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Evaluation of the topical effect of alendronate on the root surface of extracted and replanted teeth. Microscopic analysis on rats' teeth

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Abstract – The treatment of choice for tooth avulsion is replantation. The ideal replantation should be realized as quickly as possible, or at least, the avulsed tooth should be kept in an adequate solution to preserve the periodontal ligament attached to the root. If that is not possible, treatment of the radicular surface should be done in order to prevent radicular resorption. The purpose of this study was to test sodium alendronate as a substance for topical treatment of the radicular surface of avulsed teeth in an attempt to prevent the occurrence of dental resorptions. Fifty-four rat maxillary right central incisors were extracted and replanted. Group I – extra-alveolar dry period of 15 min, intracanal dressing with calcium hydroxide (CALEN®, S.S. White, Artigos Dentários LTDA, Rio de Janeiro, Brazil) and replantation; Groups II and III – extra-alveolar dry periods of 30 and 60 min, respectively, immersion in 1% sodium hypochlorite for 30 min for removal of the periodontal ligament, washing in saline solution for 5 min, and treatment of the radicular surface with 3.2 mg/l sodium alendronate solution for 10 min. Intracanal dressing with calcium hydroxide and replantation followed. At 15, 60, and 90 days post-reimplantation, the animals were killed and the samples obtained and processed for microscopic analysis. The results indicated that sodium alendronate was able to reduce the incidence of radicular resorption, but not of dental ankylosis. No significant differences were observed regarding variations in the extra-alveolar periods among the groups.

Adriana Lustosa-Pereira, Roberto Brandão Garcia, Ivaldo Gomes de Moraes, Norberti Bernardineli, Clovis Monteiro Bramante, Eduardo Antunes Bortoluzzi

Department of Endodontics, Bauru Dental School, University of São Paulo, Bauru, São Paulo, Brazil

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Adriana Lustosa-Pereira, Faculdade de Odontologia de Bauru – Disciplina de Endodontia, Al. Octávio Pinheiro Brisolla, 9-75, Vila Universitária, Bauru-SP, CEP 17012-901, Brazil
Tel.: 55 14 3235 8344
Fax: 55 14 3224 2788
e-mail: adrianalustosa2003@yahoo.com.br, adrianalustosa@hotmail.com

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Traumatic lesions in anterior permanent teeth are frequent accidents that affect mostly school-aged children, from 7 to 14 years of age, with intense physical activity and no notion of danger (1). These lesions may account for small coronary fractures or even complete dislocation of the tooth from its alveolus, characterizing a case of dental avulsion. Approximately 16% of all traumatic lesions in permanent teeth result in dental avulsion (2). The treatment recommended is dental replantation, which is the act of replacing the avulsed tooth in the alveolus (3).

When the avulsed tooth is immediately replanted or stored in a solution capable of preserving the periodontal fibers attached to the root, the chances of success in replantation increase considerably (4–7). In fact, humid storage solutions, such as milk, for example, favor the success of the replantation procedure, whereas keeping the tooth in a dry environment compromises the prognosis significantly (8).

One of the consequences of dental avulsion and posterior replantation is radicular resorption (1), and its occurrence depends on factors such as extra-alveolar period (5), storage solution (9), microbial contamination (10), and stage of radicular formation (11). Root resorptions may be classified as inflammatory or replacement. Inflammatory resorption may be further classified as internal, when occurring on the root canal wall, or external, when occurring on the external part of the root. It may even be transitory or progressive. Replacement resorption occurs when, after ankylosis, the root surface becomes gradually replaced by the bone (12).

Several procedures may be realized at the time of replantation, depending on the storage solution and extra-alveolar dry period. An extra-alveolar dry period of 15 min at most makes the prognosis favorable and endodontic treatment should be realized in a future session (4). In these cases replantation is regarded as immediate. In contrast, after 15 min, the periodontal ligament present on the root surface may become dry and, consequently, undergo necrosis, making the prognosis unfavorable (6, 13). This is what is considered late replantation, and treatment of the radicular surface of the tooth should be realized, scraping off the necrotic periodontal tissue (14) in order to preserve the root structure and prevent the action of resorptive cells (15), thus inducing the formation of new cement (16, 17).

