Effects of dietary L-arginine on orthodontic tooth movement in rats

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The purpose of this study was to investigate the effects of dietary L-arginine as nitric oxide (NO) precursor on orthodontic tooth movement in rats. 36 male ten-week old Wistar rats were randomly divided into experimental and control groups. The experimental group received 2% (w/w) dietary L-arginine in drinking water six days before the insertion of springs to elevate their blood level. On the seventh day, in both groups, maxillary incisors was moved by the insertion of springs and 12 days after insertion of springs, the rats were sacrificed, then the mesioincisal distance between maxillary incisors was measured. Afterwards, 12 and six rats from both groups were selected randomly for preparing histological section to count osteoclasts under a light microscope and for examining the surface area of root resorption lacunae under a scanning electron microscope, respectively.

The data on the extent of orthodontic tooth movement and the number of osteoclasts were analyzed by independent sample t test and findings on root resorption were analyzed by using Mann-Whitney U test. The results showed that in L-arginine group, the orthodontic tooth movement (p < 0.001) and the number of osteoclasts (p < 0.05) were significantly higher when compared with the control group. However, there was no significant difference between the two groups in terms of the surface area of resorption lacunae.

Key words: L-Arginine, dietary, orthodontic tooth movement, nitric oxide, root resorption, osteoclast, nitric oxide synthase (NOS).

INTRODUCTION

An increase in the rate of orthodontic tooth movement (OTM) without observable damage to the tooth supporting structures can shorten the active treatment period. The OTM is mediated through a slow bone remodeling process. Osteoblasts and osteoclasts have a great role in this process. Shortening the duration of orthodontic treatment is a primary goal for the majority of the researchers involved in this field (Yamasaki et al., 1989).

Various factors and mediators are involved in the remodeling process, retarding or accelerating tooth movement, depending on the factor involved. An important signaling molecule which has recently been implicated in orthodontic tooth movement is nitric oxide (NO), which is derived from the amino acid L-arginine by the nitric oxide synthase (NOS) enzyme in the human body (Marletta, 1993). Nilforoushan and Mansolon (2009) also showed that all NOS isoforms are involved in OTM with different expression patterns between tension and pressure sides, with nNOS being more involved in early OTM events. Both endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) seem to be important regulators of bone remodeling during orthodontic force application (Tan et al., 2009). NO is a short-lived, soluble, free radical gas produced by a variety of cells and capable of mediating a number of various function. The short half-life of NO indicates that its effects are regulated primarily by the rate of synthesis (Mitchell and Cotran, 2003).

The main activity of L-arginine in the human body is mediated through the production of NO molecule. L-arginine/NO pathway is involved in various biological activities in the cardiovascular, nervous, immune and...
other systems in the human body (Calver et al., 1993). NO is an important role in inflammatory processes (Roberts et al., 2004), regulating the remodeling and turnover processes of bone (Ralston et al., 1994, 1995; Jung et al., 2003). Prescribing L-arginine for human and laboratory animals has different influences such as reducing stress (Gupta et al., 1995; Wu et al., 2010), lymphocyte activation (Ochoa et al., 2001; Tan et al., 2009), increases pregnancy chances in women and animal (Battaglia et al., 1999; Liu et al., 2011; Ren et al., 2011), enhances intestinal development and expression of vascular endothelial growth factor in weanling piglets (Yao et al., 2011), retardation of atherosclerosis progression in rabbits (Boger et al., 1997), decreases human blood pressure (Siani et al., 2000) and protects against nervous damage in rats (Sharma et al., 2005). Dietary L-arginine supplementation appears to affect the metabolism of lipoproteins and might alleviate some gastrointestinal functions, commonly seen in diabetes mellitus (Miguez et al., 2004).

Local injection of L-arginine and also nitro-L-arginine as NOS precursor adjacent to teeth in rats increases tooth movement and the number of osteoclasts, which is accomplished through NO synthesis and subsequent increase in cGMP level (Shirazi et al., 2002; Akin et al., 2004). L-arginine is a non-essential amino acid and is under the influence of dietary intake. Under certain catabolic conditions such as trauma, infection and burns, it is an essential amino acid (Sy et al., 2006). After the dietary intake of this amino acid in laboratory animals, its blood level reaches the maximum after three days and after three weeks, its blood level is still higher than that in the control group (Jeremy et al., 1996). Dietary intake of L-arginine at a dose of 1.25 to 5% (w/w) in drinking water is not toxic for rats (Tsubuku et al., 2004).

Considering the fact that the local injection of L-arginine accelerates tooth movement and considering the importance of L-arginine/NO pathway in the orthodontic movement of teeth, this study was carried out to evaluate the effects of dietary L-arginine on the rate of orthodontic tooth movement, the number of osteoclasts in the area under pressure and root resorption in rats.

