The Effect of 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP) on the Regulating Factors of Tooth Extrusion in Unopposed Rat Mandibular First Molars

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Abstract: 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP) is known to have an inhibitory effect on the hard tissue metabolisms. The present study was pursued to analyze histologically the effect of HEBP on tooth extrusion in rat molar, so as to clarify the possible role of the periodontal hard tissue metabolisms in tooth extrusion.

The right maxillary molars of Wistar rats were extracted to activate extrusion of the mandibular first molar, after which HEBP was injected subcutaneously into separate groups of rats at doses of 0.25, 1.0 or 4.0 mgP/kg/day, respectively. Control rats received physiological saline solution. For the vital staining, tetracycline (6 mg/kg) was injected intravenously at start of the experiment and calcein (3 mg/kg) at 6 hours prior to sacrifice. The rats were sacrificed at 0, 2, 4, 7 and 10 days. Specimen prepared from the right mandible of each rat then was microscopically examined.

It was found that HEBP inhibited the tooth extrusion dose-dependently. Histological findings in the periapical area showed that the width of periodontal ligament decreased. The matrix formation of the alveolar bone and the dental cementum were not significantly influenced. Mineralization of the alveolar bone was only impaired in the 4.0 mgP-injected group, but mineralization of the dental cementum was found to be inhibited dose-dependently. At the lateral side of tooth socket, the labelling index of 3H-thymidine in the fibroblasts and the width of periodontal ligament were reduced. On the other hand, the width of the alveolar bone tended to increase.

These results suggest that the reduction of periodontal ligament activity and the increment in tissue resistance, brought by the HEBP-induced inhibition of bone resorption in the coronal area, were the main causes for the inhibition of tooth extrusion. However, hard tissue formation, which was particularly seen in the periapical area, seemed to be unrelated to the mechanisms of tooth extrusion.

Introduction

Recently, based on studies of continuously-erupting rodent incisors, it has come to be believed that the periodontal ligament is the source of this eruptive force, particularly the periodontal ligament fibroblast or the hydrostatic pressure within the periodontal ligament. However, other studies claimed that the rodent incisor is a special case with respect to the distribution of enamel and periodontal ligament and moreover, the growth of the end of the root never ceases. This, in turn, raises doubts about the reliability of extrapolations made from studies of the rodent incisor model system to the eruption mechanisms of the noncontinuously erupting tooth of humans.

It has been said that the eruption rate of a noncontinuously erupting tooth like the rat molar is quite low. Kinoshita et al. have reported, however, that tooth eruption of a rat
mandibular molar can be activated by the extraction of the opposed tooth. Histological changes also have been reported that atrophy of the periodontal ligament occurred in the rat mandibular molar following the removal of the occlusal contact. Further, biochemical analyses have shown that a synthesis of both types I and III collagens and an increase in the collagen turnover occurred in the unopposed (hypofunctional) rat molar periodontal ligament.

Such tooth movement under unphysiological conditions, should properly be called an extrusion. Picton and Moss, however, described that an extrusion and an eruption display a close similarity, if not an identical nature in monkey. Moreover, the structure of periodontium of the rat molar is reported to be very similar to that of human tooth and unopposed extrusion (eruption) rates are generally considered to be a full expression of the eruptive potential that is released from occlusal force. Because of these reasons, such an unopposed rat molar is thought to serve as a good model for the study of tooth eruption in the human tooth.

Bisphosphonates are analogs of pyrophosphate and have a P-C-P bonding structure instead of the P-O-P structure of pyrophosphate. Further, they characteristically manifest a high resistance against enzymatic degradation and are preferentially incorporated into the inorganic phase of hard tissues, such as bone and tooth when given in vivo. Bisphosphonates have an inhibitory effect on the formation and resorption processes of hard tissue, the bisphosphate compounds are thought to be an exogenous regulator of bone metabolism. HEBP (1-hydroxyethylidene-1,1-bisphosphonate), which was initially introduced in a study that investigated the effect of bisphosphonates on bone metabolism, is known to inhibit the precipitation of calcium phosphate crystals, thereby blocking the transformation of amorphous calcium phosphate into hydroxyapatite. As a consequence, HEBP impairs the calcification process in the bone and the tooth. Further, it also has an inhibitory effect on bone resorption. Thus, because of the biological effects of HEBP on hard tissue, it may be a useful tool in determining whether the metabolism of the alveolar bone and dental cementum is related to tooth eruption.

