#### **Original Article**

# Radiographic evaluations of molar intrusion and changes with or without retention in rats

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#### ABSTRACT

**Objective:** To describe radiographic changes caused by molar intrusion with or without retention methods in rats.

**Materials and Methods:** Thirty 12-week-old male rats were assigned to six groups (n = 5 each). Molar intrusion was achieved with an intrusion spring to two maxillary molars for 2 weeks. The control group underwent the same experimental conditions, but without the intrusion spring. The intrusion and control groups were then euthanized. In four groups, the intrusion spring was disengaged after intrusion and the new molar positions were either retained or not with an occlusal bite-block for 1 or 2 weeks. Radiographic changes were measured in the cusp tip, root apices, and alveolar crests.

**Results:** After 2 weeks of intrusion, the cusp tip and root apices had moved apically compared with the control group. However, the alveolar crests were similar in the intrusion and control groups. With retention bite-block, the new position of the intruded cusp tip was maintained, but the root apices had moved occlusally, and the alveolar crest between the two intruded molars had moved apically. Without retention, the cusp tip and root apices had moved occlusally, and the alveolar crest between the intruded cusp tip was maintained, but the root apically. Without retention, the cusp tip and root apices had moved occlusally, and the alveolar crest between the intruded molars had moved occlusally compared with the intrusion group.

**Conclusion:** Rat molars were successfully intruded and maintained at the altered position with retention bite-block. However, the apical root resorption was observed as an instant response. The alveolar crest adjacent to the intruded molars was repositioned apically, but that was a delayed response compared to root resorption. (*Angle Orthod.* 2011;81:389–396.)

KEY WORDS: Molar intrusion; Retention; Miniscrew; Openbite

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#### INTRODUCTION

Intrusion of the molar may be different from that of an incisor, because the molar is a multiradicular tooth with a furcation area, and its occlusal table is under persistent vertical force, which affects its vertical position. Orthodontic treatment by molar intrusion has rarely been reported because clinical feasibility is low. However, the introduction of temporary anchorage devices (TADs) has made it possible to achieve intrusion for the correction of openbite without surgical intervention<sup>1,2</sup> and overerupted molars at edentulous sites.<sup>3</sup>

Previous reports that described the changes that occurred after molar intrusion with TADs were mainly performed in dogs.<sup>4–8</sup> Significant amounts of intrusion were obtained,<sup>4</sup> and small but significant amounts of root resorption occurred at the apices.<sup>5</sup> Daimaruya et al.<sup>6</sup> reported that the resorbed root apices were not

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	Age (Weeks)							
Groups	12	13	14	15	16	17		
Control (n = 5)	Implantation of TAD	Occlusal bonding material	Occlusal bonding material	Sacrifice	Sacrifice	Sacrifice		
Intrusion (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Sacrifice	Sacrifice	Sacrifice		
(+)Retention-1wk $(n = 5)$	Implantation of TAD	Intrusion spring	Intrusion spring	Bite-block for retention	Sacrifice	Sacrifice		
(+)Retention-2wk $(n = 5)$	Implantation of TAD	Intrusion spring	Intrusion spring	Bite-block for retention	Bite-block for retention	Sacrifice		
(-)Retention-1wk (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Without retention	Sacrifice	Sacrifice		
(-)Retention-2wk (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Without retention	Without retention	Sacrifice		

Table 1. Experimental Design<sup>a</sup>

<sup>a</sup> TAD, temporary anchorage device.

repaired, but the alveolar bone around the root apices was remodeled. No damage to the nerves or blood vessels was observed in the inferior alveolar neuro-vascular bundle.<sup>7</sup> Clinically, the alveolar bone height changed with marginal bone remodeling during molar intrusion, but this was not associated with any increase in gingival pocket depth after molar intrusion; however, the temporary formation of a gingival pocket was not observed.<sup>8</sup>

For any treatment modality, posttreatment stability is of great importance. Clinically, the average relapse rate after molar intrusion varies from 10.3% to 30.3%.<sup>9,10</sup> Both the intruded molar and the adjacent periodontal tissue that is remodeled after molar intrusion contribute to posttreatment stability. However, it remains unclear what changes occur after molar intrusion and whether these changes are different with different retention regimens. Therefore, the aim of this study was to investigate radiographic changes of the periodontal tissue after molar intrusion and its difference with or without retention in rats.

