Powdered Anchovy (*Stolephorus sp*) Results in Higher Osteoblast Counts in the Tension Area of Orthodontic Tooth Movement in Wistar Rats

Tecky Indriana [1*], M. Rubianto[2], Retno Indrawati [3]

[1] Department of Biomedicine, Faculty of Dentistry, Jember University, Jember, Indonesia.
[2] Department of Periodontic, Faculty of Dentistry, Airlangga University, Surabaya, Indonesia.
[3] Department of Oral Biology, Faculty of Dentistry, Airlangga University, Surabaya, Indonesia.

ABSTRACT

Introduction: The prevalence of relapse in orthodontic treatment is still quite high, reaching 50% after a two-year retention period. Another study even found a relapse rate of 61.5% in 500 patients. The etiology of relapse is multifactorial and includes immature and slightly mineralized bone tissue surrounding the tooth element that was moved. Therefore, a material is needed to improve apposition or bone formation process in the tension area. One alternative that could be expected to meet the needs of bone calcium is the consumption of anchovies (Stolephorus sp), since anchovies are a high source of calcium.

Objective: To analyze the effect of powdered anchovies on increasing osteoblast count in the tension area of orthodontic tooth movement in Wistar rats.

Materials and Methods: In this laboratory animal study, we randomly divided 30 rats into six groups of five rats each. Wistar rats in the control group (K) were given orthodontic mechanical stress, standard feed and observed for 7 days (K1), 14 days (K2), and 21 days (K3). Wistar rats in treatment group (P) were provided with orthodontic mechanical pressure and extra feed of powdered anchovy at a dose of 0.0168 g/day/g BW with gastric sounding, and observed for 7 days (P1), 14 days (P2), and 21 days (P3). The rats were sacrificed on day 8 for groups K1 and P1, day 15 for groups K2 and P2, and day 22 for groups K3 and P3, and maxillary bone was taken for observation. Osteoblast cells were counted using Hematoxylin eosin (HE) staining. ANOVA and Tukey's HSD tests were used for statistical analysis of the data.

Results: There was a higher osteoblast count in the tension area of orthodontic tooth movement both in the control group and in the treatment group over time. There was also a significantly higher number of osteoblasts in the day 14 control group, and in the day 21 treatment group.

Conclusion: Intake of anchovy as a feed supplement for Wistar rats may increase osteoblast count in the tension area of orthodontic tooth movement.

Keywords: Osteoblasts, Orthodontic tooth movement, Anchovy (Stolephorus sp).

1. INTRODUCTION

Orthodontic treatment is one that uses mechanical force to move the teeth. Mechanical strength results in an alveolar bone remodeling process, resorption in the pressure area and apposition in the tension area. Remodeling involves osteoblasts in the bone formation process and osteoclasts in the bone resorption process.[1] Remodeling is required in orthodontic treatment, especially as relapse prevention. However, the prevalence of relapse in orthodontic treatment is still quite high, as much as 50% after a two-year retention period in one older study, and 61.5% in 500 patients in another study.[2,3]

The etiology of relapse is multifactorial, varying from a change in dental arch form, to an unfavorable growth

* tecky.fkg@unej.ac.id or tecky.fkg@unej.ac.id
Received on 3/10/2016 and Accepted for Publication on 8/10/2017.

pattern, to immature and slightly mineralized bone tissue surrounding the tooth element that was moved.[4] Therefore, we need material to improve the apposition process or bone formation. Bone formation may occur if there is organic material or intake from external sources, especially inorganic materials, such as calcium and phosphate, which serves as a bone nutrient intake.[5,6]

One alternative that could be expected to meet the needs of bone calcium is the consumption of anchovies (*Stolephorus* sp), since anchovies contains higher calcium.[7,8] In the preliminary study, it is known that the amount of calcium content in 100 g of anchovy fish is 800mg. Anchovies contain minerals, proteins and vitamins, and it is expected to stimulate new bone formation by enhancing the proliferation of growth factor that contributes to the increased activity of osteoblasts and inhibits the activity of osteoclasts.[5]

The purpose of this study was to analyze the effect of powdered anchovies on osteoblast count in the tension area of orthodontic tooth movement in Wistar rats.