Based on the above-mentioned reasons, this study was undertaken to investigate the effect of HEBP on tooth extrusion of an unopposed rat mandibular first molar. In addition, the possible role of the periodontal hard tissue metabolism in tooth extrusion (eruption) was also pursued.

**Materials and Methods**

1. Animals and experiment design

Eighty-seven, 10-weeks old male Wistar rats were used for this study. They were divided into 17 groups of 5 to 6 rats each. At the beginning of the experiment (day 0), so as to eliminate occlusal molar contact, three right maxillary molars were extracted under ether anesthesia. Immediately after extraction, a sodium salt of HEBP (courtesy of Lion Corporation, Tokyo, Japan) was injected subcutaneously into each rat at a dose of 0.25, 1.0 or 4.0 mg/kg/day, whereas rats used for controls were injected daily with the same volume of physiological saline solution. For the vital staining of the alveolar bone and dental cementum, tetracycline (6 mg/kg, Sigma, U.S.A.) was injected intravenously (i.v.) at the beginning of the experiment and calcein (3 mg/kg, Wako, Japan) was injected at 6 hours prior to sacrifice. Rats were then sacrificed by cervical dislocation under ether anesthesia at intervals of 0, 2, 4, 7 and 10 days (day 0, 2, 4, 7 and 10).

2. Histometric and fluorescent microscopic examinations

After each sacrifice, mandibles of rats were removed and fixed in a phosphate-buffered glutaraldehyde-formaldehyde solution adjusted to pH 7.2 for one week at 4°C. After fixation, the mandibles were washed in running tap water for 24 h, dehydrated in a graded series of ethanol and embedded in acrylic resin (LR white resin, TAAB Laboratories Equipment, Ltd., England). Each tissue block was oriented with a microtome (Leitz 1516, Ernst Leitz Wetzlar GmbH, Germany) on a standardized buccal-lingual plane of the mesial root of the mandibular first molar. The plane...
Fig. 1 1a. Schematic illustration of the sagittal section of the mesial root of the rat mandibular first molar.

The tooth axis was defined as the line connecting the midpoint of the cement-enamel junction with the midpoint of the root apex. The width of buccal (BAB) and lingual alveolar bone (LAB) which were coronal side of the root apex were analyzed by the texture analyzing system (TAS plus).

1b. Combination illustration of fluorescent and light photomicrographs in the periapical area of 1a. The width of mineralized dental cementum and mineralized alveolar bone formed after the experiment began were evaluated on fluorescent photomicrographs and they were located between two fluorescent lines. Unmineralized dental cementum, periodontal ligament and osteoid were evaluated on light photomicrographs. All measurements were performed on the tooth axis as indicated in the figure. T: Tetracycline line. C: Calcein line.

which included the entire length of the pulp chamber, canal and apical foramen, was standardized by the method of Rippin, and each block was ground manually to the thickness about 40 µm (Fig. 1a).

The ground sections were observed under a fluorescent microscope (Leitz Orthoplan, Ernst Leitz Wetzlar GmbH, Germany) and photographed. The tooth axis was defined as the line connecting the midpoint of the cement-enamel junction with the midpoint of the root apex and the amount of mineralized alveolar bone and mineralized dental cementum was determined by measuring the amount present on this tooth axis, as has been diagramed in Figure 1b. On staining the same sections with the Villanueva Goldner stain, the width of the osteoid, unmineralized dental cementum, and periodontal ligament in the periapical area was evaluated (Fig. 1b). The cementoblasts and osteoblasts were also counted, after which the relative number of osteoblasts and cementoblasts to the total surface length of the alveolar bone and dental cementum was calculated.

For the morphometrical analysis of the width of buccal and lingual alveolar bone, as shown in Figure 1a, and the width of the periodontal ligament lateral to the tooth socket, diagramed in Figure 2, a texture analyzing system (TAS
Fig. 2 Schematic illustration of measured areas of the labelled periodontal fibroblast on autoradiographic section.

The labelling index (labelled fibroblasts per area of 1 mm²) were obtained by counting labelled cells in five (Zone 1-5) areas of 500 μm in longitudinal length in the periodontal ligament. These areas were also used for the measurement of the width of periodontal ligament on Villanueva Goldner stain section. The mean width of periodontal ligament were obtained by the texture analyzing system (TAS plus).

plus, Ernst Leitz Wetzlar GmbH, Germany) was used. The mean width of alveolar bone and periodontal ligament was calculated from the parameters of area and height of the periodontal tissue.