#### MATERIALS AND METHODS

#### Materials

Thirty 12-week-old male Sprague-Dawley rats, with an average weight of 220–250 g, were maintained in stainless-steel cages and subjected to the standard 12-hour light/dark cycle. Rats were fed a pellet diet and tap water ad libitum.

The animals were assigned to six groups (n = 5 rats each; Table 1). In all groups except the control, the left maxillary first and second molars (M1 and M2) were intruded with an intrusion spring for 2 weeks. The control group underwent the same experimental procedures, but no intrusion spring was used. The intrusion and control groups were then euthanized. In the (+)retention-1wk and (+)retention-2wk groups, intruded molars were retained with occlusal bite-block for 1 and 2 weeks, respectively, after intrusion. In the

(-)retention-1wk and (-)retention-2wk groups, animals were maintained without bite-block for 1 and 2 weeks, respectively, after intrusion.

#### **Experimental Procedures**

The animals were immobilized with ether inhalation and anesthetized with an intraperitoneal injection of Zoletil (Virbac, Carros, France) and Rompun (Bayer AG, Leverkusen, Germany).

A TAD (1.2-mm diameter, 7.0-mm long, BMK Inc, Seoul, Korea) was inserted into the alveolar crest behind the maxillary left incisor. An impression of the upper arch was taken with poly-vinylsiloxane (Aquasil Ultra, Dentsply, Pa), and a dental cast (New Plastone, GC Corp, Tokyo, Japan) was fabricated. The fabricated cast was used to ensure the proper positioning of a  $0.016 \times 0.022$  inch superelastic nickel-titanium (NiTi) alloy wire (L&H Titan, Tomy, Tokyo, Japan). The wire was bent with a heat bender,<sup>11</sup> which used direct electric resistance heat. The bend was calculated to transfer an intrusive force of 0.49 N<sup>12</sup> parallel to the long axis of the teeth (Figure 1).

One week after TAD implantation, the wire was attached to the TAD and the occlusal surfaces of the M1 and M2. The space between the wire and the neck of the TAD was filled with flowable resin (Esthet-X Flow, Dentsply, Pa). Glass ionomer cement (Band-Lok, Reliance, III) was used to attach the wire to the molars. The cement was also bonded to the left maxillary third molar (M3) and the three right maxillary molars to prevent extrusion (Figure 2).

The intrusion spring was removed after intrusion. In the (+) retention groups, the three maxillary molars on each side were splinted with bonding material to form an occlusal bite-block for retention. In the (-) retention groups, all appliances, except the TAD, were removed.

Throughout the experiment, the appliances were checked every day under ether inhalation. At the end of each experimental period, the animals were





**Figure 1.** A superelastic NiTi wire after bending with a heat bender. (Top, occlusal view) the hook at the end of the wire was designed to wrap around a TAD. The shaft would be fixed to the molars that required intrusion. (Bottom, lateral view) the wire was bent to ensure the correct spring force between the molars and the TAD.

euthanized by cervical dislocation under ether inhalation, and the intrusion and retentive appliances were disengaged.

#### Measurements

The maxilla was dissected free and divided with a sagittal cut along the midline. Each half was placed on an intraoral periapical X-ray film (Insight, Kodak, NY). X-ray exposure was performed with a portable intraoral radiographic apparatus (AnyRay, E-Woo Technology, Gyeonggi-do, Korea) at a distance of 30 cm from the film. The radiographic films were processed manually.

With a digital camera connected to a microscope (Leica MZ75, Leica Microsystems Ltd, Wetzlar, Germany), the processed films were converted into digital images with a magnification of  $6.7\times$ . On the digital

image a horizontal reference plane (HRP) was created with a line drawn tangential to the cranium, below the frontal-squamosal intersection at the temporal crest.<sup>13</sup> The positional changes (described in the sections that follow) were measured with the image measuring program (Image J, National Institutes of Health, Bethesda, MD).

