Root surface defects in rat molar induced by 1-hydroxyethylidene-1,1-bisphosphonate

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Cementum surface alterations induced by 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) in the maxillary first molars in rats were studied by means of scanning electron microscopy. Single or triple injections of HEBP inhibit the formation of acellular extrinsic fiber cementum and delay the formation of cellular mixed-fiber cementum. The results indicate the importance of acellular extrinsic fiber cementum as a protection barrier against root resorption and the different mechanisms underlying the formation of the two cementum varieties. □ *Dental cementum; root resorption; scanning electron microscopy*

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Previous studies have shown that bisphosphonates, including 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), if administered in sufficient doses, inhibit the mineralization of normal calcified tissues such as bone and cartilage (1–3). With regard to the effect of HEBP on dental hard tissues, it has been shown to cause a zone of hypomineralization in the forming enamel of rat molars after the administration of a single dose (4–8). In newly erupted rat molars, gross hypoplastic lesions have been seen covering a large area of the enamel surface (9). In studies on the effect of HEBP on dentinogenesis in the developing rat molar, HEBP not only induced hypo- and hyper-mineralized incremental bands in the dentin but also caused the formation of dentinal niches containing unmineralized dentin (10). Low doses of HEBP inhibit crystal growth in the mineralization zone of predentin, resulting in crystallites of smaller size than in control animals (11). In addition, after multiple injections of HEBP no acellular cementum is deposited on the surface of the unmineralized dentin of the continuously growing mouse incisor (12). In studies with mouse molars multiple injections of HEBP have led to the formation of a massive amount of osteoid-like tissue, both on the root surface and on the alveolar bone (13). In a study on rat molars this osteoid-like tissue on the root surface has been referred to as atypical hyperplastic cementum and topographically resembles cellular mixed-fiber cementum (14).

Two types of cementum are usually recognized: acellular and cellular (15). These two types can be further subdivided, depending on the presence of cells and the presence and origin of collagen fibers (15, 16). Acellular cementum is usually found in the occlusal one-third of the roots, its organic matrix being characterized by the presence of densely packed collagen fibers and referred to as acellular extrinsic fiber cementum. The apical and interradicular parts of the roots are covered by cellular mixed-fiber cementum. They contain cementocytes with an uneven distribution and density.

Recently, studies were done in our laboratory to investigate various physical properties of the periodontal tissues of fully formed rat maxillary first molars at different time intervals. Single or multiple injections of HEBP resulted in a reduction of the extraction force (unpublished results). In this study the root surface of the maxillary first molars in HEBP-injected rats (after single or multiple administrations of the drug) was examined by scanning electron microscopy. The aim was to examine the disturbances induced in cementum by HEBP to ascertain whether these could account for the reduction in extraction force. It was also hoped that such a study would shed more light on the formation of acellular and cellular cementum.

Materials and method

Four groups of Sprague–Dawley rats, 10 to 12 days of age, were used in this experiment. Two groups of rats received either a single injection of HEBP at 12 days of age or one injection of HEBP daily at 10, 11, and 12 days of age. The other two groups served as controls and received either a single injection of physiologic saline at 12 days of age or one injection daily at 10, 11, and 12 days of age. The rats were killed by decapitation 9, 25, 35, 45, or 90 days after the injections at the age of 21, 37, 47, 57, or 102 days, respectively. The HEBP was kindly donated by Proctor and Gamble (Cincinnati, Ohio, USA). It was dissolved in distilled water at a concentration of 4 mg/ml. For each injection a volume of 0.1 ml/g body weight was given, corresponding to a dose of 10 mg phosphorus/kg body weight. Two rats from each group were killed after each observation

