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Morphometric and microscopic evaluation of the effect of solution of acetazolamide as an intracanal therapeutic agent in late reimplanted rat teeth

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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 acetazolamide, a resorption inhibitor, as an intracanal therapeutic agent for late reimplanted teeth. 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 into two groups according to the intracanal dressing: Group I, solution of acetazolamide and 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 showed that calcium hydroxide paste limited root resorption, even though not avoiding it. In contrast, no root resorption was observed after 60 days in the acetazolamide group, confirming the efficacy of the substance in inhibiting root resorption.

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Key words: acetazolamide; root resorption; teeth reimplantation; tooth avulsion

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For a successful tooth reimplantation, it is essential that the cells present on the root surface are maintained vital (1–3). Hence, immediate reimplantation (1, 4) or maintenance of the avulsed tooth in storage media compatible for survival of these cells before reimplantation (1) is fundamental.

Nevertheless, in the majority of cases, the avulsed teeth are left exposed to a dry environment or extra-alveolar for a long period of time. Thus, the treatment of the root surface and endodontic therapy of the avulsed tooth must be realized (1).

Some authors justify the use of sodium hypochlorite for removal of necrotic tissues present on the root surface before reimplantation procedures, followed by washing in saline solution (5, 6). The use of fluoride, besides strengthening dental structure, forming fluorapatite, is still toxic to the resorptive cells in the hard tissues (1, 6, 7). The use of calcium hydroxide as an intracanal therapeutic agent may avoid or limit inflammatory resorption (8), due to its alkaline pH and bactericidal effect (9), and therefore may eliminate the infection present.

Even with such treatment possibilities, there are still many cases of root resorption, and affected teeth are extracted over a period of 4–6 years (10). Hence, the search for new substances that may inhibit or delay the effects of root resorption is demanding.

The presence of specific cells and production of enzymes and ions by such cells are required for the occurrence of root resorption (1, 11, 12). The presence of acid pH in the area of resorption is

fundamental for the action of most resorptive enzymes (1, 11, 12), and decrease of the pH to nearly 4.7 at the resorption lacuna is related to the presence of hydrogen ions. Formation of hydrogen ions requires hydration of carbon dioxide (1, 11, 12), a reaction that is catalyzed by the carbonic anhydrase enzyme (13, 14). Therefore, the absence or inhibition of this enzyme alters the cycle of resorption, thereby limiting resorption (12).

Acetazolamide is a known inhibitor of carbonic anhydrase (15–17). Acetazolamide may be diluted in saline solution (17), which allows the achievement of a saturated solution that may be placed inside the root canal to act as an intracanal therapeutic agent.

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

Materials and methods

Thirty male Wistar rats (*Rattus norvegicus, albinus*), of approximately 250–300 g of 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 Ltd., São Paulo, Brazil) and xylazine (Anasedan, Agribrands do Brasil Ltd., São Paulo, Brazil), IM, in a dose of 0.05 ml per 100 g of weight for each substance. Assepsis of the anterior portion of the maxilla was realized with Periogard (Pfizer Ltd., São Paulo, Brazil). In addition, extraction of the maxillary right central incisor was realized, 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, Ballaigues, Switzerland). Root canal instrumentation was completed using Nos 20 and 25 Flexofiles. Canals were copiously irrigated with a 1% hypochlorite solution (Probem Produtos Farmacêuticos e Odontológicos Ltd., São Paulo, Brazil) using a Luer Look seringe 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 using 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 Ltd., Rio de Janeiro, Brazil) for 3 min, washed with sterile saline again, aspirated with a 30 × 4 gauge needle attached to a Luer Look seringe and dried with sterile absorbent paper points (Tanariman Industrial Ltd., Amazonas, Brazil).

The teeth were further divided into two groups according to the intracanal substance used: Group I – 10^{-5} M solution of acetazolamide (CAS 59-66-5; Deg Produtos Químicos Ltd., São Paulo, Brazil) and Group II – calcium hydroxide paste (S.S. White Artigos Dentários Ltd., 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 IU of benzatine G penicillin, via intramuscular injection.

Five animals from 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 nearby the third molar in order to loosen the hemimaxilla containing the tooth reimplanted.

