ORIGINAL ARTICLE

Effect of misoprostol, a prostaglandin E_1 analog, on orthodontic tooth movement in rats

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The purpose of this study was to investigate the effects of oral administration of misoprostol, a prostaglandin E_1 analog, on orthodontic tooth movement and root resorption in rats. Sixty-four male Sprague-Dawley rats that initially weighed 250 ± 20 g were used in this study. The animals were randomly assigned to 1 of 6 experimental and 2 control (nonappliance and appliance) groups. The experimental groups received 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 μg/kg misoprostol by gastric lavage every 24 hours for 2 weeks. A fixed orthodontic appliance consisting of a nickel-titanium closed-coil spring, 5.0 mm long was ligated between the maxillary right incisor and the maxillary right first molar. The initial activating force was 60 g. For analysis of root resorption, 99 maxillary right and left first molars from 61 animals were chosen. Serial histologic sections of the mesial root of the maxillary first molars were made, and histologic analysis of root resorption on the mesial and distal surfaces was performed. The results showed that oral misoprostol did increase the amount of orthodontic tooth movement in all the experimental groups compared with the appliance control group. This increase was statistically significant in doses of 10.0, 25.0, 50.0, and 100.0 μg/kg ($P < .001$). However, there were no statistically significant differences among these 4 different doses. There were no statistically significant differences in the amount of root resorption among the groups. However, a trend toward more root resorption was registered. On the basis of these findings, oral misoprostol can be used to enhance orthodontic tooth movement with minimal root resorption. (Am J Orthod Dentofacial Orthop 2002; 122:542-7)

Orthodontic tooth movement requires remodeling of periodontium, particularly the alveolar bone.[1-3] Bone remodeling, induced by orthodontic forces, has been suggested to be mediated by prostaglandins (PGs).[4,5] The PGs, which are synthesized and secreted by local cells in response to orthodontic mechanical stress, have been shown to stimulate the osteoclastic process of bone resorption.[6] PGE_1 and PGE_2 have been shown to stimulate bone[7-13] and root[14] resorption. However, the prevalence of root resorption varies among investigations.[14-16]

Local injection of PGE_1 or PGE_2 in humans,[17] monkeys,[5] and rats[15] has been reported to accelerate orthodontic tooth movement. A major disadvantage of the local administration of PG is pain at the site of injection,[11] which was shown to be alleviated by dissolving PG in dental lidocaine.[17] However, dental lidocaine solutions induce peripheral vasoconstriction at the site of administration and suppress local inflammatory reaction, which is necessary for bone resorption. In addition, local injection of PG is technically difficult. Some leakage occurs, and the full effect might not be achieved in all cases.[14] In comparison with local injection, systemic intravenous administration of PGE_1 has been shown to have a more marked effect on bone resorption.[11] However, rapid inactivation of PGE_1 in the lung and side effects such as local irritation and phlebitis are among the limitations of intravenous administration.[11]

Oral administration of a stable PGE_1 analog such as misoprostol, which is therapeutically available,[18] might be a simple method to increase orthodontic tooth movement without such side effects. In a study[19] of orally administered misoprostol (100 μg/kg twice daily), a significant increase in the degree and rate of orthodontic tooth movement was noted in guinea pigs. However, the effect of misoprostol on root resorption has not been reported. Because misoprostol in doses well below 100 μg/kg is pharmacologically active in rats,[20] the present study was designed to investigate whether lower doses of misoprostol can enhance orthodontic tooth movement with minimal root resorption.

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MATERIAL AND METHODS

This study used 64 male Sprague-Dawley rats of approximately the same age that initially weighed 250 ± 20 g. Six additional animals were used in a pilot study to evaluate the appliance design and placement. The 64 animals were randomly separated into 2 control groups (nonappliance and appliance) and 6 experimental groups.

The animals were exposed to the standard 12-hour light and dark cycles and were fed ground rat chow and water ad libitum. All the animals were first weighed and anesthetized with an intramuscular injection of ketamine (44 mg/kg body weight) and xylazine (2 mg/kg body weight) to complete the various procedures.[21]

Eight animals in the nonappliance control group did not receive any treatment for the 2-week period of the experiment. The animals were weighed and monitored during the course of the experiment and euthanized at the end of the experiment. The teeth were examined with light microscopy for root resorption patterns along the mesial root of the maxillary right first molar.

