Effects of Docosahexaenoic Acid (DHA) Microalgae(R) on Orthodontic Tooth Movement in the New Zealand White Rabbit

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Abstract

Docosahexaenoic Acid (DHA) microalgae(R), usually used as a diet supplement, has been reported to protect against inflammation-induced bone loss and inhibit osteoclastogenesis. Orthodontic tooth movement (OTM) is based on inflammation reaction due to biomechanical orthodontic force that triggers osteoclastogenesis to produce osteoclasts. Osteoclasts play has an important role on alveolar bone resorption, which can cause tooth movement. This study was aimed to observe the effects of DHA microalgae on OTM in New Zealand Rabbit in a quasi- experimental laboratory.

The subjects consisting of 15 New Zealand rabbits were divided into three groups: control group (OTM), DHA-diet of 750 mg (DHA-750), and DHA-diet of 1500 mg (DHA-1500). The DHA-diet was started from day one to day 14 daily per oral. During the experiment the rabbits were fed with the same rabbit basic food. Tooth movement was measured on the day 3, day 7 and day 14.

Results showed that the highest distance of OTM occurred in the control group and lowest was at DHA-1500, followed by DHA-750. There were significant OTM differences between the experimental DHA diets and the control on day 3, day 7 and day 14 ($p<0.05$). It was concluded that DHA microalgae has a potential to decrease OTM.

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Introduction

Docosehaxaenoic acid (DHA) micro-algae, a component of omega-3 fatty acids used as a diet supplement in daily life[1] has been reported to protect against inflammatory bone diseases such as rheumatoid arthritis and osteoporesis. It has a molecular formulae $C_{22}H_{32}O_2$ and is known to protect against inflammation-induced bone loss. It has also been found to inhibit osteoclasto-genesis.[1,2] Microalgae of *Schizochytrium* sp are DHA-rich Algal Oil containing 40–45 wt% DHA.[2]

The basic of orthodontic tooth movement (OTM) is inflammatory response of the periodontium to balance and continue force on the teeth. OTM is induced by orthodontic force delivered to periodontal tissue. The periodontal tissue response will create asepsis inflammation,[3,4] Orthodontic biomechanical force shifts the balance and organization of periodontal cells in favor alveolar bone remodelling.[3] The periodontal cells synthesize or produce cytokines and chemokine to stimulate periodontal ligament and bone cells to orchestrate inflammatory response, followed by osteoclastogenesis and alveolar bone resorption in compression site, resulting in tooth movement to a new position.[3,5]

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There are many expressed inflammatory cytokines stimulated by orthodontic force, such as IL-1, IL-6, tumor necrosis factor (TNF), RANKL, and M-CSF.[4,6] The RANKL and M-CSF expressed by osteoblast are the most important proinflammatory cytokines responsible for recruitment, differentiation, activation and survival of the osteoclast. Other cytokines that promote osteoclast formation and activation such as IL-1, IL-6 and TNF-α have been found in surrounding orthodontic tooth movement.[6] Osteoclastogenesis has a main role on alveolar bone resorption, and therefore can cause tooth movement.[3,5] It has been suggested that inhibiting certain inflammatory cytokines decreases osteo-

clastogenesis and lowers tooth movement rate.[4]

DHA inhibits osteoclastogenesis via receptor activator of nuclear factor-Kβ ligand (RANKL) pathway.[8] DHA suppresses macro-phage colony-stimulating factor (M-CSF)-induced proliferation of osteoclast precursors. DHA also blocks RANKL-induced osteoclast differen-tiation. Akiyama reported that DHA exerts an anti-osteoclastogenic effect by suppressing proli-feration and differentiation of BMMs and enhancing apoptosis of mature osteoclasts, thereby resulting in a diminished number of bone-resorptive cells.[2] Osteoclasts have play main roll in resorption of alveolar bone on compression site to earn tooth movement in orthodotic treatment. Decreasing amount of osteclasts can slower tooth movement.

As DHA microalgae, a safer DHA source, has anti-osteoclastogenesis effects and has been widely used as a diet supplement; it is, therefore important to evaluate whether the DHA can inhibit orthodontic tooth movement. Slower tooth movement can induce longer orthodontic treatment time, so it is important to be considered. In this study we observed the effects of DHA microalgae(R) on OTM in New Zealand Rabbit.

Methods

Fifteen 6-8 week-old males of New Zealand rabbits weighing from 1800 to 2000 g were used in this study. The study was conducted in the Integrated Research and Testing Institute Laboratory, Gadjah Mada University, Yogyakarta, Indonesia with the approval from Ethical Clearance Committee of the Faculty of Dentistry, Gadjah Mada University. The rabbits were housed one in aluminium cages and equally treated in a 12 h light/dark environment, fed with a rabbit basic food, and watered ad libitum. The rabbits were acclimated to the cage living ctreated DHA at doses of 750 mg (DHA-750) and 1500 mg (DHA-1500), so there were five rabbits within each group.

