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RESEARCH ARTICLE

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Sodium Fluoride Administration Enhances Osteocalcin and Osteonectin Expression in Alveolar Bone on the Tension Side on Orthodontic Tooth Movement

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ABSTRACT

Introduction: In the tension side on the orthodontic tooth movement, the bone formation process occurs, played by osteoblasts. Bone formation by osteoblasts in vitro can be increased by administering NaF (sodium fluoride). Speed of bone formation is one of the most important steps in orthodontic tooth movement. **Objectives:** to analyze the effect of NaF administration on the expression of osteocalcin and osteonectin in the strain area in the incisors after orthodontic tooth movement. **Methods:** The 10 Wistar adult male rats were given 10 grams of orthodontic mechanical strength on the incisors so that the incisors moved distally and were given NaF twice a day, compared to 10 mice in the control group with the same treatment without NaF administration. **Results:** NaF increase osteocalcin expression by 31.1% on day 7 and 44.8% on day 14, whereas in osteonectin the increase is 48.1% on day 7 and 26.2% on day 14. **Conclusions:** NaF increases the expression of osteonectin and osteocalcin in mice that were given orthodontic mechanical strength.

Keywords: sodium fluoride; immunohistochemistry; osteocalcin; osteonectin

INTRODUCTION

Orthodontic treatment is a long-term procedure to achieve good occlusion without tooth rotation or diastema ⁽¹⁾. After this long treatment, the success of orthodontic treatment is making a good occlusion relationship, achieving facial esthetics and not less important is maintaining the stability of the occlusion as long as possible ⁽²⁾. Even though the patient feels that the treatment has been completed when the appliance is removed, the teeth can be in an unstable position and the continuous pressure from the surrounding soft tissue can result in a tendency to relapse ⁽³⁾. To move the tooth, an application of orthodontic mechanical strength is required which results in a resorption of the alveolar bone in the pressure side of the periodontal ligament, whereas bone formation occurs in the tension side in the periodontal ligament ⁽⁴⁾.

Research on fluorine has been widely conducted, but the expression of osteocalcin and osteonectin in the orthodontic teeth movement after the administration of Sodium Fluoride for bone formation is not known well. NaF (sodium fluoride) have a potential action to modulate the proliferation of osteoblasts and osteoblast differentiation by stimulating preosteogenic embryonic mesenchyme to be mature osteoblasts which can deposit bone matrix. Osteoblasts are derived from osteoprogenitor cells in the bone marrow and the inner lining of the periosteum. When the osteoblast cells are differentiated, they will secrete ALP (alkaline phosphatase), osteocalcin, osteopontin, osteonectin ⁽⁵⁾. NaF (sodium fluoride) also can increase in osteocalcin regulation, proven by real time PCR.

Osteocalcin, also called BGP (bone gla protein), is a low molecular protein, 6 kDa and the most non-collagen proteins ingredient in the bones ⁽⁶⁾. Osteocalcin is a Ca²⁺ binding protein derived from the organic matrix of bone, dentin, and possibly other mineral tissues. Osteocalcin is expressed by osteoblasts and in the bone matrix during alveolar bone remodeling. Osteocalcin is one of the markers of gene expression of osteoblast differentiation ⁽⁷⁾. According to the research the increased osteocalcin expression indicates an increase in bone growth activity performed by osteoblasts ⁽⁸⁾. Hence, osteocalcin and osteonectin expressions of orthodontic mechanical strength with NaF (sodium fluoride) administration on alveolar bone need be analyze. The aim of this study was to investigate osteocalcin and osteonectin expression upon OTM (orthodontic tooth movement) on the tension side after NaF (sodium fluoride) administration.

METHODS

Material

The materials of research were: twenty Wistar male Rats (*Rattus norvegicus*), 3-4 month old, 200-250 gram body weight from Rats For You co (Malang, Indonesia). Ni-Ti closed coil spring light force 0,010 inch diameter and 9 mm in length (Ortho Archwire). Orthodontic Stainless Steel Ligature Wire (Protecmech USA). Tension Gauge (Ormco® Glendora, USA). Sodium fluoride for Analysis EMSURE® ACS, ISO, Reag. Ph Eur (Merck).