### Changes in the Vertical Position of Molars Due to Aging

We evaluated whether cementum apposition around the root apex, which normally occurs with aging, would cause a vertical displacement that might affect the result. The vertical positions of the right M3 were compared in the intrusion and (+)retention groups were. These positions were measured as the perpendicular distances between the HRP and three points (Figure 3): (a) the frontal-squamosal intersection at the temporal crest, (b) the M3 middle cusp tip, and (c) the M3 distal root apex. To compensate for the variance in individual skull size, we compared the ratios of each measurement to the frontal-squamosal intersection at the temporal crest, which had remained constant throughout the experiment, as follows: b/a and c/a. Thus, we compared the relative vertical positions of the M3 crown and root, respectively.

### Changes Following Molar Intrusion and With or Without Retention After Molar Intrusion

To evaluate the amount of molar intrusion, root resorption, and changes in the alveolar crest, the perpendicular distances were measured between the HRP and the following points (Figure 3): (b) the M3 middle cusp tip, (d) the M2 distal cusp tip, (e) the M2 distal (buccal) root apex, (f) the M1 mesial root apex, (g) the alveolar crest between M2 and M3, (h) the alveolar crest between M1 and M2, and (i) the alveolar crest on the mesial side of M1. Because the M2



Figure 2. Intruding appliance for molar intrusion in the rat jaws. (A) Schematic drawing of the appliance. (B) Rat upper jaw after 2 weeks of molar intrusion. The occlusal bonding material was applied to fix the intruding appliance and to prevent extrusion of other teeth.

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**Figure 3.** Measurements on a periapical radiograph. (a) Frontalsquamosal intersection at the temporal crest<sup>14</sup>; (b) middle cusp tip of maxillary third molar (M3); (c) M3 distal root apex; (d) distal cusp tip of maxillary second molar (M2); (e) M2 distal root apex; (f) maxillary first molar (M1) mesial root apex; (g) alveolar crest between M2 and M3; (h) alveolar crest between M1 and M2; (i) alveolar crest on the M1 mesial side.

distobuccal root could be confused with the distolingual root on periapical film, the M1 mesial root apex was also measured to reduce measurement error; (b) was assigned a reference value of 1, and the other measurements were normalized to (b).

Table 2. Relative Vertical Position of Molar in 15-, 16-, and 17-week-old Rats  $(P = .05)^a$ 

	15 Weeks (n = 5)	16 Weeks (n = 5)	17 Weeks (n=5)	Significance				
b/a	8.50 ± 0.31	8.48 ± 0.23	8.46 ± 0.16	NS				
c/a	$6.76\pm0.15$	$6.76\pm0.96$	$6.75 \pm 0.13$	NS				

<sup>a</sup> a indicates the perpendicular distance from the most superior point of the frontal-squamosal intersection at the temporal crest to the horizontal reference plane; b, the perpendicular distance from the middle cusp tip of the maxillary third molar (M3) to the horizontal reference plane; c, the perpendicular distance from the M3 distal root apex to the horizontal reference plane; b/a, the relative vertical position of the M3 middle cusp tip; c/a, the relative vertical position of the M3 distal root apex; NS, not significant.

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#### **Statistical Analysis**

Statistical analysis was carried out with SAS 9.1 software (SAS Institute Inc, Cary, NC). The data were evaluated with the Mann-Whitney U test for comparisons between the intrusion and control groups, and between the (+)retention and (-)retention groups. Analysis of variance (ANOVA) and the post hoc Duncan's multiple range test were performed to compare other variables. (*P* values less than .05 were considered statistically significant.)

#### RESULTS

# Changes in the Vertical Position of Molars Due to Aging

There were no statistically significant differences (P > .05) in the vertical positions of the M3 crowns and roots among 15-, 16-, and 17-week-old rats (Table 2).

