The Expression of HSP-60 and MMP-8 on Orthodontic Tooth Movement in the Alveolar Bone after Sodium Fluoride Topical Administration

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Abstract

Applying orthodontic force to teeth causes bone remodeling, namely resorption of alveolar bone in the pressure area of the periodontal ligament, while bone is formed at the tension area. Fluoride is used to stimulate cell bone formation. The purpose of this study is to find out the Sodium Fluoride effect on the HSP-60 and MMP-8 expression in the alveolar bone receiving an orthodontic force of male Wistar rats. The study used a laboratory experimental method that involved 16 male Wistar rats separated into two groups. Group C: rats obtained orthodontic tooth movement and without Sodium Fluoride. Group T: rats received orthodontic strength with Sodium Fluoride 11.75 ppm with topical application. The orthodontic strength used ligature wire with a 0.02 mm wire diameter drawn from the permanent first molars and maxillary incisors. HSP-60 and MMP-8 expression was tested using immunohistochemistry and observed on day 7 and day 14. The results of the data were tested using Anova statistical test to compare variables among groups at a level of significance of 5% ($p<0.05$).

Administration of Sodium Fluoride resulted HSP-60 and MMP-8 as expressed of osteoblast interpretation was smaller than without Sodium Fluoride administration as well as on 14th and 7th days.

Sodium Fluoride can reduce osteoblast that is expressed by HSP-60 and MMP-8 in orthodontic teeth movement.

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Introduction

Teeth that have been moved through the alveolar bone using orthodontic devices in orthodontic treatment show a tendency to return to their previous position; it is called relapse [1]. Relapse in post-orthodontic treatment commonly happens, showing that of the 500 patients treated with orthodontics, 61.5% were relapse [2]. In contrast, only 7% of patients using removable retention devices showed relapses of less than 1 mm, and 40% of relapses were less than 3 mm[3]. Relapse can make orthodontic treatment will be longer and the costs become relatively more expensive.

Orthodontic tooth movement is a complex mechanical and biological process involving a wide variety of molecules [4]. Orthodontic teeth are resorbed in the area of pressure which is made by osteoclast and opposition to the tension side which is made by osteoblast, that's called the remodeling process[5,6]. Remodelling process of bone is a dynamic interaction [6]. In this remodeling process, vascular changes occur, causing migration of leukocytes out of the capillary blood vessels, which then makes VEGF and MCSF increase. This increase is also followed by MMP-8 (Human neutrophil collagenase [7] /Matrix Metalloproteinase 8) produced by leukocyte PMN cells, gingival fibroblasts, bone, and plasma cells, which play a major role in remodeling [8]. Heat Shock Protein 60 (HSP-60) expression works as a molecular chaperon to

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maintain homeostasis, acts as anti-apoptosis, and protects cells from pathological stress [9]. Many research has been done to orthodontic tooth movement, one of the efforts that may be able is by giving Hyperbaric Oxygen (HBO) [10], Docosahexaenoic Acid (DHA) Microalgae®[11], Robusta Coffee exract [12, 4], Coffee Brew [6], Bisphosphonate (Pamidronate) [13]. Based on the description above, the problem of this research is: Does HSP-60 and MMP-8 expression decrease in male Wistar rat teeth which are given orthodontic force in the alveolar bone in subsequent to of Sodium Fluoride topical administration ?.

This study aimed to determine the effect of Sodium Fluoride administration on HSP-60 and MMP-8 expression on teeth with orthodontic force in the alveolar bone area of male Wistar rats. The benefit of this research is that it can increase scientific insights on the administration of Sodium Fluoride which can reduce HSP-60 and MMP-8 expression and make faster orthodontic treatment and relatively cheaper costs.

Materials and methods

Orthodontic Tooth Movement in Experimental Animals

The study used a laboratory experimental method that involved 16 male Wistar rats. Sixteen male Wistar rats with an age range of three to four months and body weight of 250-300 grams (0.2-0.3 kg). Sixteen Wistar rats in good condition were randomly divided into two groups: Control (C1, C2) and Treatment (T1, T2). In groups C1, C2 (n = 8) and T1, T2 (n = 8), orthodontic devices (NiTi closed coil spring) were inserted between the first maxillary molar teeth and maxillary incisors to allow orthodontic tooth movement. Group C1: orthodontic tooth movement without Sodium Fluoride for 7 days, C2: orthodontic tooth movement without Sodium Fluoride for 14 days. Group T1: orthodontic tooth movement with Sodium Fluoride for 7 days, T2: orthodontic tooth movement with Sodium Fluoride for 14 days. 10 grams/cm^2 of mechanical strength were applied to the palatal of the Wistar rat teeth in the maxillary incisors using a NiTi closed coil spring, measured using a tension gauge (Ormco® Glendora, USA). Rats were sacrificed on the 7th and 14th days for immunohistochemical examination of the expression of HSP-60 and MMP-8. The study was conducted following the rules approved by the Health Research Ethics Committee (KEPK), Faculty of Dentistry, University of Jember.

