DOI: http://dx.doi.org/10.33846/hn60206
http://heanoti.com/index.php/hn

RESEARCH ARTICLE
URL of this article: http://heanoti.com/index.php/hn/article/view/hn60206

Expression of IL-10 and Caspase-3 in Alveolar Bone after Topical Administration of Sodium Fluoride (Naf) Gel in Orthodontic Tooth Movement

Rina Sutjiati[1(CA)], Sulistyani[2], Erna Sulistyani[3], Rudy Joelijanto[4], Annisa Syifa M[5], Hasna' Fakhriyah J.[6]
[1(CA)]Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia; rinasutjiati@unej.ac.id (Corresponding Author)
[2]Department of Pedodontics, Faculty of Dentistry, Universitas Jember, Indonesia
[3]Department of Oral Medicine, Faculty of Dentistry, Universitas Jember, Indonesia
[4]Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia
[5]Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia
[6]Department of Orthodontics, Faculty of Dentistry, Universitas Jember, Indonesia

ABSTRACT

The success of orthodontic treatment can be achieved through tissue, cellular and molecular mechanisms due to mechanical stimuli formed by the pressure side and the tension side. Sodium Fluoride (NaF) is known to increase the bone formation process in the area of attraction in vitro and in vivo. The study aimed to determine sodium fluoride (NaF) against Caspase-3 by osteoblasts and IL-10 by osteoclasts in alveolar bone remodeling on orthodontic tooth movement. The research applied laboratory experiment using 16 male Wistar rats. Rats were divided into two groups; group C: given orthodontic strength without NaF; and group T: given orthodontic strength with NaF. Variable measurements were carried out after the 7th and 14th day. The data was statistically tested using One-Way Anova test analysis ($p \leq 0.05$) to compare the mean number of expressions of both groups C and T. The number of osteoclasts expressing IL-10 was significantly higher in group T when compared to that of group C while the number of osteoblasts expressing Caspase-3 was smaller in group T when compared to that of group C. NaF can increase IL-10 expression in osteoclasts and decrease Caspase-3 expression by osteoblasts on orthodontic tooth movement.

Keywords: Caspase-3; IL-10; sodium fluoride (NaF); immunohistichemistry

INTRODUCTION

Orthodontics is the first branch of dentistry that studies facial growth, tooth development, occlusion, diagnosis, prevention, and treatment of occlusion anomalies(1). The goals of orthodontic treatment are achieved through tissue, cellular and molecular mechanisms due to mechanical stimuli during orthodontic treatment. Sandstedt, Oppenhei and Schwarz state that the application of orthodontic appliances to teeth creates a mechanical force involving the pressure side and the tension side(2). These two sides are resorption by osteoclasts on the pressure side and apposition by osteoblasts on the tension side of the alveolar bone(3).

The mechanical strength of the orthodontic appliance increases the activity of caspase-3 and IL-10 on the pressure side which results in a decrease in the number of osteoblasts and an increase in osteoclastogenesis on that side. On the tension side, osteoblast proliferation and bone apposition processes occur more slowly than osteoclastogenesis and resopsy processes that occur on the pressure side(4).

The balance between osteoclasts and osteoblasts is a determinant of the success and stability of teeth that have been given orthodontic treatment. The balance and stability of orthodontic treatment can be achieved by accelerating the process of bone apposition on the tension side and by slowing down the process of bone resorption on the pressure side(5). Sodium fluoride (NaF) is one compound that is known to be able to increase the formation process in bone in the tension area and to reduce bone resorption in the pressure area(6).

In this paper, NaF is known to affect the differentiation process of bone-forming cells, the increasing activity of alkaline phosphatase in osteoblastic cells, and homeostasis of bone mineral metabolism. NaF can also act as a cumulative element that can change the tissue apposition and resorption of bone. Sodium Fluoride (NaF) is a joint compound of fluorine (F) and sodium (Na). Fluorine is mostly found in the form of fluoride (F-) in various minerals, such as in rocks and soils, volcanic and sub-volcanic rocks(7). At low doses, NaF can increase bone mass by increasing osteoblast proliferation and stimulate bone formation in vitro and in vivo(8).

This study aimed to determine the effect of NaF on IL-10 expression on osteoclasts and Caspase-3 expression on osteoblasts in alveolar bone remodeling on orthodontic tooth movement. The benefit of this study is to provide scientific information regarding the potential of NaF on IL-10 expression in osteoclasts and Caspase-3 on osteoblasts in the process of bone resorption and apposition, so that it can improve the balance and stability of bone remodeling during orthodontic treatment.

