Dose-dependent Vitamin C Supplementation Enhances Orthodontic Tooth Movement in Wistar Rats

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Abstract

The aim of study is to evaluate the effect of different doses of vitamin C supplement on amount of orthodontic tooth movement. Thirty 6-week-old male Wistar rats were divided into 3 groups according to amounts of feeding vitamin C per day: 0 (control), 500 (C500) and 1,000 (C1,000) mg/day. A 60-g mesial force was applied to maxillary right first molar by using 9-mm nickel-titanium closed-coil spring. Interproximal distance between maxillary right first and second molars was measured after 7 and 14 days. All rats were terminated at day 14, Tartrate-resistant acid phosphatase (TRAP) positive areas and remaining inter-radicular bone areas were measured. Data were analyzed by using Analysis of variance with post hoc test and Welch's test ($P<0.05$). The mean distances of tooth movement were increased in a dose dependent manner with significant difference between the control and C1,000 groups at day 14. The TRAP-positive areas were increased but the inter-radicular bone areas were decreased in both vitamin C supplement groups, there were no significant difference. This study suggests that dietary vitamin C supplement at 1,000 mg/day could enhance amounts of tooth movement during orthodontic application. However, the osteoclast profile and inter-radicular bone areas were not clearly different.

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Introduction

Accelerated tooth movement is a challenge issue due to deleterious effects such as dental caries, gingivitis, root resorption, and pulpal reaction, from long orthodontic treatment time.[1,2] There are some procedures, including surgical methods and pharmacological agents, that have been reported to improve the treatment duration. However, local pain, discomfort during the procedures and some controversies on their clinical results were patient concerns.[3,4] A new genetic manipulation technique is under a developing process and might not be able to use in most patients.[5] Thus, a less invasive, low cost and simple method is a promising approach to improve the orthodontic treatment time with no or less side effect.

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Vitamin C (ascorbic acid) has been reported that involving in bone remodeling processes, which can accelerate tooth movement.[6] Vitamin C is one of water-soluble vitamins. It is not only available in some foods, but also provided in commercial products as a nutritional supplement with low toxicity.[7] Common vitamin C supplement dosage are 500 and 1,000 mg per unit. Vitamin C plays an important role in the regulation of development, function, and survival of many cellular types, such as epithelial, endothelial, neural, chondrogenic, osteogenic and osteoclastogenic cells.[8-11] Previous reports demonstrated that vitamin C was essential to stimulate osteoclast differentiation in osteoclastogenesis. In addition, significant reduction of the osteoclast multinucleation, together with modest decline of tartrate-resistant acid phosphatase (TRAP) precursor cell formation, has been appeared in an *in vitro* study with vitamin C deficiency condition.[12]

Functions of vitamin C has been tested in many aspects of orthodontic field. Litton in 1974 revealed an essential of vitamin C to maintain

periodontal ligament status and imminently complete cessation of osteogenesis during orthodontic tooth movement (OTM) in guinea pig.[13] Moreover, a study in 2015 demonstrated that vitamin C increased rates of tooth movement and numbers of osteoclast lacunae around the compression area of rat incisor root in the group received vitamin C (1 wt%) in daily drinking water.[6] However, another study revealed insignificant rates and amounts of OTM among animals that consuming different doses of vitamin C in their daily diet.[14] Collectively, there is controversial about acceleratory effect on OTM of vitamin C that might resulted from dose of vitamin C supplement. Thus, the aim of this study is to evaluate effects of dietary vitamin C supplement in different doses on OTM accelerating during mechanical force application in Wistar rats.

Materials and methods

Animals and vitamin C administration

Thirty male Wistar rats, 6 weeks old with 150-200 g weight, were purchased from Nomura Siam International Company, Bangkok, Thailand. They were housed under controlled condition at 22±1ºC and 55±10% humidity in alternating 12-hour light-dark cycle. After orthodontic appliance insertion, the animals were offered with soft diet (082G: Perfect Companion Group Co., Ltd, Thailand) and water ad libitum. They were monitored throughout the experimental period in comply with animal welfare regulations.