# Changes Following Molar Intrusion and With or Without Retention After Molar Intrusion

Changes following molar intrusion. After intrusion of 2 weeks (Table 3), the cusp tip and root apices of M1 and M2 had moved apically significantly more than those in the control group (P < .05). The alveolar crest in the intrusion group had moved apically more than that in the control group, but the difference was not statistically significant.

Changes with retention after molar intrusion. The intrusion and (+)retention groups had similar cusp-tip heights (Table 4), but the root apices in the (+)retention groups had moved more occlusally than those of the intrusion group (P < .05). In addition, the alveolar crest between M1 and M2 in the (+)retention groups had moved more apically than those of the intrusion group (P < .05). The positions of the intruded teeth and adjacent alveolar crests were not significantly different between different retention durations.

Changes without retention after molar intrusion. The cusp tip and root apices in the (–)retention groups had moved more occlusally than those in the intrusion group (P < .05; Table 5). The alveolar crest between M2 and M3 had also moved more occlusally in the (–)retention groups than in the intrusion group (P < .05). The other alveolar crests were not significantly different between groups. There were no statistically significant differences between different durations after appliance removal.

### Comparisons Between Changes in the (+)Retention and (-)Retention Groups

The cusp tip of the (-)retention group had moved more occlusally than that in the (+)retention group (P <

 
 Table 3.
 Relative Vertical Positions of Measured Points in the Intrusion and Control Groups<sup>a</sup>

	Intrusion	Control	Significance
M2 distal cusp	0.964 ± 0.003	0.987 ± 0.002	**
M2 distal root apex	$0.748 \pm 0.008$	0.770 ± 0.017	*
M1 mesial root apex	$0.707 \pm 0.012$	0.725 ± 0.006	*
Alveolar crest between M2 and M3	0.892 + 0.006	0 900 + 0 003	NS
Alveolar crest between M1 and	0.092 - 0.000	0.900 ± 0.000	110
M2 Alveolar crest on the M1 mesial	0.878 ± 0.011	0.882 ± 0.003	NS
side	$0.868\pm0.009$	$0.870 \pm 0.007$	NS

<sup>a</sup> M1, M2, and M3 indicate maxillary first, second, and third molars, respectively; \*P < .05; \*\*P < .01; NS, not significant.

.05). However, the vertical positions of the root apices were similar between the two groups. The alveolar crests in the (-)retention group had moved more occlusally than those in the (+)retention group, but significant differences were observed only between M1 and M2.

#### DISCUSSION

In this study, we successfully induced molar intrusion with a NiTi spring anchored to a TAD. We found that the intrusion could be maintained with occlusal bite-block. However, without retention, the molars moved back to nearly the original positions, although disclusion after appliance removal might facilitate relapse.

After 2 weeks of molar intrusion, the crown moved more apically than the root apex. This indicates an apical root resorption. Root resorption was also detected during the first week of retention, but no further root shortening was observed during the second week of retention. Even without retention, the amount of occlusal movement in the root apices exceeded that of the cusp, which also indicates apical root resorption. The vertical positions of the root apices were similar between the (+)retention and (-)retention groups, but changes were noted in the cusp position. Thus, we speculated that root resorption was larger when the bite-block was used for retention after molar intrusion (Figure 4).

Stenvik and Mjor<sup>14</sup> previously hypothesized that cementum immaturity may be related to apical root resorption. Their result suggested that, after intrusive force, immature cementum might be resorbed earlier than apical alveolar bone due to the increased pressure around the apical periodontal ligament (PDL). In other studies of molar intrusion in dogs and monkeys, mild root resorptions were observed in the root apices and the furcation areas.<sup>4,5,7,15</sup> Human molars were also reported to show apical root resorption after intrusion, although the amount of resorption was not clinically significant.<sup>16</sup>

In the alveolar crest, bone resorption is not induced by increased pressure on the PDL: thus, that resorption may be a consequence of the pressure exerted against the crest by free gingival fibers as teeth are depressed. This notion is supported by studies on supracrestal fiberotomy; the fiberotomy group showed less bone resorption in the alveolar crest compared with the nonfiberotomy group.<sup>8,12</sup> In the present study, the alveolar crests did not show statistically significant changes after intrusion. However, after 1 week of retention, the alveolar crest between the two intruded molars moved apically (P < .05); this movement did not occur when the intruded teeth were not retained after intrusion. This suggested that the alveolar crest between the intruded molar was remodeled after some period of retention (Figure 4).

