Orthodontically induced root resorption in rat molars after 1-hydroxyethylidene-1,1-bisphosphonate injection

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A single injection of 1-hydroxyethylidene-1,1-bisphosphonate inhibits the formation of acellular cementum in rat molars. Instead, an atypical hyperplastic cementum is formed. In this study the resistance of this cementum to resorption was tested by applying an orthodontic force. It was found that roots lacking acellular cementum were readily resorbed. The readiness with which the atypical hyperplastic cementum was resorbed without any increase in multinucleated osteoblasts may offer useful opportunities to study the different phases of hard-tissue resorption. □ *Dental cementum; orthodontics; tooth root*

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The dental hard tissues are normally not resorbed, whereas bone is continuously resorbed. Several suggestions have been made to explain this difference (for a review see Refs. 1–3). Among the suggested protective factors against root resorption are cementoid—that is, the unmineralized cementum matrix—and the acellular cementum. Orthodontic treatment is often, if not always, associated with minor areas of root resorption. Histologic studies in orthodontic treatment report a high incidence of root resorption, whereas it is more varied in clinical studies (4). This root resorption is usually superficial and stops when the orthodontic force is removed. The resorption seems to occur when the necrotic tissue of the hyaline zone is resorbed (5, 6). Occasionally, orthodontic treatment causes extensive root resorption, which may not be detected until a major portion of the root has been eliminated. This type of root resorption, which seems to be unpredictable, usually starts at the apical region of the teeth and progresses cervically (7, 8). The mechanisms behind the root resorption and possible predisposing factors are not known. Commonly cited components are individual susceptibility, hereditary predisposition, and systemic, local, and anatomic factors associated with orthodontic treatment (9–11).

Bisphosphonates are a class of drugs with high affinity for calcified tissues (12). They have been found to inhibit both bone resorption and mineralization. Most phosphonates primarily affect resorption, but 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) has been reported to affect mineralization and resorption equally (12, 13). When HEBP is given to young rats, it inhibits the initial mineralization of the mantle dentin, and no acellular cementum is deposited on the root surface (14, 15). A single injection of HEBP is sufficient to inhibit the formation of acellular cementum. Instead, a hard tissue is formed on the root surface. This tissue is thicker than normal acellular cementum and is referred to as atypical hyperplastic cementum. The aim of this study was to investigate the role of acellular cementum in protecting against root resorption, by comparing its resistance to orthodontic forces with that of HEBP-induced hyperplastic cementum.

Materials and methods

Experiment

Thirty Sprague–Dawley rats were used in this experiment. They were divided into five groups with six rats in each group (Table 1). Three rats from each group, here called HEBP rats, were injected subcutaneously with a single injection of HEBP (Procter and Gamble, Cincinnati, Ohio, USA) at the age of 12 days. The HEBP was dissolved in distilled water at a concentration of 4 mg/ml. A volume of 0.01 ml/g body weight was injected into each rat. The dose was equivalent to 10 mg phosphorus/kg body weight. The other three rats in each group served as controls, here called Ctrl rats, and received an equal volume of physiologic saline at the same age as the experimental animals. At the age of 27 days, 15 days after HEBP injection, all six rats in group I were killed by decapitation after being anesthetized with fluanisone and fontanyl (Hypnorm®, Janssen, Pharmaceutica, Beerse, Belgica; batch 91A11/508). Orthodontic appliances were inserted in all the 24 rats in the other four groups. The animals were anesthetized with an intramuscular injection of 0.3 ml Hypnorm per 1000 g body weight. The individual orthodontic appliances

Table 1. Different groups on the basis of the insertion of the orthodontic appliance, duration of the orthodontic force, and age at death

Groups	I	II	III	IV	V
Age at injection of HEBP or saline (days)	12	12	12	12	12
Age at insertion of orthodontic force (days)	–	27	27	27	27
Duration of orthodontic force (days)	–	7	14	7	7
Resting period after removal of orthodontic force (days)	–	–	–	7	28
Age at death (days)	27	34	41	41	62

Orthodontic force was not applied to group I. The rest of the animals in groups II, III, IV, and V received an orthodontic force at the age of 27 days, 15 days after the injection of 1-hydroxyethylidine-1,1-bisphosphonate (HEBP) or physiologic saline. There were three experimental and three control rats in each group.

