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Original article

The difference of osteoblast number between day 5 and day 10 after orthodontic force in tension area and compression area

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ABSTRACT

Background: Orthodontic tooth movement can occur through the bone remodeling process. Bone remodeling is a process in which the bone undergoes resorption in the pressure area and apposition in the tension area. Cells that play a role in the process of apposition or bone formation are osteoblasts. **Objective**: This study aims to determine the difference amount of osteoblast on day 5 and day 10, the difference amount of osteoblast on the tension and pressure areas, and the interaction between the periodontal tissue area and the day after the orthodontic force application. **Method**: The subjects consisted of six Cavia cobaya which applied orthodontic wire with coil spring and metal ring to separate the two maxillary incisors. The given force is 17.5 grams on each tooth. Subjects were divided into groups: groups with treatment for 5 days and groups with treatment for 10 days. Euthanasia was done to three guinea pigs from each group on the 5th dan 10th day post orthodontic force appliance. The tissue was then taken to prepare a histological specimen and stained with Haematoxylin Eosin. Osteoblast counts were observed under a light microscope with 400X magnification and Optilab Viewer. One-Way ANOVA and Post Hoc Tukey analyzed observational data. **Results**: One-Way ANOVA test results showed a significant difference ($p < 0.05$) in the mean number of osteoblasts between groups. Post Hoc Tukey test results showed a significant difference ($p < 0.05$) in the mean number of osteoblasts between groups. **Conclusion**: There was a difference amount of osteoblast on day 5 and day 10, there were differences amount of osteoblast on the tension and pressure areas, and there was an interaction between the periodontal tissue area and the day after the application of orthodontic force.

Keywords: cavia cobaya; force; orthodontic; osteoblast

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INTRODUCTION

Orthodontic treatment is performed to move the teeth into a better arrangement. Tooth movement is the basis of orthodontic treatment. The orthodontic force will produce changes in the alveolar bone and cells in the periodontal tissue, resulting in bone formation in the region of tension and bone resorption on the pressure side. This causes the teeth to move toward a new position.[1]

The occurrence of the bone formation process can be characterized by the presence of osteoblast cells on the bone surface. The number of osteoblasts in the bone formation process will peak on the 14th day after the orthodontic wire. Osteoblasts in the paradental and interradicular areas of the molar increased on days 1, 3, 7, 10, and 14. Meanwhile, on day 5, the number of osteoblast cells decreased. The fifth day after the orthodontic force is given, there is an osteoid deposition in the tension side. Osteoid is an unmineralised bone matrix produced by osteoblasts. This shows that on day 5, osteoblasts show their activity by producing a bone matrix.[2,3,4]

Although it is known that osteoclasts peak on days 3-4 in the compression area and osteoblasts begin to increase on day 5 after orthodontic force application on the tension side,[5,6] the exact mechanism connecting these two processes is not fully understood. Furthermore, the interaction between periodontal tissue and time after orthodontic force application, particularly the difference in osteoblast numbers on days

5 and 10, as well as the ratio between tension and compression sides, remains understudied. This study aims to fill this gap by analyzing the difference in osteoblast numbers on days 5 and 10, as well as the interaction between periodontal tissue and time after orthodontic force application.

MATERIALS AND METHODS

This research has been approved by the Ethics Committee of the Faculty of Dentistry of Gadjah Mada University. This study uses an orthodontic wire coil spring with a force of 35 grams. The 35-gram force is applied to both maxillary incisors of the subject. The research subjects used are 6 male Cavia cobayas. The experimental animals were then divided into 2 groups: the treated group for 5 days and the group treated for 10 days, with each group having 3 animals.

Cavia cobaya were acclimatized for 1 week before the experiment. A customized coil spring welded to a metal ring was installed to separate both maxillary incisors. The given wire force is 35 grams for both teeth. Animals were sacrificed on day 5 or day 10. The histological section was taken 5 mm from the free gingiva. Guadruple cross-sectionally, with a thickness of 5 microns, stained by Hematoxylin Eosin (HE). HE-colored tissue was then observed using a light microscope with 400X magnification and OptiLab Viewer®. The osteoblast cell count is then performed using a J-cell counter image.