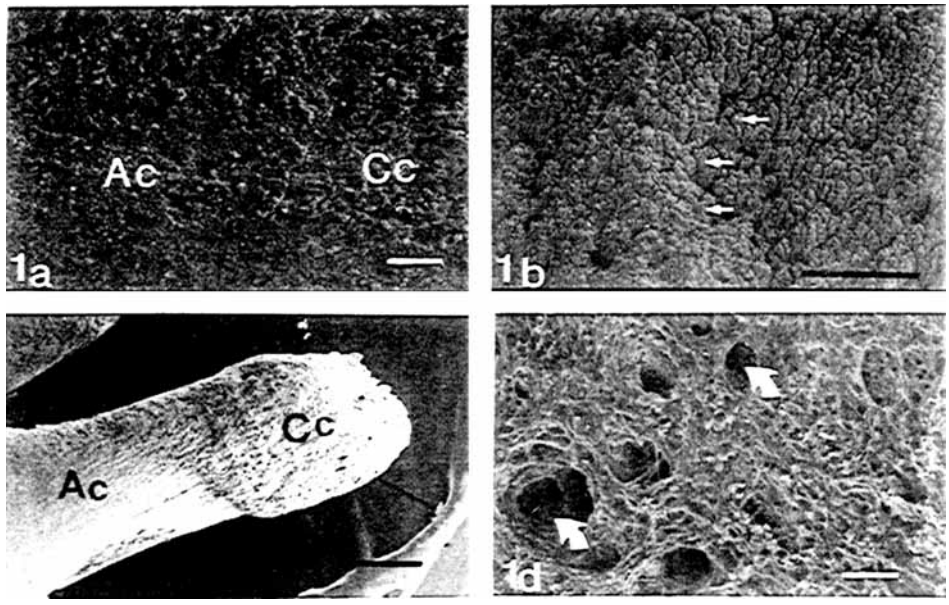

Fig. 1a. Mesial surface of mesial root of maxillary first molar in a 21-day-old control rat. Note accumulation of mineral nodules at acellular extrinsic fiber cementum surface and the rather rough surface of cellular mixed-fiber cementum. No mineralized ends of extrinsic fibers are seen at the root surface. Ac = acellular extrinsic fiber cementum; Cc = cellular mixed-fiber cementum (undifferentiated at this age). Bar = 1 μm. 1b. Acellular extrinsic fiber cementum surface in 102-day-old control rat, showing closely packed, well-organized mineralized ends of extrinsic fiber bundles (arrows). Bar = 5 μm. 1c. Cellular mixed-fiber cementum covering apical two-thirds of mesial root surface and elevated above acellular extrinsic fiber cementum surface level in 57-day-old control rat. Ac = acellular extrinsic fiber cementum; Cc = cellular mixed-fiber cementum. Bar = 40 μm. 1d. Cellular mixed-fiber cementum surface in 102-day-old control rat, showing cementocyte lacunae (arrows) and intrinsic fibers surrounding them. Bar = 1 μm.

period. The maxillary first molars from both sides, of both experimental and control rats, were dissected free and fixed for 24 h in a solution of 2.5% gluteraldehyde and 4% paraformaldehyde dissolved in phosphate buffer solution at pH 7.4. The specimens were further immersed in Dakin's solution for 20 min to remove any superficial organic material. The teeth were rinsed in distilled water, dehydrated in increasing concentrations of ethanol, and air-dried overnight. The specimens were then mounted on aluminum stubs in a position that enabled the mesial surface of the mesial root to be studied. The specimens were then coated with 20 nm Au-Pd alloy (Polaron Equipment Ltd, E 5150) and examined in a scanning electron microscope (Joel JSM-820 SEM) at an accelerating voltage of 10 kV.

Results

The investigation focuses on the topographic changes of the mesial surface of the mesial root of maxillary first molars. There were no differences between the two control groups. At 21 days of age in the control group the root surface was covered by accumulations of mineral nodules in the form of globular deposits (Fig. 1a). With increasing age the surface of the cervical part of the root was characterized by short projections, apparently consisting of extrinsic fiber bundles. At the age of 102 days the closely packed mineralized ends of the extrinsic fibers created the homogeneous appearance of an 'acellular extrinsic fiber cementum' surface (Fig. 1b). In the most apical part, at 21 days of age, an unevenly spread accumulation of mineral nodules coalesced around small cementocyte lacunae and created a rough structure of the root surface, giving the appearance typical of cellular cementum (Fig. 1a). With increasing age, the mineralized ends of the closely packed extrinsic fibers were seen as elevations around the cementoblast lacunae. At 57 days of age, apically, almost two-thirds of the mesial root was covered by rough cellular mixed-fiber cementum (Fig. 1c). The mineralized ends of the extrinsic fibers were surrounded by intrinsic fibers. These intrinsic fibers lay around the extrinsic fibers at a depressed level, in the plane parallel to the root surface at a right angle to the extrinsic fibers. At 102 days of age the intrinsic fibers mineralized more extensively, reaching to the mineralized ends of the extrinsic fibers, and forming a picture typical of 'cellular mixed-fiber cementum'. The lumpy appearance of the cellular mixed-fiber cementum was due to a porous root surface