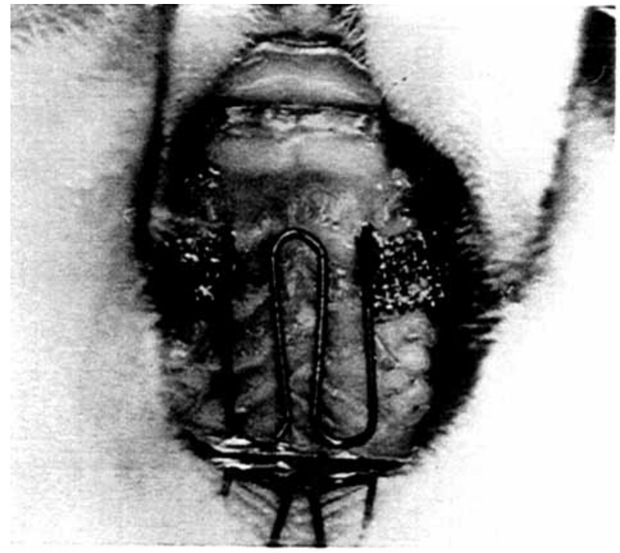

Fig. 1. Orthodontic appliance attached with bonding material over the wire netting covering the upper first molar.

were attached with Bonding Adhesive (Ormco, System 1+, DAB, Malmö, Sweden) to the palatal surface of the maxillary first molars. The appliances were left in place for 7 or 14 days.

Orthodontic appliance and force

A fixed lingual appliance was constructed for the buccal movement of the maxillary first molars. The appliance comprised a 0.28-cm Australian light wire (Wilcock, Ortopto, Göteborg, Sweden), which was bent to fit in between the molars and the curvature of the palate. A wire mesh was welded on the wire to cover the occlusal surfaces of the first molars. The measured distance between the first molars was approximately 4.0 mm in all animals (Fig. 1). At the start of the orthodontic treatment the appliance was expanded 1 mm more than this distance. An initial force was delivered to the maxillary first molars. The initial force was 150 mN as measured with a force-measuring gauge (16).

The animals were given a powdered diet (Evos R3, Stockholm, Sweden) during the orthodontic treatment. They were weighed at the start and at the end of the experiment. They were kept in cages on net floors to prevent the cutter from interfering with the orthodontic appliances. The orthodontic force was applied for 7 days to the rats in groups II, IV, and V and for 14 days to the rats in group III. The rats in groups II and III were killed immediately after the appliances were removed. After removal of the appliances, the rats in group IV had a resting period of 7 days, and the rats in group V 28 days, before being killed. All rats were anesthetized with Hypnorm before decapitation.

After the animals had been killed, the maxillae were dissected out and fixed in a solution of 4% paraformaldehyde in phosphate buffer (pH 7.4) for 24 h, demineralized in 24% ethylenediaminetetraacetic acid (EDTA) solution for 2 weeks, dehydrated in increasing concentrations of ethanol, and embedded in paraffin. Buccolingual sections were taken at the level of the mesial root of the first molar as parallel as possible to the long axis of this root. The section thickness was 5 μm. The sections were stained with hematoxylin–eosin, mounted with Eukitt, and examined under a light microscope.

Results

All animals followed the normal weight curve for young rats during the experimental period and did not seem to be disturbed by the treatment. The orthodontic appliances were examined regularly during treatment. They were all stable at the end of the treatment, with residual force acting on the maxillary first molars.

