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# Overeruption of periodontally affected unopposed molars in adult rats

Fujita T, Montet X, Tanne K, Kiliaridis S. Overeruption of periodontally affected unopposed molars in adult rats. J Periodont Res 2010; 45: 271–276. © 2009 John Wiley & Sons A/S

*Background and Objective:* In clinical practice, anterior teeth with periodontal disease exhibiting signs of overeruption are occasionally encountered. However, the influence of periodontitis on unopposed teeth needs to be further elucidated. This study investigated, in rats, the overeruption pattern of unopposed mandibular molars with experimentally induced periodontitis.

*Material and Methods:* Sixty adult male rats were divided equally into four groups. In two groups, periodontitis was induced by a silk thread placed around the cervix of the right mandibular molar. In two groups with and without experimentally induced periodontitis, the crowns of the right maxillary molars were reduced occlusally by grinding to simulate unopposed teeth. After 4 wk, the animals were killed and scanned using micro-computed tomography to measure the vertical position of molars and the buccal and lingual alveolar bone levels.

*Results:* There were no significant differences in the overeruption of opposed molars with and without periodontitis. However, the alveolar bone level of opposed molars with periodontitis was lower than that of healthy molars. Healthy unopposed molars were extruded when compared to molars with an antagonist. The alveolar bone level of healthy unopposed molars was not influenced by molar overeruption. Unopposed molars with periodontitis exhibited significantly larger extrusion than healthy unopposed molars. The lingual alveolar bone level of unopposed molars with periodontitis was lower than that of other healthy and periodontally affected teeth.

*Conclusion:* The loss of antagonist causes overeruption of the unopposed tooth, which becomes more prominent in the presence of periodontitis.

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JOURNAL OF PERIODONTAL RESEARCH doi:10.1111/j.1600-0765.2009.01230.x

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Key words: unopposed molar; overeruption; alveolar bone; periodontitis; malocclusion

Accepted for publication April 13, 2009

The prevalence of pathologic tooth migration in patients with periodontal disease is reported to range from 30 to 56% (1), demonstrating a mean attachment loss of migrated teeth which is significantly greater than that of contralateral control teeth (2). Extrusion of periodontally affected teeth is a common finding in patients with periodontal problems (2).

There is a general belief that molars without antagonists overerupt, leading to long-term dental problems (3). Meanwhile, it is indicated that the degree of overeruption differs for molars without antagonists (4). Craddock & Youngson (5) examined a sample group of 120 subjects. They concluded that 83% of the unopposed teeth exhibited overeruption, ranging from

0.5 to 5.4 mm, whereas the remaining molars (17%) showed no overeruption at all. Possible explanations for this variation were the length of time that these teeth were unopposed, the age of the subject when the antagonist was lost (6,7) and possibly the periodontal status of the unopposed teeth (8). In fact, Compagnon & Woda (8) demonstrated that the periodontal condition

is a critical factor in vertical tooth migration. Similar findings, but in a small number of cases, were reported in a 10-year longitudinal study of 12 adults in whom periodontally affected unopposed molars showed an average overeruption of 2 mm, while healthy molars demonstrated a mean overeruption of 0.5 mm (6).

Two animal experiments tested a hypothesis that unopposed teeth overerupt substantially in the presence of periodontitis (9,10). Kohno et al. (9) based their findings on the results obtained from three monkeys and concluded that no significant differences were found in the degree of overeruption between molars with and without periodontitis, while Iwakawa (10) reported, in his investigation using five dogs, that periodontally affected teeth showed a significantly larger eruption than healthy control teeth. It is considered that these different findings are a result of the small number of subjects and the lack of precise methodology in these studies.

Therefore, this study evaluated the overeruption pattern of unopposed mandibular molars in rats, with and without experimental periodontitis, by measuring the molar displacement on skull reconstruction after scanning these animals using micro-computed tomography.

#### Material and methods

#### **Experimental animals**

Sixty, 26-wk-old male rats of the Wistar strain were divided equally into four groups (n = 15 in each group). In two groups, periodontitis was induced by a silk thread placed around the cervix of the right mandibular molar, according to Lindhe & Ericsson (11), until the end of the experiment. In two groups with and without experimentally induced periodontitis, the crowns of the right maxillary molars were reduced occlusally by approximately 0.8 mm (i.e. all of the clinical crown) by grinding with a dental hand-piece under general anesthesia (Nembutal; Dainippon Sumitomo Pharma, Osaka, Japan) (7), establishing a 'healthy unopposed' molar group and a 'periodontitis unopposed' molar group. The remaining two groups were the 'healthy opposed' molar group and the 'periodontitis opposed' molar group. During the experiment, the animals were fed a soft diet and water ad libitum. Body weights were measured every 2 d as an indicator of the general physical condition of the rats. The study was approved by the Ethics Committee of Hiroshima University.