Immunohistochemical Procedure

This procedures were consist of mandibular dissection, formalin fixation,decaficification using EDTA, dehydration, embedding, and paraffin samples. Tissue excision of less than 180 µm from labial-palatal root furcation of the maxillary incisor teeth was colored for HSP-60 and MMP-8 expression. Tissue fragment was deparaffinized, blocked with hydrogen peroxide, given antigen, and then incubated with serum and monoclonal antibodies anti-HSP-60 and MMP-8.

Observations were carried out with a light microscope on a 400x magnification scale. The field of view was taken on the tension side of the osteoblasts. Interpretation of results to obtain rough data, processed using the immunoRatio application. This application interprets the percentage of results from the density of brown color in the appearing photos or images.

Results

Research Group	Control		Treatment	
	7thday	14thday	7thday	14thday
	Mean ±SD	Mean ± SD	Mean ± SD	Mean ± SD
MMP-8	71.625±2.812	64.525±2.273	32.275±1.180	27.225±1.710
HSP-60	66.700±8.697	44.600±3.253	55.000±4.608	39.825±4.951

Table 1. The number of HSP-60 and MMP-8 expressions expressed by osteoblast.

Group		Mean ± SD	F	%
Control	7th day	66.700±8.697	4.203	0.030
	14th day	44.600±3.253		
Treatment	7th day	55.000±4.608		
	14th day	39.825±4.951		

Table 2. Average Description, Standard Deviation (SD), and Difference Test between HSP-60 Expression Groups in Control Groups and Treatment Groups (%).

Sixteen male Wistar rats in good condition were randomly divided into two groups: Control (C1, C2) and Treatment (T1, T2). In groups C1, C2 (n = 8) and T1, T2 (n = 8), orthodontic devices (NiTi closed coil spring) were inserted between the first maxillary molar teeth and

maxillary incisors to allow orthodontic tooth movement. 10 grams/cm^2 of mechanical strength were applied to the palatal of the Wistar rats teeth in the maxillary incisors using a NiTi closed coil spring, measured using a tension gauge, (Ormco® Glendora, USA). Immunohistochemical examination and interpretation were performed based on immune Ratio, The mean of osteoblast expressed by HSP-60 and MMP-8 are shown in Table 1.

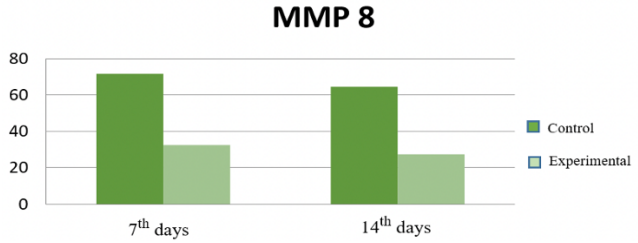

Figure 1. The average number of osteoblast in HSP-60 expression on 7thday and 14thday.

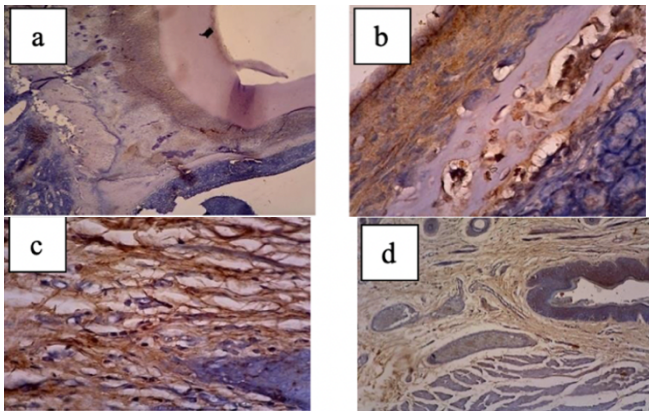

Figure 3. Osteoblast cell on 7thday 14thday, MMP-8 expression. (a): 7 days control group and (b): 14 days control group, (c): 7 daystreatment group (d): 14 days treatment group.