One week after acclimatization, orthodontic tooth movement was performed by 100 g-Nickel Titanium (Niti 0.011 x 0.012, American Orthodontic, USA) open coil spring placed on archwire (SS 0,016 x 0,016, American Orthodontic, USA) between two brackets bonded on the right and left lower incisors, under general anesthesia by ketamine hydrochlorine at a dose of 35 mg/kg (Ketalar 50, Dankyo Co Ltd, Tokyo, Japan) in combination with xylasin at a dose of 13 mg/kg (Celactal 2%, Bayer-Japan Co Ltd, Tokyo, Japan). The reciprocal force of the spring was measured with a gouge, and the spring was fixed to exert approximately 100 g of force which corresponded to 6 mm of length by compressed 50 %. These springs were to move the left and right lower insicors reciprocal distally.

Diet DHA microalgae (DHA Microalgae *Schizochytrium* sp, Pioneer Biotech, Shaanxi Pioneer Biotech Co.Ltd, China) were set at DHA doses of 750 mg and 1500 mg daily from day one to day 14 at 9 am. The DHA addition per oral by sonde for two experimental groups that were started immediately after orthodontic appliances were set. The OTM were measured on day 3, 7 and 14 by digital Vernier Caliper (Mitutoyo Digimatic Caliper 500-197-30 81N/0.01 mm, Indonesia). After 14 days all subjects were sacrificed by over doses of anesthesia. Analysis of variance (Anova) was used to test the mean differences, followed by Turkey post-hoc test for multiple comparisons at the probability values of statistically significant consideration was less than 0.05.

Results

In this study the effects of DHA microalgae on the orthodontic tooth movement in rabbits were examined. Figure 1 shows that after 14 days of DHA administration the experimental groups (DHA-750 and DHA 1500) had lower tooth movement than the control group. It was observed in Figure 2 that the highest movement after 14 days was on the control group (5.50 $\pm$0.21 mm) and the lowest was on DHA-1500 (4.63$\pm$0.37 mm) followed by DHA-750 (4.80$\pm$0.14 mm).

This study demonstrated that DHA significantly decreased rates of tooth movement on day 3, day 7 and day 14 ($p<0.05$). On the day 3, the highest reductions were observed in the two experimental groups compared to control group. The distance of tooth movement at DHA-1500 was significantly lower than that at DHA-750 on the day 7 and day 14 ($p<0.05$) but not significantly different from that on day 3 ($p> 0.05$) (Figure 2). The average OTM values were 3.50 mm (dose 1500 mg) and 3.94 mm (dose 750 mg) on the day 7; 4.63 mm (dose 1500 mg) and 4.80 mm (dose 750 mg) on the day 14; and 2.58 mm (dose 15 00mg) and 2.66 mm (dose 750 mg) on

the day 3.

Figure 1. The distance of tooth movement (red line) after 14-day addition of docosahexaenoic acid (DHA) microalgae(R) at the doses of 750 and 1500 mg and control. Experimental groups show lower distance of orthodontic tooth movement (OTM) than the control.

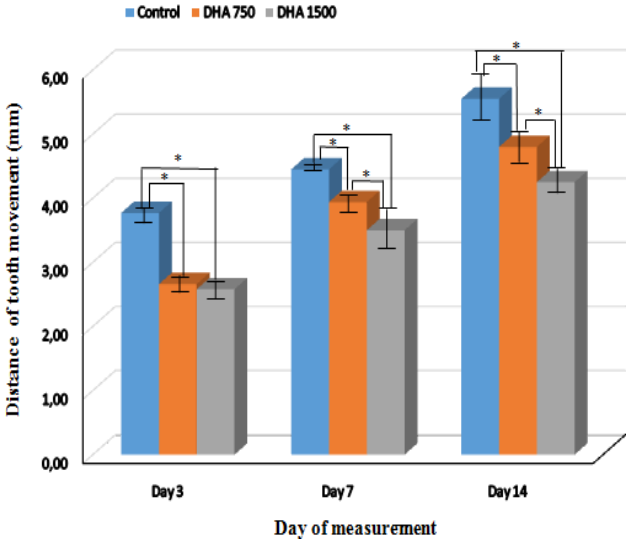

Figure 2. Effects of Docosahexaenoic Acid (DHA) microalgae(R) on the distance of tooth movement. The ordinate shows the distance of tooth movement versus day of measurements. Each bar shows mean value ±SD, *p<0.05.