Eight animals in the appliance control group did not receive misoprostol. Each animal received 0.5 mL distilled water daily as vehicle by gastric lavage.

Forty-eight animals were divided into 6 experimental groups (8 rats for each group). These groups received different doses of misoprostol, ie, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 μg/kg, every 24 hours for 2 weeks. Serial concentration solutions of the drug were prepared, and each animal in the experimental groups received 0.5 mL of the relevant solution.

For induction of orthodontic tooth movement, the method of Leiker et al[15] was followed and will be described later. The appliance design was similar to that described by Bridges et al,[22] which was a nickel-titanium closed-coil spring (0.010 × 0.030-in), 5.0 mm long, ligated between the maxillary right first molar and the maxillary right incisor with 0.010-in ligature wire. The initial orthodontic force measured 60 g[23] and was not reactivated during the course of the study. This method was used for all the animals in appliance control and experimental groups.

Leiker et al[15] prepared a groove along the distolingual line angle of the maxillary first molar to prevent slippage of the ligature wire. However, in this study, we found that this groove was not necessary because the maxillary first molar had a bulge in approximately the gingival third of its crown, and when the ligature wire was tied, it was fixed under the bulge. Therefore, the ligature wire would not be displaced once the tooth started to move.

Misoprostol stock solution (10 μg/mL) was prepared by dissolving the compound (Misoprostol:HPMC dispersion [1:100]; G. D. Searle Co, Skokie, Ill) in distilled water. The stock solution was stored at 2°C to 8°C.

The first dose of misoprostol was administered 1 hour before placement of the appliance. All animals in the experimental groups received the drug by gastric lavage with a 1-mL disposable syringe and stainless steel feeding needle (perfectum size 13; Popper and Sons, Inc, New Hyde Park, NY) every day at 10:00 AM for 2 weeks.

During the study, the weight and general condition of all the animals and the status of the appliances were evaluated. At the end of experimental period, the animals were killed, and the maxillae were sectioned and prepared for analysis and evaluation. Tooth movement was determined by measuring the space created between the maxillary right first and second molars with a standard feeler gauge (Mitutyo Corp, Paramus, NJ) calibrated to 0.01-mm increments. The space was measured before the appliance was removed to prevent discrepancy in the tooth relapsing. Because the insertion of the interproximal gauges might cause an increase in the space between the first and second molars, ie, a wedging effect, intervention was necessary to prevent this effect. The distance between the mesial surface of the maxillary right first molar and the distal surface of the maxillary right third molar was measured with a digital caliper (resolution: 0.01 mm; code no. 500-320, model CD4; Mitutyo Corp, Japan), and care was taken not to increase the distance by feeler gauge insertion.

Block sections of the maxillae from the remaining animals were made, perfused, placed in 10% formalin for 10 days, and decalcified with 5% formic acid. The maxillary right and left first molars for each experimental group and the maxillary right first molar for each control group were chosen. In experimental animals, the maxillary left first molar was considered to evaluate the effect of misoprostol on root resorption without any orthodontic force. The sample in the present study included 107 maxillary right and left first molars. Eight of the 107 teeth were excluded from the study because of sectioning error, leaving a total sample of 99 teeth. Parasagittal sections of the mesiodistal aspect of the teeth were cut at 6-μm intervals and stained with hematoxylin and eosin.

Histologic analysis of root resorption on the mesial and distal surfaces of the mesial root of the maxillary first molars was performed. The mesial root was selected for several reasons.[15,16] First, it was by far the largest of the 5 roots on the maxillary first molars.

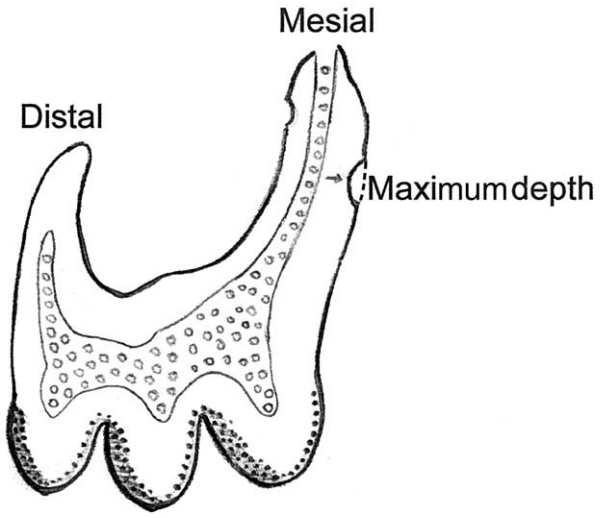

Fig 1. Method of measuring root resorption.

