Morphometric and microscopic evaluation of the effect of a solution of alendronate as an intracanal therapeutic agent in rat teeth submitted to late reimplantation

Mori GG, Garcia RB, de Moraes IG, Bramante CM, Bernardineli N. Morphometric and microscopic evaluation of the effect of a solution of alendronate as an intracanal therapeutic agent in rat teeth submitted to late reimplantation.

Abstract – The use of substances that inhibit root resorption may be an alternative for cases of unsuccessful reimplants. Hence, the purpose of this study was to test a solution of alendronate, a resorption inhibitor, as an intracanal therapeutic agent for teeth submitted to late reimplantation. Thirty rat maxillary right central incisors were avulsed and kept dry for 30 min. The teeth were instrumented, and the root surfaces treated with 1% hypochlorite solution followed by application of 2% sodium fluoride. Thereafter, the teeth were divided in two groups according to the intracanal dressing: (i) group I, solution of alendronate and (ii) group II, calcium hydroxide paste. Teeth were then reimplanted in their respective sockets. The animals were killed at 15, 30 and 60 days after reimplantation and the samples processed for morphometric and microscopic analysis. The results demonstrated that the solution of alendronate and the calcium hydroxide paste limited the root resorption, yet did not impair its occurrence. It may be concluded that alendronate and calcium hydroxide paste demonstrated similar behavior.

Graziela Garrido Mori[1], Roberto Brandão Garcia[2], Ivaldo Gomes de Moraes[2], Clóvis Monteiro Bramante[2], Norberti Bernardineli[2]

[1]Integrate Clinic, Dentistry School of Adamantina, São Paulo, Brazil; [2]Endodontics of Dentistry School of Bauru, University of São Paulo, Bauru, São Paulo, Brazil

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Graziela Garrido Mori, Rua Oscar de Toledo Cezar, 111, Pirapozinho-SP, Brazil CEP 19200–000
E-mail: grazielagm@uol.com.br

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Accidents affecting the teeth are very common. Their consequences may range from small tooth fractures to complete displacement from the socket, characterizing tooth avulsion (1). After reimplantation, there may be ankylosis and root resorption, which frequently lead to tooth loss in <4–6 years (2).

Because of the similar morphology, enzymatic properties and function of the cells leading to resorption of dentin, cement and bone, the processes of root and bone resorption may be considered similar (3). Thus, drugs or other substances that may inhibit bone resorption may also be effective for the treatment of tooth resorption.

A known inhibitor of resorption is the alendronate (4–11). This drug is employed for the treatment of diseases involving resorption, such as osteoporosis (4,5,7), Paget's disease (4,5), hypercalcemia (4,5), osteogenesis imperfecta (5), complications of bone metastases (4,5), periodontal diseases (5,10) and in roots of teeth kept in dry environment before reimplantation (9).

The alendronate may directly or indirectly act on the clasts (5,7,8,10). Solutions containing different concentrations of alendronate may inhibit the bone resorption; moreover, the higher the concentration, the higher the effect (6,8). However, concentrations above 10^{-4} M are toxic not only to the clasts, but also to other cells in the organism (6).

Kum et al. (11), conducted an investigation with culture of bone cells from skulls of mice and demonstrated that 10^{-5} M solution of alendronate may inhibit the clasts. These authors suggested

utilization of this solution as intracanal therapeutic agent. In 2004, Mori et al. *(concluded work)* revealed the diffusion capacity of the solution of alendronate through the dentinal tubules, reaching the external root surface. However, investigations should be conducted on teeth with history of trauma to confirm its ability to inhibit root resorption when used as an intracanal therapeutic agent.

Based on the aspects presented, the purpose of this study was to evaluate the effect of a solution of alendronate as an intracanal therapeutic agent in avulsed and further reimplanted teeth, in comparison with calcium hydroxide paste.

Material and methods

Thirty male Wistar rats (*Rattus norvegicus, albinus*), of approximately 250–300 g weight were used for this study. The animals received a grained solid diet and water *ad libitum*. For surgical interventions, the animals were anesthetized with a mixture of ketamine (Dopalen, Sespo Indústria e Comércio Ltda, São Paulo, Brazil) and xylazine (Anasedan, Agribrands do Brasil Ltda, São Paulo, Brazil), i.m., in a dose of 0.05 ml $(100\ g)^{-1}$ for each substance. Assepsis of the anterior portion of the maxilla was carried out with Periogard. In addition, extraction of the maxillary right central incisor was carried out, simulating a case of tooth avulsion.