The acceleration rate of tooth movement shows descriptively in Figure 3. There were similar trends of acceleration rates of the tooth movemen t as affected by DHA additions. All experimental groups reached highest rates on the day 3 (2.58 mm and 2.66 at DHA doses of 1500 mg and 750 mg, respectively) then the retes slowed down on day 7 (0.82 mm and 1.21 mm at DHA doses of 1500 mg and 750 mg, respectively), and were relatively stable on day 14 (0.84 mm at DHA dose of 1500 mg and 0.86 mm at DHA dose of 750 mg).

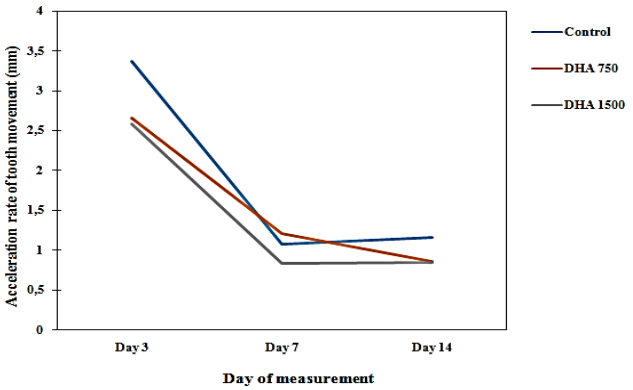

Figure 3. Effects of docosahexaenoid acid (DHA) on the acceleration rate of tooth movement.

Discussion

Group of DHA-750 as well as DHA-1500 had slower tooth movement than the control (Figure 1 and 2). This indicates that DHA addition had inhibited the tooth movement. DHA might have decreased the number of osteoclasts which were responsible for the alveolar resorption on compression side to result tooth movement. Osteoclasts formation performed via osteoclasto-genesis. In the treated groups DHA might have inhibited the osteoclastogenesis.

William[9] and Ashiry[10] reported in previous histology studies showed that the formation of osteoclasts was induced at compression side during orthodontic tooth movement. OTM is induced by orthodontic force delivered to periodontal tissue and creates inflammation,[3,4] The periodontal cells synthesize cytokines (IL- 1, IL-6, TNF-alfa, RANKL, M-CSF) and chemokine to stimulate periodontal ligament and bone cells to orchestrate inflammatory.[3,5] These compounds regulate inflammatory response and induce osteoclastogenesis followed by alveolar bone resorption, resulting in tooth movement.[3,4,7,10] The rate-limiting step in an orthodontic tooth move-ment is considered to be bone resorption at the compression side.[11]

In this study DHA was suggested to affect bone metabolism of remodelling and to protect against inflammatory-induced bone resorption by inhibiting osteoclastogenesis at the compression side. Teixeira[4]and Yakiyama[2] reported that DHA inhibited osteclastogeneses mediated by RANKL.[4,2] RANKL bonded RANK–a receptor protein in the precursor osteoclast surface membrane promotes differentiation, proliferation and maturation of osteoclast. The RANKL/RANK binding is crucial for differentiation,

function, and survival of osteoclast.[2,10] DHA blocked the cytokines of RANKL-induced osteoclast differentiation.[2,7] Differentiating and mature osteoclasts show high level of trap activity which is commonly used as marker for osteoclasto-genesis. Kasonga *et al*. reported that DHA suppressed TRAP activity in differentiating osteoclasts and mature osteoclasts. DHA significantly reduced RANKL-induced osteoclast formation in differentiating osteoclasts, as treated-DHA yielded fewer large multinucleated osteoclasts.[7]

DHA could play inhibitory by exerting an anti-osteogenic effect by inhibiting M-CSF-induced proliferation of osteoclast precursors, suppressing the proliferation and differentiation of BMMs, and enhancing mature osteoclast apoptosis. These result in a lower number of osteo-clasts.[5,2] M-CSF as well as RANKL play key roles in orthodontic tooth movement. Mechanical compressive force increases the expression of M-CSF[2]. RANKL and M-CSF, which are produced by osteoclasts, induce osteoclasts precursors to differentiate and fuse into resorbing osteoclasts.[12] M-CSF is responsible for the proliferation, differentiation, and survival of osteoclast precursor. RANKL stimulates osteoclastogenesis and prevents osteoclast apoptosis.[12,13] Study in macrophage-stimulated LPS expressing inflam- matory cytokines like IL-1β, IL-6, TNF-α, M-CSF reported that the expression of the inflammatory cytokines was suppressed after DHA addition. DHA disturbed macrophage to synthesize IL-1β, IL-6, TNF-α, M-CSF.[13] Cytokines IL-1β, IL-6, TNF-α, and M-CSF act to promote formation and activation of osteoclasts which play a significant role in alveolar bone resorption. Decreased expression of the inflammatory cytokines can reduce in orthodontic tooth movement;[6,7] therefore the results of this study showed significant lower tooth distance in the experimental groups of DHA-750 as well as DHA 1500 compared to the control.