At the start of the experiment, before the insertion of orthodontic appliances, a distinct layer of acellular cementum covered the root surface in the Ctrl rats. This was in contrast to the HEBP rats, in which a layer of atypical hyperplastic cementum of various thicknesses was present at the root surface. A thin, irregular basophilic line covered the atypical hyperplastic cementum, and no distinct resorption lacunae were seen within the cementum layer. After application of the orthodontic appliances, root resorption was noticed in the HEBP rats, both on the pressure and tension sides. Root resorption also took place in the Ctrl rats, but it was limited to the area adjacent to the hyaline zone on the pressure side. After a resting period of 28 days after the removal of the orthodontic appliances, the re-

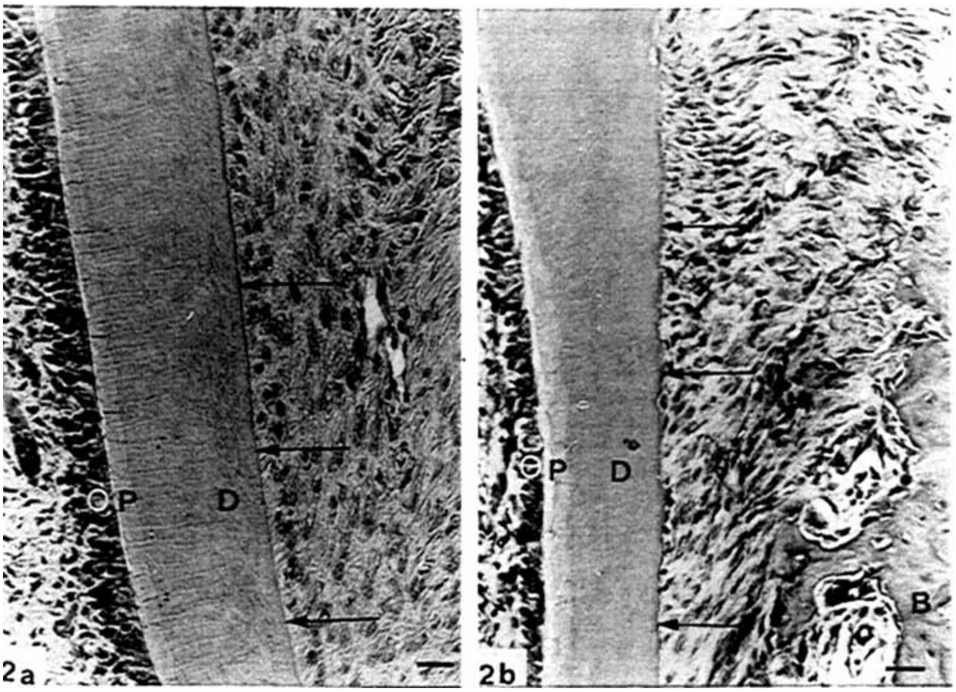

Fig. 2a. Buccolingual section of maxillary first molar in 27-day-old Control rat (group I). Arrows indicate acellular cementum along the root surface. O = odontoblasts; P = predentin; D = dentin. (Hematoxylin and eosin; bar = 40 μm.) 2b. Buccolingual section of maxillary first molar in 27-day-old 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) rat, 15 days after single injection of HEBP, without orthodontic treatment (group I). Note the irregular, basophilic reversal line and the absence of acellular cementum along the root surface (arrows). O = odontoblasts; P = predentin; D = dentin; B = bone. (Hematoxylin and eosin; bar = 40 μm.)

sorption lacunae were replaced by some hard tissue apposition in both HEBP and Ctrl rats. The appearance of periodontium at different observation periods is described in detail as follows.

Group I

In the sections from Ctrl rats that were killed at the age of 27 days without any orthodontic appliances being inserted, the acellular cementum appeared as a straight, darkly stained, distinct line covering most of the root surface (Fig. 2a). In the most apical part cellular cementum was seen. Bundles of collagen fibers extended from both the acellular and cellular cementum towards the alveolar bone. The alveolar bone had an uneven surface with few reversal lines.