Doses of vitamin C were calculated on the basis of a dose that human taking vitamin C supplement in one day as previously described.[15] The rats were randomly and equally divided into three groups (n = 10 per group): (1) the control group, receiving only distilled water (DW) without dietary vitamin C supplement, (2) the C500 group, obtaining 44.05 mg/kg rat body weight of vitamin C supplement equal to 500 mg in a 60-kg man, and (3) the C1,000 group, getting vitamin C supplement as 88.10 mg/kg rat body weight comparable with 1,000 mg in a man with 60 kg weight. The dietary vitamin C solutions were freshly prepared from commercial vitamin C (Mega We Care Nat C Ester, Thailand) with DW. The solutions were protected from light and temporarily stored at 4ºC in the dark environment until force feeding was performed. Proper amounts of the solutions were given directly into stomach with feeding needle daily for a 21-day period (Figure 1a).

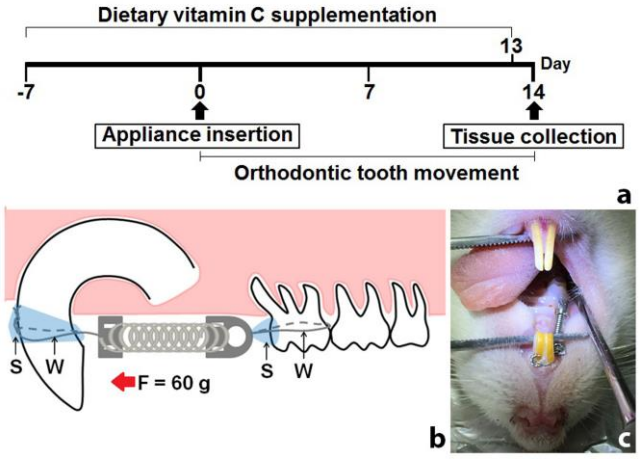

Figure 1. Application of the orthodontic force system in a rat model. (a) Schematic of OTM time course for oral vitamin C supplement (day -7 to 13), appliance insertion (day 0), tooth movement measurement (day 7, 14) and tissue collection (day 14). (b) An illustration of mesially movement of the maxillary right first molar by a NiTi closed coil spring (F = 60 g), red arrow indicating direction of force, W, ligature wire; S, resin composite. (c) A photograph of intra-oral NiTi closed-coil spring application before fixed with composite resin.

All procedures associated with animals were conducted with the approval of the Animal Welfare Committee of Center for Animal Research Naresuan University (NU-AE600916).

OTM assessment

After 7 days of vitamin C administration (Day 0), animals were anesthetized by intraperitoneal administration with an optimal dose of ketamine and xylazine mixture. In addition, tramadol was injected subcutaneously for improving efficacy of anesthesia and relieving pain.[16] Tooth movement mechanic was modified from Sodagar and co-workers.[17] Briefly, a 9-mm nickel-titanium closed-coil spring (0.010 inch wire diameter and 0.030-inch coil diameter; Ormco Corp., Glendora, CA, USA) was placed between the maxillary right first molar (M1) and both maxillary incisors with a 0.009 inch stainless steel ligature wire. A cervical groove was prepared in the gingival third of each incisor to increase the ligature wire retention. A force gauge was used to ensure that a 60-g force was produced by a 1-mm activation of the spring

(Figure 1b and 1c). Then, ligature wires were fixed in place by flowable light-cured resin composite (Filtek Z350; 3M ESPE, USA). Rat's incisor was growing continuously although appliance was inserted.[18] Any potential damage of appliance. Thus, the spring was repositioned cervically on day 7. The distance of OTM was defined from the gap between M1 and second maxillary molar (M2). The measurements were performed at day 7 and 14 of the experimental period by using a feeler gauge (Mitutoyo 184-303S, Kanagawa Japan).

Quantitative analyses of TRAP-positive osteoclasts and inter-radicular bone

The animals were euthanized with overdose thiopental intraperitoneal injection following by decapitation on day 14. The maxillae were dissected and fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 4º C for 48 hours and subsequently decalcified in 10% EDTA solution, pH 7.4, at 4º C for 8 weeks. The decalcified tissues were processed and embedded in paraffin wax. All specimens were cut on horizontal serial section of 5 µm in thickness and decided sections, were apical to the furcation as described below, were gathered. The section were stained with hematoxylin and eosin (HE) or TRAP, as previously described.[19] Images of each section were captured using light microscopy (Olympus BX50; Olympus, Tokyo, Japan) and analyzed with micro-imaging software (Olympus cellSens Dimension, version 1.6).