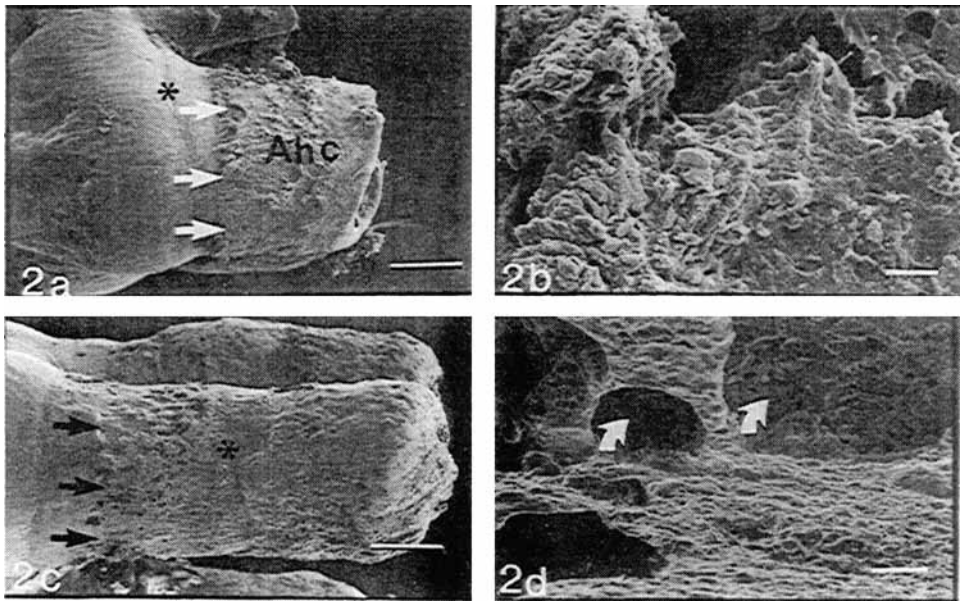

Fig. 2a. Mesial surface of mesial root of maxillary first molar 9 days after a single injection of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), in 21-day-old rat, showing demarcation line (arrows) separating root surface formed before and after HEBP injection, and HEBP-induced atypical hyperplastic cementum. Cementoenamel junction indicated by an asterisk. Ahc = atypical hyperplastic cementum. Bar = 40 μm. 2b. Detail of root surface formed after a single injection of HEBP showing surface structure of HEBP-induced atypical hyperplastic cementum. Bar = 1 μm. 2c. Mesial root of maxillary first molar, 90 days after single injection of HEBP, in 102-day-old rat. Arrows indicate root resorption at surface of HEBP-induced atypical cementum. Note smooth zone apical to root resorption (asterisk). Bar = 40 μm. 2d. Detail of root resorption. Note absence of extrinsic fibers. Arrows indicate resorption lacunae. Bar = 1 μm.

with cementocyte lacunae, which were surrounded by the intrinsic fibers (Fig. 1d).

In the experimental animals 9 days after a single injection of HEBP at the age of 21 days an atypical cementum was seen. This was elevated above the level of the acellular extrinsic fiber cementum, thus forming a demarcation line that separated the atypical cementum from the root surface formed before the HEBP injection. The surface structure of the atypical cementum was characterized by elevated spikes and knobs, giving the root surface a rough appearance (Fig. 2a, b).

Twenty-five and 35 days after a single injection of HEBP there was some additional accumulation of mineral nodules fusing with each other, and some minor resorption lacunae were seen. Forty-five days after a single injection of HEBP, almost the entire surface of the atypical cementum was covered by resorption lacunae. The resorption did not penetrate into the dentin, and some mineralized tissue lined the resorption lacunae. Apical to the resorption zone a smooth zone was observed. This was situated between the already resorbed atypical cementum and the most apical part of the root, where the cellular mixed-fiber cementum was located. Ninety days after a single injection of HEBP some recovery had taken place; the width and depth of the resorption lacunae seemed to be decreased, and some more mineralized tissue was deposited both in the resorption lacunae and on the root surface. No mineralized ends of extrinsic fibers were seen on the surface of the atypical cementum at any of the observation periods (Fig. 2c, d).

Apically, 9 and 25 days after a single injection of HEBP there was no accumulation of mineral nodules, and the root surface was rather smooth compared with the controls. However, 35 days after the administration of the drug, evenly dispersed, closely packed, small mineral nodules were observed. At 102 days of age, 90 days after HEBP injection, the mineralized ends of the closely packed extrinsic fibers had an appearance typical of the acellular extrinsic fiber cementum in the control animals at the age of 47 days. However, there were no mineralized intrinsic fibers around the extrinsic fibers (Fig. 3a, b, c).