In the HEBP rats, the most cervical part of the mesial root of the maxillary first molar (approximately 100 μm) was covered by a distinct, thin layer of acellular cementum. Apically to this area, at the root surface, there was an atypical hyperplastic cementum of various thicknesses, which covered the dentin with no distinct separation between the two tissues. An irregular basophilic line, which was thinner than acellular cementum, was observed on the surface of the atypical hyperplastic cementum. The staining intensity of this line was similar to that of the acellular cementum. At the apical area some cellular cementum was formed. Collagen fibers extended from the root surface formed before and after the injection of HEBP to the alveolar bone. Their orientation was less regular than that of the Ctrl rats of the same group. Numerous reversal lines were observed in the alveolar bone, more than in the Ctrl rats (Fig. 2b).

Group II

These rats had the orthodontic appliance for 7 days and were killed immediately after the removal of the appliances. In the Ctrl rats, on the pressure side, there was a large hyaline zone at the level of the alveolar crest (Fig. 3a). Multinucleated giant cells covered part of the hyaline zone. The root surface covered by the hyaline zone seemed to be intact (Fig. 3c), but root resorption was found at the periphery of this zone. No root resorption was seen in the remaining part of the root. On the tension side the acellular cementum was stained as a continuous, distinct, dark line. The periodontal ligament was well organized, and the alveolar bone had an even surface (Fig. 3a,b).

In the HEBP rats, on the pressure side, some hyaline zones could be observed in the periodontal ligament at