In contrast to human jaws, in the rat model, it was not possible to deliver intrusion force directly from a TAD placed in the interdental alveolar bone. Instead, the alveolar crest behind the maxillary incisor was chosen for TAD implantation because it provides sufficient cortical bone and harbors no critical structures. The loading to TAD was initiated 1 week after implantation. TAD failure (loosening) was not observed throughout the experiment.

Table 4. Relative Vertical Positions of Measured Points in the Intrusion and (+)Retention Groups<sup>a</sup>

	Intrusion	(+)Retention-1wk	(+)Retention-2wk	Significance
M2 distal cusp	$0.965 \pm 0.003$	$0.965 \pm 0.006$	$0.968 \pm 0.008$	NS
M2 distal root apex	$0.748 \pm 0.008 \text{ A}$	$0.759 \pm 0.006 \text{ A/B}$	0.761 ± 0.011 B	*
M1 mesial root apex	$0.707 \pm 0.012 \text{ A}$	0.721 ± 0.006 B	$0.720 \pm 0.007 \text{ B}$	**
Alveolar crest between M2				
and M3	$0.892 \pm 0.006$	$0.897 \pm 0.002$	$0.896 \pm 0.003$	NS
Alveolar crest between M1				
and M2	$0.878 \pm 0.011 \ \text{A}$	$0.865 \pm 0.007 \text{ B}$	$0.866 \pm 0.003 \text{ B}$	*
Alveolar crest on the M1				
mesial side	$0.868\pm0.009$	$0.867 \pm 0.011$	$0.863\pm0.002$	NS

<sup>a</sup> M1, M2, and M3 indicate maxillary first, second, and third molars, respectively; \*P < .05; \*\*P < .01; different letters show post hoc results after ANOVA, indicating significant differences among experimental groups at .05 significance level; NS, not significant.

Table 5.	Relative	Vertical	Positions	of	Measured	Points	in th	ne	Intrusion	and	(-	)Retention	Groups <sup>a</sup>
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	Intrusion	(-)Retention-1wk	(-)Retention-2wk	Significance
M2 distal cusp	0.964 ± 0.003 A	0.979 ± 0.006 B	0.979 ± 0.002 B	**
M2 distal root apex	$0.748 \pm 0.008 \text{ A}$	0.766 ± 0.010 B	$0.768 \pm 0.007 \text{ B}$	*
M1 mesial root apex	$0.707 \pm 0.012 \text{ A}$	0.726 ± 0.009 B	$0.724 \pm 0.007 \text{ B}$	*
Alveolar crest between M2				
and M3	$0.892 \pm 0.006 \text{ A}$	$0.899 \pm 0.004 \text{ B}$	$0.901 \pm 0.002 \text{ B}$	*
Alveolar crest between M1				
and M2	0.878 ± 0.011	$0.878 \pm 0.004$	$0.879 \pm 0.008$	NS
Alveolar crest on the M1				
mesial side	$0.868\pm0.009$	$0.868 \pm 0.009$	$0.866 \pm 0.008$	NS

<sup>a</sup> M1, M2, and M3 indicate maxillary first, second, and third molars, respectively; \*P < .05; \*\*P < .01; different letters show post hoc results after ANOVA, indicating significant differences among experimental groups at .05 significance level; NS, not significant.

The Japanese NiTi wire is known for its superelasticity and low hysteresis.<sup>12</sup> With superelasticity, the stress value remains fairly constant; therefore, additional activation of the wire was not required after initial application. With low hysteresis, the wire changes very little between loading and unloading; thus, the wire delivered constant intrusive force to the teeth throughout the 2 weeks of intrusion.