METHODS

Orthodontic Tooth Movement in Experimental Animals

The research used experimental-quantitative research type applying Randomized Posttest Only Control Group Design, by selecting the experimental group and the control group randomly. The experimental group would further be subject to treatment. At the end of the activity after giving the treatment, a post test was carried out on both groups, so that the effect of the treatment on the experimental unit could be seen. Variable measurements were carried out after the 7th and 14th day. The experimental animals used were 16 male Wistar rats weighing 200-250 grams which were divided into two groups: the control group (C1, C2) (n = 8) and the treatment group (T1, T2) (n = 8). The amount of orthodontic mechanical pressure was applied to the maxillary incisors of male Wistar rats to move the incisors palatally using a closed coil spring (NiTi) of 10 grams/cm2 which was measured using a tension gauge (Ormco® Glendora, USA). The mice were decaputated on the 7th and 14th day for immunohistochemical staining, so that IL-10 expression on osteoclasts and Caspase-3 expression on osteoblasts were identified. This research was conducted under the approval of the ethics commission of the Faculty of Dentistry, University of Jember. Procedure of the study was approved by KEPK Faculty of Dentistry, Jember University No. 581/UN25.8/KEPK/DL/2019.

Immunohistochemical Procedure

The right maxilla of rats that had been resected was fixed using 10% Neutral Formalin Buffer, decalcified using 10% Formic Acid, dehydrated using alcohol, cleared using xylol, impregnated and embedded using paraffin. Tissue incision was carried out with a thickness of 5 μm from the labial-palatal root furcation of the maxillary incisors stained immunohistochemically, so that IL-10 and Caspase-3 expressions were shown. Tissue was deparaffinated using xylol, rehydrated using absolute ethanol, blocked with peroxidase blocking solution and prediluted blocking serum, immersed in IL-10 and Caspase-3 antibodies, soaked with secondary antibodies, soaked with horseradish peroxidase (HRP), soaked with diaminobenzidine tetrahydrochloride (DAB), dropped with mayer's hematoxylin and a bluing reagent.

Positive expression results on IHC staining would show a brown color in the cytoplasm of osteoclasts and osteoblasts under microscope. Preparation images with a magnification of 400 × were taken with an optilab camera and then processed in the Immunoratio Software (IRS) application. This observation was carried out by two observers to avoid subjectivity.

The data was statistically tested using One-Way Anova test analysis ($p \leq 0.05$) to compare the mean number of expressions of both groups C and T.

RESULTS

The results of calculating the average expression of IL-10 on osteoclasts and Caspase-3 expression on osteoblasts can be seen in Table 1.

Table 1. Mean of IL-10 expression by osteoclasts and Caspase-3 expression by osteoblasts

Group	Control		Treatment	
	7th day	14th day	7th day	14th day
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
IL-10	8.72 ± 0.841	15.26 ± 2.194	17.006 ± 3.39	29.60 ± 2.670
Caspase-3	87.5 ± 12.024	80.95 ± 14.621	72.75 ± 16.556	31.625 ± 7.763

Table 2. Average description, standard deviation (SD), and difference test between IL-10 expression groups in control groups and treatment groups

Group		Mean ± SD	Frequency	Percentage
Control	7th day	8.72 ± 0.841		
	14th day	15.26 ± 2.194	50.593	0.000
Treatment	7th day	17.006 ± 3.39		
	14th day	29.60 ± 2.670		

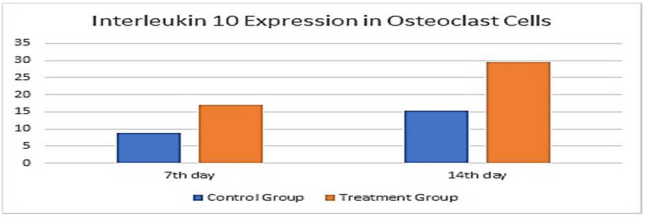

Figure 1. The average of IL-10 expression on the 7th day and 14th day

Table 3. Average description, standard deviation (SD), and difference test between Caspase-3 expression group in control groups and treatment groups