The specimens were kept 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 from 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 realized, with IMAGELAB 2000 version 2.4 (Diracom 3, Vargem Grande do Sul), specific for measuring areas and perimeters. Fifteen histologic sections of each experimental period were photographed using a digital camera (JVC TK-1270 Color Video Camera, JVC, Tokyo, Japan) attached to a microscope (Axiolab; Carl Zeiss, 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 (acetazolamide)

Analysis of histologic sections of specimens in Group I at 15 days, revealed the formation of a dense connective tissue oriented sometimes in a parallel or perpendicular direction to the root surface. This tissue was present in the other experimental periods also, and at 60 days the percentage of the occurrence of periodontal ligament-like tissue perpendicularly to the root surface was greater ($P < 0.05$) (Table 1). Areas of ankylosis were observed in all experimental periods, however, most significantly at 15 days ($P < 0.05$) (Table 1).

Examination of the radicular surface revealed the presence of cement recovering great part of the root surface at 15 and 30 days. At 60 days, cement was present in 100% of the specimens (Table 1). Small inflammatory resorption lacunae were observed at 15 and 30 days; however, at 60 days no resorption lacunae were observed in any of the specimens (Fig. 1 and Table 1).

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

	Experimental period		
Histologic events	15 days (%)	30 days (%)	60 days (%)
Inflammatory resorption	0.68	0.46	0
Replacement resorption	0	0	0
Presence of cement	85.93[a]	94.20[a]	100[b]
Ankylosis	25.30[c]	2.37[d]	8.85[d]
Connective tissue parallel	49.74[e]	86.42[f]	62.90[g]
Connective tissue perpendicular	14.44	8.64[h]	28.24%[i]

[a]With significant statistical difference from [b]($P < 0.05$).
[c]With significant statistical difference from [d]($P < 0.05$).
[e]With significant statistical difference from [f]($P < 0.05$) and [f] from [g]($P < 0.05$).
[h]With significant statistical difference from [i]($P < 0.05$).

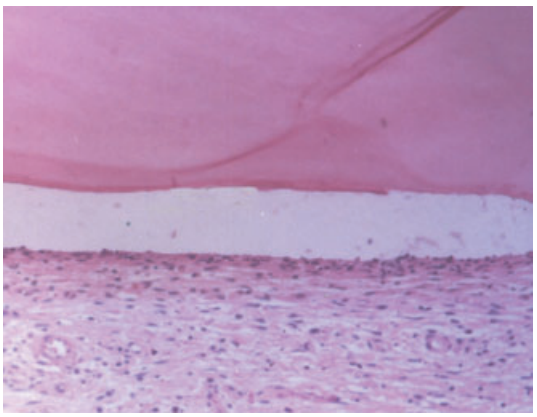

Fig. 1. Group I (acetazolamide). Sixty days, middle third: integrity of dental structure with no resorption lacunae. Original magnification 157.5×.

Group II (calcium hydroxide)

At 15 days, a dense connective tissue oriented sometimes parallel sometimes perpendicular to the root surface was observed. However, the presence of a periodontal ligament-like connective tissue decreased over time and at 60 days it was not observed anymore (Table 2). Dental ankylosis was observed in all experimental periods (Table 2).

Examination of the root surface revealed the presence of cement covering almost the whole extension of the root. Lacunae of inflammatory and replacement resorptions were also present in the specimens of this group (Fig. 2). No significant differences were observed regarding the histologic events observed throughout the experimental periods (Table 2).

Differences were observed when comparing the results from Groups I and II. The periodontal ligament-like connective tissue perpendicular to the root surface was increased at 60 days in Group I ($P < 0.05$) (Table 3). The amount of connective tissue parallel to the root surface and the amount of ankylosis was similar in both groups at 15 and 60 days and different at 30 days (Table 3). The presence of cement covering the root surface and

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

	Experimental period		
Histologic events	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]

[a]With significant statistical difference from [b]($P < 0.05$).

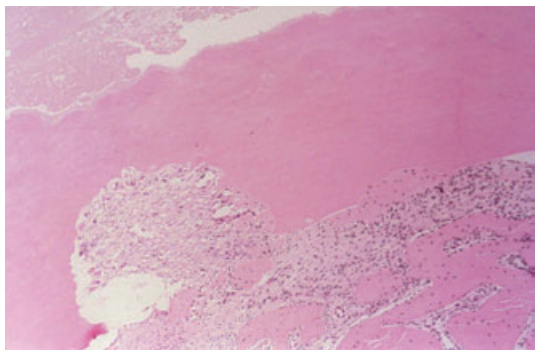

Fig. 2. Group II (calcium hydroxide). Sixty days, middle third: connective tissue parallel to the root and lacunae of inflammatory and replacement root resorption. Original magnification 63×.