Therefore, quality serial histologic sections of the root could be obtained. Second, it was typically the 1 root that remained intact during dissection of the teeth from the alveolar bone. Third, the mesial root, which is located buccolingually in the middle of the tooth, was in approximately the same plane as the applied force. Fourth, this root has been examined in previous studies and therefore could be compared with findings from the previous investigations.

We measured the maximum depth of each resorption lacunae at the deepest point by using the distance from the bottom of the cavity tangent to the line passing the intact root surface from both sides of the cavity (Fig 1). Resorbed surfaces were measured directly by means of an ocular micrometer and a Zeiss microscope (475200-9901; Gttingen, Germany). Six sections per molar were prepared, and the mean value for each tooth was achieved by averaging them. The summed amount of linear surface resorption along the mesial and distal surfaces was recorded and evaluated statistically for differences between groups at a significance level of $P < .05$.

The error in measurements concerning the amount of tooth movement and root resorption were calculated by 1 examiner repeating the measurements in 12 randomly selected experimental animals. One-way ANOVA test was conducted to determine differences between the recordings and was not found to be significant for the amount of tooth movement or root resorption.

One-way ANOVA was conducted to determine differences in the amount of tooth movement among the groups, and the Student *t* test was performed to determine differences in the root resorption of all the experimental groups compared with the appliance control group.

Table I. Tooth movement in experimental and appliance control groups

Group	*No. animals*	*Mean (mm)*	*SD (mm)*	*Minimum (mm)*	*Maximum (mm)*
Appliance control	7	0.2629	0.1487	0.10	0.55
I (2.5 μg/kg)	6	0.2667	0.1239	0.10	0.40
II (5.0 μg/kg)	8	0.2675	0.1330	0.10	0.48
III* (10.0 μg/kg)	8	0.4788	0.2766	0.10	0.85
IV* (25.0 μg/kg)	8	0.5263	0.0595	0.45	0.60
V* (50.0 μg/kg)	8	0.5588	0.1156	0.40	0.75
VI* (100.0 μg/kg)	8	0.5738	0.1057	0.45	0.75

*Tooth movement in groups III, IV, V, and VI was significantly higher at $P < .001$ compared with appliance control group.

RESULTS

All groups receiving oral misoprostol had increased orthodontic tooth movement when compared with the appliance control group. This increase was statistically significant in doses of 10.0, 25.0, 50.0, and 100.0 μg/kg ($P < .001$). However, there were no statistically significant differences among these 4 different doses. Data are presented in Table I and Figure 2.

The data were evaluated as absolute root resorption in millimeters. A mean increase in the amount of root resorption in the experimental groups I, III, IV, V, and VI compared with the appliance control group was found. However, this increase was not statistically significant. No significant differences were observed on the maxillary left first molars in the different experimental groups when compared with the maxillary right first molars in the nonappliance control group. Therefore, misoprostol alone could have not caused significant root resorption. Mean root resorption in the experimental group III (10.0 μg/kg) was higher than the other groups, with a *P* value of .09, which was in the borderline range ($.05 < P < .1$).

The amount of root resorption along the distal surface of the mesial root of the maxillary first molar was less than the mesial surface in all the specimens, and these amounts were included in the data. Interestingly, some animals in the nonappliance control group also had some root resorption (Table II; Fig 3).

