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Original Article

Evaluation of intracanal acetazolamide in late reimplanted rat teeth

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ABSTRACT

Objective: The aim of this study was to evaluate the use of acetazolamide combined with different agents as intracanal medication in late reimplanted rat teeth. **Materials and Methods:** In 100 Wistar rats, divided into 5 groups of 20, one of the following medications was used: Acetazolamide liquid (AL); AL with calcium hydroxide powder (ALHC); acetazolamide powder with AL; acetazolamide powder with physiological solution; and calcium hydroxide with physiological solution (control). At 30 and 60 days after reimplantation, the animals were sacrificed, tissues were processed, and cuts were stained with hematoxylin and eosin. An optical microscope was used to determine the following: percentage of inflammatory root resorption (RRI); percentage of substitute root resorption (RRS); and presence of ankylosis. The data obtained was submitted for statistical analysis. **Results:** Group ALHC had a significantly higher RRS than the control group at 60 days ($P = 0.01$). Group AL showed significantly less ankylosis than the other groups, including the control, at 30 days. AL showed results similar to those of the control group with respect to RRS. **Conclusion:** Acetazolamide has the potential to be an effective intracanal medication.

Key words: Acetazolamide, calcium hydroxide, intracanal medication, resorption

INTRODUCTION

Root resorption is a frequent complication in tooth reimplantation.[1-3] Because all or part of the periodontal ligament may be missing, the bone tissue grows toward the surface of the tooth root and ankylosis occurs. As a result of this fusion, resorption occurs by replacement: The tooth is replaced by bone tissue.[1,4]

Because the process of bone resorptions similar to that of dental resorption, substances used in bone therapy may be effective in the treatment of root resorption. Acetazolamide, is a nonbacteriostatic sulfonamide with distinct chemical structure and pharmacological activity from sulfonamides, which inhibits carbonic anhydrase and consequently prevents bone resorption, has been studied for its effects on root resorption. The substance was first suggested by Mori and Garcia[5] for treatment of the root surface. Since then, multiple forms of acetazolamide have been examined.

In 2006, Mori *et al*.[6] investigated the use of intracanal liquid acetazolamide in late reimplanted rat teeth and used calcium hydroxide paste as a control. At 60 days, no resorption was observed in the group treated with intracanal liquid acetazolamide.

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Mori *et al*.[7] used rat subcutaneous tissue to evaluate the biocompatibility of 2 experimental acetazolamide pastes, with physiological solution and propylene glycol used as vehicles. The experimental paste used with the physiological solution was biocompatible.

Mori *et al*.[8] also tested an experimental intracanal acetazolamide powder in physiological solution on avulsed and late reimplanted rat teeth and found it limited root resorption more effectively than calcium hydroxide paste.

Having reviewed the findings by Mori *et al*.,[5-8] the authors of this study sought to evaluate the effects of acetazolamide on late implanted rat teeth as well to examine acetazolamide in different forms and combinations and to compare its effects to those of calcium hydroxide.

MATERIALS AND METHODS

This experimental study was approved by the Local Research Ethics Committee. Five intracanal medications were evaluated by introducing them in the right central incisors of 100 Wistar rats, divided into 5 treatment groups of 20 rats each, as follows:

Group 1: Acetazolamide liquid (AL)
Group 2: AL and calcium hydroxide powder (ALHC)
Group 3: Paste of acetazolamide powder and AL (APAL)
Group 4: Paste of acetazolamide powder and physiological solution (APSF)
Group 5: Paste of calcium hydroxide powder and physiological solution (control).

The calcium hydroxide powder and AL were manufactured by Dermo Ervas Comércio de Produtos Químicos Limitada, Curitiba, Brazil. The concentration of acetazolamide was 10^{-5} M. The acetazolamide powder consisted of 0.02 g acetazolamide with 1% nipagim, 0.04% sodium benzoate, and recipient Q_{sp} 10 g. The liquid consisted of 0.02 g acetazolamide, 0.1% of nipagin, 0.4% sodium benzoate, and 100 mL water Q_{sp}.[6]

Liquid ratio tests of the powder were performed by a single operator. The initial portions of powder were weighed on a precision balance and then gradually added to 150 μL of the liquid until a dense paste with the appearance of toothpaste was obtained.