Rats divided to 4 groups, 4 rats each group, 2 control groups (C1, C2) and 2 treatment groups (T1, T2). Rats feeded standard pellet (RatBio, Citrafeed). Procedure of the study was approved by *Komite Etik Penelitian Kesehatan (KEPK) Fakultas Kedokteran Gigi Universitas Jember No. 155/UN25.8/KEPK/DL/2018*.

Methods

Rats were acclimatized for one week before being treated at the Microbiology Laboratory, Universitas Jember. After acclimatization, ni-ti closed coil spring diameter of 0.25 mm installed in and activated. Before installation, Rats were anesthetized with ketamine solution (PT. Guardian Pharmatama) and xylazine (Interchemie, Holland) with a ratio of 1 : 2 each – 0.15 – 0.18 ml intramuscular injection (i.m).

Next step was adjustment the ni-ti closed coil spring strength using tension gauge to produce power of 10 gr/cm². Closed coil spring ni-ti wire is installed between maxillary central incisor and the right maxilla first molar (I1 and M1) to move the incisor tooth to palatal direct. On incisor teeth, wire is attached at a hole made by a round bur on the distovertical side and a stainless steel ligature wire 0.1 mm mounted around the first molar tooth. Glass ionomer cement was applied to increase retention.



Figure 1. Instalation of closed coil spring

On treatment groups, not in control groups, NaF (sodium fluoride) gel 11.34 ppm were given topically using modified syringe on areas near the Wistar rat's gingiva sulcus. NaF (sodium fluoride) gel administered 0.2 – 0.3 ml twice a day, morning and afternoon, for 7 days in group 1 and 14 days for group 2 until a day before rats sacrificed with intramuscular injection (i.m) of ketamine overdose. Rats in group 1 (C1, T1) were decapitated in on day 8th and group 2 (C2, T2) on 15th day and anterior part of maxilla tissue were separated by scalpel.

The tissue that has been taken was fixed using Buffered Neutral Formalin 10% solution for 24 hours and decalcification was carried out using EDTA (Ethylenediaminetetraacetic acid) 10% for 30 days. Next step was tissue dehydration using alcohol graded from low to high concentrations, 70%, 80%, 95% and 100%. After this tissue undergo clearing using xylol and embedding in paraffin block.

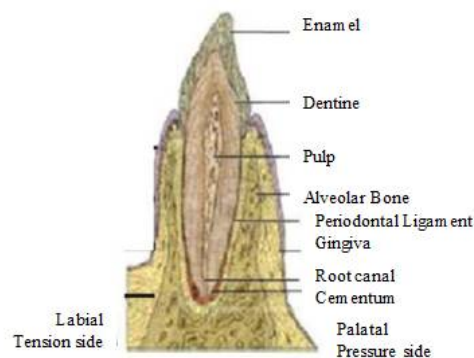


Figure 2. Illustration of tissue labial-palatal dissection

Slide were made from the maxillary incisors which were cut in direction labial to palatal in of 4-6 μm thickness. Slide deparaffinized using xylol, then blocked using peroxidase blocking solution (Scytek Laboratories, USA) followed by prediluted blocking serum (Scytek Laboratories, USA). The next step was incubating using osteocalcin monoclonal antibody (Rabmab® EPR3690) and osteonectin monoclonal antibody (Aviscera Bioscience 72-303) followed by a secondary antibody UltraTek Anti-Polyvalent Biotinylated Antibody (Scytek Laboratories, USA).

Observation on osteoblasts with osteocalcin and osteonectin expression was carried out using a light microscope with 1000x magnification in the tension side at 10 fields of view. Observations were performed by four people to avoid subjectivity.