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

	Experimental period					
	15 days (%)		30 days (%)		60 days (%)	
Histologic events	Group I	Group II	Group I	Group II	Group I	Group II
Inflammatory resorption	0.68	0.26	0.46	2.84	0[a]	2.5[b]
Replacement resorption	0	0	0[c]	0.11[d]	0[c]	0.56[d]
Presence of cement	85.93	96.05	94.20	90.58	100[e]	82.78[f]
Ankylosis	25.30	28.71	2.37[g]	24.58[h]	8.85	17.29
Connective tissue parallel	49.74	46.74	86.42[i]	55.82[j]	62.90	67.38
Connective tissue perpendicular	14.44	20.59	8.64	10.17	28.24[k]	0[l]

[a]With significant statistical difference from [b]($P < 0.05$).
[c]With significant statistical difference from [d]($P < 0.05$).
[e]With significant statistical difference from [f]($P < 0.05$).
[g]With significant statistical difference from [h]($P < 0.05$).
[i]With significant statistical difference from [j]($P < 0.05$).
[k]With significant statistical difference from [l]($P < 0.05$).

the occurrence of root resorption were different between the groups (Table 3).

Discussion

The occurrence of root resorption in the experimental groups in this study was low. However, we observed an increase in the resorption process in specimens of Group II. This confirms the characteristic of calcium hydroxide in interfering and limiting the process of root resorption (18, 19).

The superiority of the solution of acetazolamide (Group I) regarding inhibition of root resorption when compared with calcium hydroxide can be found in Table 3. The results of Group I demonstrated the occurrence of small lacunae of inflammatory resorption at 15 and 30 days, whereas at 60 days no resorption lacunae could be observed (0% of occurrence). The resorption lacunae detected at the initial periods may be classified as areas of transitory inflammatory resorption as they did not persist over time. These areas, in general, cease and repair (1, 8, 20), fact that we observed at 60 days in this group.

The absence of dental resorption in the specimens of Group I may be explained by the mechanism of action of the active substance in the solution. Acetazolamide is known as a potent inhibitor of carbonic anhydrase (15–17), an enzyme present in clasts. This enzyme catalyzes the reaction between carbonic acid and water, resulting in the formation of hydrogen ions (1, 11, 12), which in turn are responsible for the decrease in pH in the Howship lacunae (11, 12, 20). The acid pH allows liberation and action of other enzymes, which will participate along in the resorption process (12). Therefore, with carbonic anhydrase inhibition, there will be no decrease in pH and resorption is not likely to occur. Several studies comfirm the efficacy of acetazolamide in inhibing bone resorption (15–17, 21, 22).

The idea of using acetazolamide as a substance to inhibit dental resorption arose in 2002, in a study by Mori and Garcia (6). Briefly, solution of acetazolamide was used for the treatment of the radicular surfaces of rat teeth that had been intentionally avulsed and reimplantated late. The results demonstrated the occurrence of root resorption, misadvising its use. However, for treatment of the root surface, the tooth was immersed in the solution for a period of 20 min only. The hypothesis of using acetazolamide as an intracanal therapeutic agent was tempting, once it could be used for a prolonged period of time (6). According to the results of this study, the solution of acetazolamide proved to be effective in inhibiting dental resorption when used as an intracanal therapeutic agent.

Examination of the radicular surfaces allowed us to verify a direct proportion between the occurrence of radicular resorption and presence of cement. The latter was present in most cases, showing the efficiency of sodium hypochlorite for removal of necrotic periodontal ligament with no prejudice to the radicular cement (23). At 60 days, however, there was less cement present in Group II than in Group I ($P < 0.05$), which is probably related to the occurrence of root resorption.

The presence of a periodontal ligament-like connective tissue in the periodontal space was low at 30 and 60 days despite being considerably high at 15 days. This can be explained by the reinsertion of remainder periodontal ligament in the alveolar wall and not by the formation of a new tissue (6). This periodontal ligament had been replaced, over time, by a newly formed connective tissue, oriented in a parallel direction to the root, as shown. On the contrary, at 60 days, the amount of connective tissue perpendicular to the root was greater in

specimens of Group I ($P < 0.05$). Analyzing these events in their respective experimental periods for Group I, it was possible to observe the formation of a periodontal ligament-like connective tissue, suggesting an initial repair of the area, considering that at 60 days the amount of such tissue was greater than at 15 and 30 days.

In conclusion, we emphasize that the calcium hydroxide paste limited radicular resorption, despite the low frequency of occurrence observed. Instead, the solution of acetazolamide was efficient in inhibiting root resorption, favoring the repair of avulsed and further reimplanted teeth.

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