3. Autoradiographic analysis of the periodontal ligament fibroblasts

An autoradiographic analysis was conducted to investigate the labelling index of periodontal ligament fibroblasts, and for this purpose, 21 Wistar rats were used (each group has 5–6 animals). The extraction of the molars and the HEBP injections were accomplished through the protocol previously described and the rats were maintained until day 7. The rats then were injected with 0.5 μCi/g of ³H-Thymidine intravenously, 1 hour prior to sacrifice, under ether anesthesia. After removal, the right mandibles were fixed, decalcified in a neutral, 10% ethylene diamine tetaacetic acid (EDTA) solution, and embedded in glycol metaacrylate resin (Technovite 7100, Kulzer & Co. GmbH, Germany). Then, 2 μm-thick sections from the right mandible were cut on the standardized plane of mesial root of the first molar and separately mounted on glass slides. These sections then were dipped in an autoradiographic emulsion (SAKURA, NR-M2, Konishiroku Co. Tokyo) at 40°C and exposed for 8 weeks at 4°C. Next, the sections were developed (SAKURA, KONIDOL-X, Konishiroku Co. Tokyo), fixed (SAKURA, KONIFIX), rinsed with distilled water and stained with azure-eosin. The number of labelled periodontal ligament fibroblasts was counted in five zones of the periodontal ligament on the tooth axis, each zone 500 μm long, as indicated in Figure 2.

4. Statistical analysis

The statistical significance of differences between the experimental and control groups was evaluated by the Mann-Whitney U test.

Results

1. Body weight of rats

At start of the experiment, the mean body weight of the rats was 317±9 g (mean±S.D., n=87). On day 1, however, the body weights of every group decreased temporarily, perhaps because of the trauma associated with the tooth extraction, but they quickly recovered and their body weights then increased at a fairly constant rate until the end of the experiment. In the group of rats injected with 4.0 mgP/kg of HEBP, the body weight growth tended to slow after day 8, although significant difference could not be seen throughout the experimental period (control group: 329±8 g (n=6), 4.0 mgP-injected group: 318±10 g (n=5) on day 10).

2. Histological observation of the periodontal tissue in the periapical area

Figure 3 and 4 show the fluorescent photomicrographs of the periapical area of a rat mandibular first molar and photomicrographs of section stained with the Villanueva Goldner stain on day 10. Part of the tooth socket and root apex were surrounded by tetracycline and calcein labelling lines in the periapical area. The finding indicates that these tissues were formed during the experimental period.

The fluorescent microscopy in the control
Fig. 3 Fluorescent photomicrographs of the periodontal tissues in the periapical area on day 10.

After 10 days of molar extraction, mineralized dental cementum (MDC) and mineralized alveolar bone (MAB) formations were observed remarkably in control group (3a). In 4.0 mgP-injected group, calcein line could not be seen, however, significant amount of osteoid (OS) and unmineralized dental cementum (UDC) could be observed at tooth socket and root apex, respectively (3b). The width of periodontal ligament (PDL) in 4.0 mgP-injected group (3b) decreased remarkably as compared with the control (3a). T: Tetracycline line. C: Calcein line.

group (Fig. 3a) indicated that, new mineralized alveolar bone can been seen occupying all the area at the bottom of tooth socket, with some portions of the bone showing a comb-like morphology. The new mineralized dental cementum deposited only at the root apex. The light microscopic observation (Fig. 4a and 4c) showed that the unmineralized dental cementum was also seen covering the mineralized dental cementum. Further, the width of periodontal ligament in the periapical area increased remarkably, as compared with the width at the beginning of the experiment (day 0).

In the 0.25 and the 1.0 mgP-injected groups, there was no change in the amount of alveolar bone produced during the experimental period. In the 1.0 mgP-injected group, however, mineralization in the alveolar bone was partially inhibited and a thin osteoid layer was occasionally observed at part of alveolar bone facing to the periodontal ligament. The amount of mineralized dental cementum decreased and the thick layer of the unmineralized dental cementum was observed by the administration of HEBP. Also, the width of the periodontal ligament reduced, in comparison to the width of the control group.