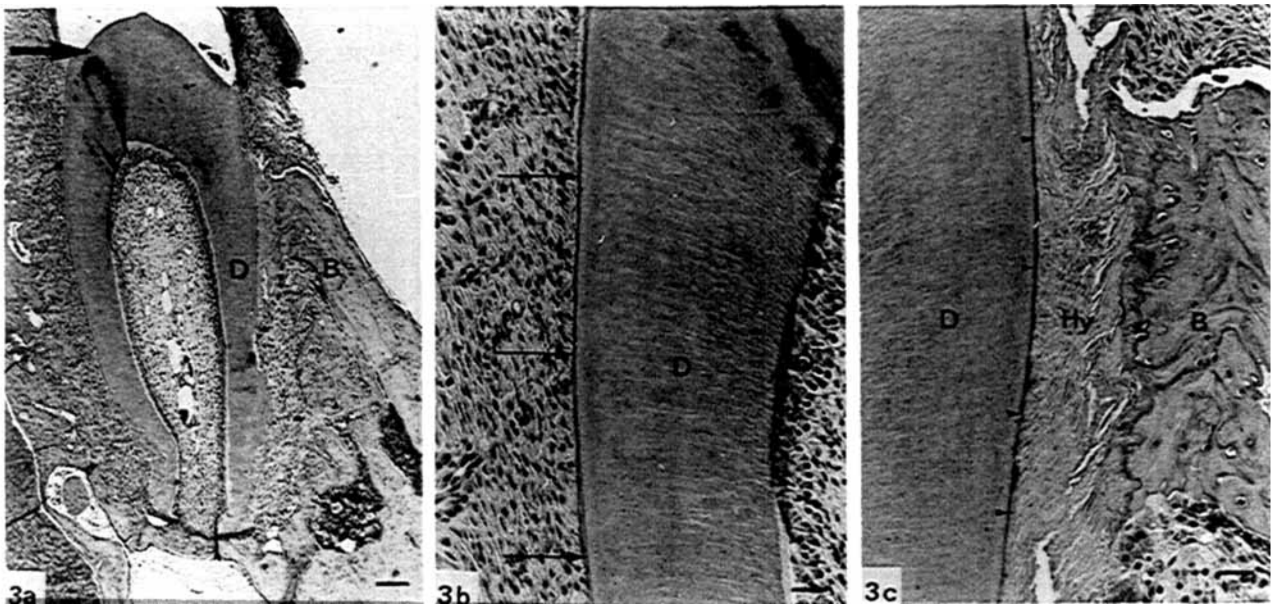

Fig. 3a. Buccolingual section of maxillary first molar in 34-day-old Control rat, after 7 days of orthodontic treatment (group II). Arrow indicates the direction of the force. D = dentin; B = bone. (Hematoxylin and eosin; bar = 200 µm.) 3b. Detail of tension side. No root resorption is seen along the root surface. Note darkly stained acellular cementum (arrows). D = dentin; O = odontolasts. (Hematoxylin and eosin; bar = 40 µm.) 3c. Detail of pressure side. A darkly stained line of acellular cementum (arrowheads) is seen under the hyaline zone. There is no root resorption under the hyaline zone on the root surface. D = dentin; Hy = hyaline zone; B = bone. (Hematoxylin and eosin; bar = 40 µm.)

the level of the alveolar crest. The hyaline zones were generally small. Resorption lacunae were observed both at the periphery and under the hyaline zones. In addition, numerous resorption lacunae were present along most of the root surface. The root resorption had penetrated into the dentin. The periodontal fibers were not well oriented, and the presence of macrophages in a wide area along the pressure side of the root gave the impression that a large hyaline zone was being resorbed. The alveolar bone showed numerous reversal lines and resorption lacunae at the periodontal surface (Fig. 4a,c). On the tension side, resorption lacunae were found in the root surface immediately apical to the normal acellular cementum. The atypical hyperplastic cementum was resorbed. The remaining part of the root was delineated by an irregular, thin, darkly stained line. The periodontal ligament fibers were better organized than on the pressure side. The alveolar bone was characterized by thin trabeculae and numerous reversal lines. No bone resorption was seen (Fig. 4b).

Group III

These rats had the orthodontic appliance for 14 days and were killed immediately after. In the Ctrl rats, on the pressure side, numerous multinucleated giant cells were found around the remnants of the hyalinized tissue (Fig. 5a). The root surface in this region showed wide but shallow resorption lacunae. The periodontal ligament, outside the hyalinized area, was well organized. Some remnants of hyalinized tissue were found at the bone surface. The bone had an uneven periodontal surface. On the tension side the periodontal tissue had a normal appearance, including acellular cementum, an organized periodontal ligament, and newly formed osteoid on the periodontal side.

In the HEBP rats, no hyaline zones were found on the pressure side. Numerous resorption lacunae containing multi- or mono-nuclear cells and small areas of atypical hyperplastic cementum could be seen along the root surface (Fig. 5b). The periodontal ligament fibers were more regularly organized than in the HEBP rats of group II. Numerous osteoclasts were found along the periodontal side of the alveolar bone surface. On the tension side no root resorption was found with the exception of a couple of small resorption lacunae. No typical acellular cementum layer could be seen, but in some areas there was a layer of atypical hyperplastic cementum. At the surface of this cementum there was an irregular, darkly stained line. The periodontal ligament was well organized (Fig. 5c). Osteoid tissue was found at the alveolar bone surface facing the periodontal ligaments.

Group IV

These rats had the orthodontic appliance for 7 days and were killed 7 days after the removal of the

