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Effect of dietary n-3 Polyunsaturated Fatty Acids on Bony Remodeling during Eruptive Tooth Movement

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The aim of this study was to investigate the effect of n-3 polyunsaturated fatty acids (PUFAs) on eruptive movement during tooth development.

Sprague-Dawley (SD) rat pups were randomly divided into two groups; control group and experimental group. The experimental group was administered daily with n-3 PUFA by intraperitoneal (IP) injection. After 10 days postpartum, rat pups were sacrificed to evaluate the effect of n-3 PUFA on eruptive tooth movement. Histological analyses were by hematoxylin-eosin (H&E) staining. Tartrate-resistant acid phosphatase (TRAP) assay was performed to compare the osteoclast distribution in the bone matrix above the developing molar teeth. Incisor teeth eruptions were noticeably observed in IP group, as compared to control group. Rat pups in IP group showed faster tooth eruption on day 8 after birth. Through histological analyses, IP group showed thinner bone matrix and more osteoclasts above the 1st molar teeth, as compared to control group. TRAP assay showed significantly stronger stained pattern that the osteoclast above the 1st molar teeth in IP group, as compared to control group. The results suggested that n-3 PUFA could affect osteoclastic activity involved in bony remodeling during eruptive tooth movement.

Key words: Eruptive tooth movement, Osteoclast, Bone matrix, n-3 PUFAs

Introduction

PUFAs, their metabolites and lipid mediators induced from PUFAs are known as important substances in the regulation of lots of biological processes; inflammatory responses [1,2], regulation of stem cell proliferation and differentiation [3], cell cycle progression [4], signal transduction [5] and bone metabolism [6-11].

PUFAs are fatty acids and categorized into 1 of 2 families: n-3 PUFAs and n-6 PUFAs. n-3 PUFAs are essential fatty acids. The structure of n-3 PUFAs may be too complicated, but it shares the part of common structure that the double bond exists at the 3rd carbon from the omega end. Thus, it is usually called omega-3. Although n-3 PUFAs are classified under essential fatty acids, it can not be synthesised by human body. Therefore, n-3 PUFAs should be taken by many dietary supplements. The best dietary food of n-3 PUFAs is fish oil [12,13]. The dietary supplements for n-3 PUFAs are very close to our life and flowing over − nutritional jelly, milk power, and so on. However, the entire effect with the human

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body is not clear.

Relation with the bone metabolism, there have been increasing reports that various PUFAs have crucial effects in regulating bone remodeling. There have been also many studies involved with the effect of n-3 PUFAs on hard tissue, and which are almost concentrated on the bone tissue [14-19]. Especially, although n-3 PUFA and their metabolites in vivo have down-regulated on osteoclastogenesis [20], it is not clear.

The previous report related with tooth demonstrated that effect of dietary n-3 PUFAs involved in experimental orthodontic tooth movement were lower in rats fed a fish oil enriched diet compared to control [21]. Another group reported that the number of osteoclasts on pressure side during experimental orthodontic tooth movement was lower in the fish oil group compared to control [22]. Although the influence of n-3 PUFAs in the regulation of bone metabolism has been studied, the effect of n-3 PUFAs on bone remodeling during tooth development has not been studied yet.

Tooth development is accompanied by the dynamic processes and starts with the formation of tooth bud. At the tooth bud stage, the epithelium goes into the connective tissue and it is called bud stage as it looks like a bud. Then as the embryonic development proceeds, it turns into the morphological change of the cap, and the bell. Therefore, it is called as the cap stage and bell stage. After these processes, the hard tissue formation is followed and then, finally, the tooth shows its shape into the oral cavity via the tooth movement for eruption [23].

Tooth movement for eruption is explained with three categories of movement; preeruptive, eruptive and posteruptive tooth movement. Each of three categories is continuous and complicate processes for tooth eruption [24-26]. Although tooth movements are very important processes for tooth eruption, the eruption mechanism is not fully identified into an integrated theory.

Especially, among three categories of tooth movement, the mechanisms related with eruptive tooth movement is known well. These are generally described of four factors which are composed of root formation, hydrostatic pressure, bony remodeling and periodontal ligament. All of developing and growing teeth have to retain their own pathway of eruption. It is obvious that the bone matrix and epithelium should be degenerated to open the way when the tooth follicle is pushed upwards. In this connection, bony remodeling during eruptive tooth movement is existed in one of the crucial processes. Existence of lots of osteoclasts differentiated from monocytes may be elevated bone resorption during eruptive tooth movement. Therefore, osteoclast is seen as a indicator during bone resorption for bony remodeling.