DISCUSSION

The results of this study showed that administration of oral misoprostol, a PGE_1 analog, enhanced orthodontic tooth movement with minimal root resorption in rats. The involvement of PGs in mediating orthodontic

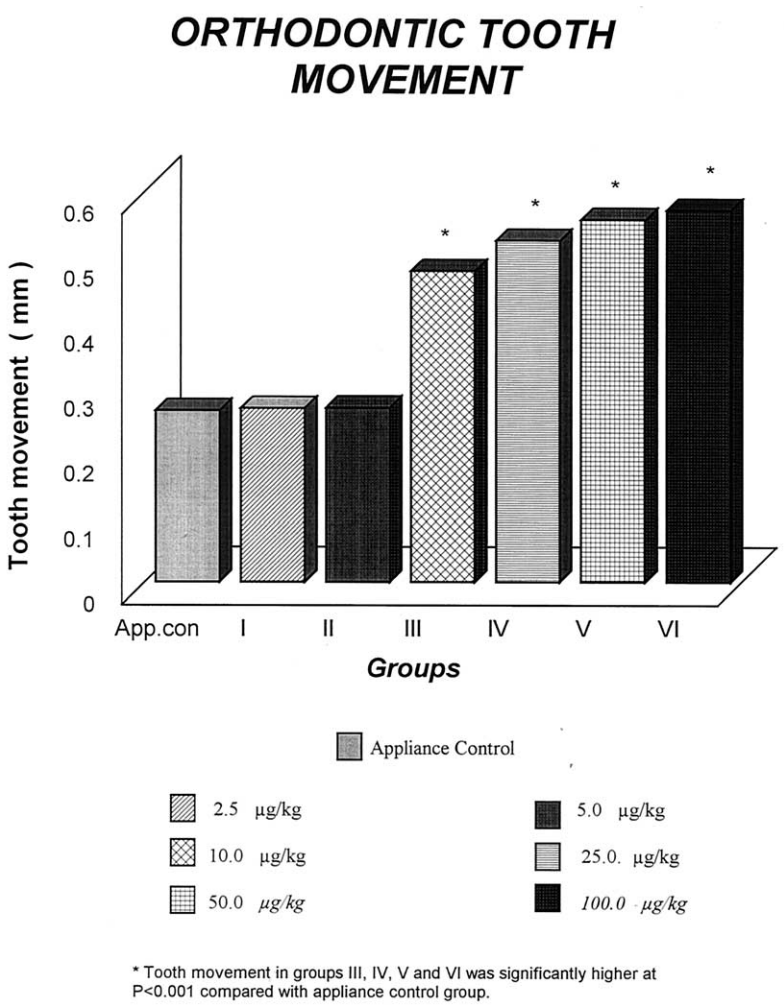

Fig 2. Mean tooth movement measurements.

tooth movement is well established.[24] An acceleration of orthodontic tooth movement in conjunction with exogenous PG administration has been reported previously[5,15,17,19] and is consistent with the results of the present study.

The local injection of PGs has drawbacks, such as pain and leakage of the drug at the site of injection. Systemic intravenous administration of PGs also has limitations, such as phlebitis and local irritation. Therefore, oral administration of a PG analog, such as misoprostol, might be a simple way to increase orthodontic tooth movement without encountering these limitations.

The results of the only study[19] in which the effect of misoprostol (100.0 µg/kg/12 h) on orthodontic tooth movement has been evaluated showed that the drug did significantly enhance the degree and rate of orthodontic tooth movement. There were fundamental differences

Table II. Summed amount of surface resorption along mesial and distal surfaces

Group	*No. teeth*	*Mean (mm)*	*SD (mm)*
Nonappliance control	8	0.0109	0.0124
Appliance control	7	0.0286	0.0225
I (2.5 µg/kg R)	6	0.0396	0.0320
I (2.5 µg/kg L)	6	0.0208	0.0246
II (5.0 µg/kg R)	7	0.0214	0.0213
II (5.0 µg/kg L)	7	0.0214	0.0213
III (10.0 µg/kg R)	8	0.0578	0.0377
III (10.0 µg/kg L)	8	0.0187	0.0188
IV (25.0 µg/kg R)	7	0.0500	0.0382
IV (25.0 µg/kg L)	7	0.0214	0.0224
V (50.0 µg/kg R)	7	0.0357	0.0292
V (50.0 µg/kg L)	7	0.0250	0.0280
VI (100.0 µg/kg R)	7	0.0536	0.0529
VI (100.0 µg/kg L)	7	0.0357	0.0429

R, Right; *L*, left.

