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Histological and histochemical quantification of root resorption incident to the application of intrusive force to rat molars

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SUMMARY This study was conducted to investigate the nature of root resorption resulting from intrusive forces applied to the rat lower molars, by means of histological and histochemical techniques with tartrate resistant acid phosphatase (TRAP). Thirty-eight 13-week-old Wistar strain male rats were used. Intrusive force was created by a fixed appliance which was adjusted to exert an initial force of 50 g for the duration of 1, 2, and 3 weeks. The degree of root resorption and distribution of TRAP positive cells were evaluated.

On the root surface, the TRAP positive scores were low in the apical regions. Significant differences in the scores were found in the inter-radicular region of the roots between the experimental and control groups for the 2- and 3-week groups. More active resorption of bone occurred during the experimental period, as denoted by greater TRAP positive scores on the bone than on the root surface. Root resorption scores in the apical root region were larger in the 2- and 3-week groups than in the 1-week group. Significant differences in the root resorption scores were also found between the 1- and 3-week groups in the inter-radicular region, indicating that intrusive force application of a longer duration may lead to a higher frequency of root resorption. It is shown that, irrespective of the level of TRAP positive cells and root resorption scores, the degree of root resorption activity is higher in the apical root region than in the inter-radicular area. These results indicate that cellular cementum may be resorbed more easily because of its richer organic components and low mineralized structure.

Introduction

Root resorption is an idiopathic problem, occasionally observed in association with orthodontic tooth movement. Various factors relevant to root resorption are roughly divided into biological and mechanical factors or a combination of both (Brezniak and Wasserstain, 1993a,b). For the mechanical factors, it is generally accepted that extensive tooth movement, root torque, jiggling and intrusive forces are responsible for root resorption. In particular, intrusive force is probably the most detrimental to the root because of stress concentration at the apical root region (Tanne and Sakuda, 1983), although tooth intrusion is an important mode of tooth movement and inevitable in clinical orthodontics (Reitan and Rygh, 1994).

A previous experimental study on maxillary molar intrusion indicated some association between degenerative tissue and root resorption observed in the pressure area (Bondevik, 1980). However, co-existence of hyalinized tissue and root resorption was not found in another report (Sato *et al*., 1984). Thus, there has been much controversy about tissue changes incident to experimental tooth intrusion, and the mechanisms of root resorption still remain unclear.

For a possible explanation of the controversy, two problems in previous experimental systems may be pointed out. First, root resorption was observed in histological examinations. However, the results were based only on qualitative findings without any quantitative evaluation (Bunch 1942; Dellinger 1967; Reitan 1974; Bondevik,

1980, 1984; Sato *et al*., 1984). Secondly intrusion of the upper molar, which served frequently as the experimental tooth in previous studies, is essentially followed by either the resorption or remodelling of a thin bone layer on the nasal floor. Therefore, the bone remodelling may influence the actual phenomena of apical root resorption.

With these considerations, this study was designed to investigate the influence of intrusive forces on biological responses of rat lower molar periodontal tissue, in terms of the degree of root resorption and distribution of tartrate resistant acid phosphatase (TRAP) positive cells.

Materials and methods

Experimental animals

Twenty-six 13-week-old Wistar strain male rats were used as experimental animals, and twelve served as the controls. These animals were divided into three groups according to the duration of force application, 1-, 2-, and 3-week groups (Table 1). All the animals were fed a standard laboratory diet consisting of pellets and water *ad libitum*.

Table 1 Number of animals.

Force duration	Experimental group	Control group
1 week	9	4
2 weeks	8	4
3 weeks	9	4

Experimental tooth movement

Intrusive force was created using a modification of Bondevik's appliance (1980), which consisted of orthodontic bands on the mandibular incisors, 0.7 mm main wire, and 0.25 mm cantilever spring with helix (Figure 1). The spring was calibrated by a tension gauge (Shinpo Corp., Tokyo, Japan) to exert an initial force of 50 g applied in an apical direction and placed into an artificially made groove on the occlusal surface of the left lower first molar without producing interference to the original occlusal contact between the upper and lower dentitions. For the controls, the same appliance was placed on the lower dentition without activation of the spring. In this