The extracted teeth were kept dry, attached by the crown to a sheet of pink wax for 30 min. The dental papillae of the teeth were excised with a no. 11 surgical blade (Embramac Exportação e Importação, São Paulo, Brazil), for exposure of root canals. The pulp was removed via apical foramen, with a slightly curved no. 15 Flexofile (Dentsply-Maillefer, Switzerland). Root canal instrumentation was completed using no. 20 and no. 25 Flexofiles. Canals were copiously irrigated with a 1% hypochlorite solution (Probem Produtos Farmacêuticos e Odontológicos Ltda, São Paulo, Brazil, using a Luer Lock syringe and a 30 × 4 gauge needle.

After preparation, teeth were immersed for 30 min in 50 ml of 1% hypochlorite solution, washed in sterile saline and then immersed in 20 ml of sodium fluoride solution (pH 5.5) for 20 min. The sodium fluoride solution was prepared from a mixture of 0.1 M phosphoric acid (pH 2), and 2% sodium fluoride (pH 8).

Root canals were further irrigated with sterile saline and filled with EDTA (Odahcam Dentsply Indústria e Comércio Ltda, Rio de Janeiro, Brazil) for 3 min, washed with sterile saline again, aspirated with a 30 × 4 gauge needle attached to a Luer Lock syringe and dried with sterile absorbent paper points (Tanariman Industrial Ltda, Amazonas, Brazil).

The teeth were further divided in two groups according to the intracanal substance to be used: group I – 10^{-5} M solution of alendronate (Pharmácia Específica–Bauru, São Paulo, Brazil) and group II – calcium hydroxide paste (S.S. White Artigos Dentários Ltda, Rio de Janeiro, Brazil).

Reimplantation procedures followed with each tooth in its respective socket. No contention was applied. The animals received a single dose of 20 000 U.I. benzatine G penicillin, via intramuscular injection.

Five animals of each group were killed at 15, 30 and 60 days after reimplantation with an excessive dose of anesthetic. The right side of the maxilla was separated from the left in the median line with a no. 15 surgical blade. The maxilla was further cut near the third molar in order to loosen the hemimaxilla containing the tooth reimplanted.

The specimens were fixed in 10% buffered formalin for 7 days and decalcified in 4.13% EDTA (pH 7). The specimens were then processed and embedded in paraffin to show transversal cuts of the cervical, middle and apical thirds of the tooth. Sections of 5 μm were taken at each 100 μm with a microtome, totaling 20 sections for each specimen. The sections were stained by hematoxylin-eosin.

For microscopic analysis, the following parameters were considered: integrity of dental structure, presence of cement, characteristics of the connective tissue formed in the periodontal space, presence of dental ankylosis and occurrence of inflammatory or replacement resorption.

In addition, a morphometric analysis was also carried out, with ImageLab 2000 version 2.4 (Diracom 3, São Paulo, Brazil), specific for measuring areas and perimeters. Fifteen histologic sections of each experimental period were photographed with a digital camera (JVC TK-1270 Color Video Camera, Tokyo, Japan) attached to a microscope (Carl Zeiss, Axiolab, Jena, Germany) connected to a PC. The images obtained were stored as figures (Tif 24) for further interpretation in the ImageLab program. The perimeter of the areas showing ankylosis, connective tissue formed in the periodontal space, the amount of cement remaining as well as areas of inflammatory or replacement resorption were measured.

The results obtained were organized in tables and used for statistical analysis by the Kruskal–Wallis test, with a significance of 5%. Whenever significant differences were found, Dunn's test was used to assay individual comparisons.

Results

Group I (alendronate)

Analysis of histological sections of specimens in group I revealed formation of a dense connective tissue parallel to the root surface in most cases at 15 days. This tissue presented few collagen fibers,

Table 1. Mean percentage values of the histologic events according to each experimental period in group I (alendronate)

Histologic events	Experimental period		
	15 days	30 days	60 days
Inflammatory resorption	0[a]	0.77[a]	14.21[b]
Replacement resorption	0	0	0.02
Presence of cement	97.58[c]	97.86[c]	76.89[d]
Ankylosis	14.73	2.46	9.23
Connective tissue parallel	85.24	96.25	84.04
Connective tissue perpendicular	0	1.27	1.28

The values with different superscript letters in the same row differ significantly ($P < 0.05$).

areas of blood clot and inflammatory cells. At 30 days, this tissue was better organized, presented a larger amount of collagen fibers and absence of inflammatory cells. The same was observed at 60 days (Table 1). Tooth ankylosis was observed at all study periods, yet with a low occurrence (Table 1).

Examination of the root surface revealed the presence of cementum covering great part of the root surface, being significantly reduced at 60 days ($P < 0.05$; Table 1). Lacunae of inflammatory resorption were observed after 30 days, and at 60 days their occurrence was significantly increased (Table 1). Lacunae of replacement resorption were present at 60 days, contrarily to the 15 and 30-day periods, when no replacement resorption was observed (Table 1).