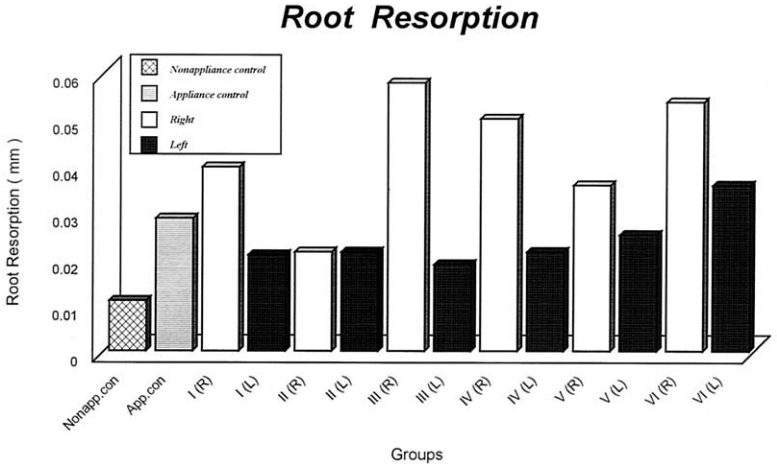

Fig 3. Mean root surface resorption (mesial and distal surfaces). *Group I*, 2.5 μg/kg; *group II*, 5.0 μg/kg; *group III*, 10.0 μg/kg; *group IV*, 25.0 μg/kg; *group V*, 50.0 μg/kg; *group VI*, 100.0 μg/kg.

between our study and that of Kehoe et al,[19] who used a single 0.016 titanium-molybdenum alloy spring to separate guinea pigs' maxillary incisors with an initial force of 25 g. In the present study, a nickel-titanium closed-coil spring with an initial force of 60 g for induction of orthodontic tooth movement in the maxillary first molar of rats was used, which is similar to other investigations.[15,16] Although 100.0 μg/kg/12 h misoprostol is well tolerated by and produces no side effects in guinea pigs,[19] lower doses of this drug are also pharmacologically active[20] and can be administered once a day in rats.[25] In addition, the rat is the most common animal model and has been used in previous investigations.[4,11,15,16,21]

Therefore, we decided to evaluate every 24 hours the effects of lower doses of this drug on the amount of orthodontic tooth movement and root resorption in rats. We found that the threshold dose of misoprostol necessary to increase orthodontic tooth movement was 10 μg/kg/d. However, there were no statistically significant differences among 10.0, 25.0, 50.0, and 100.0 μg/kg/d.

The effects of PGE_1 or its analogs on root resorption have not yet been reported. On the basis of our results, different doses of misoprostol in the experimental groups I, III, IV, V, and VI increased the amount of root resorption compared with the appliance control group. However, this increase was not statistically significant. Our finding is consistent with that of Brudvik and Rygh,[14] who used local injection of PGE_2 to evaluate root resorption. In other studies,[15,16] local injection of PGE_2 significantly increased root resorption, which is not consistent with the results of the present study.

In the present study, to evaluate root resorption, the maxillary right first molars in all the groups and the maxillary left first molars in all the experimental groups were considered. By choosing the maxillary left first molar, we were able to evaluate whether misoprostol alone can increase root resorption without any orthodontic force. Because mean root resorption did not increase significantly for the maxillary left first molars in all the experimental groups compared with the maxillary right first molars in the nonappliance control group, it was concluded that misoprostol accelerates orthodontic tooth movement without the untoward effect of enhanced root resorption.

The finding that the amount of root resorption along the distal surface of the mesial root of the maxillary first molar was less than that along the mesial surface is consistent with the results of the previous studies.[15,16] The amount of root resorption in the experimental group III (10.0 μg/kg) was more than the other groups. This finding could not be explained by the authors.

CONCLUSIONS

The following conclusions can be drawn from this study:

- A lower dose of misoprostol (10.0 or 25.0 μg/kg/d) appears to be as effective in enhancing orthodontic tooth movement as are the higher doses of 50.0 and 100.0 μg/kg/d used in this study or even 100.0 μg/kg/12 h, as used in previous investigations.
- In all doses used, misoprostol did not significantly increase the amount of root resorption. However, mean root resorption in the experimental group III

(10.0 μg/kg) was statistically in the borderline range ($.05 < P < .1$).

- The optimum dose of misoprostol to enhance orthodontic tooth movement with minimal root resorption in rats appears to be 25.0 μg/kg/d.

To confirm the effect of misoprostol on root resorption, scanning electron microscopic and quantitative histomorphometric analyses are suggested, and a clinical trial with oral misoprostol to facilitate orthodontic treatment is strongly recommended.

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