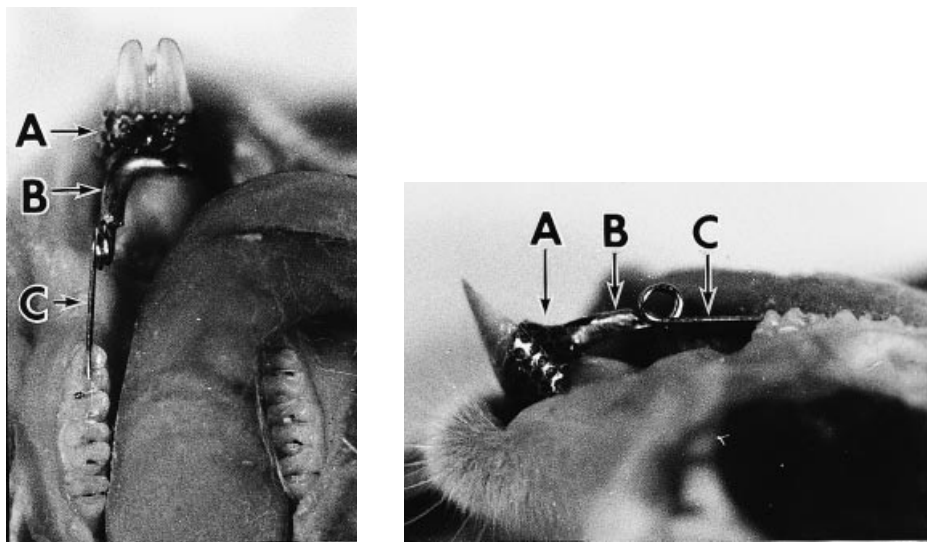

Figure 1 Occlusal (left) and lateral (right) views of appliance for experimental tooth intrusion. (A) Orthodontic band. (B) 0.7 mm wire. (C) Cantilever spring with helix (0.25 mm wire).

study, root resorption was the main focus and the magnitude of force was established at a higher level than usual. Duration of force application was 1, 2, and 3 weeks (Table 1).

Measurement of the amount of tooth intrusion

Before and after intrusion, impressions were taken of the lower dentitions with Xantopren L (Bayer Dental, Germany) and models manufactured. Photographs were then taken of the plaster models of the left lower molar region from an exact lateral direction perpendicular to the lower dentition under the following condition common to all the models, i.e. the artificial groove fabricated on the buccal surface of the crown was designed to be in an upright position to the camera. The buccal cusps of the molars on the photographs were traced on acetate paper. The distances between the mesiobuccal cusps of lower first molars before and after intrusion were then measured with digital calipers (Mitsutoyo Co., Osaka, Japan) on the tracings superimposed at the buccal cusps of the second and third molars.

Preparation of tissue sections for histological observation

At the end of each experimental period, the animals were sacrificed under general anaesthesia with pentobarbital of 0.1 ml/100 g body weight, and the mandible, including the first, second and third molars, was dissected and fixed in 10 per cent neutral buffered formalin for 24 hours. After fixation, the samples were decalcified in 14 per cent EDTA for 3 weeks, dehydrated in ethanol, and then embedded in paraffin. The embedded specimens were cut into parasagittal sections of 4.5 μm thickness.

Eight comparable sections, including the apical foramen of the distal root, the greatest root, of the lower first molar, were stained with TRAP. The histochemical staining for TRAP was carried out according to the methods described by Cole and Walters (1987) and Farrell *et al*. (1990). The remaining eight sections were stained with haematoxylin and eosin (H&E), and observed microscopically.

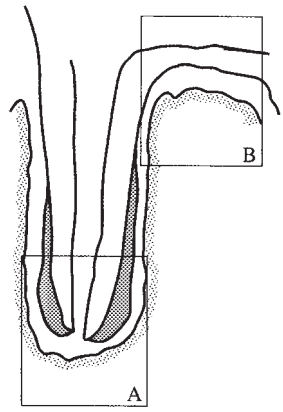

Figure 2 The location of histological observation. Apical root (A) and inter-radicular (B) regions.

Evaluation of TRAP positive scores

On the eight stained sections, TRAP positive scores were examined on the root and bone surfaces in the apical root and inter-radicular regions (Figure 2A and B). On the magnified (×120) photograph of each region, a transparent sheet with 10 × 10-mm grids was superimposed. For the root surface in both the apical and inter-radicular regions, the numbers of grids with and without TRAP positive cells were counted. TRAP positive scores (percentage of TRAP positive grids) were obtained by dividing the number of positive grids by the total number of grids along the root surface. On the alveolar bone surface, TRAP positive scores were similarly determined (Figure 3).