Materials and Methods

This study was a true experimental study with multifactorial design using male Wistar white rats (*Rattus norvegicus L*), each 2 months old and weighing from 250 to 270 g. Prior to the study, ethical feasibility for manipulating the experimental animals was assessed by the Health Ethics Committee, Faculty of Dentistry, Airlangga University, Surabaya. A total of 30 male rats were divided into two groups randomly, namely the control group (K) and the treatment group (P). The K group was subdivided into three groups randomly (K1, K2, K3), and the P group was subdivided into three groups randomly (P1, P2, P3).

Before treatment, all experimental animals in the control and treatment groups were prepared by intraperitoneal injection of an anesthetic, 50 mg/kg of ketamine hydrochloride, for easy installation of NiTi closed coil springs (Ormco, Orange, USA). These mechanical stressors were fastened between the maxillary first permanent teeth and maxillary central incisive tooth using 0:01-inch stainless steel ligature (3M Unitek, Monrovia, CA, USA) and were fixed with a type I FUJI (3M Unitek, Monrovia, CA, USA).[9]

In the control group (K), rats were treated with orthodontic mechanical stress and fed with standard feed as well as sterile distilled water *ad libitum* and *carboxymethylcellulose natrium* (CMC Na) with sonde and observed for 7 days (K1), 14 days (K2) and 21 days (K3). In the treatment groups (P), rats were treated with orthodontic mechanical stress and given extra feed of powdered anchovy at a dose of 0.0168 g/day/g BW. This dose was based on the amount of calcium content in 100 g anchovy to the needs of calcium in the body in the stomach using sonde, standard feed and sterilized distilled water *ad libitum* and observed for 7 days (P1), 14 days (P2) and 21 days (P3). The body weight of each rat in each group was obtained once a week as a guideline for determining the anchovy dose.

On days 8, 15 and 22, the respective rat groups were sacrificed by anesthesia using intraperitoneal injection of ketamine hydrochloride at a lethal dose.[9] Operation and removal of maxillary bone were performed for periodontal tissue examination to count expressed osteoblasts using hematoxylin eosin staining (HE).[10]

Results

Mean osteoblast counts in control and treatment groups were found, and results were statistically analyzed (Table 1).

Table 1 suggests that there was a significantly higher number of osteoblasts ($p < 0.05$) in the P group compared to group K. In group K, there was a higher number of osteoblasts that was insignificant ($p \geq 0.05$) in the day 14 group compared to the day 7 group. However, there was a significantly higher number of osteoblasts in the day 21 group. In group P, there was a significantly higher number of osteoblasts on day 14.

Table 1. Mean and standard values of osteoblast counts between study group on days 7, 14 and 21

Osteoblast count (cell/mm^2)					
Groups	**n**	**Day 7**	**Day 14**	**Day 21**	**p-value**
Control (K)	5	7.6±1.516$^{a.a}$ (n=5)	8.2±1.102$^{a.a}$ (n=5)	10.8±2.236$^{a.b}$ (n=5)	0.000*
Treatment (P)	5	10.4±1.427$^{b.a}$ (n=5)	12.2±1.923$^{b.b}$ (n=5)	13.4±2.073$^{b.b}$ (n=5)	0.000*
p-value		0.003*	0.000*	0.000*	

* There is a significant difference between groups (P <0.05)