Data Analysis

The data were statistical analysis using SPSS version 20. The normality test of the data was carried out using the Kolmogorov Smirnov Test and the homogeneity test using the Levene Test (with normal distribution, $n=16$), $P>0.05$. Statistical differences between groups were tested using One Way Anova followed by Post Hoc LSD analysis, $P<0.05$.

RESULTS

The Effect of NaF Administration on Osteonectin Expression in Rat OTM Model

The mean and standard deviation of osteocalcin and osteonectin expression using immunohistochemical staining on day 7 and day 14 are shown in Table 1. The result showed an increase in osteocalcin and osteonectin expression from day 7 and day 14 as well as the average expression of osteocalcin and osteonectin was higher in the NaF (sodium fluoride) -given group than in the NaF-given group on day 7th and day 14th.

Table 1. The result of osteocalcin and osteonectin expressions

| Groups | Control Day 7 average \pm SD | Control Day 14 average \pm SD | Treatment Day 7 average \pm SD | Treatment Day 14 average \pm SD |
|-------------|--------------------------------|---------------------------------|----------------------------------|-----------------------------------|
| Osteocalcin | 7.87 \pm 0.07 | 9.14 \pm 0.15 | 10.36 \pm 0.33 | 13.24 \pm 0.18 |
| Osteonectin | 7.72 \pm 0.20 | 10.32 \pm 0.21 | 11.44 \pm 0.47 | 13.02 \pm 0.28 |

* significant at $\alpha = 0.05$

The result of this research showed that, the 7th day control group Rats (C1) had fewer osteoblast cells than the 14th day control group (C2). This difference was consistent with histological studies which show that the initial stage of bone remodeling occurs on day 7th and the final stage occurs on day 14th.

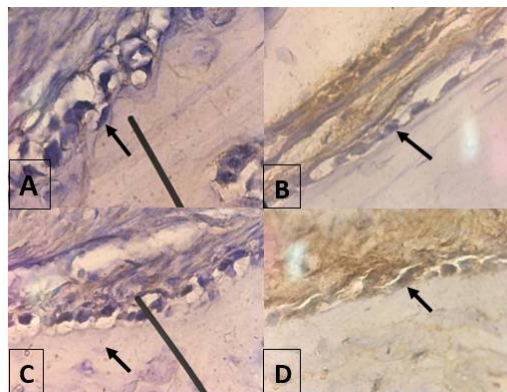


Figure 3. Expression of osteonectin osteoblasts on day 7th and day 14th. (A) and (C) were the control groups day 7th and day 14th, while (B) and (D) are the treatment groups of NaF day 7th and day 14th.

The treatment group also had the same result, there was an increase on day 14th compared to day 7th. Comparison between the treatment compared to the control group, there was a significant increase of osteonectin expression in both rats given NaF (sodium fluoride) on day 7th and on day 14th (figure 4). The increase in osteonectin expression is due to that NaF (sodium fluoride) can increase osteoblast activity. Provision of NaF (sodium fluoride) in certain concentrations can increase mineralization ability, as well as stimulate ALP (alkaline phosphatase) activity, besides that Fluorine ion has an affinity for bones so that it can stimulate osteoblasts and increase bone apposition and mineralization processes.

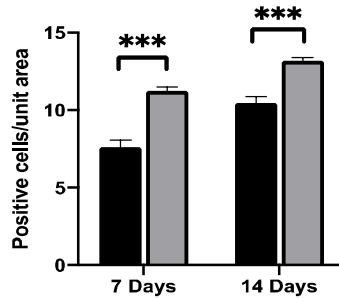


Figure 4. Osteonectin expression on day 7 and day 14. Expressions were assessed as outlined in materials and methods. Graphs represent means \pm SD for 5 repeated observations, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The Effect of NaF Administration on Osteocalcin Expression in Rat OTM Model

The results of this study showed that the osteoblast cells number in the control group without NaF (sodium fluoride) administration on day 14th had a higher than on day 7th. This result consistent with the fact that the last stage of osteoblast differentiation, osteoblasts will overexpress osteonectin on days 14-28 followed by calcium and phosphate deposition.