RESULTS

After orthodontic force application, osteoblasts were examined on day 5 and day 10. The osteoblasts were counted on the compressed and tension sides of the maxillary incisors of Cavia cobaya. The research results are the average value and standard deviation from the two treatment groups.

The osteoblast number (Table 1) shows that the mean cell count on day 10 is higher than the average number of osteoblast cells on the 5th day. The average number of osteoblast cells on day 5 and day 10 was higher on the tension side, while the mean osteoblast cell counts on the compressed side were lower. The normality and homogeneity tests showed that the data could be analyzed parametrically

The normality test is performed to see whether the data is normally distributed. The normality test used was Shapiro-Wilk because the number of samples used in this study was less than 50. The normality test results showed the significance value of the 5th day of the interested group was 0.637, the 10th day in tension groups was 0.220, while the 5th day and the 10th day in compressed groups were 0.463 and 0.637, respectively; the analysis of the whole group shows that the data is normally distributed ($p > 0.05$). The homogeneity of the data was tested with Levene's test. Homogeneity test results showed that the data have a homogeneous variance because they have a significance

Table 1. The mean and standard deviation of osteoblast cell number

	X ± SD tension side	X ± SD pressure side
day-5	6.17 ± 1.04	2.83 ± 0.38
day-10	8.75 ± 0.38	5.33 ± 1.09

Table 2. One-Way ANOVA Test result of osteoblast cell formation

	Sum of squares	Degree of freedom	Mean of squares	F	p
Tension side	10.010	1	10.010	15.016	0.018
Pressure side	9.375	1	9.375	15.254	0.017
Day 5	16.667	1	16.667	27.119	0.006
Day 10	17.510	1	17.510	26.266	0.007

Table 3. Post Hoc tukey test result of osteoblast number

	day 5 pressure side	day 10 tension side	day 10 pressure side
day 5 tension side	0.001*	0.006*	0.042*
day 5 pressure side	-	0.007*	0.005*
day 10 tension side	-	-	0.002*

of 0.129 and 0.083 ($p > 0.05$); the data were analyzed using One-Way ANOVA.

The results of the One-Way ANOVA test (Table 2) show a significant difference ($p < 0.05$) in osteoblast cell count on the 5th-day group and 10th-day in tension or pressure sides. Post Hoc Tukey test (Table 3) shows a significant difference in the number of osteoblasts between groups.

DISCUSSION

A microscopic cascade process occurs when the orthodontic force is applied. First is the response of blood flow changes that cause an increase in oxygen levels on the tension side and a decrease in the compression area. Another theory is the presence of piezoelectric signals—more precisely, bioelectric potentials in the form of small current voltages—released as a result of bone bending and deformation of the crystal structure. In addition, the theory of neurotransmitters (including substance P, intestinal polypeptide (VIP), and calcitonin gene-related peptide) can be released as a result of physical deformation caused by peripheral stressors on paradental tissues, such as nerve fibers and terminals. Therefore, PDL and bone cells can react to primary stimuli, such as orthodontic forces, by producing bioelectric signals as a result of bone bending, inflammatory chemical mediators, such as PGE2, cytokines, such as nitric oxide (NO), and neurotransmitters. PDL cells, such as fibroblasts, and bone cells, such as osteoblasts, have receptors for these chemicals.[7]

