SHORT COMMUNICATION

Involvement of nitric oxide in orthodontic tooth movement in rats

Keigo Hayashi, DDS,[a] Kaoru Igarashi, DDS, PhD,[b] Kotaro Miyoshi, DDS, PhD,[c] Hisashi Shinoda, DDS, PhD,[d] and Hideo Mitani, DDS, MS, PhD[e]
Akita and Sendai, Japan

Nitric oxide (NO) is an important regulatory molecule in bone formation and resorption. The purpose of this study was to examine the role of NO in orthodontic tooth movement in rats. We used specific inhibitors of NO synthases (NOS). Upper first molars of 9-week-old male Wistar rats were moved buccally for 21 days. The local administration of N^G-nitro-L-arginine methyl ester · HCl (L-NAME), a general inhibitor of NOS activity, significantly reduced tooth movement. On the other hand, N^6-(1-iminoethyl)-L-lysine · 2HCl (L-NIL), a selective inhibitor of the inducible isoform of NOS, had no effect. These results suggest that NO is an important biochemical mediator in the response of periodontal tissue to orthodontic force and is produced primarily through the activity of constitutive NOS. (Am J Orthod Dentofacial Orthop 2002;122:306-9)

Orthodontic tooth movement results from the response of periodontal tissue to orthodontic force, which leads to modeling and remodeling of the surrounding alveolar bone. These responses are considered to occur through the activation of specific signaling pathways. Although several paracrine-autocrine mediators, including neurotransmitters,[1,2] cytokines,[3-5] and arachidonate metabolites,[3,6-8] have been suggested to be involved, the signal transduction pathways of orthodontic mechanical stimuli are not yet clear. Identification of such pathways might lead to a pharmacologic intervention to control the rate of orthodontic tooth movement.

Nitric oxide (NO) is a short-lived free radical that is involved in cardiovascular homeostasis, neurotransmission, and immune function. NO is also an important regulatory molecule in bone formation and resorption.[9] Previous studies have shown that NO production is necessary for bone responses to mechanical stimulation.[10-12] However, it is not known whether NO is involved in the response of periodontal tissue to orthodontic force. The purpose of the current study was to examine the role of NO in orthodontic tooth movement with specific inhibitors of NO synthases (NOS).

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[a]Research Fellow, Division of Orthodontics, Department of Life-Long Oral Health Science, Tohoku University Graduate School of Dentistry.
[b]Assistant Professor, Division of Orthodontics, Department of Life-Long Oral Health Science, Tohoku University Graduate School of Dentistry.
[c]Instructor, Department of Oral Surgery, Akita University Medical School.
[d]Professor, Division of Pharmacology, Department of Oral Biology, Tohoku University Graduate School of Dentistry.
[e]Vice President, Tohoku University; Professor, Division of Orthodontics, Department of Life-Long Oral Health Science, Tohoku University Graduate School of Dentistry.
Reprint requests to: Kaoru Igarashi, Division of Orthodontics, Department of Life-Long Oral Health Science, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan; e-mail: igarashi@mail.cc.tohoku.ac.jp.
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MATERIAL AND METHODS

Twenty-two male Wistar rats, 9 weeks old, were used. Experiments were performed according to the methods of our previous study.[13] Briefly, both the right and left upper first molars of the animals were moved buccally with a standardized expansion spring, which was made of 0.012-inch nickel-titanium wire and initially generated an average force of 165 millinewtons on each side. The expansive force was applied without adjustment for 21 days. Fifty microliters of treatment solution were injected into the subperiosteum area adjacent to the left upper first molar every 3 days during the experimental period. N^G-nitro-L-arginine methyl ester · HCl (L-NAME) (Calbiochem, San Diego, Calif), a general inhibitor of NOS, at concentrations of 1 mg/mL and 10 mg/mL, and N^6-(1-iminoethyl)-L-lysine · 2HCl (L-NIL) (Sigma, St Louis, Mo), a selective inhibitor of inducible NOS, at concentrations of 1 mg/mL and 5 mg/mL, were used in this study. These compounds were dissolved in 0.9% NaCl solution (saline). The right first molar served as a control with an injection of only saline. The general condition of each animal was monitored during the experimental period. Animals that had been injected with only saline, L-NAME (10 mg/mL), or L-NIL (5 mg/mL) were weighed every third day. Treatment of the animals was in accordance with the guidelines for the use of experi-