MATERIALS AND METHODS

36 approximately ten-week-old male Wistar rats with an average weight of 200 ± 22 g were selected and randomly divided into the control and L-arginine groups. To adapt the rats to the environmental conditions, they were kept in standard 12 h light and dark intervals at 24°C for a week.

The rats in both the experimental and control groups received drinking water and standard laboratory food ad libitum during the study period. The drinking water in the experimental group contained 2% (w/w) L-arginine as a solute (Jeremy et al., 1996; Boger et al., 1997; Miguez et al., 2004). To elevate the blood level of L-arginine, the experimental group had access to this kind of drinking water six days before the placement of the tooth-moving springs until the day when the rats were sacrificed (12 days after spring placement). The rats were monitored during the study and were weighed at the beginning and at the end of the study.

On the 7th day, the tooth-moving spring were placed on the maxillary incisors in both the experimental and control groups. Holes were prepared on maxillary incisors, and 30 g of reciprocal force was applied to the teeth with a spring bent from 0.35-mm stainless steel wire, modified with respect to Akin et al. (2004). The springs were placed on a grid and activated on a single arm with a plier. The force was measured with a gauge, and the springs were not reactivated during the experiment. Prior to spring placement, the rats were anesthetized using 85 mg/kg ketamine (Alfasan, Woerden, Holland) in a peritoneal procedure. After being sure of anesthesia depth and fixing the animals, a special device that is specifically designed for this study was used to open the mouths of the rats and the springs were place (Figure 1).

Considering the results of the previous studies, 30 g force was used to move the maxillary incisors of the rats (King et al., 1991; Akin et al., 2004). This amount of force is less than 90 g force used to open the maxillary incisors in rats (Chang et al., 1997). 12 days after spring placement, the rats were sacrificed with an overdose of the anesthetic agent. Then, the distance between the mesiodiscal line angles of maxillary incisors in both the control and the L-arginine group was measured using digital calipers, with a measuring accuracy of 0.01 mm (Figure 2). Subsequent to measuring the amount of orthodontic movement, 12 rats were randomly chosen from each group and their premaxillae were removed along with the incisor teeth and placed in 10% formalin for four days for fixation so that the number of osteoclasts could subsequently be counted. Then, the specimens were placed in 10% nitric acid for two days for decalcification. After decalcification, the specimens were rinsed for 2 h and again placed in 10% formalin for three days for fixation. Finally, the specimens were placed in special molds in a specific direction. The specimens were finally placed in paraffin molds and were ready to be sectioned. The specimens were sectioned serially by a microtome at 5 µm thicknesses perpendicular to the long axis of the incisor teeth in the bone. Five serial sections were prepared at 5 µm thicknesses below the alveolar crest.

The specimens were stained with Hematoxylin and Eosin. To standardize the area for osteoclastic count, a tangential line was drawn on the histological plate on the upper border of the teeth (toward the nasal cavity) and perpendicular to the middle septum. Another line was drawn parallel to the line 2 mm away from that on the lower side of the teeth. The third line was drawn tangential to the external border of the alveolar and perpendicular to the two earlier mentioned lines. The fourth line was drawn 1 mm away from the third line and parallel to it on the nasal septum side. This way, we had a 2 mm² surface area on the external side of the alveolus for each incisor tooth, which added up to 4 mm² of the alveolar surface on both sides for osteoclastic count (Figure 3).

Therefore in each rat, five sections and in each section the distance of the right and left incisors were evaluated. On the whole, ten pressure areas with a total surface area of 20 mm² were measured and evaluated in each rat. Osteoclastic count was carried out only on the internal surface of the alveolus, which was under pressure, and resorption lacunae with multinuclear cells were considered as osteoclasts. Two histologists counted the osteoclasts twice at different times under a light microscope.

The incisors in the six remaining rats from each group, which had not been histological studied, were prepared to evaluate the resorption lacunae under a scanning electron microscope. The images of the scanned specimens were stored at 30x magnification and then the surface areas of resorption lacunae were calculated in mm² using AutoCAD software and the results were compared between the experimental and control groups (Figure 4). To evaluate the normal distribution of data, one sample Kolmogorov-Smirnov analysis was used. Levene’s test was used to evaluate the
Figure 1. Device for fixation and opening of rat’s mouth.

homogeneity of variances.

Finally, independent sample t test was used to compare the rate of tooth movement, the number of osteoclasts and the weight of the animals before and after the experiment between the two groups. Mann-Whitney U test was used to compare the surface area of the resorption lacunae on the root surface which was under pressure (the average of resorption lacunae at distal surface of the left and right roots in each specimen).

RESULTS

The average weight of the rats prior to the experiment was 200 ± 22 g. There was no significant difference between the weights of the rats before the experiment or after the experiment between two groups.