Four sections at level 150, 200, 250 and 300 µm were stained with TRAP. The TRAP-positive osteoclast areas were assessed under range of color threshold in a region of interest (ROI) 600 x 1200 pixel^2 under 100x magnification and calculated as percentage of TRAP$^+$ osteoclasts areas.

Three sections at level 155 and 255 and 355 µm were stained with HE. Histomorphometric analysis was evaluated according to Dibart and colleagues.[20] A pentagon-shaped ROI was created under 40x magnification. Each angle was determined by the center of the five roots. The total amount of bone remaining in ROI was counted in pixel and calculated as percentage of inter-radicular bone area.

Statistical analyses

A one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons was used to determine whether there were inter-group difference of OTM distance on day 14, the TRAP-positive osteoclast areas and the intra-radicular bone areas. Due to the homogeneity of variance was not assumed, Welch's test was used to determine whether there were inter-group difference of OTM distance on day 7. The significance level was set at $P<0.05$.

Results

During experimental period, animals were health with gradually increasing body weight (data not shown). All orthodontic close coil springs remained in the appropriated position.

Amount of tooth movement

High dietary vitamin C supplement provided more effect in orthodontic tooth movement in an animal model. At day 7, distance of tooth movement in was highest in C1,000 group with 0.27±0.09 mm (mean±SD) among three groups. The gaps in control and C500 groups were 0.21±0.06 mm and 0.18±0.05 mm, respectively. Although it is a trend that the distance in C1,000 group was greater than the other two, there is no statistically difference($P>0.05$). At day 14, the mean distances of tooth movement were increased as a dose dependent manner. The highest amount of tooth movement was also found in C1,000 group (0.53±0.14 mm) and significantly difference compared to control group (0.36±0.12 mm). However, there is no statistically significance both between C1,000 and C500 groups and C500 and control groups (Figure 2).

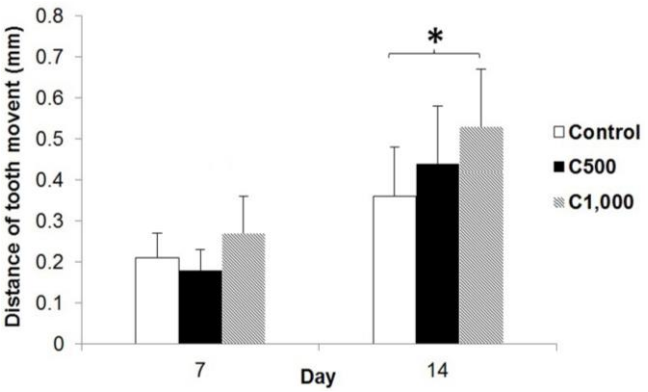

Figure 2. Distances of tooth movement on day 7 and 14 (n=10). Data shows as mean±S.D. Statistically significance is considered when P-value is less than 0.05 (*$P<0.05$).

TRAP-positive areas

TRAP staining was used to detect TRAP-positive areas on pressure side during OTM (Figure 3a). The TRAP-positive areas were increased in both vitamin C supplement groups (C500 = 0.81±0.71%, C1,000 = 0.76±0.69%) compared to the control group (0.62±0.43%). However, there were no statistically differences among those three groups (Figure 3b).

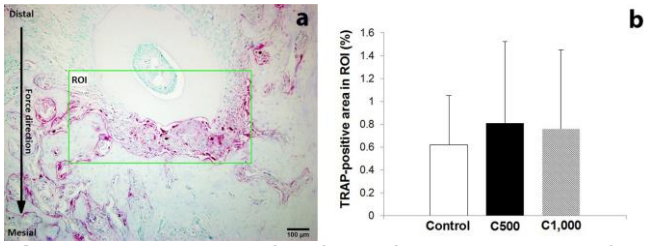

Figure 3. TRAP-stained section at compression side on day 14. (a) TRAP staining (red color) on the horizontal section of distobuccal root of the maxillary right first molar. Measurement of the TRAP-positive area only in the ROI (a green rectangular), black arrow indicating direction of force. (b) Quantitative of TRAP-positive areas (n=10), data presenting in mean±S.D.