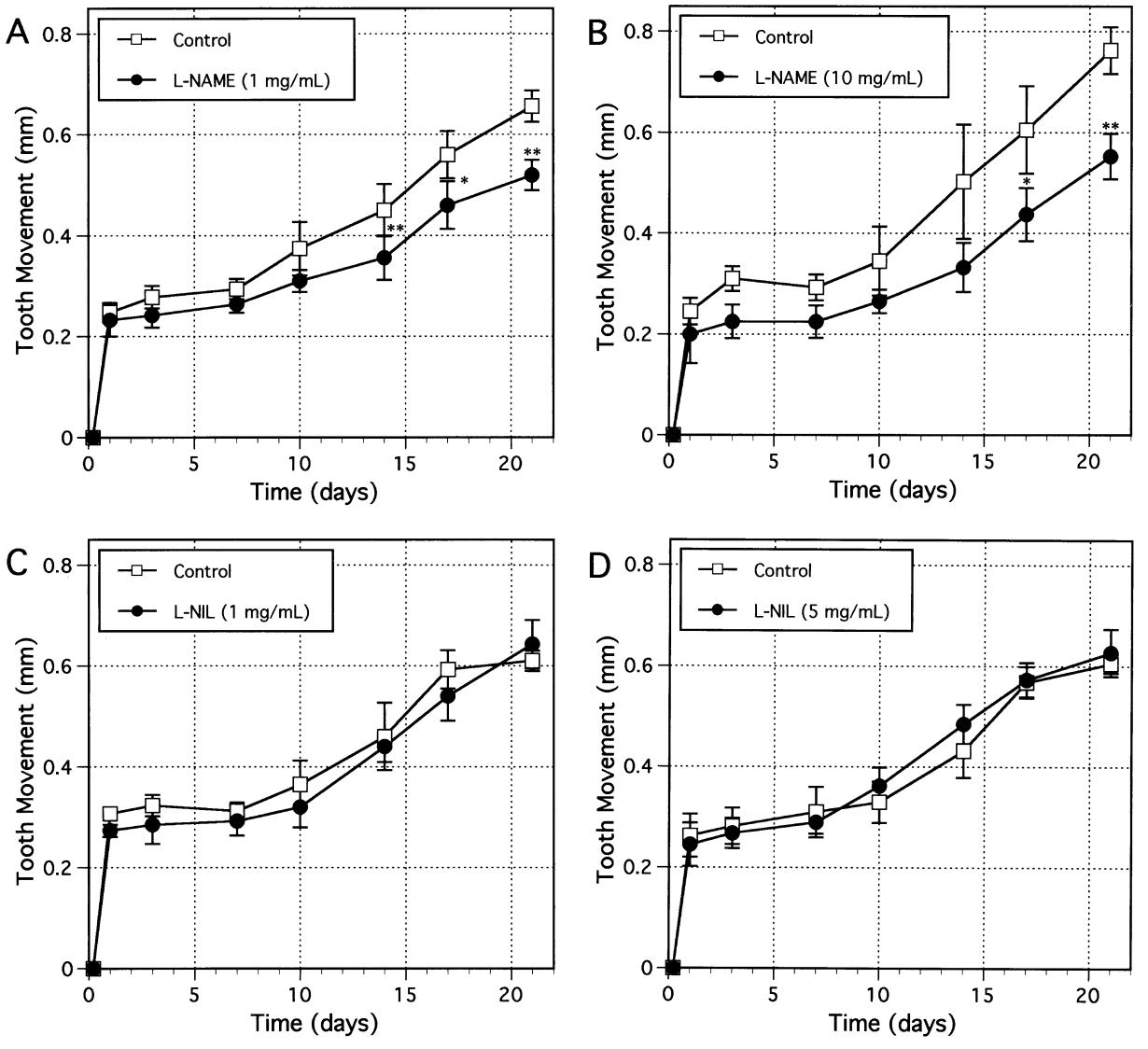

Fig. Time course of tooth movement in animals injected with NOS inhibitors. Each point represents mean ± SEM (n = 4 or 5). Two-way ANOVA indicated that time-related changes in tooth movement were highly significant ($P < .001$) in all 4 experiments. **A**, L-NAME (1 mg/mL). Effect of treatment was highly significant ($P < .001$ by 2-way ANOVA). Tooth movement on treated side was significantly less than that on control side on days 14, 17, and 21. **B**, L-NAME (10 mg/mL). Effect of treatment was highly significant ($P < .001$ by 2-way ANOVA). Tooth movement on treated side was significantly less than that on control side on days 17 and 21. *$P < .05$ and **$P < .01$ vs control side by paired *t* test. **C**, L-NIL (1 mg/mL). **D**, L-NIL (5 mg/mL). Local administration of L-NIL did not affect tooth movement.

mental animals of the Animal Care and Use Committee of Tohoku University Graduate School of Dentistry.