Evaluation of root resorption

On the eight H&E stained sections, root resorption scores were determined. On the magnified (×120) photographs of the apical root and inter-radicular regions, the grid-sheet used for the preceding evaluation was superimposed in the same way, and the numbers of grids with or without resorption lacunae were counted separately. Root resorption scores (percentage of resorption grids) were determined by dividing the number of grids with resorption lacunae by

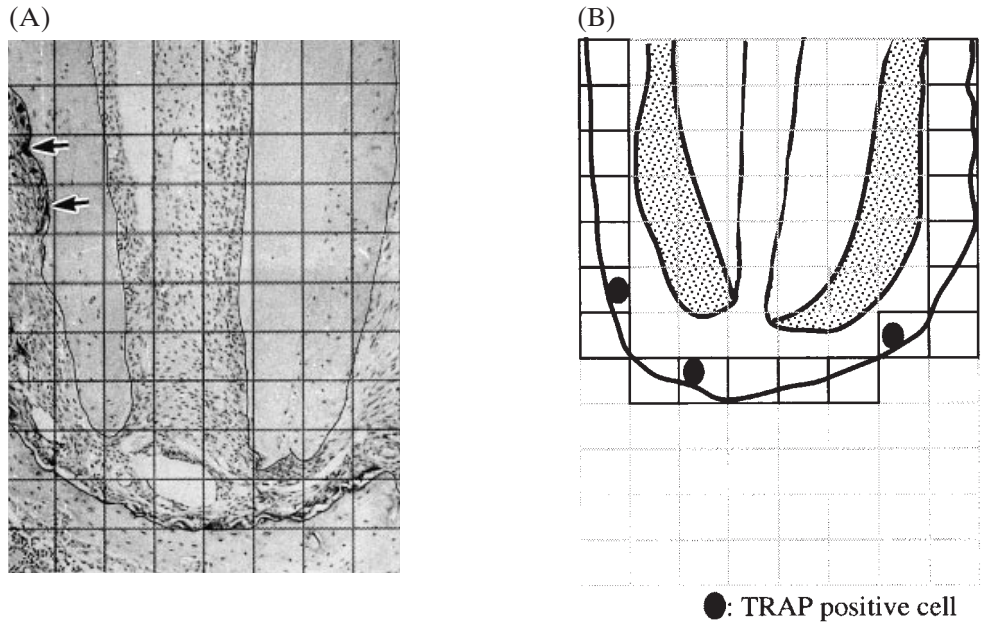

Figure 3 Evaluation of TRAP positive cells. (A) Photomicrograph (×120). (B) Schematic representation TRAP positive score = (number of girds containing TRAP positive cells/total number of grids) ×100.

the total number of grids along the root surface (Figure 4).

Finally, root resorption activity as a secondary parameter was calculated in the experimental group by dividing the root resorption scores by the corresponding TRAP positive scores on the root surface.

Statistical analysis

Means and standard deviations were calculated for each group. For the comparison of experimental and corresponding control groups, a Student's *t*-test was used to examine the differences. For the experimental groups, analysis of variance (ANOVA) was used to determine differences in variance among groups with different experimental periods. Pairwise comparisons (Scheffe) were then executed between groups when significant differences in the variances were found ($P < 0.05$). These calculations were performed with a statistical program 'StatView 4.11J' (Abacus Concepts Inc., CA, USA) on a Power Macintosh 7100 computer (Apple Computer Corp., CA, USA).

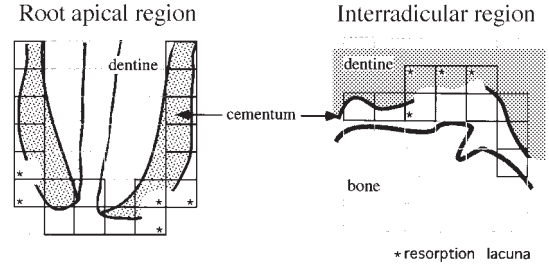

Figure 4 Evaluation of root resorption. Root resorption score = (number of girds containing resorbed lacunae; with asterisk/total number of grids) ×100.

Results

The amount of tooth intrusion (Table 2)

For the 1-, 2-, and 3-week groups, the amount of intrusion was 1.01, 1.36, and 1.75 mm, respectively. A significant difference was found between the 1- and 3-week groups, indicating a greater amount of tooth intrusion for the longest duration of intrusive force application, although the amount was not proportional to the duration.

Table 2 The amount of tooth intrusion.

Force duration	Tooth intrusion (mm)	Significance		
1 week	1.01 ± 0.22	NS		($P < 0.05$)
2 weeks	1.36 ± 0.29	NS		
3 weeks	1.75 ± 0.52			

NS, not significantly different.

TRAP positive score (Table 3)

On the root surface in the apical root and inter-radicular regions. For all the experimental groups, TRAP positive scores varied slightly without any regular tendency in relation to the force duration. The scores were relatively lower in the apical root area than in the inter-radicular region, indicating resorptive change was more active in the inter-radicular region. In the apical root region, no significant differences in the scores were found between the experimental and control groups. However, significant differences were found in the inter-radicular region between experimental and control groups for the 2- and 3-week experimental periods.