In 4.0 mgP-injected group, calcein only weakly labelled the bone. The amount of mineralized alveolar bone produced during the experimental period decreased to 15% level found in the control group and the mineralized dental cementum formation was completely inhibited (Fig. 3b). Moreover, a thick osteoid layer and unmineralized dental cementum was observed throughout the periapical area of the tooth socket and root apex, respectively. Simi-
Figure 4c and 4d are large magnification of the periapical areas of Figure 4a and 4b, respectively. After 10 days of molar extraction, mineralized alveolar bone (MAB) was irregular in shape at the bottom of tooth socket in control group (4a and 4c). In 4.0 mgP-injected group, significant amount of osteoid (OS) and unmineralized dental cementum (UDC) could be observed at tooth socket and root apex, respectively (4b and 4d). The width of periodontal ligament (PDL) in 4.0 mgP-injected group (4d) remarkably decreased as compared with the control (4c).
Fig. 5 The changes of periodontal tissues in the periapical area by the administration of HEBP.

Each column is composed of mineralized dental cementum, unmineralized dental cementum, periodontal ligament, osteoid and mineralized alveolar bone. The amount of tooth extrusion was calculated as the height of each column minus control column on day 0 and it was inhibited dose dependently by the administration of HEBP on day 10. Alveolar bone formation (mineralized alveolar bone plus osteoid) was not affected by the administration of HEBP, however, mineralization of alveolar bone were inhibited in 4.0mg P-injected group. Dental cementum formation (mineralized dental cementum plus unmineralized dental cementum) was reduced only in 4.0 mgP-injected group and mineralization was inhibited dose dependently. Each vertical bar represents the mean ± S.D., (n=5-6).

In Figure 5, the height of each column represents the changes of the periodontal tissue in the periapical area of the rat mandibular first molar during the experimental period and it represents the amount of the tooth extrusion. Each column is composed of five tissue areas: the mineralized dental cementum, the unmineralized dental cementum (cementoid), the periodontal ligament, the osteoid and the mineralized alveolar bone.

On receiving daily injection of HEBP, tooth extrusion decreased dose dependently by day 10, which appears to be mainly due to the decrease in the width of the periodontal ligament. At the start of the experiment, especially in the 4.0 mgP-injected group, tooth extrusion decreased dose dependently by day 10.
Increased and this tendency continued until day 4. However, the total amount of the dental cementum (mineralized dental cementum plus unmineralized dental cementum) and alveolar bone (mineralized alveolar bone plus osteoid) did not change among the groups throughout the experimental period. One exception occurred in the 4.0 mgP-injected group in which, as can be noted in Figure 5 on day 10, dental cementum formation ceased in comparison to the other groups. Mineralization of the dental cementum was also completely disturbed in this group.

Figure 6 also graphically shows the changing pattern of the periodontal ligament width in the periapical area. In the control group, the augmentation of the width of the periodontal ligament was accelerated soon after the molar extraction and almost reached its plateau level by day 7. In the lower dose of HEBP-injected groups (0.25 and 1.0 mgP/kg), the augmentation rate decreased during the experimental period. In 4.0 mgP-injected group, the pattern of periodontal ligament augmentation was differed. Augmentation was rapid soon after the molar extraction, and reached a maximum level at day 2, after which augmentation decreased to the level of the lower dose groups on day 10.

4. Number of cementoblasts and osteoblasts in the periapical periodontal tissue

Cementoblasts and osteoblasts significantly increased soon after the extraction of molar in the control group (cementoblasts, day 0: 37.1 ±3.6 cells/mm; day 2: 52.2 ±3.1 cells/mm, p<0.05; osteoblasts, day 0: 37.3 ±5.9 cells/mm; day 2: 64.7 ±10.3 cells/mm, p<0.05). In control group, the number of cementoblasts was continued to increase until day 10 (83.9 ±3.0 cells/mm), however, the number of osteoblasts was quickly reached plateau level by day 4. In the experimental groups, number of these cells also increased to almost the same level of the control group, and no significant difference was noted between the control and experimental groups during the experimental period. Thus, the effect of HEBP on cementoblasts and osteoblasts in this region was negligible.

5. Histological observation of lateral periodontal tissue

The fluorescent microscopy indicated that, new mineralized alveolar bone observed on both lateral sides of tooth socket in coronal area in the control group. The shape of bone surface was rather smooth at lingual side, however, it was irregular at buccal side. The fluorescent lines of tetracycline and calcine were irregularly deposited and disappeared in some part in buccal side, indicating the extensive remodeling of bone during the experimental period. The new mineralized dental cementum can not be identified on the coronal root surface. By the light microscopic observation (Fig. 4a), osteoid was rare on the surface of mineralized alveolar bone both at buccal and lingual side.