A family of such bisphosphonated compounds have been used or developed for treatment of bone diseases. Of these, sodium alendronate has been shown to be very effective in the clinical treatment of osteoporosis (18) and presented an enhanced inhibitory effect on root resorption through osteoclast inhibition (19). Some studies have also demonstrated a direct effect on bone formation (20, 21).

Based on the aforementioned characteristics, bisphosphonated compounds have been used topically in dental research and demonstrated satisfactory results (22, 23).

Material and methods

Fifty-four male rats, of approximately 250–300 g weight each were used for this study. For surgical interventions, the animals received a preanesthetic medication consisting of xylazine chloride in a dose of 15 mg/kg of weight, via intramuscular injection in the back of the thigh. Further anesthesia followed with a combination of ketamine chloride (25 mg/kg of weight) and xylazine chloride (10 mg/kg of weight), via intramuscular injection in the same region. Asepsis of the anterior portion of the maxilla was realized with 2% chlorexidine solution and the maxillary right central incisor was extracted simulating a case of dental avulsion.

The extracted teeth were allowed to dry attached by the crowns to a sheet of pink wax for a predetermined time according to each experimental period of 15, 30, and 60 min. This condition simulated a dried periodontal ligament, as frequently observed by dentists when receiving a patient with dental avulsion.

After each experimental period, the dental papilla was excised with a #11 surgical blade for exposure of root canals. The pulp was removed via apical foramen, with a slightly curved #15 Flexofile (Dentsply-Maillefer, Switzerland). Canals were copiously irrigated with a 0.9% saline solution and aspirated using a Luer Lock syringe and a 25 × 6 gauge needle.

The teeth were divided into three groups according to the experimental extra-alveolar period and specific treatment, as follows: Group I – extra-alveolar dry period of 15 min; root canals were dried with sterile paper points, dressed with calcium hydroxide paste, and replanted in their respective alveoli simulating immediate replantation; Groups II and III – extra-alveolar dry period of 30 and 60 min, respectively; teeth had their radicular surfaces treated by immersion in 50 ml of 1% sodium hypochlorite for 30 min for the removal of periodontal ligament, washed in 50 ml of 0.9% saline solution for 5 min, and then immersed in 50 ml sodium alendronate (3.2 mg/l) for 10 min. Root canals were aspirated, dried, and dressed with calcium hydroxide paste and teeth replanted in their alveoli, simulating a case of late replantation.

The calcium hydroxide paste was composed of calcium hydroxide, polyethyleneglycol, zinc oxide, and colophonium (Calen®, S.S. White, Artigos Dentários LTDA, Rio de Janeiro, Brazil) and was introduced in the root canal with a proper syringe (ML®, S.S. White). The replanted teeth did not receive any immobilization. The animals received a single dose of benzatine penicillin 20 000 UI, via intramuscular injection, in the back of the right thigh.

At 15, 60, and 90 days post-replantation, six animals from each group were killed with a massive dose of anesthetics. The maxilla was separated from the mandible and the hemimaxilla containing the replanted tooth recovered.

Samples were fixed in 10% buffered formalin for 7 days, decalcified in 4.7% EDTA pH 7.0 until a fine needle could be introduced without resistance, histologically processed, and embedded in paraffin wax. Twenty-seven samples were embedded to provide transversal cuts of the cervical, middle, and apical thirds, and another 27 were used for longitudinal cuts. Alternate sections of 5 μm each were obtained and stained by hematoxylin–eosin for evaluation by light microscopy.

Results

A descriptive microscopic analysis of the sections was realized as follows:

1 *Group I* (extra-alveolar dry period of 15 min): A periodontal ligament-like, newly formed connective tissue with signs of normality occupying the space between the root surface and the alveolar bone was observed in a major part of the radicular surface at 15 days (Fig. 1). Some sites of radicular resorption were observed in the middle and apical thirds, and discrete bone formation was observed in close contact to the root near the apex, characterizing dental ankylosis (Fig. 2). In addition, sites of inflammatory reaction with several polymorphonuclear neutrophils, macrophages, multinucleated giant cells, and proliferating granulation tissue were observed extending into the alveolus. At 60 days, ankylosis was predominant along the radicular surface, and replacement as well as communicating inflammatory resorptions were present, especially in the middle and apical thirds. Sites of intense inflammatory resorption communicating with the root canal space were observed along the radicular surface at 90 days, mainly in the middle and apical thirds (Fig. 3). Some specimens in this group presented large areas of ankylosis and some areas of replacement resorption (Fig. 4). Nevertheless, preserved periodontal spaces with formation of periodontal ligament parallel to the radicular surface were also observed.