In these days, n-3 PUFAs were included in lots of food for growing children as the additives. Therefore, the food additives for use as nutritional supplements should be considered for the normal pattern during the growth phase including of the tooth development and eruptive movement.

Although the roles of dietary n-3 PUFAs in bone biology have been progressively reported, effect of n-3 PUFAs on the bony remodeling during eruptive tooth movement has not been elucidated. Therefore, the purpose of this study was to investigate the effect of n-3 PUFAs supplied with menhaden fish oil on bony remodeling during eruptive tooth movement.

Materials and Methods

Menhaden fish oil (Sigma-Aldrich Co, MO, USA), which is rich in n-3 PUFAs, was used. SD rats were purchased from the Damul Science (Daejeon, Korea) and housed in laboratory animal care-approved facilities. After SD rats mating, the birthday of the pups was designated as 0 day (0 d) postpartum. The experimental protocol, including the use of animals in the research, was approved by the Institutional Animal Care and Use Committee, Wonkwang University, Korea.

Experimental design

Newborn SD rat pups were divided randomly into control or n-3 PUFAs injection group (25 and 28 rats, respectively). Each batch of experiments in five times was used a litter of rats. The n-3 PUFAs injection group was daily administrated 5.075$\mu\ell$ of menhaden fish oil per 1 gram of body weight by injecting into IP. Rat pups were sacrificed on 10 d after their birth to examine the result. The result was determined in following aspects: time taken to eruption and histology analyses.

Histological observation and histomorphometric analyses

The maxillae were isolated at 10 d and immersion-fixed in

4% paraformaldehyde(PFA) solution (pH 7.4) overnight, followed by decalcification in 10% (w/v) ethylenediaminetetraacetic acid (EDTA, pH 7.4) over several weeks. They were routinely dehydrated and embedded in paraffin, after which the histological sections were prepared for H&E and TRAP staining. The effect of n-3 PUFA on the eruptive tooth movement were examined by analysing the bone matrix above the developing molar tooth from the histological sections of the maxillae at 10 d. After H&E and TRAP staining both of control and n-3 PUFAs injection groups, the specimens were examined and photographed under a digital microscope (Nikon eclipse 90i, Tokyo, Japan).

Results

Survival rates

All rats from control and n-3 PUFAs injection groups in the whole experiment had survived until the day of sacrifice (10 d after birth).

Changes in weight of body

Changes in weight of body during 10 d are shown in Fig. 1. No significant differences in weight of body were found between control and n-3 PUFAs injection groups at the beginning of feeding. The condition of different diets did not severly change general growth of rats. At the end of 10 d, mean value in weight of body was slightly greater in n-3 PUFAs injection group.

Tooth eruption

Rats in n-3 PUFAs injection group showed faster tooth eruption compared to control group when observed in 8d after birth (Fig. 2). Tooth eruption is already started in n-3 PUFAs injection group. n-3 PUFAs injection group displayed that the maxillary and mandibular incisor teeth erupted very prominently, while control group did not show any tooth eruption at oral mucosa. Therefore, it is plausible that the dietary n-3 PUFAs during tooth development may facilitate the eruptive tooth movement.

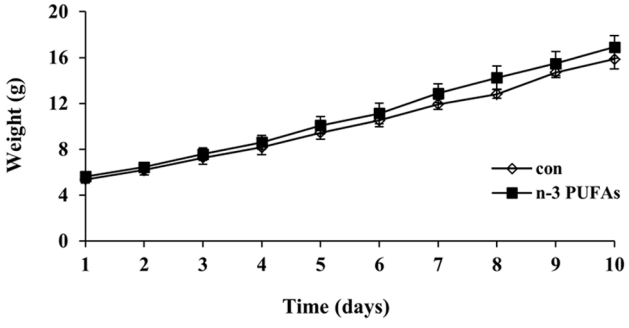

Fig. 1. Changes of mean value in weight of body in control and n-3 PUFAs injection groups during 10 d. No significant differences between control group and n-3 PUFAs injection group were found by mean values of Student's *t*-test.