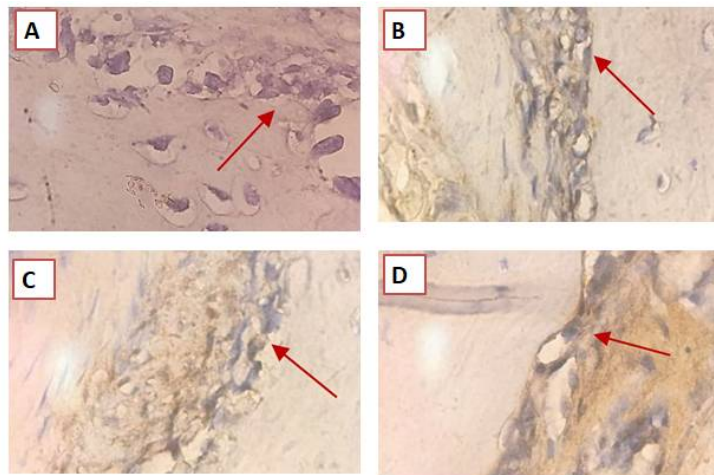


Figure 5. Expression of osteocalcin of osteoblasts on day 7th and 14th. A and C are the control groups day 7th and day 14th, while B and D are treatment groups of NaF day 7th and day 14th. Osteocalcin expressions of Osteoblasts on the alveolar bone marked with a red arrow, 1000x magnification

An increase in the number of osteoblast cells also occurred in the group of rat OTM (orthodontic tooth movement) model given NaF (sodium fluoride) administration on day 7th and day 14th. These results were also supported by various studies i.e. the administration of NaF (sodium fluoride) resulted in active proliferation of osteoblast cells and extracellular bone matrix biosynthesis (collagen type I), the period of cell differentiation, cell proliferation and regulation of collagen and protein synthesis associated with bone cell phenotypes such as ALP (alkaline phosphatase), osteocalcin and osteonectin.

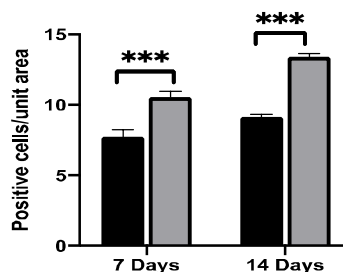


Figure 6. Osteocalcin expression on day 7 and day 14. Expressions were assessed as outlined in Materials and Methods. Graphs represent means \pm SD for 5 repeated observations, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

DISCUSSION

Treatment group exhibited higher osteocalcin expression than control. In addition, the effect of fluoride on osteoblast activity where it was shown to have a positive effect on the differentiation of osteoblasts, bone nodule formation and bone regeneration, followed by cell differentiation period and collagen synthesis regulation and protein of bone cell phenotype such as alkaline phosphatase, osteocalcin, osteonectin, osteopontin⁽⁹⁾. Fluoride has a role in increasing bone mass as an indicator of bone remodelling⁽¹⁰⁾. Through one-way anova test, table 3 exhibits significant difference ($p < 0.05$) which refers to significant difference between treatment group and control indicating that NaF has confirmed to induce a significant increase of osteocalcin and osteonectin expressions.

During OTM (orthodontic tooth movement), 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⁽¹¹⁾. Osteoblast is formed by osteoprogenitor cell from cartilage bone and inside layer of periosteum. Osteoprogenitor differentiation is influenced by BMP-2 (bone morphogenetic proteins-2), PDGF (Platelet-derived Growth Factor) produced by limfosit and TGF- β 1 (transforming growth factor beta-1). During differentiation, the release of compound such as alkaline phosphatase, osteocalcin and osteonectin occurs⁽¹²⁾. During bone inflammation, on the recovery process, TGF- β 1 (transforming growth factor beta-1) is expressed on the first phase and during recovery and works as factor of chemotaxis progenitor mesenchymal cell with macrophage to the location of wound recovery. Further induced proliferation and enable collagen production towards matrix extracellular by osteoblast (Dimitriou et al, 2005). TGF- β 1 (transforming growth factor beta-1) also signalized BMP-2 (bone morphogenetic proteins-2) synthesis by osteoprogenitor cell. This will enable osteoblast differentiation and support osteoclast apoptosis and hinder bone resorption⁽¹³⁾. Bone growth was resulted by progenitor which triggers Osteoblastogenesis with BMP (bone morphogenetic protein). BMP (bone morphogenetic protein) will enable OSF2 (osteoblastic specific factor 2) or Cbfa 1 (core binding factor 1). Cbfa 1 (core binding factor 1) activates osteoblast specific genes such as osteopontin, sialoprotein, collagen type 1 and osteocalcin, osteonectin⁽¹⁴⁾.