Bondevik<sup>12</sup> reported that rat molar intrusion reacted similarly to different magnitudes of force (0.29 to 0.98 N), but the incidence of cell-free zones and root resorption lacunae seemed to increase as the force increased. Based on that finding, we decided to use an intrusion force of 0.49 N, which was considered the lowest force level possible with the heat-bent NiTi wire spring.

The vertical position of rat molars may be influenced by alveolar bone growth, cementum apposition, and the application of bite-block. It was reported that the bone maturity score in rats does not increase after 9 weeks of age,<sup>17</sup> and that bone formation rates in the rat alveolus and jaw bones significantly decrease after 9 weeks of age.<sup>18</sup> Therefore, we used 12-week-old rats for this study to minimize the effects of alveolar bone growth. Cellular cementum forms rapidly on the apical portion of the root after 40 to 60 days of age in rats.<sup>19</sup> However, as shown in Table 2, cementum apposition with aging did not significantly affect the results over the experimental periods studied. Bresin<sup>20</sup> reported that in 4-week-old rats, the lower molars had intruded after they had been bonded to bite-block for 4 weeks. However, 12-week-old rats have nearly completed their skeletal growth, unlike 4week-old rats, which are in their most active period of skeletal growth.<sup>17,18</sup> To evaluate whether the bite-block that attached the wire to the occlusal tables could, on its own, cause the vertical displacement of teeth, we performed a pre-experimental study and found that after 4 weeks of bonding there was no effect on the relative vertical positions of teeth (P > .05). Therefore, alveolar bone growth, cementum apposition, and occlusal bite-block were not considered to be major influencing factors in our study.

Intrusion may be unstable because the intrusive tooth movement must counteract the physiologic movement of extrusion; in addition, periodontal fibers, which are generally thought to resist occlusal forces, can also strongly resist intrusive forces.<sup>9</sup> Based on our study, an occlusal bite-block could effectively retain intruded molars, and the alveolar crest was remodeled apically after the retention, although apical root resorption occurred. In many clinical situations for openbite correction, an occlusal stop is maintained after molar intrusion, which may serve as a natural form of retention. Future histologic evaluations will be essential to gain a better understanding of the periodontal tissue changes that occur in retention after molar intrusion.

**Table 6.** Comparisons Between Positions of Measured Points in the (+)Retention and (-)Retention Groups According to the duration After Molar Intrusion<sup>a</sup>

	(+)Retention-1wk	(-)Retention-1wk	Significance	(+)Retention-2wk	(-)Retention-2wk	Significance
M2 distal cusp	$0.965 \pm 0.006$	0.979 ± 0.006	*	$0.968 \pm 0.008$	0.979 ± 0.002	*
M2 distal root apex	$0.759 \pm 0.006$	$0.766 \pm 0.010$	NS	0.761 ± 0.011	$0.768 \pm 0.007$	NS
M1 mesial root apex	$0.721 \pm 0.006$	$0.726\pm0.009$	NS	$0.720 \pm 0.007$	$0.724 \pm 0.007$	NS
Alveolar crest between M2						
and M3	$0.897\pm0.002$	$0.899\pm0.004$	NS	$0.896 \pm 0.003$	$0.901 \pm 0.002$	NS
Alveolar crest between M1						
and M2	$0.865\pm0.007$	$0.878\pm0.004$	*	$0.866 \pm 0.003$	$0.879\pm0.008$	*
Alveolar crest in M1 mesial						
side	$0.867\pm0.011$	$.0868\pm0.009$	NS	$0.863\pm0.002$	$0.866\pm0.008$	NS

<sup>a</sup> M1, M2, and M3 indicate maxillary first, second, and third molars, respectively; \*P < .05; NS, not significant.



**Figure 4.** Relative vertical position changes of tooth components and alveolar crest after molar intrusion with or without retention. Results are shown for the three places in the jaw that showed changes after intrusion. \* P < .05; \*\* P < .01.

#### CONCLUSION

• Rat molars were successfully intruded and maintained at the altered position with retention biteblock. However, apical root resorption was observed as an instant response. The alveolar crest adjacent to the intruded molars was repositioned apically, but that was a delayed response compared with root resorption.

#### ACKNOWLEDGMENT

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