increased osteoblast activity will increase OPG levels, thus accelerating bone formation (16). Blue–LED exposure can promote osteoblast differentiation and proliferation for bone formation, followed by an increased OPG activity (17).

The 30–second exposure time in this study was based on the photon law conversion proposed by Ekizer et al. (9), in which Arndt–Schulz's Law converted: exposure to 1000 mW LEDs for 30 seconds is optimal in increasing cell proliferation. This is in line with research by Rahmah et al. (18) showing that there was an increase in the ALP levels in the gingival crevicular fluid after exposure to

blue–LED and the optimal exposure time was 30 seconds with the intensity of 1000 mW/cm^2 and a wavelength of 490 nm. In this study, blue–LED exposure was used to induce osteogenic cells that were found deep within the soft tissue. The control group had lower OPG levels due to the absence of an external stimulus to enhance cell metabolism. The blue–LED exposure group experienced a photobiomodulation effect, i.e., stimulating osteoblast proliferation and differentiation, characterized by an increased OPG level (18, 19). On the other hand, the control group without light exposure did not receive any external stimulus that could help activate bone–forming cells.

This study showed that there was a significant difference in the OPG levels between the group e xposed to blue LED and the group not exposed to light ($p<0.05$). This is consistent with research by Chang et al. (19), showing that there was an increase in the OPG levels in the group with light exposure compared to the group without light exposure. Blue–LED provides a photobiomodulation effect, thereby increasing electrons for molecular oxygen reduction and accelerating ATP production for the metabolism of cells involved in bone remodeling (20). High ATP levels help cells have more efficient bone turnover during bone remodeling.

There was an effect of exposure to blue–LED with the intensity of 1000 mW/cm2 and a wavelength of 490 nm and observation periods on days 0, 3, 7, and 14 on the OPG levels in the side of the pressure of the gingival crevicular fluid after the stabilization phase of Wistar rats. The highest OPG level was found on day 14 compared to other days of observation. Blue–LED increased mitochondrial activity, thereby increasing ATP production (10). Photons from blue–LED waves are absorbed by cytochrome c, a mitochondrial photoreceptor (21). The absorption of photons will stimulate electron excitation, thus increasing the capacity of mitochondria to produce ATP by increasing the electron transport rate (10). Increased ATP levels can induce cells to process bone formation due to increased metabolic activity. This study used male Wistar rats to prevent any intervention from uncontrollable factors such as the hormone estrogen. The effect of exposure to blue–LED with the intensity of 1000 mW/cm^2 and a wavelength of 490 nm on cells in vitro has not been carried out, so the effect on primary culture cells is unknown.

The results of the study showed that exposure to blue–LED could increase OPG levels, evident from the different OPG levels between the treatment and control groups on days 7 and 14 after the orthodontic appliance was removed. OPG, a glycoprotein component of TNF, is generated by osteoblast cells (22). It competes with RANK to bind to RANKL to prevent the recruitment, differentiation, and maturation of preosteoclasts into osteoclasts by inhibiting osteoclastogenesis (23). OPG inhibits osteoclast activation by suppressing the osteoclast matrix activation and inducing apoptosis of preosteoclast cells. With OPG levels higher than RANKL levels, OPG will bind to RANKL, thereby inhibiting the bonds between RANKL and RANK and slowing down the formation of new osteoclasts. (23,24).

Local OPG gene transfer could prevent osteoclastogenesis activity by RANKL, thus inhibiting orthodontic tooth movement (25). Local injection of OPG has been known to potentially serve as a stabilizing agent after orthodontic treatment. Local injection of OPG can restore the volume and density of bone tissue and reduce the relapse rate in experimental animals that were treated to have orthodontic tooth movement (26). Increased OPG levels can inhibit osteoclast differentiation, induce osteoclast apoptosis, and stimulate osteoblast proliferation; it can also serve as a reference to prevent relapse after orthodontic treatment.

The GCF was collected on days 0, 3, 7, and 14 of observation to identify OPG activity which was used as a biomarker of orthodontic tooth relapse. The results demonstrated that there was an increase in OPG levels from day 0 to day 3; however, it was not statistically significant ($p>0.05$). This is likely because osteoclasts increased from day 1 to day 4 while osteoblasts decreased. In the initial phase, bone resorption was higher than bone apposition (27). The OPG levels increased along with the days of observation in each group on day 7 and day 14. This shows that observation periods affected the OPG levels. Osteoblasts increased from day 7 to day 14. On day 14, cellular activity increased, and hyaline tissue started to form (27,28).

The limitation of this study was that the toxicity effect of blue-LED intensity of 1000 mW/cm^2 and a wavelength of 490 nm was unknown. Further study may be required on the toxicity effect to understand better the effect of blue-LED exposure in alveolar bone and underlying periodontal tissue during the relapse phase.

CONCLUSION

Blue–LED exposure intensity of 1000 mW/cm^2 and a wavelength of 490 nm increased the OPG levels

on the side of pressure in the GCF during the relapse phase in Wistar rats. OPG levels in the blue–LED group have increased from days 0, 3, 7, and 14, with the highest peak on the 14th day.

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