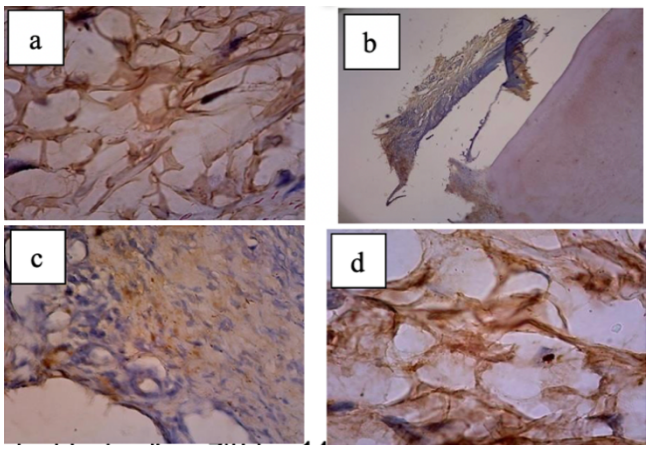

Figure 4. Osteoblast cell on 7thday 14thday, HSP-60 expression. (a): 7 days control group and (b): 14 days control group, (c): 7 days treatment group (d): 14 days treatment group.

Discussion

This experimental study on the orthodontic tooth movement used Sodium Fluoride 11.34 ppm in gel form given to the incisor sulcus of Wistar rats which were moved by using tensile strength of 10 grams/cm^2, to see the expression of HSP-60 and MMP-8 in the tension side. The Sodium Fluoride active substance 11.34 ppm in the form of a topical gel given to the gingival sulcus of the right upper incisor of male Wistar rat enters the cell plasma membrane through active transport by flowing ion channels [14].

Cellular and molecular responses of tooth movement results in this study were evaluated through osteoblast cell research. This research method used immunohistochemical techniques as staining and immunoRatio software as a technique for counting the number of cells in the percentage of HSP-60 and MMP-8 expression. The percentage of HSP-60 expression in each group was tested using the Kolmogrov- Smirnov normality test with significance ($p>0.05$). Normality test results obtained were equal to 0.96, so it can be said that data were normally distributed. The next test was homogeneity test by using Levene's test with significance ($p>0.05$). Levene test results obtained were equal to 0.41, so it can be said that data were homogeneous. Based on the results of normality and homogeneity tests, One way Anova parametric test was then applied with significance ($p<0.05$). The One way Anova test aimed to determine the significant difference in the average of all groups. The results of one way Anova test indicated a significance value of 0.03, so it can be said that the data were significantly different.

HSP-60 marker examination is done reading osteoblast seen through immunoRatio techniques. The results of studies on osteoblast expressed by HSP- 60 show a lower percentage in the group with Sodium Fluoride administration (T1 and T2) compared to the group without Sodium Fluoride administration (C1 and C2). This is because HSP 60 is a chaperon molecule (the formation of protein structures) which functions to protect the organism from severe stress resulting in cell death.[11]

HSP play a important role in protection from stress-induce celluler damage. Increased stress on cells also increases HSP expression[15]. Likewise, the examination of rats with the administration of Sodium Fluoride for 14th days (T2) showed lower expression than that for 7th days (T1). This is because on the 14th day the application of orthodontic pressure will occur lag phase, i.e. there is no or minimal movement of the teeth because the surrounding cellular components around it are activated due to tooth movement in the previous stage.[1]

The percentage of expression of *Metalloproteinase*-8 (MMP-8) matrix in each group was then tested using the Kolmogrov-Smirnov normality test with the results were equal to 0.51, so it can be said that the data were normally distributed. The next test is homogeneity test using the Levene's test with the results were equal to 0.57, so it can be said that the data were homogeneous. Based on the results of normality and homogeneity tests that have been carried out, One way Anova parametric test was then performed with the results 0.00, so that it can be said that the data is significantly different.

Metalloproteinase-8 (MMP-8) matrix marker examination is performed by examining osteoblast seen through the immunoRatio technique. The results of research on osteoblast expressed by the Matrix *Metalloproteinase*-8 (MMP-8) showed a significantly lower percentage in the group given Sodium Fluoride compared to that without Sodium Fluoride. This is because the *Metalloproteinase*-8 (MMP-8) matrix also works on the bone during orthodontic tooth movement, which starts with degrading collagen and the extracellular matrix. Matrix metalloproteinases (MMPs) have a significant role in PDL remodeling both physiologically and pathologically. [16]

The present Sodium Fluoride induced alters enzyme activities (of intrinsic antioxidant SOD, CAT, GST) confirming that the imbalance triggered by ROS production results in oxidative stress. Excessive ROS profusion can lead to lipid peroxidation [17], for which MDA is considered an important indicator. The study shows the enhancement of MDA content and carbonyl content after NaF-treatment in adult flies. The high content of protein carbonyl and elevated MDA level in adult flies might cause the excess cell death which stimulates higher protein degradation and the increased peroxidation of membrane lipid. Thereby, this process disrupts body homeostasis. That way, sodium fluoride can cause apoptosis of osteoblast. [18]

Conclusion

Giving Sodium Fluoride (NaF) in the form of gel on orthodontic teeth movement will reduce the expression of HSP-60 and MMP-8 in the alveolar bone of male Wistar rats.

Declaration of Interest

The authors report no conflict of interest.

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