The highest tooth movement reductions observed in the two experimental groups compared to control group were on the day 3, but the distance of tooth movement between the DHA groups was not significantly different (Figure 2). Orthodontic tooth movement with continuous forces is divided into 3 stages, initial phase, and lag phase post larger phase. Initial phase runs from after force application until day 3-6. The day 3 period apparently was in the initial orthodontic tooth movement called initial phase when the teeth moved fast. Compressive pressure was conducted in periodontal ligament. RANKL level increased in periodontal specially by compressive force as early as 3 hours after orthodontic force application and remained elevated after at least 5 days.[15,17] Previous study showed RANKL level became significantly higher after 24 hours of continuous force and remained to increase after 5 days.[18] High RANKL level, as a specific mediator for osteoclast formation and initiation of bone resorption, had more influence on osteoclastogenesis to cause tooth movement. It was demonstrated on the day 3 both DHA doses 1500 mg and 750 mg had the same level of effects to inhibit tooth movement. DHA treatment in these two doses might have disturbed the synthesis of RANKL and M-CSF in the same level as that in the initial phase of orthodontic tooth movement, therefore it induced osteo-clastogenesis of formation, proliferation and activation of the clast cells. Akiyama[2] analyzing DHA treatment during osteo-clastogenesis in cell cultures reported that DHA addition after 48 hours strongly inhibited osteoclastogenesis. DHA inhibited the cell-cell fusion process during osteo- clastogenesis. DHA-treated BMMs were shown to be TRAP-positive, but not multinucleated.

This study showed that on the day 7 and 14 different doses had significant different effects on the tooth movement (Figure 2). The decrease in the tooth movement due to DHA- 1500 was higher than DHA-750. Proffit[19] stated after initial phase OTM will enter to a lag phase when tooth moves less or stops for about 2-3 weeks. The results of this research in lag phase showed less tooth movement and was induced by the dose of DHA. The addition of 1500 mg DHA had higher inhibition effects on tooth movement than 750 mg addition. Similar finding was reported by Kasonga[6] when he treated CD+4 cells with different DHA concentrations of 20 uM, 40 uM, 60 uM, and 80uM. He observed no osteoclastogeneis CD+4 at the lowest concentration of 20 uM, but doses of 40 uM and above had caused osteoclastogenesis.This researcher suggested that there was an inhibitory effect to osteoclastogenesis at concentration as low as 40 uM and a complete inhibition of osteo-clast formation at 80 uM.

The acceleration rates of all groups

reached highest on the day 3 (Figure 3) which was considered as the initial phase of orthodontic tooth movement. During this phase, the teeth moved fast and finished for 3-4 days or 7 days after force application. On the day 7 and day 14 acceleration rates were lower than on the day 3, as this was in a lag phase period of orthodontic tooth movement. Most *et al*. stated that orthodontic tooth movement usually enters to lag phase after initial phase finishes and runs for about 2-3 weeks. In the lag period RANKL level decreases and results in much less or no tooth movement.[20] Results of DHA- 750 and DHA-1500 acceleration rate values were still under the control value (Figure 3). Allam et al. reported that DHA could downregulate mediator proinflammatory IL-1β, IL-6, TNF-α, and M-CSF, and the RANKL then inhibits osteo-clastogenesis.[15] This inhibition of osteoclasto-genesis might reduce osteoclast formation which had important role in orthodontic tooth movement, and therefore resulted in a relatively lower rate of acceleration rate of the tooth movement than the control.

These study showed that DHA administration inhibited tooth movement. However the average distance of the tooth movement decreased relatively low. The total distance of OTM treated with DHA at doses of 750 mg, 1500 mg, and control were 4.80 mm, 4.63 mm, 5.55 mm respectively, while the OTM decreases were 0.75 mm and 0.92 mm at DHA-diet at doses of 750 mg and 1500 mg or 13.5% and 16.5% compared to the control, respectively. However, these values are still clinically acceptable and reasonable and should be considered in improving orthodontic tooth movement. As it has been widely used for daily health supplement and prevention of bone resorption supplement, DHA microalgae could be considered when ones carry out an orthodontic treatment. In the future, the research of DHA microalgae in effect on OTM during orthodontic active treatment in patients will be conducted. Based on the results of this study DHA microalgae has a potential biomaterial for inhibiting orthodontic root resorption, therefore it will become a challenging research topic in the future.

Conclussion

The current study clearly demonstrated that dietary DHA microalgae is an important factor in inhibiting orthodontic tooth movement. DHA microalgae at doses of 750 mg and 1500 mg has a potential to decrease orthodontic tooth movement. From a clinical translation perspective, this study provides insights to modulation to improve tooth movement in orthodontic treatment.

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Declaration of Interest

The authors declare that there are no conflicts of interest.

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