There are multiple routes for conveying mechanical stress that operate on cells and their surrounding matrix. In addition, there are numerous interactions and connections between these cells. These interactions cause short-term increases in intracellular levels of second messengers such as calcium, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and inositol phosphatase 3 (IP3). These second messengers transmit signals to the nucleus via a series of kinases. Variations in gene expression, protein synthesis, and patterning are explained by the different second messengers found in the nucleus of each cell. Extracellular matrix gamma carboxyglutamic acid (GLA) protein, C-fos, C-jun mRNA AP-1, Egr-1, and growth differentiation factor SP-1 9B are recently discovered immediate early gene expression protein transcription factors. Transcription factors appear to be increased in response to mechanical stimuli, cytokines, and growth factors. These transcription factors can result in interactions that cause osteoclastic bone resorption or cellular proliferation or differentiation that leads to osteoblastic bone creation. Osteoblasts are integral in bone remodelling; they produce prostaglandins, RANKL, and OPG, which are the two latest ligands, either promoting or preventing osteoclastogenesis.[8,9]

In the current study, osteoblasts were formed in the tension and pressure sides. Osteoblasts on the tension side were higher than on the pressure side ($p < 0.05$). Showed that the process of bone formation was higher on the tension side. The pressure side, known as an area of osteoclast bone resorption during orthodontic tooth movement,[4] does not mean osteoblast is not formed. Though it was at a lower number, it supports the reversal theory: a decrease in osteoclasts, the osteoblasts will

increase, and vice versa; if osteoclasts decrease, osteoblasts will increase.[10, 11]

Based on the results obtained, it can be seen that the alveolar bone likely continues to experience remodeling post-orthodontic force actively. There was no active bone formation process on the fifth day after the orthodontic force application in the area of tension; only a small number of osteoblasts were formed on the pressure side because the area is more dominated by osteoclast cells, suggesting that on day 5, there is still an active bone resorption process on the pressure side. On day 10, after the orthodontic force was applied, the osteoblast number was increased on the tension side, suggesting active bone formation. The cell formation also increased on the pressure side compared to day 5. These results suggest that on the 10th day after orthodontic force, the alveolar bone will actively perform the bone formation process.

The number of osteoblasts appears more noticeable on the tension side than on the pressure side. When tensile orthodontic forces occur, the cells in the periodontal ligament will increase the number of specific secondary messengers, the Extracellular Signal Regulated Protein Kinase (ERK).[12] Extracellular Signal Regulated Protein Kinase (ERK) will induce the expression of RUNX-2, leading to an increase in osteoblast activity and bone production. The number of periodontal ligament cells does not increase in the area of tension. However, there is an increase in the number of osteoblasts, indicating that RUNX-2 induces fibroblasts to differentiate into osteoblasts.

Alkaline phosphatase (ALP) showed an increment on day 0 and day five after orthodontic force, but on the 10th day, it decreased. In contrast, osteocalcin appears to have decreased on days 4-5 and increased on day 10. *Alkaline phosphatase* is a bone-forming biomarker that actively works to mineralize bone.[2] Meanwhile, osteocalcin works by inhibiting the activity of osteoblasts.[12] Based on this, compared with the results obtained, it is known that the 10th day is still an active bone formation process, so it does not indicate the existence of osteocalcin activity to inhibit osteoblasts. On the contrary, it also indicates that an active bone mineralization process is still occurring, one of which is played by an alkaline phosphatase (ALP) biomarker.

CONCLUSION

Based on the results obtained from the observation, it is known that there is a relationship between the duration of orthodontic force and the area of teeth (tension and pressure sides). On the 5th day, the treatment group showed that on the tension side, the bone formation process had not likely occurred; it might still be dominated by bone resorption processes on the pressure side. Meanwhile, on the 10th day, there was a process of active bone formation by osteoblasts on the tension and pressure side.

The duration of orthodontic force application influenced osteoblast formation in the tension and pressure areas. This study was not done with orthodontic appliance reactivation, and no measurements of the distance of movement of teeth occurred. So, it is yet to be known whether the force given to the teeth is still working maximally until day5-to-10. The orthodontic force may have decreased as it reached the 10th day; however, the results showed the number of osteoblasts increased until the 10th day, suggesting that there is still an active process of bone formation

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