Nine days after triple injections of HEBP, at 21 days of age, SEM observation of the root was not possible because of the dissolution of the unmineralized dentin during the preparation. Twenty-five days after triple HEBP injections the whole root surface was characterized by unevenly dispersed, scanty mineral nodules in the form of elevated spikes representing atypical

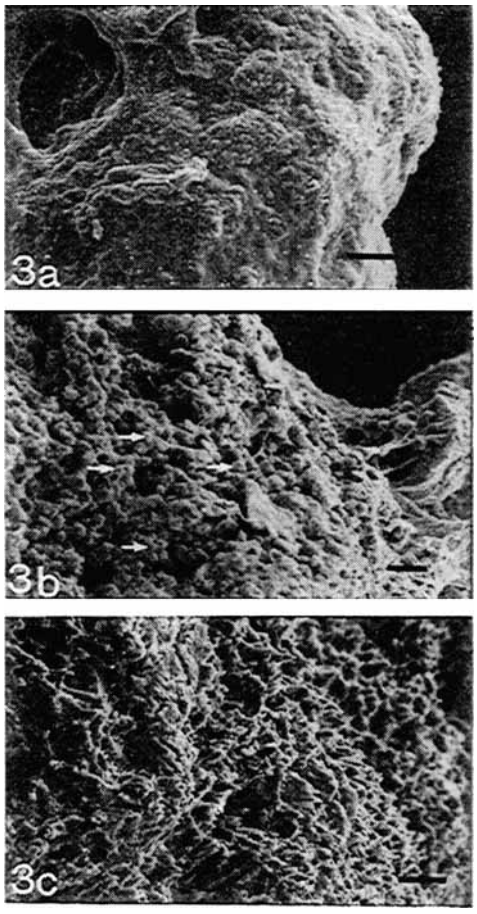

Fig. 3a. Most apical part of mesial root of maxillary first molar, 9 days after single injection of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), in 21-day-old rat. Note absence of mineralized nodules. Bar = 1 μm. 3b. Surface of most apical part of root showing evenly dispersed, closely packed, small mineralized nodules (arrows), 35 days after single injection of HEBP, in 47-day-old rat. Bar = 1 μm. 3c. Ninety days after single injection of HEBP, in 102-day-old rat, mineralized ends of closely packed extrinsic fibers at the most apical part of root. Bar = 1 μm.

cementum (Fig. 4a). Thirty-five days after triple injections of HEBP some resorption lacunae were found at the atypical cementum surface. Forty-five days after the injection of the drug the resorption lacunae had penetrated into the dentin, exposing the dentin tubules. The resorption zone was bordered both cervically and apically by a smooth zone. This smooth zone, with no resorption lacunae at the most cervical part of the root closer to the cementoenamel junction was narrower than the one located apical to the resorption zone (Fig. 4b, c). Ninety days after triple injections of HEBP, at the age of 102 days, some mineralized hard tissue seemed to be deposited at the root surface in the resorption lacunae. No mineralized ends of extrinsic fiber bundles were seen (Fig. 4d).

Observation of the most apical part of the root, 25 days after triple injections of HEBP, was precluded because the preparatory procedure led to dissolution of the tissue. Thirty-five days after the administrations of the drug the apical part of the root was rather smooth, and no resorption lacunae were noticed. Forty-five days after the HEBP injections an accumulation of mineral nodules and some mineralized ends of extrinsic fibers were seen apically; 90 days after the injections, there were bundles of mineralized extrinsic fibers at the root surface. Between these extrinsic fiber bundles, cementocyte lacunae were observed but no mineralized intrinsic fibers were found (Fig. 5a, b, c).

Discussion

The present results show that the SEM is a valuable tool in studying the fine details of the organization of normal and HEBP-induced cementum surfaces. However, we should note that the specimen preparation procedures that we used made it difficult to investigate the HEBP-induced atypical cementum surface a short time after the injections.

The prominent feature of these results is that the formation of acellular extrinsic fiber and cellular mixed-fiber cementum was inhibited by single or triple injections of HEBP. However, a recovery was possible 90 days after the administration of the drug.