2 *Groups II and III* (extra-alveolar dry period of 30 and 60 min, respectively, and surface treatment with 3.2 mg/l sodium alendronate): The results obtained for these groups were very similar to those of the previous group. At 15 days, reinsertion of the periodontal ligament was observed in the middle and apical thirds, in a parallel or perpendicular direction to the radicular surface (Fig. 5). Formation of new bone in close contact to the periodontal ligament was observed in the apical third, which may turn into dental

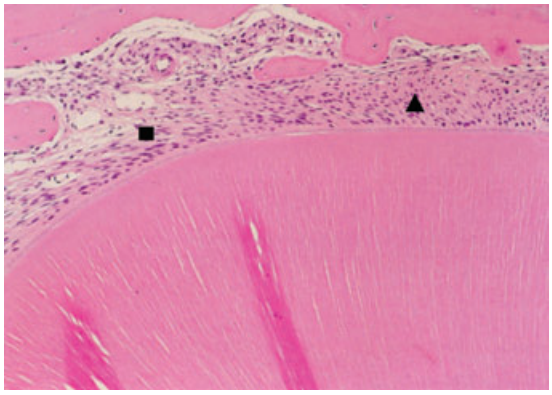

Fig. 1. Group I, 15 days. Periodontal ligament reinsertion in parallel (■) or perpendicular (▲) direction.

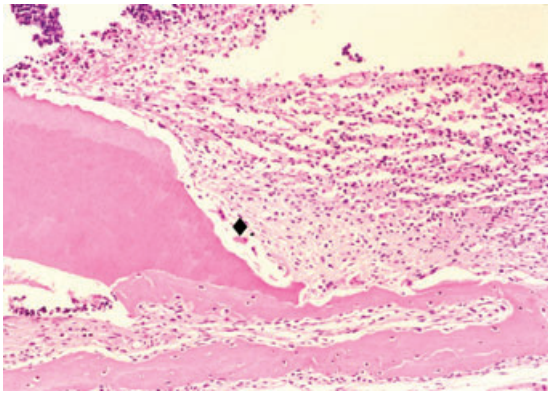

Fig. 3. Group I, 90 days. Communicating inflammatory resorption (♦).

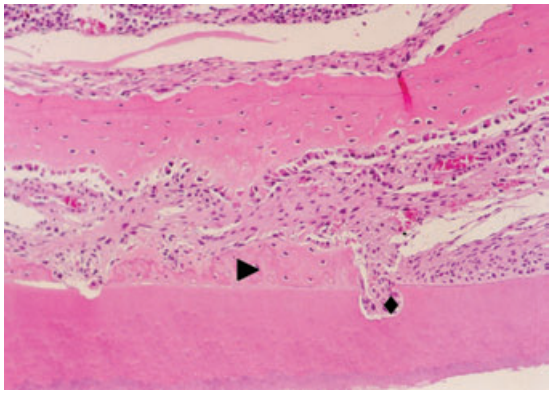

Fig. 2. Group I, 15 days. Presence of new bone (▶) and resorption lacunae (♦).

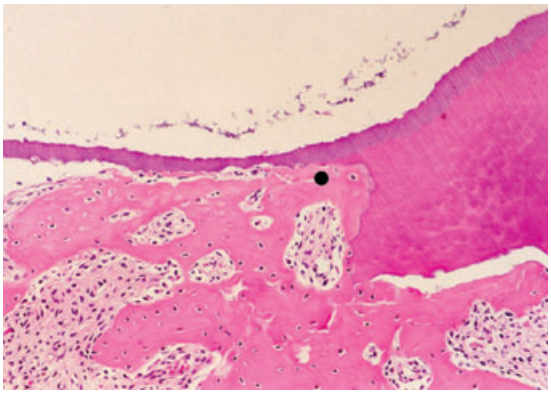

Fig. 4. Group I, 90 days. Intense replacement resorption (●).