In the 0.25 and the 1.0 mgP-injected groups, mineralized alveolar bone was being produced during the experimental period as indicated by the two fluorescent lines, however, in the 1.0 mgP-injected group, mineralization in the alveolar bone was partially inhibited. The light microscopic observation showed that a thin osteoid layer was occasionally observed on the lateral side of the tooth socket. In 0.25 mgP-injected group, the shape of bone surface...
at buccal side was rather smooth compared with the control. Calcein labelling can be observed as a smooth, non-interrupted line running on the surface of the buccal and lingual side of bone. The fluorescence seemed to be more intense compared with the control.

In 4.0 mgP-injected group, the mineralization of alveolar bone decreased and calcein labelling line can be rarely observed at both sides. A thick osteoid layer covered throughout the tooth socket (Fig. 4b). Similar to the periodontal tissue in the periapical area, there was a reduction in the width of the periodontal ligament as compared with the control (Fig. 4b).

6. Changes in the buccal and lingual alveolar bone

The mean width (area divided by the height of the bone) of the buccal and lingual alveolar bone in the experimental groups did not differ from that of the control group on day 10 (Fig. 7). However, a tendency towards an increment in the width was observed, especially on the lingual side of the 4.0 mgP-injected group.

7. Changes in the periodontal ligament and labelling index of the periodontal ligament fibroblasts

On day 7, the width of the periodontal ligament (Zones 1, 3 and 4, Fig. 2) reduced significantly in the 4.0 mgP-injected group in comparison to the width of the control group on day 7 (Fig. 8). Further, in the 0.25 mgP-injected group, a slight augmentation was noted in the buccal periodontal ligament (Zones 1, 2 and 3), whereas a significant reduction was observed in the lingual periodontal ligament (Zone 4).

Figure 9 illustrates the effect of HEBP on the number of labelled periodontal ligament fibroblasts on day 7. In each counted area (Zones 1 to 5), the labelling index decreased significantly in the HEBP-injected groups and the reduction seemed to be dose dependent. The periodontal ligament fibroblasts close to the periapical area (Zones 3 and 4) appeared to be more affected than the fibroblasts in the other three coronal areas (Zones 1, 2 and 5).

**Discussion**

Numerous theories have been proposed for interpreting the mechanisms and regulating factors responsible for tooth eruption. Up to the present, tooth eruption is thought to be regulated by the following three main factors:

1. occlusal force, 2. eruption force and 3. tissue resistance.

With respect to the eruption force (a prime mover), a variety of causative mechanisms have been proposed, which can be generally classified into two groups\(^{32-35}\). The first group of causative mechanisms involve the periodontal connective tissue, including the periodontal collagen, periodontal fibroblasts and the vascular pressure or fluid elements around the tooth. Second group of causative mechanisms involve the alveolar bone growth, root growth and pulp cell proliferation.

Despite considerable research, there is conflicting evidence for and against all the above
Fig. 8 The effect of HEBP on the width of periodontal ligament in lateral side of the tooth socket.

The width of periodontal ligament was significantly reduced in 4.0 mgP-injected group in Zone 1, 3 and 4 on day 7. Remarkable changes could not be seen in other administration groups. Values are the mean ± S.E., (n = 5-6).

Fig. 9 The effect of HEBP on the labelling index of periodontal ligament fibroblasts.

The labelling index in the experimental group was significantly reduced as compared with the control on day 7. The effects of HEBP were more remarkable in the periapical area (Zones 3 and 4 in Fig. 2) than the coronal areas. Values are the mean ± S.E., (n = 5-6).
mechanisms as being responsible for tooth eruption. Although in the continuously erupting tooth the periodontal ligament is widely accepted as a prime mover of tooth eruption, it has not been yet determined how closely the metabolism of the hard tissue, including the dental cementum and alveolar bone, is related to the eruption of noncontinuously erupting teeth.