#### Procedure of image processing

After 4 wk, the 60 animals were killed under general anesthesia (Nembutal; Dainippon Sumitomo Pharma), decapitated and the heads scanned at a pixel size of 18 µm using micro-computed tomography (SkyScan 1076; SkyScan, Kontich, Belgium). Threedimensional reconstruction was performed using SkyScan's volumetric reconstruction software, and an Open-Source Imaging platform (Osirix) was used for morphological analyses.

#### Morphological analyses

From a three-dimensional image of the rat's head, standardized frontal sections of the mandible were obtained and measurements were made on the sections according to Fujita et al. (7) (Fig. 1). Supraposition of the first mandibular molar was estimated by measuring the distance from a stable structure (the centre of the mandibular canal) to the buccal and lingual cusps on nine standardized frontal sections. These consisted of three consecutive sections (the center of the cusp, and 90 µm anterior and 90 µm posterior from the center) in each long axis of the anterior, central and posterior cusps of the first molar. The molar position is the average of both the distances to the buccal and lingual cusps in all sections. In these sections, the buccal and lingual alveolar bone levels around the mandibular first molar were also measured with reference to the centre of the mandibular canal. The supraposition and the buccal and lingual alveolar bone heights around the left mandibular molar were used as intra-individual controls.

The supraposition was estimated as the difference in the distances described

	ΗU				ΡU				РО				ОН			
	Mean	SD	Median	95% CI	Mean	SD	Median	95% CI	Mean	SD	Median	95% CI	Mean	SD	Median	95% CI
Supraposition	98.6	109.2	130.0	39.2 to 158.0	226.7	176.1	268.1	134.4 to 319.0	5.0	87.6	13.9	-40.9 to 50.9	12.9	124.9	-2.8	-52.5 to 78.3
Buccal bone level	-29.3	171.8	-34.4	-122.7 to 64.1	-252.6	222.2	-200.6	-369.0 to -136.2	-167.8	158.5	-180.0	-250.8 to -84.8	-41.5	157.6	-48.9	-124.1 to 41.1
Lingual bone level	-35.6	124.0	-32.2	-103.0 to 31.8	-273.3	130.7	-239.4	-341.8 to -204.8	-140.0	116.2	-128.3	-200.9 to -79.1	-1.0	132.5	-2.8	-70.4 to 68.4

Changes in mean, standard deviation, median and 95% confidence interval of each group

Ι. Table HO, healthy opposed molar group; HU, healthy unopposed molar group; PO, periodontitis opposed molar group; PU, periodontitis unopposed molar group



Unopposed molar

Molar with antagonist

*Fig. 1.* Upper panel: three-dimensional image of a rat's head. Lower panel: frontal section of a rat's mandible in the region of the first molar. Estimation of molar supraposition: distance on the right side (unopposed molar) minus distance on the left side (molar with antagonist). In control animals possible molar supraposition was estimated by calculating the value of the distance on the right side minus the distance on the left side (both molars with antagonists).



*Fig. 2.* Body weight gain in the four groups of rats during the experimental period (mean  $\pm$  standard deviation). HO, healthy opposed molar group; HU, healthy unopposed molar group; PO, periodontitis opposed molar group; PU, periodontitis unopposed molar group.

above on the right side with unopposed molars minus the corresponding distances on the left side where the molars had an antagonist. Differences in the level of alveolar bone were evaluated in a similar manner. In the control animals with opposed molars on both sides, differences in the molar position, or in the alveolar bone level, between the right and left sides were evaluated.

#### Statistical analyses

An unpaired *t*-test was performed to examine between-group differences in the values measured, after checking all variables for the presence of normal distribution and equality of variance (*F*-test). All statistical analyses were performed using Microsoft Excel 2003 (Microsoft, Redmond, WA, USA). Differences were considered significant at a *p*-value of < 0.05.

#### Error of the method

The error of the method was estimated by remeasuring 24 animals from each of the four groups, at least 3 mo following the initial measurement, using Dahlberg's formula: Se =  $\sqrt{\sum d^2/2n}$ , where  $\sum d^2$  is the sum of squares of the differences between the first and second measurements, and n is the number of measurements (12). The sections for second measurements were selected again before remeasuring. The error of the method (Se) for the evaluation of the supraposition of the molars on the frontal sections was 30.3 µm, and for the evaluation of the differences of the level of the buccal and lingual bone the Se values were 46.5 and 40.0 µm respectively.