Movement of the upper first molars was measured on days 0, 1, 3, 7, 10, 14, 17, and 21 after the application of force. A tracing of the occlusal view of a precise plaster model of the right and left upper jaw was magnified 10 times with a profile projector. The contours of the palatal cusps of the second and third molars of these tracings were then superimposed on those of the second and third molars on tracings from a pretreatment plaster model. The distance between the crests of the mesiopalatal cusps of the first molars before and after tooth movement was measured with sliding calipers. The method has been previously described in detail.[14]

Data were subjected to 2-way analysis of variance (ANOVA). The paired *t* test was used to evaluate the significance of differences in tooth movement between the treated side and the contralateral control side.

RESULTS

During the course of the experiment, there were no differences in weight gain between animals that had been injected with only saline and those injected with either of the NOS inhibitors at higher concentrations. The mean weight gain plus or minus SEM for 21 days was 38.4 ± 3.4 g, 38.0 ± 3.8 g, and 37.5 ± 2.2 g in animals injected with saline, L-NAME (10 mg/mL), and L-NIL (5 mg/mL), respectively.

In saline-injected control animals, there was no difference between the movement of the left molar and the right molar. The average tooth movement (mean ± SEM) for 21 days in these animals was 0.53 ± 0.06 mm on the left side and 0.52 ± 0.05 mm on the right side. The Figure shows the time course of tooth movement in animals injected with L-NAME at concentrations of 1 mg/mL (*A*) and 10 mg/mL (*B*) and those injected with L-NIL at concentrations of 1 mg/mL (*C*) and 5 mg/mL (*D*). Tooth movement on both the control and treated sides in these animals exhibited 3 typical phases, ie, a phase of rapid movement within 1 day, a lag phase lasting for several days, and a phase of progressive movement. In L-NAME-injected animals, there was a significant reduction in tooth movement on the treated side compared with that on the contralateral control side on days 14 (at a concentration of 1 mg/mL), 17, and 21. The percent of control value (mean ± SEM) on day 21 was 79.4% ± 3.8% at a concentration of 1 mg/mL and 72.4% ± 3.9% at a concentration of 10 mg/mL. On the other hand, there was no difference in tooth movement in L-NIL-injected animals between the treated side and the control side throughout the experimental period.

DISCUSSION

The present results clearly demonstrate for the first time that NO production in the local environment is necessary for the maximum response of periodontal tissue to orthodontic mechanical stimulation in rats. The local administration of L-NAME caused a significant reduction in tooth movement. Because the initial tooth movement within 1 day was considered to be due to compression of the periodontal ligament,[15] the net tooth movement caused by bone responses could be estimated to be 67.3% and 58.7% of the control at concentrations of 1 mg/mL and 10 mg/mL, respectively. Thus, NO apparently played an important role in orthodontic tooth movement.

NO can be produced by the oxidation of L-arginine by any of 3 distinct isoforms of NOS: a neuronal form (nNOS), an endothelial form (eNOS), or an inducible form (iNOS). Both nNOS and eNOS are constitutively expressed and are collectively referred to as constitutive NOS enzymes (cNOS). In the present study, we used L-NAME, a general inhibitor of NOS, and L-NIL, a selective inhibitor of iNOS, which exhibits a 20-fold to 30-fold selectivity over cNOS.[16] In contrast to the considerable inhibitory effect of L-NAME, the local administration of L-NIL did not affect orthodontic tooth movement. This suggests that cNOS, but not iNOS, plays a role in the response of periodontal tissue to orthodontic force in rats. Previous studies have shown that eNOS acts as the major NOS isoform that regulates NO production in bone.[17-19] Recent in vitro studies have revealed that cultured human periodontal cells can produce NO in response to cyclic tension force through the activation of eNOS.[20,21] Together with these findings, our results suggest that eNOS might act as the major NOS isoform that regulates NO production in periodontal tissue in response to orthodontic mechanical stimuli. Further studies with histochemical approaches such as immunohistochemistry or in situ hybridization are necessary to identify the responsible isozyme(s).

CONCLUSIONS

The present results suggest that NO is an important biochemical mediator in the response of periodontal tissue to orthodontic force and is produced primarily through the activity of cNOS.

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