Table 2. Mean percentage values of the histologic events according to each experimental period in group II (calcium hydroxide)

Histologic events	Experimental period		
	15 days	30 days	60 days
Inflammatory resorption	0.26	2.84	2.50
Replacement resorption	0	0.11	0.56
Presence of cement	96.05	90.58	82.78
Ankylosis	28.71	24.58	17.29
Connective tissue parallel	46.74	55.82	67.38
Connective tissue perpendicular	20.59[a]	10.17	0[b]

The values with different superscript letters in the same row are differing significantly ($P < 0.05$).

Group II (calcium hydroxide)

Analysis of histological sections of specimens revealed the formation of a dense connective tissue parallel or perpendicular to the root surface at 15 days. The presence of a periodontal ligament-like connective tissue (perpendicular direction) decreased over time and at 60 days it was no longer observed (Table 1). Tooth ankylosis was observed at all experimental periods (Table 2).

Examination of the root surface revealed the presence of cementum covering a large part of the root surface, which decreased over time (Table 2). Lacunae of inflammatory resorption were observed in all experimental periods. The lacunae of replacement resorption were present at 30 and 60 days (Table 2).

Comparing the results obtained in groups I and II, the presence of tooth ankylosis was more evident in group II at 30 days ($P < 0.05$). This difference was not observed at 60 days (Table 3). Analysis of the events occurring at the periodontal space reveals the difference between groups as to the presence of type of connective tissue (Table 3). In group I, the tissue parallel to the root was more frequent than in group II at 15 and 30 days, with no difference between groups at 60 days. On the other hand, the tissue perpendicular to the root was more significant at 15 days in group II. However, its occurrence was low in both groups.

Analysis of the root surface reveals that a larger amount of cementum was present after 30 days in group I. The presence of cementum was equal between groups at 60 days (Table 3). Concerning the inflammatory root resorption, there was difference at 30 days and at 60 days the lacunae of resorption were statistically similar (Table 3). The same was true for lacunae of replacement resorption (Table 3).

Table 3. Mean percentage values of the histologic events according to each experimental period in groups I and II

Histologic events	Experimental period					
	15 days		30 days		60 days	
	Group I	Group II	Group I	Group II	Group I	Group II
Inflammatory resorption	0	0.26	0.77[a]	2.84[b]	14.21	2.5
Replacement resorption	0	0	0[c]	0.11[d]	0.02	0.56
Presence of cement	97.58	96.05	97.86[e]	90.58[f]	76.89	82.78
Ankylosis	14.73	28.71	2.46[g]	24.58[h]	9.23	17.29
Connective tissue parallel	85.24[i]	46.74[j]	96.25[i]	55.82[j]	84.04	67.38
Connective tissue perpendicular	0[i]	20.59[m]	1.27	10.17	1.28	0[m]

The values with different superscript letters in the same row are differing significantly ($P < 0.05$).

Discussion

The frequency of occurrence of root resorption in the experimental groups of this study was low, although resorption events were observed with increasing time. This emphasizes the characteristic of alendronate (4–11) and calcium hydroxide (12–15) in limiting the resorption process.

Table 2 shows the effectiveness of alendronate in inhibiting root resorption at 15 and 30 days, since at 60 days the resorption was greatly increased ($P < 0.05$). This observation may indicate the need for successive dressings, which might inhibit the occurrence of root resorption for a longer period of time.

The presence of cement could be detected on the root surface of most of the specimens in both groups. This suggests the efficacy of sodium hypochlorite for removal of necrotic periodontal ligament present on the root surface with no injuries to the cement layer (16). A direct correspondance between the occurrence of root resorption and the absence of cement was also noticed (Table 3).

Regarding the histological events occurring on the periodontal space, both groups exhibited dental ankylosis. The occurrence of dental ankylosis may be explained by the loss of Mallassez epithelial rests present in the periodontal ligament as a consequence of tooth avulsion, once these cells are responsible for maintaining the periodontal space (17). The low frequency of dental ankylosis affecting the experimental groups of this study can be seen in detail in Table 3. This finding may have influenced the occurrence of substitutive resorption once it is a consequence of dental ankylosis (1,3,18).

The results of group II also demonstrate the discrete presence of periodontal ligament-like connective tissue at 30 days and absence in 60 days (Table 2), whereas at 15 days it was considerably higher. This may be explained by the replacement of periodontal tissue remnants from the alveolar wall onto the root surface of the reimplanted tooth and not by the formation of a new tissue. Such periodontal tissue remnants were substituted over time by a newly formed connective tissue disposed in a parallel direction to the root surface, as observed.

According to the results of this study, both substances tested presented the capacity of limiting root resorption in teeth reimplanted late, nevertheless not limiting its occurrence. Concerning the periodontal space, the formation of a periodontal ligament-like connective tissue was not favored by any of the groups. Hence, it may be concluded that alendronate and calcium hydroxide paste demonstrated similar behavior.

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