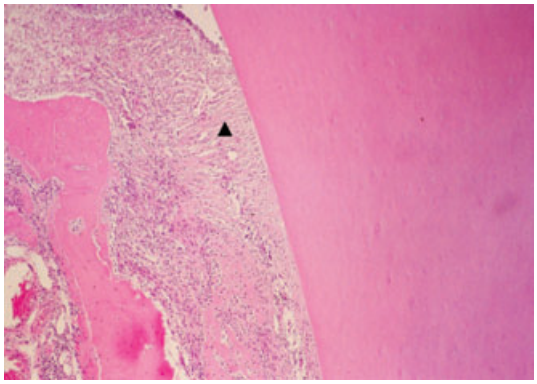

Fig. 5. Group III, 15 days. Small segment of periodontal ligament (▲).

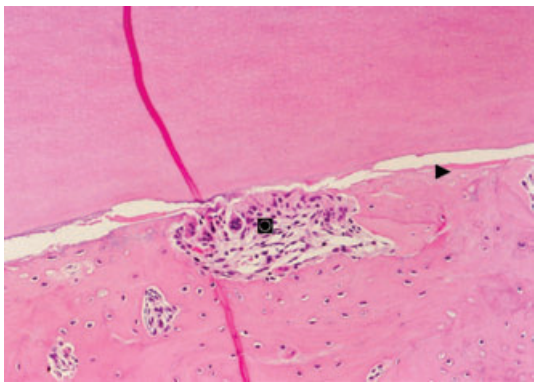

Fig. 6. Group III, 90 days. Ankylosis (▶) and discrete areas of connective tissue with chronic inflammatory infiltrate and giant cells (◙).

ankylosis in a more advanced stage. Extensive areas of ankylosis covering the cervical and middle thirds with areas of mild inflammatory resorption and the presence of multinucleated giant cells were observed at 90 days (Fig. 6). Despite the existence of a periodontal ligament-like connective tissue in the apical third, it presented as very unorganized.

The frequencies of occurrence of the main microscopic events observed in the different groups and experimental periods are best represented in Table 1.

Discussion

As immediate replantation is realized, the periodontal fibers of the alveolus are expected to reinsert into the surface of the avulsed tooth so that it might develop its normal functions (24). Therefore, it is essential that replantation procedures are realized as fast as possible, at most after 15 min (4, 25). The aggressive incidence of resorptive events detected in specimens replanted after 15 min (Group I), especially at 60 and 90 days, demonstrated the failure of the procedure, denying the advantages of immediate replantation in monkeys (26) and humans (4, 25). Controversies in these results should be interpreted with caution, considering the experimental rat model used in this study. These animals present an accelerated metabolism when compared to other animals or humans (27). For this reason, probably, the maximum extra-alveolar period of 15 min is considered acceptable for the success of dental replantation procedures in humans, but may have been excessive for rats.

A calcium hydroxide paste was used as intracanal dressing in all specimens in an attempt to minimize or cease the process of inflammatory resorption, and also the effects of bacterial contamination (12, 25, 28, 29). However, it did not avoid the occurrence of communicating inflammatory resorption in specimens of Group I at 60 and 90 days.

The reinsertion of the periodontal fibers in a parallel direction to the root surface, as observed in Group I, has also been reported in other works (14, 30). When the periodontal ligament is maintained over the cement surface, an adequate environment for bone-derived undifferentiated mesenchymal cell proliferation is formed. However, removal of this ligament would lead to exposure of a mineralized

Table 1. Summary of the microscopic events observed in the different groups and experimental periods

	Extra-alveolar dry period								
	15 min (Group I)			60 min (Group II)			90 min (Group III)		
Microscopic events	15 days	60 days	90 days	15 days	60 days	90 days	15 days	60 days	90 days
Inflammatory resorption	+	+++	+++	+	+	+			
Replacement resorption		+++	+++		+	+			
Communicating resorption		+++	+++						
Periodontal space with parallel fibers	+++	+	+	+++	++	+	+++	+	+
Periodontal space with perpendicular fibers	++			++			++		+
Ankylosis	+	+++	+++	+	++	+++		+++	+++

Note: Blank cells, absent; +, mild; ++, moderate; +++, intense.

surface that compromises the attachment of the mesenchymal cells, essential for periodontal ligament regeneration. Hence, the periodontal connective tissue attached to the alveolar wall after replantation would tend to form a capsule involving the root of the replanted tooth, and the expected reinsertion of collagen fibers onto the cement surface is impossible. In the present study, a similar situation was observed; regardless of the experimental period, when chemical removal of periodontal ligament was realized, a dense connective tissue surrounded the radicular surface of every specimen. At 60 and 90 days, the periodontal space was gradually occupied by bone, characterizing the occurrence of dental ankylosis. This observation is in agreement with the hypothesis of Ehnevid et al. (30), that the periodontal ligament-like connective tissue formed after replantation would favor the occurrence of dental ankylosis.