On the bone surface in the apical root and inter-radicular regions. The scores were significantly greater in all the experimental groups than in the corresponding controls, excluding those of the inter-radicular area in the 1-week experimental and control groups. Furthermore, the scores in the experimental groups were greater in the 3-week group than in the 1- and 2-week groups. More active resorptive change occurred on the bone surface during the experimental period, as noted by larger TRAP positive scores for the alveolar bone than for the root surface.

Root resorption score (Table 4)

Apical root region. All the experimental groups exhibited significantly greater scores than the corresponding controls. The values were larger in the 2- and 3-week groups than in the 1-week group. However, no significant differences were found among the three experimental groups with different durations of force application.

Inter-radicular region. Root resorption scores were also significantly greater in all the experimental groups than in the corresponding controls. For the experimental groups, the scores increased gradually according to the duration of force application. A significant difference in the scores was found between the 1- and 3-week groups, indicating that intrusive force application of a longer duration may lead to a higher frequency of root resorption.

Root resorption activity (Table 5)

In the apical root region, resorption activity gradually increased up to 3 weeks during

Table 3 TRAP positive scores.

Anatomical site	Force duration	Experimental group	Control group
Apical root surface	1 week	2.71 ± 3.64	1.58 ± 2.65
	2 weeks	3.52 ± 6.06	0.19 ± 0.38
	3 weeks	2.97 ± 3.13	0.37 ± 0.74
Inter-radicular root surface	1 week	25.29 ± 12.39	11.75 ± 13.21
	2 weeks	22.10 ± 6.79*	4.98 ± 7.77
	3 weeks	13.99 ± 11.15*	0.47 ± 0.95
Apical bone surface	1 week	26.05 ± 8.26*	11.19 ± 10.27
	2 weeks	25.63 ± 13.45*	10.24 ± 5.71
	3 weeks	35.12 ± 7.56*	3.26 ± 0.91
Inter-radicular bone surface	1 week	28.67 ± 8.01	18.93 ± 8.06
	2 weeks	26.33 ± 11.21*	8.00 ± 4.80
	3 weeks	30.49 ± 9.86*	10.18 ± 4.12

*Significantly different ($P < 0.05$).

Table 4 Root resorption score.

Anatomical site	Force duration	Experimental group	Control group
Apical region	1 week	18.83 ± 12.27*	0.94 ± 1.89
	2 weeks	29.52 ± 9.94*	0.49 ± 0.98
	3 weeks	25.45 ± 8.70*	0.78 ± 1.57
Inter-radicular region	1 week	36.93 ± 9.53*	1.53 ± 1.99
	2 weeks	* 53.53 ± 10.42*	5.52 ± 3.35
	3 weeks	60.90 ± 18.01*	3.51 ± 4.43

*Significantly different ($P < 0.05$).

Table 5 Root resorption activity.

Anatomical site	Force duration	Experimental group
Apical region	1 week	6.95
	2 weeks	8.38
	3 weeks	8.57
Inter-radicular region	1 week	1.46
	2 weeks	2.42
	3 weeks	4.35

the experiment and was greater than in the inter-radicular region for all experimental periods, although the values in both anatomical regions exhibited similar patterns of increase. Irrespective of the level of TRAP positive cells (Table 3) and the root resorption scores (Table 4), the degree of root resorption activity was more substantial in the apical root region than in the inter-radicular area.

Discussion

The present study demonstrates that distribution of TRAP positive cells is higher on the alveolar bone than on the root surface, which is in agreement with a previous report that alveolar bone is more readily resorbed than root (Lindskog and Hammarstrøm, 1980). This may be one reason why rich recruitment of clast cells, including osteoclasts derived from haemato-poietic progenitors of bone marrow, may be induced quickly on the alveolar bone surface facing the periodontal space by mechanical stimuli.

In the present study, TRAP positive scores were extremely low on the apical root surface throughout the experiment. This may be due to the fact that an increase of local pressure was inhibited by thick and soft cellular cementum layers in the apical region (Sato *et al.*, 1984). Furthermore, since the lower first molar is not a single-rooted tooth, it is likely that concentration of compressive stresses may occur in the inter-radicular region, incident to the application of intrusive forces. It seems a reasonable assumption that compressive stresses from intrusive forces interfere more markedly with the blood supply and create more extensive cell-free zones in the inter-radicular region than in the apical area (Bondevik, 1980). These differences in biomechanical tissue reaction may cause differences between resorptive changes in the apical root and inter-radicular regions. It has been stated that maturity of the cementum is of great significance in the progress of root resorption (Stenvik and Mjør, 1970), although dense mineralized tissue is resistant to removal (Reid, 1986; Jones *et al.*, 1996). Another explanation for resorption is cellular cementum with organic components commonly found in the apical root region, which undergo resorption more easily. However, the level of TRAP positive clast cells was relatively low in this apical region.