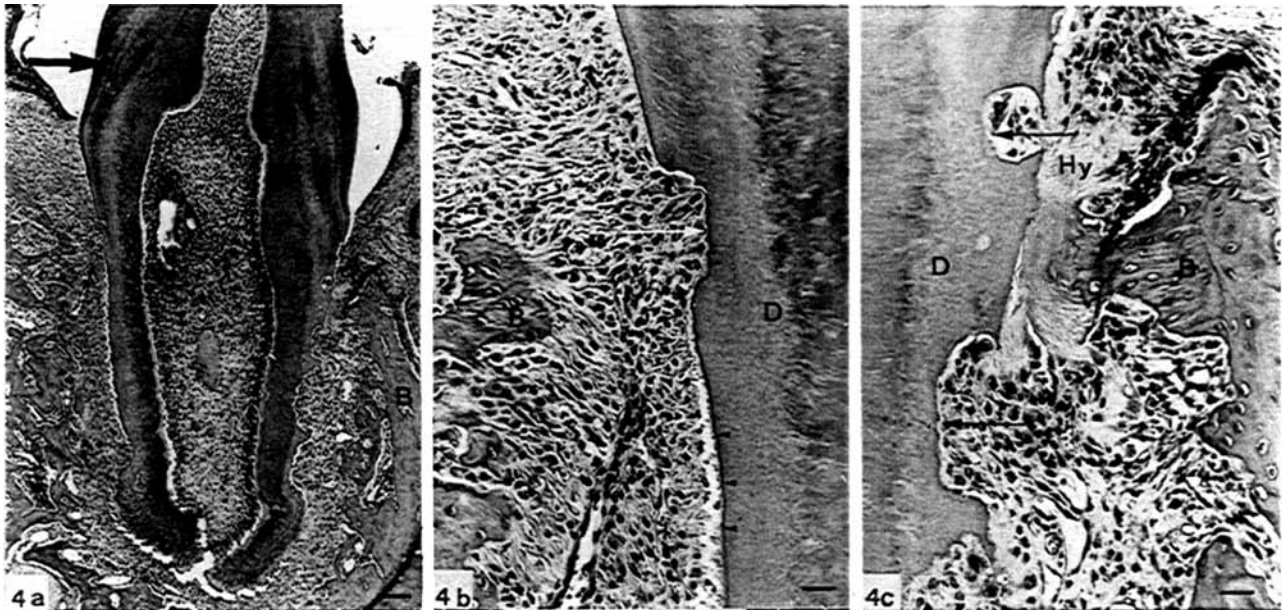

Fig. 4a. Buccolingual section of maxillary first molar in 34-day-old 1-hydroxyethylidine-1,1-bisphosphonate (HEBP) rat, after 7 days of orthodontic treatment (group II). Arrow indicates direction of force. A single injection of HEBP has caused a heterogeneous staining of dentin formed after injection of HEBP. D = dentin; B = bone. (Hematoxylin and eosin; bar = 200 µm.) 4b. Detail of tension side. Note root resorption (arrow) and basophilic reversal line along the root surface (arrowheads). D = dentin; B = bone. (Hematoxylin and eosin; bar = 40 µm.) 4c. Detail of pressure side. Note the extensive root resorption along the root surface and under the hyaline zone (arrows). Hy = hyaline zone; D = dentin; B = bone. (Hematoxylin and eosin; bar = 40 µm.)