The occurrence of inflammatory resorption is related mainly to prolonged extra-alveolar periods in a dry condition (12, 25, 31), and also to the presence of bacterial contamination (25, 31). In the rat experimental model, an extra-alveolar period of 15 min may be excessive. In fact, the resorption events were intense at 60 and 90 days, as inflammatory and replacement resorptions were observed concomitantly with the occurrence of ankylosis.

Ankylosis and replacement resorption are related to initial damages on the radicular surface, caused by prolonged extra-alveolar periods in dry condition compromising the radicular surface and further the cement layer (12, 31). The absence of Malassez epithelial rests in the dried periodontal ligament would lead to the occurrence of ankylosis because they are responsible for maintaining the periodontal space (32).

Traumas such as dental avulsion are enough to cause the death of cementoblasts and elimination of Malassez epithelial rests present on the periodontal ligament, and this could explain the process of replacement resorption (26). Replacement of cementoblasts by osteoblasts, which present a receptor for parathyroid hormone allied to the interface bone/dentine/cement, will allow the tooth to participate in the process of bone remodeling, being gradually replaced by bone, and causing the exposure of sequestered antigens, further triggering the immunopathologic response (33).

When immediate replantation is not realized (a fact commonly observed in the daily dental practice) (34), a late replantation must be performed, including treatment of the radicular surface using substances that attempt to avoid or reduce the occurrence of radicular resorptions (15, 34). Despite the great number of substances tested for treatment of the radicular surface, none has yet completely avoided the occurrence of resorptions (35). The most favorable results were obtained with the use of fluorides (15) in dogs, monkeys, and rats. Fluorides provide the formation of fluorapatite crystals over the radicular surface, inhibiting the action of osteoclasts and thus reducing the incidence of resorption.

Similar to fluoride, sodium alendronate is a substance used for treatment of osteoporosis (36). It inhibits osteoclast activity during bone resorption (19) binding to hydroxyapatite in bone, mainly in the surfaces affected by osteoclastic resorption (18).

The results for Groups II and III (30 and 60 min of extra-alveolar period in dry storage, respectively) were similar, demonstrating that variations in extra-alveolar periods were not significantly different. The newly formed periodontal tissue, inserted in a parallel direction to the root, most frequently at 15 days, was gradually replaced by bone until attachment to the root surface, characterizing dental ankylosis. Nevertheless, large areas of radicular resorption were not observed in Groups II and III.

Removal of periodontal ligament of the avulsed tooth with sodium hypochlorite exposes the radicular surface rich in hydroxyapatite, which is easily recognized by osteoclasts, thus triggering the resorption process (30). Sodium alendronate, on the other hand, presents the property of binding itself to hydroxyapatite crystals in bone (18). Similarly, it would bind to hydroxyapatite crystals on the exposed radicular surface, preventing the action of osteoclasts and subsequently the occurrence of resorption (19).

Sodium alendronate was not able to prevent the occurrence of ankylosis as observed in Groups II and III at 60 and 90 days. However, extensive resorptions did not occur in those experimental periods, especially in Group III. According to Selby (18) the alendronate used for surface treatment was probably incorporated into the hydroxyapatite crystals exposed by the action of sodium hypochlorite, thus inhibiting osteoclastic activity and radicular resorption.

Comparing the results of this study with previous reports, we consider our findings very promising regarding sodium alendronate as a substance for topical treatment of the radicular surface of avulsed teeth. Sodium alendronate was not able to prevent the occurrence of dental ankylosis; however, it reduced the occurrence of radicular resorption significantly. In addition, variations in extra-alveolar periods of Groups II and III did not interfere significantly in the results. Additional studies are necessary, using longer experimental periods and also comparing these results with those with 2% sodium fluoride, considered the ideal substance for treatment of the radicular surface of avulsed teeth (25).

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