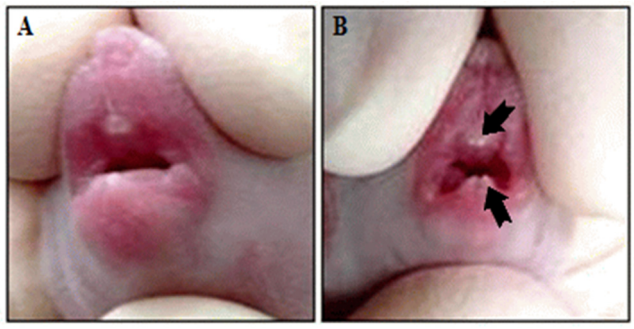

Fig. 2. Tooth eruption at postnatal 10 d. This result displayed the representatives that was the general tendency of each group (A; control, B; n-3 PUFAs injection group). Arrows: incisor.

Histological findings

Fig. 3 shows the histological appearance of bone matrix above the developing maxillary 1st molar tooth at 10 d. Through H&E staining, the pinkish layer above the 1st molar tooth indicated the bone matrix. The bone matrix layer in n-3 PUFAs injection group is thinner than control group (Fig. 3B). The higher magnification analysis revealed that lots of osteoclast crowding existed in the bone matrix around the tooth follicle of n-3 PUFAs injection group (Fig. 4). The appearance of many osteoclasts may be the reason of the early eruption, opening the erupting way by degenerating the bone matrix.

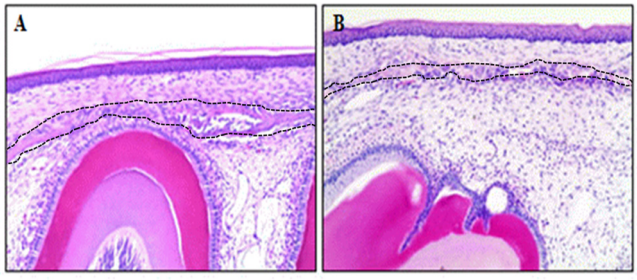

Fig. 3. Histological analyses. Histoligical changes in were shown that the bone matrix layer above the maxillary 1st molar was thinner than control group (A; control, B; n-3 PUFAs injection group). x 40.

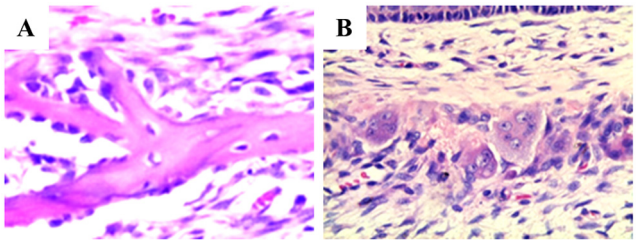

Fig. 4. The higher magnification of histological image in n-3 PUFAs injection group. As shown Fig. 3B, the bone matrix was thinner in n-3 PUFAs injection group. Through higher magnification, lots of osteoclast were in the bone matrix above the tooth follicle of n-3 PUFAs injection group (A; control, B; n-3 PUFAs injection group). x 400.

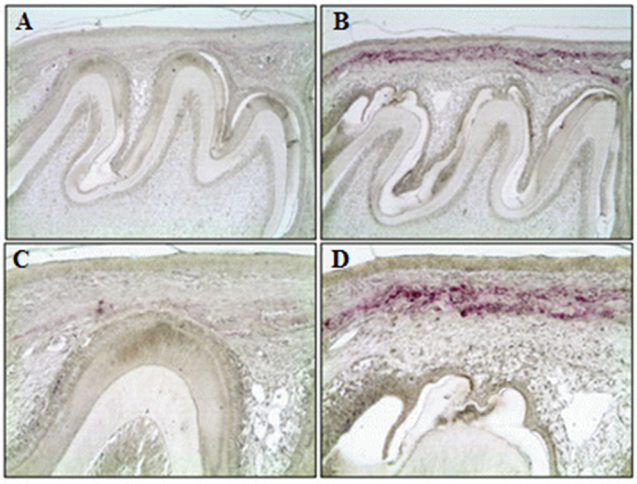

Fig. 5. TRAP assay. The bone matrix was on the upper side of the tooth follicle and, in n-3 PUFAs injection group, lots of osteoclast activity appeared to be in the same place (A, C; control, B, D; n-3 PUFAs injection group).