#### **Results**

#### Body weight

There were no significant differences in the body weight of rats between groups with and without unopposed molars, among either healthy animals or those with periodontitis (Fig. 2). These findings indicate that there was no substantial effect of the series of experimental procedures on the general condition of the rats.



*Fig. 3.* Changes in the molar position (m), and in the buccal bone (b.b.) and the lingual alveolar bone (l.b.), in the healthy opposed molar group (HO) and in the periodontitis opposed molar group (PO). \*p < 0.01, \*p < 0.05. Box plot: horizontal lines represent 75 percentile, median and 25 percentile. The vertical bars indicate the maximum and minimum values of the sample. NS, not significant.



*Fig.* 4. Changes in the molar position (m), and in the levels of buccal bone (b.b.) and lingual alveolar bone (l.b.), in the healthy opposed molar group (HO) and in the healthy unopposed molar group (HU). \*p < 0.05. Box plot: horizontal lines represent 75 percentile, median and 25 percentile. The vertical bars indicate the maximum and minimum values of the sample.

# Induction of experimental periodontitis

We initially determined whether the silk thread placed around the cervix of the right mandibular molar induced periodontitis (Fig. 3, Table 1). There were no significant differences in supraposition and alveolar bone levels between the right and left sides of the healthy opposed molar group. The buccal and lingual alveolar bone levels in the periodontitis and opposed molar group ( $-168 \pm 159 \mu m$ ,  $-140 \pm 116$ 

µm) were lower than those in the healthy opposed molar control group (-42 ± 158 µm, p < 0.05; -1 ± 133 µm, p < 0.01, respectively). There were no significant differences in supraposition between the periodontitis and opposed molar and healthy opposed molar groups (5 ± 88 µm and -13 ± 125 µm in periodontitis and opposed molar and healthy opposed molar groups, respectively).

# Difference in vertical tooth position (supraposition)

After the 4-wk experiment, the healthy unopposed molar group exhibited a supraposition of 99  $\pm$  109 µm more than the contralateral molars with antagonists, which was greater than that in the healthy opposed molar group (-13  $\pm$  125 µm, p < 0.05) (Fig. 4, Table 1).

The periodontitis unopposed molar group demonstrated a higher supraposition  $(227 \pm 176 \,\mu\text{m})$  than that in the group with periodontitis and opposed molars  $(5 \pm 88 \,\mu\text{m}, p < 0.05)$  (Fig. 5, Table 1). The molar supraposition was greater in the periodontitis and unopposed molar group than in the healthy unopposed molar group (p < 0.01) (Fig. 6, Table 1).

# Difference in buccal alveolar bone level

The buccal alveolar bone level of the healthy unopposed molars was not higher than that of the contralateral molars with antagonists  $(-29 \pm 172 \ \mu m)$ , and there were no significant differences when compared with the buccal alveolar bone level of the healthy opposed molar group  $(-42 \pm 158 \,\mu\text{m})$  (Fig. 4, Table 1). There was no significant difference between the buccal alveolar bone level  $(-253 \pm 222 \ \mu m)$  in the periodontitis unopposed molar group and that in the periodontitis opposed molar group  $(-168 \pm 159 \ \mu m)$  (Fig. 5, Table 1). The buccal alveolar bone level in the periodontitis unopposed molar group was lower than that in the healtchy unopposed molar group (p < 0.01)(Fig. 6, Table 1).



*Fig. 5.* Changes in the molar position (m), and in the levels of buccal bone (b.b.) and lingual alveolar bone (l.b.), in the periodontitis opposed molar group (PO) and in the periodontitis unopposed molar group (PU). \*\*p < 0.01. Box plot: horizontal lines represent 75 percentile, median and 25 percentile. The vertical bars indicate the maximum and minimum values of the sample.



*Fig.* 6. Changes in the molar position (m), and in the levels of buccal bone (b.b.) and lingual alveolar bone (l.b.), in the healthy unopposed molar group (HU) and in the periodontitis unopposed molar group (PU). \*p < 0.01, \*p < 0.05. Box plot: horizontal lines represent 75 percentile, median and 25 percentile. The vertical bars indicate the maximum and minimum values of the sample.