^{ab} The same superscript showed no difference between groups

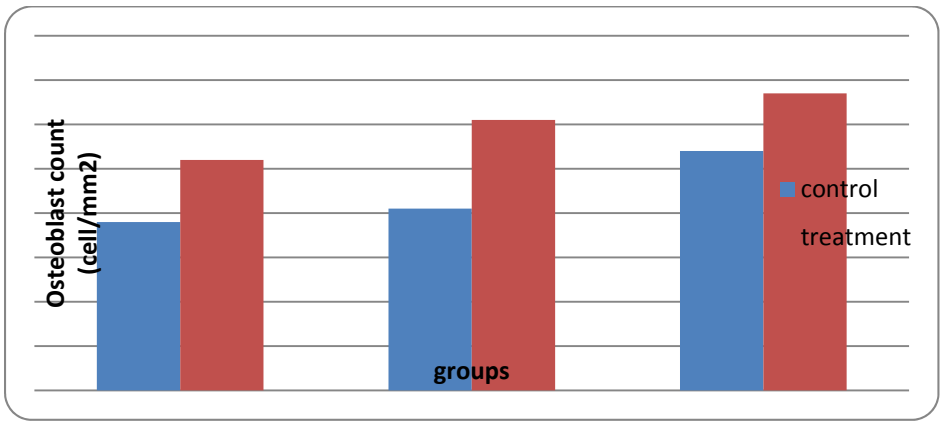

Figure 1: Mean osteoblast count in the tension area of orthodontic tooth movement in all groups

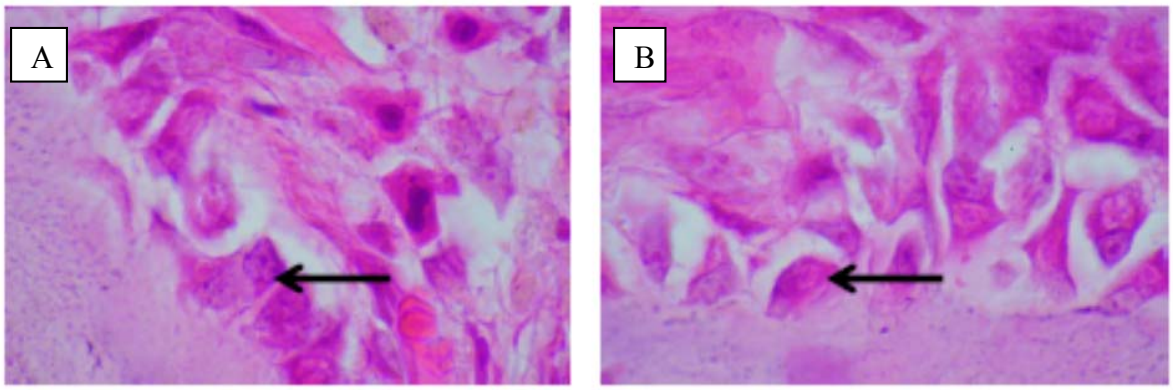

Figure 2: Example of results of immunohistochemical examination of alveolar bone osteoblasts in the tension area of orthodontic tooth movement

Notes:
A. Osteoblast in control group (K) (indicated by arrow)
B. Osteoblast in treatment group (P)
⟶ : Osteoblasts

Discussion

Overall, the osteoblast count was higher ($p<0.05$) in the treatment group receiving anchovies compared to that of the control group. In the control group, there was a significantly higher number of osteoblasts in the day 21 rats compared to the day 14 rats (Table 1). In the treatment group, a significantly higher numbere was observed on day 14 compared to day 7. But there was not a significant change on day 21 compared to day 14. This supports the supposition that the provision of anchovy may increase the number of osteoblasts on day 14.

High anchovy intake may increase extracellular calcium, intracellular calcium and osteoblast proliferation. These results correspond with previous studies reporting that increased extracellular calcium significantly stimulates bone formation, increasing osteoblast proliferation. A high calcium intake positively affects bone.[11]

The mechanism of the higher osteoblast formation is likely due to the fact that mechanical pressure applied to orthodontics causes mechano-transductions signaling, resulting in extracellular ATP release. This activates P2X7 purinergic osteoblast receptor cells to bind to calcium ions derived from anchovies. Thus, calcium infiltrates the cells and stimulates the production of growth factor TGF-β, which activates MAPK and affects NFkb via RUNX-2, thus increasing the activity of the osteoblast progenitor cells that trigger osteoblast differentiation.[12]

Anchovy also contains protein. Protein has positive and negative effects on calcium balance, and the positive effects of dietary protein on bone mass and fracture depends on dietary calcium. Dietary protein administered in patients with bone fractures may stimulate the production of growth factors such as insulin-like growth factor 1 (IGF-1), which stimulates osteoblasts for bone formation.[13]