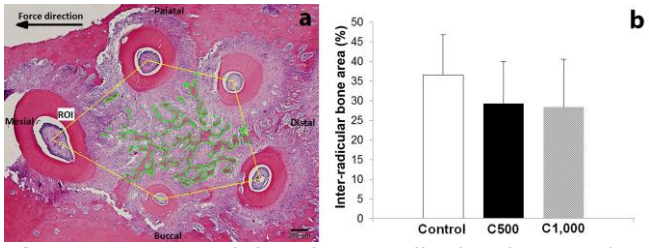

Figure 4. Remaining inter-radicular bone after force application on day 14. (a) HE staining on the maxillary right first molar shows inter-radicular bone areas traced with green line in the ROI (a yellow pentagon), black arrow indicating direction of force. (b) Quantitative analysis of inter-radicular bone (n=10), data presenting in mean±S.D.

Inter-radicular bone area changes

Alveolar bone remaining after 14-day of tooth movement was assessed as inter-radicular bone area by histomorphometric method (Figure 4a). Percentage of inter-radicular bone in certain ROI areas was maximum in control group (36.60±10.18%). The bone proportion was gradually decreased when animal received greater vitamin C amount (C500 = 29.31±10.71%, C1,000 = 28.35±12.18%). However, no statistically significance was observed among the groups (Figure 4b).

Discussion

In this study, we demonstrated that vitamin C supplement could increase rate of OTM when it reached to the optimal level. Vitamin C has been tested for accelerating rate of OTM for long time, but its effect is still controversy. Miresmaeili and coworkers reported that 1 wt% oral vitamin C supplement (about 1,000 mg/kg/day) could enhance OTM in rats,[6] but McCanlies and colleagues showed amount of OTM was slightly increased but was not different when animals consumed 0.2 and 1 mg per day of vitamin C.[14] Our study showed that OTM enhancement was observed in both rats obtaining two different vitamin C concentrations with significant improvement in C1,000 group at day 14. Thus, we assumed that effect of vitamin C on OTM depended on its concentration. When it reached sufficient vitamin C level, increased OTM distance was clearly observed in this animal study.

It has been accepted that tooth movement occurs in a postlag phase which is a result from alveolar bone resorption.[21] Osteoclasts play an important role in bone resorption processes and TRAP expressed by the cells is a key biomarker to detect osteoclast profile.[22] Hence, TRAP staining was applied in this study and our data found the TRAP-positive areas were no statistically significance. The intensity of the TRAP-positive areas reached the maximum level in C500 group. This data was not the same aspect observed in the amount of tooth movement, and also was unrelated results from the previous study in 2015.[6] King and colleagues reported that osteoclast numbers reached their peak level at 5 days after force application and decreased afterward,[23] However, another study in the same animal model demonstrated the matching expression timing of osteoclast features seen in our experiment.[24] In addition, it has been reported that the effect of vitamin C on osteoclastogenesis depended on its concentration.[12, 25] Since results from C500 group showed the highest osteoclast expression level, it could be that the cells were activated and finished their functions by the time of the experiment termination (day 14). Conversely, in the same period of time, it was possible that osteoclast activity in C1,000 group has already faded out after the cells finished their functions. Thus, a proper time period for osteoclast profile

investigation would be included in future study to has better point of view of vitamin C effects on osteoclast activity.

Inter-radicular bone can be used as a representative feature of alveolar bone change during OTM.[20] Considering that no remarkable difference of the inter-radicular bone in this study was detected, it was probable that widely bone formation during remodeling processes occurred. A recent study which investigated the effects of vitamin C concentrations on bone microstructure in ovariectomized Wistar rats presented that dietary vitamin C supplement could stimulate osteoblastogenesis but inhibit osteoclastogenesis.[26] Nonetheless, our results revealed bone reduction tendency in the same manner with osteoclast profile in vitamin C treatment groups. Although, the bone reduction data was relevant to OTM and osteoclast function in this study, bone formation occurred in the same remodeling process which could be a reason why results between three groups were not statistically difference.

Despite the fact that optimal levels of vitamin C to accelerate OTM and particular biological effects of vitamin C on OTM remain unclear, it is feasible to apply dietary vitamin C in clinical use to accelerate OTM because of low toxicity and commercial availability of the vitamin. Thus, a clinical evaluation of the vitamin C effect on OTM would be provide not only essential information about tooth acceleration, but also other aspects of the treatment such as time and complications.

Conclusions

1) Vitamin C supplement at a 1,000 mg/day dose could increase distance of OTM in Wistar rats.
2) At day 14 after orthodontic force application, osteoclasts and inter-radicular bone are no remarkable different in animals getting dietary vitamin C supplement.

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Declaration of Interest

The authors report no conflict of interest.

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