It has been observed in other studies that, after HEBP administration, a large amount of osteoid-like tissue is formed on the root surface and on the alveolar bone (12, 13, 17). This osteoid-like tissue on the root surface has been referred to as atypical hyperplastic cementum (14). However, mineral accumulation on the surface of this atypical cementum is different from osteoid and other unmineralized hard tissue matrices, particularly in its resistance to the enzymes and chemicals used during the SEM preparatory procedures. This cementum also differs from normal acellular extrinsic fiber cementum in that no extrinsic fiber bundles were observed at its surface. The mechanism behind the development of this atypical cementum is not known, but it may be a selective effect of HEBP on specific cell populations invading the periodontium. There was a delay in the formation of the atypical cementum in the rats who received triple injections of HEBP. This delay might have occurred in relation to the inhibitory effect of HEBP on the mineralization of mantle dentin or directly on the mineralization of cementum. It has been suggested that the disturbance in acellular cementum formation in HEBP-treated rats is related to the mineralization disturbances of the underlying root dentin (14).

There was no atypical cementum formation in the apical part of the molar roots in HEBP-injected rats; the formation of normal cellular mixed-fiber cementum was only delayed by the HEBP injections. Ninety days

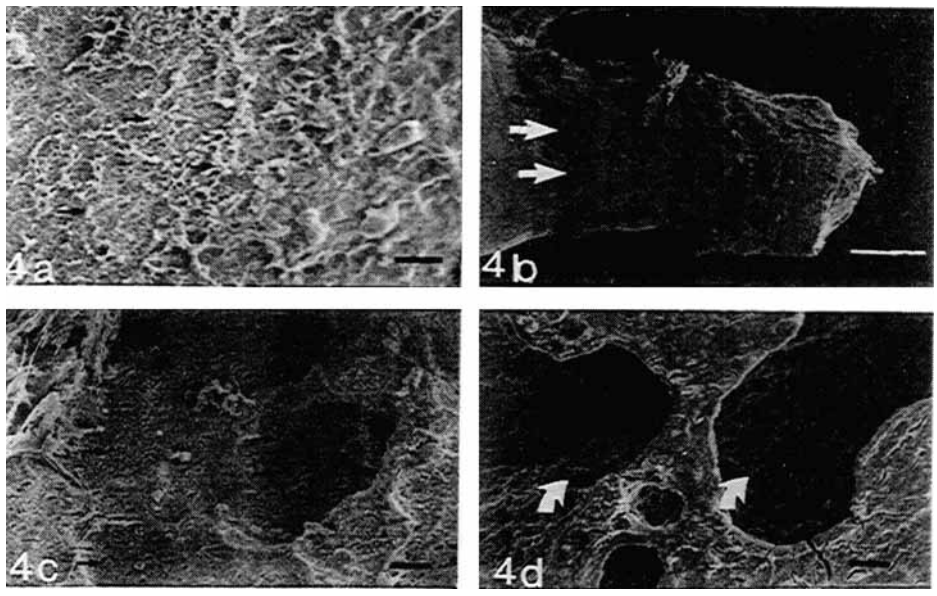

Fig. 4a. Twenty-five days after triple injections of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), in 37-day-old rat, showing scanty mineralized nodules at root surface formed after HEBP injections (arrows). Bar = 1 μm. 4b. Root resorption, 45 days after triple injections of HEBP, in 57-day-old rat (arrows). Note smooth zone at cementoenamel junction and apical to root resorption (asterisk). Bar = 1 μm. 4c. Detail of Fig. 4b, showing penetration of root resorption into dentin. Arrows indicate dentin tubules. Bar = 1 μm. 4d. Ninety days after triple injections of HEBP, in 102-day-old rat, showing some mineralized hard tissue deposition in resorption lacunae (arrows). Bar = 1 μm.

after both single and triple injections of HEBP the surface of the most apical part of the molar roots consisted of only mineralized ends of extrinsic fibers, without intrinsic fibers surrounding them, similar to what is seen in the control rats at the age of 37 days. This delay in the formation of cellular mixed-fiber cementum can also be attributed to the temporary effect of HEBP on mineralization.

It may be assumed that most of the defects on the developing dental tissues were related to the inhibition of mineralization. It has been shown that dentin mineralization inhibited by HEBP in rat molars displays an abnormal mineralization pattern (18). Some surface lesions have been reported on the enamel surface of maxillary incisors in rats who received a single injection of HEBP (8). These enamel surface lesions were of three different types, indicating that a single injection of HEBP interferes with different stages of enamel formation.