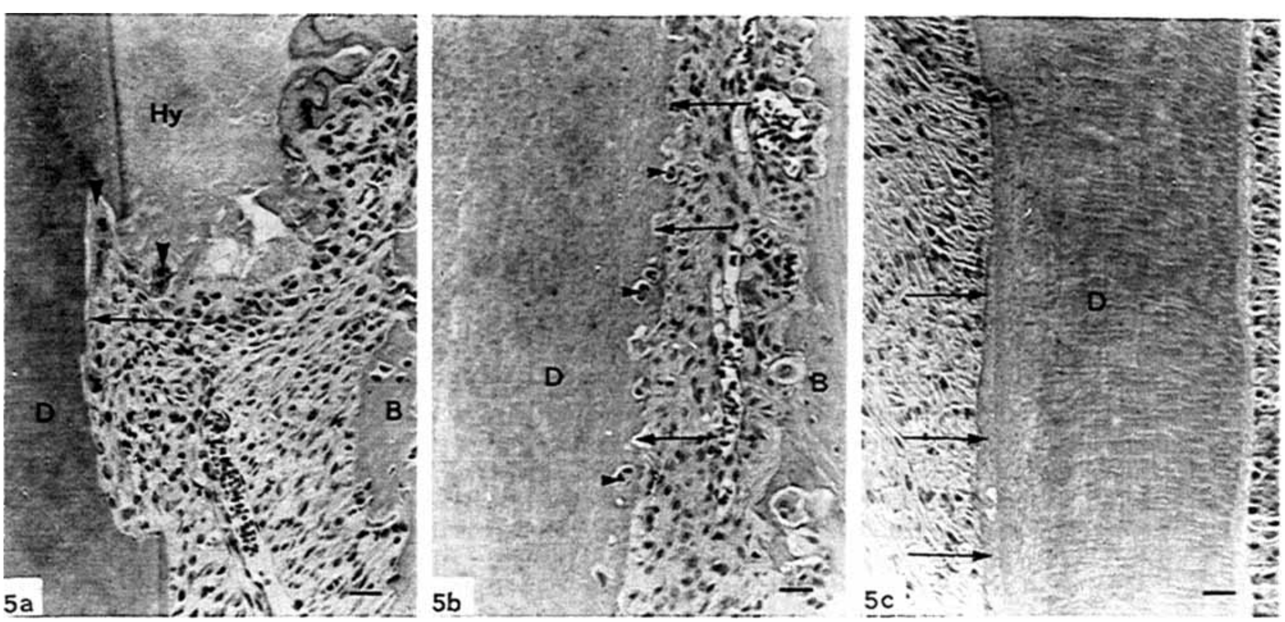

Fig. 5a. Pressure side in 41-day-old Control rat (group III), showing elimination of the hyaline zone by resorption advancing from the periphery and simultaneous resorption of the adjacent root surface (arrow). Arrowheads showing multinucleated cells resorbing dentin and hyalinized tissue. D = dentin; Hy = hyaline zone; B = bone. (Hematoxylin and eosin; bar = 40 µm.) 5b. Pressure side in 1-hydroxyethylidine-1,1-bisphosphonate (HEBP) rat at the age of 41 days, after 14 days of orthodontic treatment (group III). There is no visible hyaline zone. Note small resorption lacunae within the remnants of HEBP-induced atypical hyperplastic cementum along the root surface (arrows). Some cell inclusions are seen within HEBP-induced hyperplastic cementum (arrowheads). D = dentin; B = bone. (Hematoxylin and eosin; bar = 40 µm.) 5c. Tension side in HEBP rat, at the age of 41 days, after 14 days of orthodontic treatment (group III), showing remnants of HEBP-induced atypical hyperplastic cementum along the root surface (arrows). D = dentin. (Hematoxylin and eosin; bar = 40 µm.)

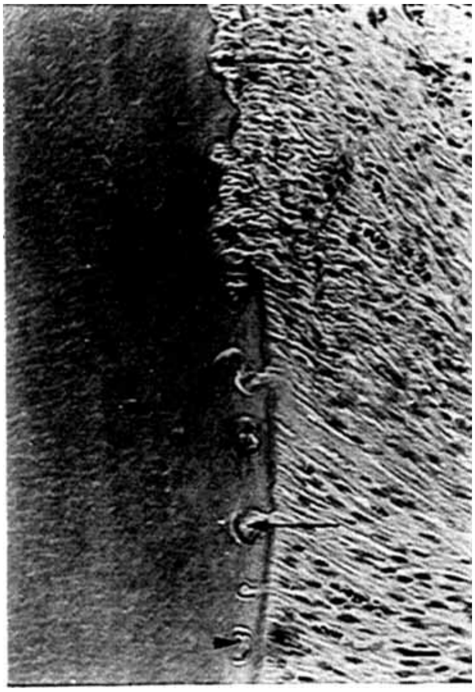

Fig. 6. Pressure side in 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) rat after 7 days with appliance followed by 28 days without appliance (group V), showing repair after root resorption. Periodontal ligament fibers extend from the hard tissue that has been formed in the resorption lacunae (arrows). Some cell inclusions are seen within HEBP-induced hyperplastic cementum (arrowheads). D = dentin. (Hematoxylin and eosin; bar = 40 μm.)

appliances. In the Ctrl rats, on the pressure side, root resorption with no hyaline zone was seen. The periodontal ligament was well organized except at the root resorption area. On the tension side the acellular cementum was stained as a continuous, distinct, dark line. The periodontal ligament was well organized, and no resorption was seen in the bone.