TRAP assay

To investigate the distribution of osteoclast in the bone matrix above the molar tooth follicle at 10 d, TRAP assay was performed. The data significantly showed the elevated osteoclast activity in n-3 PUFAs injection group compared to control group (Fig. 5).

Discussion

In the present study, the developmental period related with tooth eruption in rat pups was altered by n-3 PUFAs supplied with menhaden fish oil. The results demonstrate that menhaden fish oil diets, rich in n-3 PUFAs, elevated eruptive tooth movement in this experimental model. During 10 d for this experiment, all rat pups did not show any growth change compared to control group. However, the increase of osteoclast in the bone matrix above tooth follicle in n-3 PUFAs injection group would not overlook if the assigned period for experiment would be a more longer than 10 d. Because, in n-3 PUFAs injection group, another bone compartments except the bone matrix above tooth follicle may be affected by numbers of osteoclasts. Actually, the appearance of osteoclast in lots of bone matrix formed by the endochondral or intramembranous ossification was the increased pattern compared to control group (data not shown).

PUFAs are fatty acids which has at least 18 carbons over 2 double bonds. The classification of PUFAs is representative of n-3 and n-6 families. Alpha-linolenic acid (ALA) (18:3) and linoleic acis (LA) (18:2) are the precursor compounds for the n-3 and n-6 series of PUFAs. These crucial compounds have to take the dietary system because ALA and LA can not be endogenously synthesized in the human body. The best dietary material of n-3 PUFAs is fish oil and, for getting n-6 PUFAs, a number of edible plant oils, such as soybean and corn, is the best source [27].

The PUFAs metabolites are oxidative products obtained by the activities of various enzymes and non-enzymatic oxidation. There are 2 kinds of PUFAs metabolites: eicosanoids and docosanoids. The eicosanoids, induced from the 20 carbons n-3 and n-6 PUFAs, include the lipoxins, prostaglandins, leukotrienes, thromboxanes, and E-series resolvins. Docosanoids, induced from the 22 carbons PUFAs, were the DHA, docosatrienes, protectins and D-series resolvins [28].

In the previous study, different families of PUFAs revealed to have differing regulation related with the bone metabolism. The dietary n-3 and the n-6 PUFAs using the animal models appeared to be bone protective. However, it was unclear because there were no consensus as to the proper amount of n-3 and n-6 PUFAs. Furthermore, it is assumed that individual PUFAs in both the n-3 and the n-6 PUFAs families have differing activities in bone metabolism. Therefore, there is a need further studies to confirm the relative effects of individual PUFAs in bone metabolism.

In these days, several studies have focused on determining the ratio of n-3 : n-6 fats in the dietary food for the bone metabolism [29]. The reason is that it has been reported that not only is the total contents of PUFAs

in the diet important for regulating bone tissue, but the composition of dietary PUFAs also reveals to be important.

PUFAs and their metabolites on calcium balance and bone metabolism were generally known as the positive regulators for osteoblastogenesis and osteoblast activity. On the contrary, several metabolites of AA could stimulate osteoclastogenesis and promote bone resorption. Specifically, leukotrine 4 inhibits osteoblast activity and induces osteoclastogenesis by RANKL-independent mechanism [30, 31].

There was reported that researches on PUFAs and their metabolites might have relevance to osteoblastogenesis and osteoclastogenesis. Therefore, it is also required to determine the effects of the composition and amount of dietary n-3 and n-6 PUFAs on bone metabolism.

The present study was to examine the effect of dietary n-3 PUFAs on bony remodeling during eruptive tooth movement. Our findings support that dietary n-3 PUFAs in growing rat pups could affect the regulation of bony remodeling related with eruptive tooth movement. In n-3 PUFAs injection group, the thinner bone matrix layer above the 1st molar tooth compared to control might facilitate the tooth eruption. Through TRAP assay, osteoclast activity in bone matrix of the 1st molar tooth was significantly shown in n-3 PUFAs injection group. Therefore, it is plausible that the number of osteoclasts has relationship with facilitation of tooth eruption since degradation of upper bone matrix is essential and should be ahead of tooth eruption.

In conclusion, our results confirmed the relationship between n-3 PUFAs and tooth eruption in histological level. However, in order to investigate the complete mechanism behind this, molecular study should be followed.

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Conflict of interest

The author declares that they have no conflicting interest.

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