Osteoblasts play a crucial role in the process of bone formation, in the induction and regulation of extracellular matrix mineralization and in the control of bone remodeling. During bone formation, mature osteoblasts synthesize and secrete type I collagen (which represents the greatest part of the organic extracellular bone matrix) and various non-collagen proteins such as osteoclasin, osteopontin and bone sialoprotein. These proteinsh exert various essential functions, including the regulation of bone turnover, the control of bone mineral deposition and the regulation of bone cell activity. Osteoblasts also synthesize IGF-1, which control bone cells in an autocrine and or paracrine manner. IGF-1 secreted from osteoblasts in the bone tissue has been demonstrated to be a potent chemotactic factor that might play a major role in the recruitment of osteoblasts during bone formation.[14,15]

Conclusion

This study found that anchovy (*Stolephorus* sp) intake as additional feed for Wistar rats (*Rattus norvegicus L*) results in higher osteoblast counts in the tension area of orthodontic tooth movement.

REFERENCES

(1) Nakanishi H, Seki Y, Kohno T, Muramoto T, Toda K, Soma K. Changes in Response Properties of Periodontal Mechanoreceptors after Experimental Orthodontic Tooth Movement in Rat. *Angle Orthod*. 2004; 74: 93-99.

(2) Alyami EA, Kuijpers-Jagtman AM, Van't Hof MA. Stability of Orthodontic Treatment Outcome: Follow up Until 10 Years Postretention. *Am J Orthod Dentofacial Orthop.* 1999; 72: 90-99.

(3) Sheibani A, Valaei N, Vasooghi M, Noorbakhsh M (). Incidence of Relapse in Orthodontic Treatments and Related Factors. *Journal of Research in Dental Sciences*. 2010; 7 (2): 32-41.

(4) Hsu JT, Chang HW, Huang HJ, Yu F, Tu MG. Bone Density Change Around Teeth During Orthodontic Treatment. *Clin Oral Invest.* 2011; 15 (4): 511-51

(5) Tara S. Rogers, Marjorie G. Garrod, Janet M. Peerson A, Darren J. Hillegonds, Bruce A. Buchholz E, Elieke Demmer A, Christine Richardson A, Erik R. Gertz B, Marta D. Van Loan A, B. Is bone equally responsive to calcium and vitamin D intake from food vs.

supplements? Use of calcium tracer kinetic model. *Bone Reports.* 2016; (5): 117-123.

(6) Charles TP, Joshua RL, Frank AL. Essential Nutrients For Bone Health and a Review of This Availability in the Average North American Diet. *The Open Orthopaedics Journal.* 2012; (6): 143-149.

(7) Kadam, SU and Prabhasankar P. Marine Food as Functional Ingredients in Bakery and Pasta Products. *Food Research International.* 2010; 43: 1975-1980.

(8) Larsen R, Eilersten KE and Elvevol EO. Health Benefits of Marine Foods and Ingredients. *Biotechnology Advances.* 2011; 29: 508-518.

(9) Mohsen S, Mahin K, Ahmad Reza D. The Effect of Chronic Renal Insufficiency on Orthodontic Tooth Movement in Rats. *Angle Orthodontist.* 2001; (71): 494-498

(10) Shibata, Y, Fujita S, Takahashi H, Yamaguchi A, Koji T. Assessment of Decalcifying Protocols for Detection of Specific RNA by Non-Radioactive in Situ Hybridization in Calcified Tissues. *Histochem Cell Biol*. 2000; 113: 153 – 159.

(11) Zayzafoon M. Calcium/Calmodulin Signaling Controls Osteoblast Growth and Differentiation. *Journal of Cellular Biochemistry.* 2006; 97 (1): 56-70.

(12) Dixon SJ, Sims S.M. P2 purinergic receptors on osteoblasts and osteoclasts. Potential target for drug development. *Drug Development Research*. 2000; 49: 187-200.

(13) Bess Dawson-Hughes. Interaction of dietary calcium and Protein in Bone health in Humans. *Am J Clin Nutr*. 2003; 852-854.