Group		Mean ± SD	F	%
Control	7th day	87.5 ± 12.024		
	14th day	80.95 ± 14.621		
Treatment	7th day	72.75 ± 16.556	14.585	0.000
	14th day	31.625 ± 7.763		

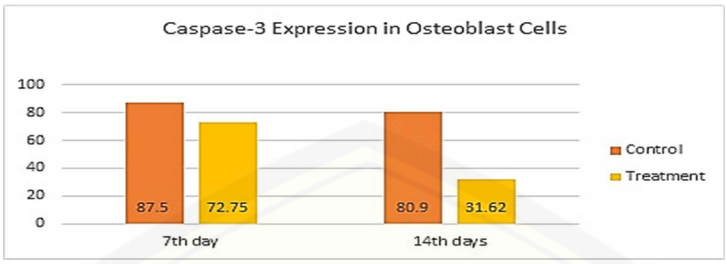

Figure 2. The average of Caspase-3 expression on the 7th day and 14th day

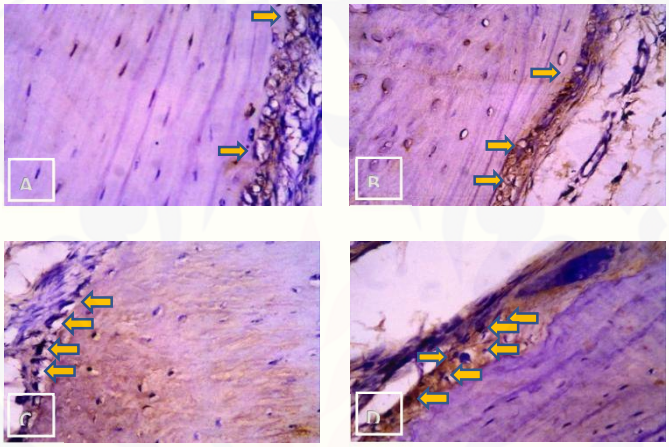

Figure 3. Osteoclast cell on 7th day and 14th day in IL-10 expressions. (A) C1: control group 7th day, (B) C2: control group 14th day, (C) T1: treatment group 7th day, (D) T2: treatment group 14th day.

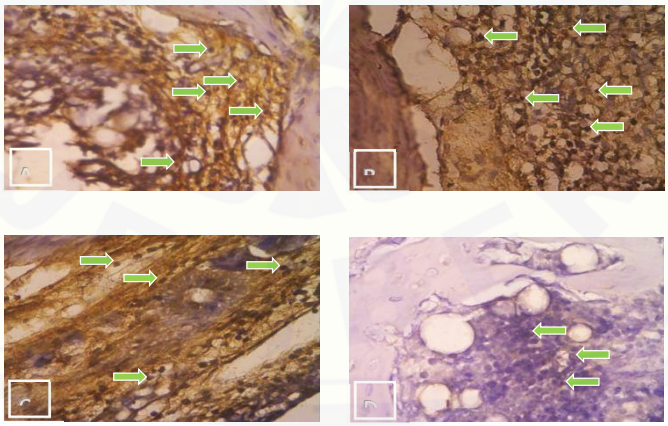

Figure 4. Osteoblast cell on 7th day and 14th day in Caspase-3 expressions. (A) C1: control group 7th day, (B) C2: control group 14th day, (C) T1: treatment group 7th day, (D) T2: treatment group 14th day.

DISCUSSION

Fluoride in NaF affects osteoblast differentiation, bone nodule formation and bone regeneration which leads to bone formation and resorption and reduces osteoclast cell formation[8]. The dose of NaF gel used was calculated by multiplying the optimal dose of fluorine of 2.16 mg/kg/day with the body weight of the experimental animal by 200-250 grams or 0.2-0.25 kg and multiplying the length of treatment the experimental animal and obtained the result of 11.34 mg. The dose was made into a gel and given to the gingival groove of male Wistar rats as much as 0.2-0.3 ml twice a day in the morning and evening. This volume was obtained by multiplying the

optimal dose of fluorine 2.16 mg with the weight of the experimental animal from 0.2 to 0.25 kg divided by the optimal concentration of fluorine 0.2% or 2 mg/ml so that the result was 0.216-0.27 ml.

The results of the study on IL-10 expression showed that in the C1 control group the mean IL-10 expression was 8.72% and then in the C2 group the mean IL-10 expression was 15.26%. The T1 treatment group showed a mean IL-10 expression of 17.006% and T2 of 29.60%. This shows that the IL-10 expression in the C1 group was lower than that in the C2 group as well as the T1 treatment group was lower than the T2 group.