Root formation also has long been considered as being a prime mover in the eruption process. However, it has been reported that the ability of tooth eruption was not affected by irradiation that stopped root formation, or by surgical manipulations of the root. These reports have indicated that root formation is not necessary in the processes of tooth eruption. In this study, tooth extrusion was inhibited in HEBP-injected rats, however, root formation (e.g. mineralized dental cementum plus unmineralized dental cementum formation shown in Fig. 5) still continued at the same rate as in the control group. This finding indicates, in accord with the previous reports, that tooth extrusion is regulated by mechanisms other than root formation.

Alveolar bone growth also has been reported to be responsible for the tooth eruption. In this regard, using a dog model, Marks and Cahill have described tooth eruption as being a series of metabolic events in the alveolar bone characterized by bone resorption and formation. In this study, on the other hand, the alveolar bone formation (mineralized alveolar bone plus osteoid formation in Fig. 5) in the periapical area did not appear to have any causal relationship in tooth eruption, based on the following two observations: At the first, 2 days after the extraction of molar, the tooth extrusion was not accord with the formation of the alveolar bone in the periapical area. At the second, at the end of the experiment, the tooth extrusion was inhibited dose-dependently by the administration of HEBP, whereas the amount of bone formation (mineralized alveolar bone plus osteoid) in the HEBP-injected groups was almost equal to that of the control group (Fig. 5). These observations would appear to indicate that bone formation was the result rather than the cause of tooth extrusion.

As for the periodontal ligament, which is believed to be the source of the eruptive force, the changing pattern of the periodontal ligament in the periapical area was coordinate with tooth extrusion in all groups of rats (Fig. 5 and 6). These results appear to indicate that augmentation of the periodontal ligament is mainly responsible for tooth extrusion. Further, with reference to our observations of the lateral side of tooth socket, HEBP injections significantly reduced the labelling index of the periodontal ligament fibroblasts as compared with the control group. Simultaneously, there was a reduction in the width of the periodontal ligament, especially in the 4.0 mgP-injected group. Such results indicated that HEBP has an inhibitory effect on the proliferative activity in the periodontal ligament of the rat mandibular molar, resulting the decrease of the eruption force.

However, there is another factor that also should be considered, that is the increment of the periodontal tissue resistance, and it may be that the low proliferation rate of the periodontal ligament fibroblasts or the consequent decrease in the periodontal matrix (e.g. the collagen fiber) turnover might have caused a reduction in the motility of tooth.

On day 2, the augmentation rate of the periodontal ligament remarkably increased in the high dose (4.0 mgP/kg) HEBP-injected group. As it has been recently reported that a high dose of HEBP reduces collagenous matrix formation in bone tissue and teeth, this HEBP impairment of collagen formation may have decreased the tissue resistance, which then lead to the initial acceleration in the widening of the periodontal ligament that was seen. Whatever the influence of HEBP on the periodontal connective tissue in the present study, its effect on hard tissue must be more important than that on other tissue, because of its pharmacological nature. The tooth extrusion that occurred after the HEBP injection seem to be prevented by the same mechanisms which were seen in the osteopetrotic (ia) rat. In this rat, incisor and first molar were rare, because unresorbed bone blocked the eruption pathway for emergence of a molar, thus suggesting that the removal of the overlying bone may be a necessary part of tooth eruption.
As seen in the present results, the mean width of the alveolar bone increased on both sides of the tooth by HEBP injection (Fig. 7) and this widening may have been due to the inhibition of bone resorption by HEBP. Such phenomenon was confirmed by the histological observation of alveolar bone, showing a smooth bone surface and non-interrupted fluorescent line, especially at buccal side.

Tissue resistance is thought to be regulated with the integrity of the periodontium. The anchorage associated with the bone-related region of the periodontal ligament, and by the bone itself. Therefore, tissue resistance seemed to be increased by HEBP administration because bone resorption was inhibited in the coronal area and because the rapid remodeling of the bone-related region of the periodontal ligament was impaired as discussed before. It is very likely that this inhibition of bone remodeling caused by HEBP injection is the main reason for the inhibition of tooth extrusion in the unopposed rat mandibular first molar and this phenomenon was very similar to that happen in osteopetrotic rat.

The present study have shown that tooth extrusion is inhibited by an injection of HEBP. It is suggested that the formation of the alveolar bone and dental cementum in the periapical area are unrelated to the tooth extrusion. Finally, the reduction of periodontal ligament activity and the increment in the tissue resistance mainly by the HEBP-induced inhibition of bone resorption in the coronal area seem to be the causes for the inhibition of tooth extrusion.

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