### Difference in lingual alveolar bone level

The lingual alveolar bone level of the healthy unopposed molars was not higher than that of the contrala teral molars with antagonists  $(-36 \pm 124 \ \mu\text{m})$ , and there were no significant differences compared with the buccal alveolar bone level of the healthy opposed molar group  $(-1 \pm 133 \ \mu\text{m})$  (Fig. 4, Table 1). The lingual alveolar bone level in the period ontitis unopposed molar group

 $(-273 \pm 131 \mu m)$  was lower than that in the periodontitis opposed molar group  $(-140 \pm 116 \mu m, p < 0.01)$ (Fig. 5, Table 1). In the periodontitis unopposed molar group, the lingual alveolar bone level was lower than in the healthy unopposed molar group (p < 0.01) (Fig. 6, Table 1).

#### Discussion

The present findings confirm the clinical observations that periodontally affected unopposed molars erupt more substantially than periodontally healthy teeth (6). During the overeruption of unopposed molars, the alveolar bone level was lower mainly in the group with periodontitis than in the healthy group. The results of our study agree with previous findings reported by Iwakawa (10) in dogs, but differ from the results reported by Kohno et al. (9). The latter may be a result of differences in the severity of experimental periodontitis between the two studies (9). These authors reported that the experimentally induced periodontitis around the molar was mild because they could not clearly observe alveolar bone resorption around the molar, which raises doubts about the success of their intervention to induce experimental periodontitis. In our study, we clearly observed resorption of the alveolar bone in the periodontitis opposed molar group and in the periodontitis unopposed molar group. We found that the lingual alveolar bone level around the unopposed molar with periodontitis was lower than that around the opposed molar with periodontitis. This indicates that overeruption may induce a further decrease in the alveolar bone level around teeth with periodontitis. Our findings are in agreement with previous studies stipulating that the periodontal condition is a critical factor associated with vertical tooth migration (8). A hypothesis was suggested by Waerhaug (13) that the pressure in the vessels, or the interstitial pressure, is increased by inflammation in the furcation area and thus the teeth may overerupt. However, this hypothesis

was not validated by our findings because the experimentally induced periodontitis in this model did not reach the furcation area. In general, healthy teeth have the potential for vertical displacement over time as a result of the eruption potential that exists in the periodontal ligament (14). In healthy conditions, the eruption of the tooth is in pace with the vertical augmentation of the alveolar process. The bone modelling of the alveolar process takes place with slower pace in adults than in growing individuals, which in its turn decreases the eruption rate of the tooth. A possible explanation for our findings could be that in the presence of periodontitis, the loss of coronal periodontal attachment can facilitate the complete expression of the eruption potential of the tooth, dissociated from the slow bone turn-over of the alveolar crest (15). However, the biological mechanism inducing moderate or severe overeruption in the presence of periodontal disease requires further investigation.

Many studies have reported using a silk thread placed around the cervix of the molar to induce periodontitis in rats (16,17). This method was used in the present study to induce periodontitis because of its simplicity and reliability. In our study, we maintained the periodontal condition until the end of the experiment. Thus, periodontitis was induced experimentally, and the level of alveolar bone in the periodontally affected group was clearly lower than that in the controls. However, it is unclear when the overeruption took place. In this study, we selected a method of grinding the molar crowns using a dental handpiece to eliminate occlusal contacts with the antagonist (7). As it was assumed that this intervention may have made it difficult for rats to eat a diet consisting of hard food, a diet of soft food was provided for both the experimental and control groups. However it seems that the grinding was not a serious disturbing factor for the animals because a similar body weight was maintained in all groups.

In previous studies, vertical displacement was measured clinically using calipers (9,10). However, this method has problems in the accuracy of measurement. Recently, microcomputed tomography has been used for precise analysis of the bone structure in the rat mandible (18,19). Thus, we employed this technology and reconstructed the three-dimensional image in each rat, which allowed us to observe three-dimensional changes in the tooth position. Moreover, we could obtain virtual sections of any part of the sample without destroying the specimen itself. Ideally, we should have measured these changes longitudinally but there is no method currently available to achieve that goal. Therefore, we estimated the overeruption of the unopposed molar, measuring the positional differences between the left side and the right side in rats.

In conclusion, periodontitis induced in adult rats may have promoted further overeruption of the unopposed molars, and the lack of an antagonist tooth may have complicated the periodontal conditions of the unopposed tooth.

#### Acknowledgements

This investigation was supported, in part, by Grants-in-Aid for Scientific Research of the Ministry of Education, Culture, Sports, Science and Technology (20791577). We would like to thank Dr J. Ohtani for his help during the experimental procedure of the study and Dr G. S. Antonarakis for linguistic correction of the manuscript.

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