IL-10 expression in osteoclasts increased in both control and treatment groups. This is in accordance with the research conducted by Zorlu et al., (2019) and Yao et al., (2019) who reveal that the number of osteoclasts decreases by giving NaF to mice given orthodontic strength[9]. The effect of NaF on osteoclasts according to Kuang et al., (2017) and Kuang et al., (2019) is due to an increase in the number of cytokines IL-10[10, 11].

Molecularly, giving NaF will increase IL-10 expression which plays a role in reducing osteoclast formation both directly and indirectly. IL-10 directly decreases the expression of NFATc1 which has an important role in osteoclast differentiation[12]. Meanwhile, IL-10 indirectly increases OPG expression and decreases RANKL expression. Osteoclast cell formation occurs when RANKL binds to RANK. With the increase in OPG, the bond between RANKL and RANK is inhibited so that osteoclast formation is reduced. In addition to going through the OPG-RANKL pathway, IL-10 indirectly reduces the expression of pro-inflammatory cytokines that play a role in the formation of osteoclasts such as TNF-α, IL-6, and IL-1β and reduces M-CSF which plays an important role in osteoclast formation[13]. Based on the results of the One Way Anova test analysis, it was found that the significance value was less than 0.05, so the difference in each group was significant, so that the NaF results could increase IL-10 expression in osteoclasts.

The use of an optimum force of 10 g/cm2 is essential to obtain the maximum rate of orthodontic movement without damage to the roots, periodontal ligaments, and alveolar bone[14]. Research by Minato et al (2018) states that the increase in Caspase-3 expression appears more stable at a power of 10 gr/cm2 starting from the first day to the seventh day compared to the Caspase-3 expression which tends to increase and reaches a peak on the fifth day then decreases sharply in the seventh day.

The results of this study on the expression of caspase-3 in the osteoblast cells of the T1 group with a mean of 72.75% showed lower results than that of the C1 group with a mean of 87.5%. The mean number of osteoblast Caspase-3 expression in the T2 group was 31.62%, also lower than C2 at 80.9%. The decrease in the expression of Caspase-3 osteoblast cells in the control group (C1, C2) compared to the treatment group (T1, T2) is in accordance with Gruber's statement (Oweis, 2018) that the administration of NaF can increase the number of osteoblasts and the bone apposition process by suppressing Caspase-3 expression[15].

NaF decreases Caspase-3 expression through suppression of TNF-α synthesis[16]. Zheng et al. report that TNF-α induces cleaved-caspase-3 expression which is a marker of apoptosis in osteoblasts[17]. These findings explain the linkage between caspase-3 and TNF-α. Mitsuhashi et al. also found that TNF-α mRNA expression appeared to be significantly increased after application of orthodontic mechanical loads[18].

The results of difference tests for group C1 and group C2 were not significantly different in relation to the occurrence of inflammation due to mechanical strength of orthodontics which continued since the beginning of the orthodontic appliance application until the 14th day. Çevik argues that the high amount of TNF-α synthesized at the time of inflammation stimulates Caspase-3 activity[19].

Research by Minato et al. revealed that the expression of Caspase-3 and RANKL was observed in the application of heavy orthodontic mechanical loads as well as optimal orthodontic mechanical loads even though the expressions of both of them appear higher under heavy mechanical loads. The difference in peak time of increase in Caspase-3 positive cells and RANKL positive cells also showed that the number of Caspase-3 positive cells peaked earlier than the number of RANKL positive cells[20].

The application of orthodontic mechanical loads causes the alveolar bone osteoblasts to activate Caspase-3 which initiates the activation of the osteoblast apoptotic pathway itself. Furthermore, RANKL will induce osteoclastogenesis which will lead to alveolar bone resorption. The bone remodelling process involves RANKL in bone resorbtion by osteoclasts and caspase-3 in the mechanism of osteoblast apoptosis on opposite sides. The function of NaF in reducing Caspase-3 expression is obtained by suppressing TNF-α synthesis. Suppression of these cytokines inhibits activation of Caspase-3 by osteoblasts [21].

CONCLUSION

Sodium Fluoride increases IL-10 expression on osteoclasts and decreases Caspase-3 expression on osteoblasts during orthodontic tooth movement.

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