The finding of the OTM in the L-arginine and control groups is presented in Table 1. As shown in Table 1, the rate of the OTM in L-arginine group exceeds that in the control group and the difference is statistically significant (P < 0.001), and also, the number of osteoclasts in L-arginine group was more than that in the control group and the difference was statistically significant (P < 0.05).

Scanning electron microscope evaluation of the maxillary incisors in rats in both groups to estimate the extent of root resorption revealed that the mean surface area of resorption lacunae difference between two groups was not statistically significant (Table 2).

DISCUSSION

In animals, arginine improves protein synthesis, microvascular development, immune function, antioxidant activity and cell proliferation by regulating some intracellular signaling pathway (Yin and Tan, 2010).

Various factors and mediators including prostaglandins, cAMP, interleukin 1-beta and neurotransmitters are involved in the orthodontic tooth movement (Davidovitch et al., 1975; Shirazi et al., 2002). A mediator which has recently attracted attention as being involved in orthodontic tooth movement and bone remodeling is NO, which is derived from the amino acid L-arginine in the human body by the action of the enzyme NOS (Ralston et al., 1995). This molecule is a key messenger involved in the synthesis of the cyclic guanosine monophosphate (cGMP) (MacIntyre et al., 1991; Hsu et al., 2003), subsequently increasing cellular activity and osteoclasts recruitment. NO can have a direct role in the activation and synthesis of other messengers such as prostaglandin E2 (PGE$_2$). Under mechanical stress, human fibroblasts and osseous cells demonstrate an increase in NO synthesis (Pitsillides et al., 1995; Nakago-Matsuo et al., 2000).

The impact of various factors on the orthodontic movement of the teeth in rats five to 14 days after the
application of force has been investigated in various studies and it has been demonstrated that the difference in tooth movement between experimental and control groups is more significant from day seven onward (Yamasaki et al., 1984; Engstron et al., 1988; Kale et al., 2004; Akin et al., 2004).

It is shown in this study that 12 days after force application, the rate of orthodontic tooth movement in oral L-arginine group was 1.6 times more than that in the control group (P < 0.001). It was concluded that dietary L-arginine accelerates orthodontic tooth movement in rats which is similar to local L-arginine as Shirazi et al. (2002) demonstrated in a research study. Hayashi et al. (2002) demonstrated a decrease in the OTM in group receiving NOS inhibitor as compared to the control group. Akin et al. (2004) in their study showed an increase in the tooth movement by the use of nitro-L-arginine as NOS precursor.

Movement of the teeth during orthodontic treatment involves osteoclastic resorption of the alveolar bone around the tooth root in the direction of the movement. Recruitment of osteoclasts and an increase in their number is considered as an important factor in the evaluation of the rate of orthodontic movement (Kaku et al., 2001; Shirazi et al., 2002; Akin et al., 2004; Mavragani et al., 2005). In addition, the maximum osteoclastic activity and concentration during orthodontic force application is usually observed five to seven days after force application (Tanne et al., 1998; Ren et al., 2005). In this study, the premolars of 12 rats, which contained the central incisors, were prepared for osteoclastic count under a light microscope. The results demonstrate that the number of osteoclasts in L-arginine group was more than that in the control group (p < 0.05). This finding is consistent with the results of a study carried out by Akin et al. (2004) and Shirazi et al. (2002).

Considering the results of this study, the difference in the number of osteoclasts and the rate of orthodontic tooth movement can only be attributed to the presence of L-arginine in the drinking water in the experimental group.

Resorption is a cell-mediated process; various mediators and messengers are involved in the recruitment and differentiation of the cells in the periodontal ligament (PDL) during orthodontic tooth movement. Therefore it is logical to believe that cell-mediated factors in tooth movement can also influence root resorption (Rygh et al., 1986; Leiker et al., 1995).

The result demonstrate that the difference in root
Figure 3. Area of osteoclasts count.

Figure 4. SEM view of root and resorption lacunae.
resorption between two groups was not statistically significant. This finding is contrary to the finding of the study carried out by Shirazi et al. (2002), in which root resorption was less in L-arginine group as compared to the control group.

The relationship between NO and OTM was shown in previous studies (Shirazi et al., 2002; Hayashi et al., 2002; Akin et al., 2004). In light of these studies and our findings, it can be said that NO and L-arginine as NO precursor increases the number of osteoclasts in compression site and result to increase in the rate of orthodontic tooth movement.

### Conclusion

Adding 2% (w/w) L-arginine to the drinking water of rats during orthodontic movement of the teeth leads to the following results:

1) Increase in orthodontic tooth movement.
2) Increase in the number of osteoclasts around the tooth in the movement direction in rats.
3) No significant increase in root resorption during orthodontic tooth movement in rats.

**REFERENCES**