In the HEBP rats minor resorption lacunae at the root surface were found on the pressure side. No hyaline zones were seen in the periodontal ligament. There was a thin, basophilic, irregular line covering the root surface. The periodontal ligament was not well organized. Numerous thin, basophilic reversal lines were found in the bone. On the tension side some resorption lacunae were seen at the root surface, and the root was delineated by a thin, irregular line. The periodontal ligament was well organized. There was no resorption in the bone.

Group V

These rats had the orthodontic appliance for 7 days and were killed 28 days after the removal of the appliances. In the Ctrl rats, on the pressure side, there were some minor resorption lacunae at the root surface. No hyaline zone could be seen. The resorption lacunae were not deep and wide, like those seen in the Ctrl rats in group III. The periodontal ligament was regularly organized. On the tension side the acellular cementum was stained as a distinct, darkly stained line. The periodontal ligament and the bone had the same appearance as that seen on the tension side in group II.

In the HEBP rats no hyaline zones were found on the pressure side. The resorption lacunae were outlined by a darkly stained, irregular line. Bundles of collagenous fibers extended from the root surface to the alveolar bone (Fig. 6). The periodontal fibers were well organized, and the alveolar bone had a smooth and mineralized periodontal surface. On the tension side no root resorption could be seen. The root surface was delineated by an irregular, darkly stained line. The periodontal ligament was well organized.

Discussion

The present study confirms previously published results that a single injection of HEBP inhibits the formation of acellular cementum (14). The cementoid or atypical hyperplastic cementum that is formed instead has been found to resorb with time (15). This may be why only irregular areas of this tissue remained after 15 days at the time the orthodontic appliances were inserted. The mechanisms behind the initiation and cessation of the resorption of this tissue are essentially unknown. The reproducibility of this process, however, may be valuable in studying the different phases of hard tissue resorption. This study shows that the roots of HEBP rats were much more readily and extensively resorbed than those of normal teeth.

When the resorption had stopped, the root surface was outlined by a darkly stained, irregular, thin line. This line was similar to the reversal line in bone, indicating the cessation of the resorption in that specific area. Studies of the bone remodeling sequence have shown that, at the reversal phase, a hard tissue matrix is deposited and evens out the resorption lacunae (18). In contrast to the reversal lines in bone, there was no new hard tissue formed on top of the 'radicular reversal line', either on the pressure or on the tension side.

The hyaline zone is described in the literature as a sterile necrotic area that causes cellular reactions in the adjacent alveolar bone and root surface after the application of an orthodontic force (19–21). The development of the hyaline zone, root resorption, and the healing process could be observed in all groups in this study. The hyaline zone has been shown to resist degradation and to persist for a long time, the duration depending on the magnitude of the force (22, 23). The peripheral areas of the hyalinized compressed tissue are removed by an invasion of cells and blood vessels from the adjacent undamaged periodontal membrane. The hyalinized material is ingested by the phagocytes (6, 24). In the rats that received a single injection of HEBP the elimination of the hyaline zone was faster than that seen in the control rats. A possible explanation for the faster elimination of the hyaline zone is that a more massive resorption in the surrounding root and bone with better access to the necrotic tissue for the invading cells results in a different elimination time. It is also possible that the hyaline zone in the HEBP rats was less dense than in the Ctrl rats, and this might play a role in its elimination.

In agreement with other studies, there was no root resorption immediately under the hyaline zone in the Ctrl rats (19, 25, 26). In the HEBP rats, however, resorption lacunae were observed under the hyaline zone. Moreover, the resorption lacunae penetrated deeply into the dentin. This might partly be due to a HEBP-induced developmental defect of the dentin.

In conclusion, the experimental model used in this investigation made it possible to study the root resorption from a different approach, that of the developmental disturbances in mineralized tissues. The readiness with which the atypical hyperplastic cementum is resorbed without any increase in multinucleated osteoblasts may be valuable in studying hard-tissue resorption.

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