Based on the research results on day 7 and day 14, the treatment group with topical NaF (sodium fluoride) administration indicated significant difference due to different treatment towards each group. The improvement of osteoblast with osteocalcin expression of treatment group is higher than that of control as anticipated through research hypothesis. On day 14, the number of Osteoblast cells with osteocalcin expression is higher than that of the same group on day 7. This is in line with previous researches that osteocalcin will reach the top after two-weeks administration of topical NaF (sodium fluoride)⁽¹⁵⁾.

The research results exhibited higher average of osteonectin expression towards treatment group rather than control group. Azarpira, 2008 suggests that ion fluor possessed affinity towards bone stimulates osteoblast and improves apposition process and bone mineralization. Moreover, nodul osteoblas formation towards cultured bone occurred due to osteoblastic differentiation stimulated by NaF (sodium fluoride) in concentration 10⁻⁸ to 10⁻⁵ M and NaF (sodium fluoride) towards 10⁻⁸ to 10⁻⁵ M can improve osteoblast proliferation, improve mineralization process, and stimulate ALP (alkaline phosphatase) activity. It has role as a marker returning phase from resorption process of apposition (10). One-way Anova in table 3 show a significant difference ($p < 0.05$) referring to significant difference between treatment group and control group. Thus, NaF (sodium fluoride) is confirmed can induce a significant increase of osteonectin expression. Immunohistochemistry revealed that Ihh, Smo, Gli2, and Runx2 were all positively expressed in different degrees in osteoblasts of metaphysis, the expressions were all increased after exposure to fluoride. After exposure to fluoride, the bone cortex thickened, the bone trabecular thickened, and bone trabecular density increased⁽¹⁶⁾.

Furthermore, osteoblastic differentiation can be induced by fluoride through signalling path Wnt/ β -catenin. Fluoride can be found in osteoblast and significantly supports osteoblast proliferation and ALP (alkaline phosphatase). mRNA (messenger ribonucleic acid) expression improvement was also found in bone differentiation marker including Collagen type I, ALP (alkaline phosphatase) and Osteonectin. Moreover, fluoride also induced transcription expression of Runx-2 (Runt-related Transcription Factor-2). Overall, the findings indicated that fluoride induced osteoblastic differentiation through activation of Akt- and GSK-3 β of Wnt/ β -catenin signalling pathway in primary rat osteoblast⁽¹⁷⁾.

Based on the research results on day 7 and day 14, a significant difference was indicated after NaF (sodium fluoride) topical administration. The difference result confirmed that different treatments carried out on each group. The number of osteoblast via osteonectin expressions was higher in treatment group, but lower than that in control; as stated in the hypothesis stage. The number of osteoblast-osteonectin expressions in the group of day 14 was higher than that of day 7. The difference was relevant to the histological research showing that resorption occurred in the first phase in 3-5 days and the last phase of bone remodelling was around 7-14 days⁽¹⁸⁾.

CONCLUSION

NaF 11.75 ppm topical administration towards orthodontic teeth movement indicates an effective impact to improve osteocalcin and osteonectin expressions rather than that without NaF. NaF 11.75 ppm topical administration towards orthodontic teeth movement indicates an effective impact on day 7 and day 14.

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