The crystals formed during the mineralization of acellular extrinsic fiber cementum are known to be composed of hydroxyapatite and appear both between and within the collagen fibers in the shape of platelets (15). HEBP is known to inhibit the transformation of amorphous calcium phosphate into hydroxyapatite (19) and to delay the aggregation of apatite crystals into larger clusters in vitro (20). The surface lesions of the dental hard tissues induced by this drug might therefore be a result of a direct effect on crystal growth. However, there could be a direct effect of HEBP on the dentinal matrix-forming cells—that is, ameloblasts, odontoblasts, and cementoblasts—resulting in changes of synthesis and composition of the matrix proteins. HEBP affects the synthesis and the biologic properties of several other matrix components, such as glycoaminoglycan and proteoglycan in cartilage in vitro (21), dentin collagen in vivo (5, 22), and phospholipids in vitro (23). In addition, it has been shown that HEBP interferes not only with the deposition of minerals but also with the deposition of the matrix components of acellular cementum (24). Alternatively, it has been suggested that the deposition of these components is coupled to the deposition of the mineral phase (25). In this study HEBP might have prevented the deposition of acellular extrinsic fiber cementum matrix by preventing the formation of minerals. However, the difference in the effect of HEBP on the two cementum varieties may be due to different mechanisms underlying cellular and acellular cementum formation.

It is well known that HEBP inhibits bone resorption. Two possible mechanisms have been suggested: i) a physiochemical interaction with apatite crystals, and ii) an interference with cellular metabolism, affecting the ability of osteoclasts to resorb bone (25).

In this study, however, the HEBP-induced atypical cementum was easily and extensively resorbed. This

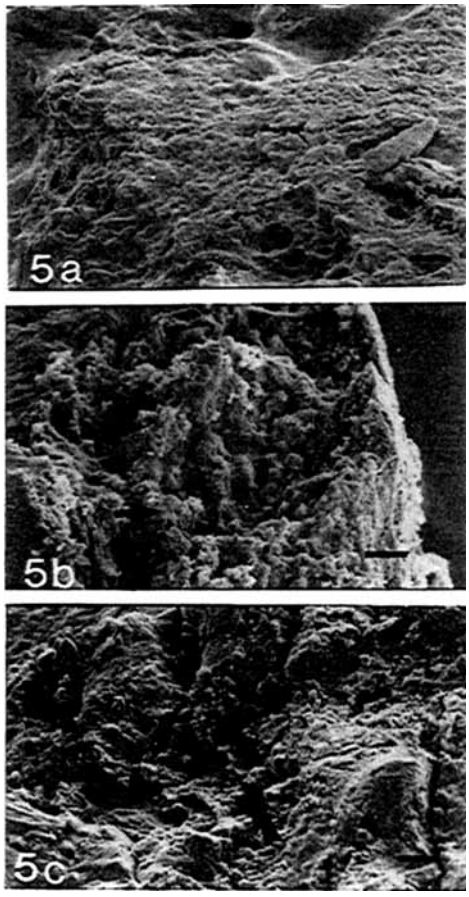

Fig. 5a. Detail of the most apical part of the mesial root, 35 days after triple injections of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), in 47-day-old rat, resembling the root surface seen 9 days after a single injection of HEBP shown in Fig. 3a. Bar = 1 μm. 5b. Forty-five days after triple injections of HEBP, in 57-day-old rat, showing some mineralized ends of extrinsic fibers at the surface of the most apical part of root (arrows). Bar = 1 μm. 5c. Ninety days after triple injections of HEBP, at the surface of the most apical part of the root, showing mineralized ends of extrinsic fiber bundles with cementocyte lacunae in between (arrows). Note absence of mineralized intrinsic fibers. Bar = 1 μm.

spontaneous resorption stopped at the peripheral surface level of dentin after a single administration of HEBP. After triple injections of HEBP there was a delay in the formation of atypical cementum and in the accumulation of mineral nodules. The atypical cementum formed after triple injections of HEBP was resorbed spontaneously a short time after its formation, and resorption continued into the dentin. The mechanism behind the initiation and cessation of the resorption of this tissue is essentially unknown. The inhibition of osteoclast activity does not satisfactorily explain the action of this drug in vivo. It may involve other cell systems that are related to bone resorption. In another study on mice molars, after 50 days of HEBP administration resorption was seen along the acellular cementum surface (26). The authors suggested that cells of the periodontal ligament were killed by HEBP, and this in itself might have resulted in cementum resorption, perhaps by the local release of factors that would attract resorbing cells to the root surface. One of the mechanisms involved in the protection or modification of the resorption of the root is thought to be that cementum, being more resistant to resorption than the dentin, to some extent protects the root from resorption (27). The obvious and extensive resorption of the root surface in HEBP-injected rats found in our study supports this idea.

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