Most previous studies have reported that the severity of root resorption is directly related to the duration of force application, or treatment time. In a histological study, Stenvik and Mjør (1970) reported that between 34 and 56 per cent of the examined teeth experienced resorption lacunae after 15 and 20 days of tooth movement, respectively. In the present study, a greater

amount of intrusion was achieved 3 weeks after the beginning of tooth intrusion. For apical root resorption, there were no significant differences among the 1-, 2-, and 3-week groups. However, in the inter-radicular region, the degree of root resorption was greater in the 3-week than in the 1-week group. Therefore, it may be concluded that a longer duration of intrusive force application will cause more extensive resorptive change and may be a significant risk factor for root resorption.

A previous study has reported that the cell-free zone disappeared more rapidly after application of lighter than heavier forces, and the resultant root resorption was less extensive (Kyomen and Tanne, 1997). In the cellular cementum layer, apposition of new cementum may be expected as tissue reformation, if the external stimulation is not excessive, to repair minor resorption lacunae on the cellular cementum surface. From a clinical point of view, minor root resorption cannot be detected by radiographic examination (Reitan and Rygh, 1994). Since root resorption may be progressive in nature, it is not clear whether or not unloading of intrusive force, when root shortening is first detected, is effective for preventing the progress of the root resorption. However, it is essentially necessary for clinical practice that excessive force application should be avoided thereafter.

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References

Bondevik O 1980 Tissue changes in the rat molar periodontium following application of intrusive force. European Journal of Orthodontics 2: 41–49

Bondevik O 1984 Tissue changes in the rat molar periodontium following alteration of normal occlusal forces. European Journal of Orthodontics 6: 205–212

Brezniak N, Wasserstein A 1993a Root resorption after orthodontic treatment: part 1. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 62–66

Brezniak N, Wasserstein A 1993b Root resorption after orthodontic treatment: part 2. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 138–146

Bunch W B 1942 Tissue changes occurring in dogs incident to depressing movements. Angle Orthodontist 12: 177–183

Cole A, Walters L M 1987 Tartrate-resistant acid phosphatase in bone and cartilage following decalcification and cold-embedding in plastic. Journal of Histochemistry and Cytochemistry 35: 203–206

Dellinger E L 1967 A histologic and cephalometric investigation of premolar intrusion in the *Macaca speciosa* monkey. American Journal of Orthodontics 53: 325–355

Farrell L, Yen E, Brudvik P, Rygh P, Suga D M 1990 Identification of orthodontically induced root resorptive cells using TRAP stain. Journal of Dental Research 69: 201 (Abstract)

Jones S J, Arora M, Boyde A 1996 The rate of osteoclastic destruction of calcified tissues is inversely proportional to mineral density. Calcified Tissue International 56: 554–558

Kyomen S, Tanne K 1997 Influences of aging changes in proliferative rate of PDL cells during experimental tooth movement in rats. Angle Orthodontist 67: 67–72

Lindskog S, Hammarstrøm L 1980 Evidence in favor of an anti-invasion factor in cementum or periodontal membrane of human teeth. Scandinavian Journal of Dental Research 88: 161–163

Reid S A 1986 Effect of mineral content of human bone on *in vitro* resorption. Anatomical Embryology 174: 225–234

Reitan K 1974 Initial tissue behavior during apical root resorption. Angle Orthodontist 44: 68–82

Reitan K, Rygh P 1994 Biomechanical principles and reactions. In: Graber T M, Vanarsdall R L Jr (eds) Orthodontics. Current principles and techniques, 2nd edn. C.V. Mosby, St Louis, pp. 165–181

Sato T, Iida J, Kurihara S 1984 A histological investigation on the periodontal tissue changes during molar depression in rats. Japanese Journal of Orthodontics 43: 361–372 (in Japanese)

Stenvik A, Mjør J 1970 Pulp and dentine reactions to experimental tooth intrusion. A histologic study of the initial changes. American Journal of Orthodontics 57: 370–383

Tanne K, Sakuda M 1983 Initial stress induced in the periodontal tissue at the time of the application of various types of orthodontic force: three-dimensional analysis by means of the finite element method. Journal of the Osaka University Dental School 23: 143–171