(14) Carvalho RS, Bumann A, Schaffer JL, Gerstenfeld LC. Predominant Integrin Ligands Expresses by Osteoblasts Show Preferential Regulation in Response to Both Cell Adhesion and Mechanical Perturbation. *J. Cell Biochem.* 2002; 84: 497-508.

(15) Corrado A, Cantatone FP, Grano M, Colucci S. Neridronate and Human Osteoblasts in Normal, Osteoporotic and Osteoarthritic Subjects. *Clin Rheumatol.* 2005; 24: 527-534.

أثر مسحوق سمك الانشوجي الجاف على زيادة عدد خلايا اوستيوبلاست في منطقة الضغط لأسنان جرذ نوع وستار

تيكي اندريانا[1]، روبيانتو،[2] إندراواتي[3]

[1] قسم الطب الحيوي، كلية الأسنان، جامعة جمبر، إندونيسيا.

[2] قسم اللثة، كلية الأسنان، جامعة إيرلانجا، إندونيسيا.

[3] قسم بيولوجيا الفم، كلية الأسنان، جامعة إيرلانجا، إندونيسيا.

ملخص

خلفية: انتشار الانتكاس في المعالجة التقويمية لا تزال مرتفعة جدا، حوالي 50٪ بعد فترة الاحتفاظ لمدة عامين، وفقا لباحثين آخرين لا يزال الانتكاس من 61.5٪ من 500 مريض. يحدث الانتكاس، لأن ضغط العظام في التوتر ليست الأمثل، وبالتالي تحتاج إلى مادة لتحسين عملية تكوين أو تشكيل العظام في منطقة الشد. البديل الوحيد الذي يزعم بأنه يمكن تلبية احتياجات الكالسيوم العظام عن طريق تناول أسماك الأنشوجة (Stolephorus س)، لأن الأنشوجة هي مصدر للكالسيوم عالية.

الهدف: تحليل تأثير مسحوق الأنشوجة على عملية زيادة خلايا العظم العظمي في مجال حركة تقويم الأسنان.

المواد والطريقة: باستخدام دراسة الحيوان، عشوائيا 30 الفئران ويستار جانتان مقسمة إلى 6 مجموعات مع كل مجموعة تتكون من 5 الفئران. في السيطرة على المجموعة (K) أعطيت إجهاد تقويم الأسنان، الغذاء القياسية، ولاحظ لمدة 7 أيام ((K1، 14 يوما (K2) و21 يوما (K3)؛ أعطيت فئران ويستار في مجموعة العلاج (P) عن طريق الضغط الميكانيكي والمزيد من الطعام مسحوق الأنشوجة تقويم الأسنان عند تناول جرعة مقدارها 0.0168 جم / يوم / ز الفئران من خلال المعدة السبر، في الملاحظة لمدة 7 أيام ((P1، 14 يوما ((P2، 21 يوما (P3). في يوم 8 مجموعات الفئران (K1 و P1)، يوم 15 مجموعة الفئران (K2 و P2)، يوم 22، تمت التضحية مجموعات الفئران (K3 و P3) واتخذت العظام خلايا الفك عدد قابلية الملاحظة العظمية باستخدام تلطيخ Hematoxyllin يوزين (HE). تم تحليل البيانات التي تم الحصول عليها باستخدام أنوفا وتوكي هسد.

النتائج: كانت هناك زيادة في عدد الخلايا العظمية في مجال حركة تقويم الأسنان في المجموعة الضابطة ومجموعة العلاج. في مجموعة العلاج كان هناك زيادة كبيرة في الخلايا العظمية في يوم 14، واليوم 21 في المجموعة الضابطة.

الخلاصة: تناول الأنشوجة كمكمل غذائي في الفئران ويستار (الجرذ norvegiccus L) يمكن أن تزيد من عدد الخلايا بانية العظم في سحب المنطقة على حركة الأسنان تقويم الأسنان.

الكلمات الدالة: أوستيوبلاست، تقويم الأسنان حركة الأسنان، الأنشوجة (ستوليفوروس سب).

تاريخ استلام البحث 2016/10/3 وتاريخ قبوله للنشر 2017/10/8.