One hundred male rats (Rattus norvegicus albinus, Wistar breed) weighing 180–200 g were used. The rats were housed in groups of 4 animals each in clean cages and identified according to treatment groups and study time points. During the period of the experiment, the animals were fed with crumbed rations and water *ad libitum*.[6,8]

The animals were anesthetized with an intramuscular injection of ketamine hydrochloride (Dopalen, Sespo Indústria e Comércio Ltda., São Paulo, Brazil) and xylazine hydrochloride (Anasedan, Sespo Indústria e Comércio Ltda, São Paulo, Brazil). The dose of each drug was 0.05 mL/100 g of body weight.[5,8]

Asepsis was performed in the anterior portion of the maxilla with Periogard (Pfizer Ltda., São Paulo, Brazil), and the maxillary right central incisors were extracted with extractor forceps for rat teeth developed and patented by the researcher (PI 10201-2011567) to simulate tooth avulsion. The teeth remained exposed to the environment for 60 min. After this, the dental papillae were excised with Number 11 scalpel blades (Solidor Ltda., São Paulo, Brazil), and retrograde pulp tissue was removed with precurved Number 15 Flexofile files (Dentsply Maillefer, Ballaigues, Switzerland).[6,8]

Retrograde canals were prepared with Number 15, 20, and 25 Flexofile files and irrigated with 5.0 mL of 1% sodium hypochlorite (Biodinâmica Química e Farmacêutica Ltda., Paraná, Brazil).[6,8]

After the pulp was prepared, the teeth were immersed in 50 mL of 1% sodium hypochlorite for 30 min to remove the periodontal ligament. The teeth were then washed in physiological solution and immersed in 20 mL of 2% sodium fluoride solution for 20 min.[6,8]

After the root surfaces were treated, the canals were irrigated with physiological solution and 17% EDTA (Biodinâmica Química e Farmacêutica, Ltda., Paraná, Brazil) for 3 min to remove the smear layer. Next, the teeth were rinsed again with physiological solution, aspirated, and dried with absorbent paper points.[6,8]

Subsequently, each tooth was filled with the corresponding intracanal medication and reimplanted in its respective alveolus. No splinting was performed after reimplantation because of the curved and retentive anatomy of the tooth.[6,8] The animals were medicated with 1 intramuscular prophylactic dose of 20,000 IU of G benzylpenicillin antibiotic, with the aim of avoiding infections after surgery that could invalidate the samples.[6,8]

After 30 and 60 days, 10 animals from each group were sacrificed with 180 mg/kg thiopental intraperitoneally administered until cardiorespiratory failure occurred. The right hemimaxilla containing the reimplanted teeth were removed, fixed in 10% formalin solution for 24 h, and decalcified in EDTA 4,13% for 2 months.[6,8] With a microtome blade (Leica Biosystems, Nussloch, Germany), the maxilla was sectioned in two ways: (a) in the vestibular-palatine direction so that it contained only the reimplanted tooth and the adjacent alveolar bone and (b) transversally in 3 equal parts (corresponding to the cervical, middle, and apical thirds of the teeth). For histological analysis, the third middle root was selected because the coronal and apical portions may have been affected by the surgical and endodontic procedures.[9] In addition, because the root apex was open, the apical portions of specimens could present differences in tissue response because of the eventual escape of the intracanal medication. Therefore, semi-serial cross-sectional cuts were made in the middle third of each specimen. Four cross-sections of 4 μm thickness were obtained and stained with hematoxylin and eosin. For each section, images of the entire root were captured using an Olympus BX-50 microscope (Olympus, Tokyo, Japan) coupled to a Dinolite® AM 423X microcamera (AM Eletronics Corporation, New Taipei, Taiwan) with a 10X lens.

The images were analyzed with the morphometry program Image Pro-Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA).The "Create Trace" option of the measurement module was used and the following were measured in micrometers: (a) total perimeter of the root; (b) perimeter of inflammatory resorption (RRI); and (c) perimeter of replacement resorption (RRS). In addition, the presence of ankylosis was recorded. To calculate the percentages, the perimeter RRS or RRI (r) was divided by the total perimeter of the root (t), and the quotient (q) was multiplied by 100. ([r/t = q] × 100). Finally, the mean value of the sections was calculated.[10]

For microscopic analysis, the following were taken into consideration: presence of dental ankylosis, RRI, and RRS. The presence of cement on the integral dentin surface was considered favorable. The presence of RRI or RRS was considered an unfavorable prognosis.[5,6,8,11]

Statistical analysis

The data were statistically analyzed with the SPSS 19.0 program (SPSS Inc., Chicago, IL, USA). For the RRI and RRS values, the Kruskal–Wallis test was used. When statistically significant differences were found, the Dunn multiple comparison test was used. To analyze ankylosis results, the test of difference between two proportions was used. The level of significance adopted for all statistical tests was 5% ($P < 0.05$).

RESULTS

All groups are shown in Figure 1a-j. There were no statistical differences in RRI and RRS between the two-time points –30 days and 60 days. For RRI, there was no statistical difference between the groups at either time point.

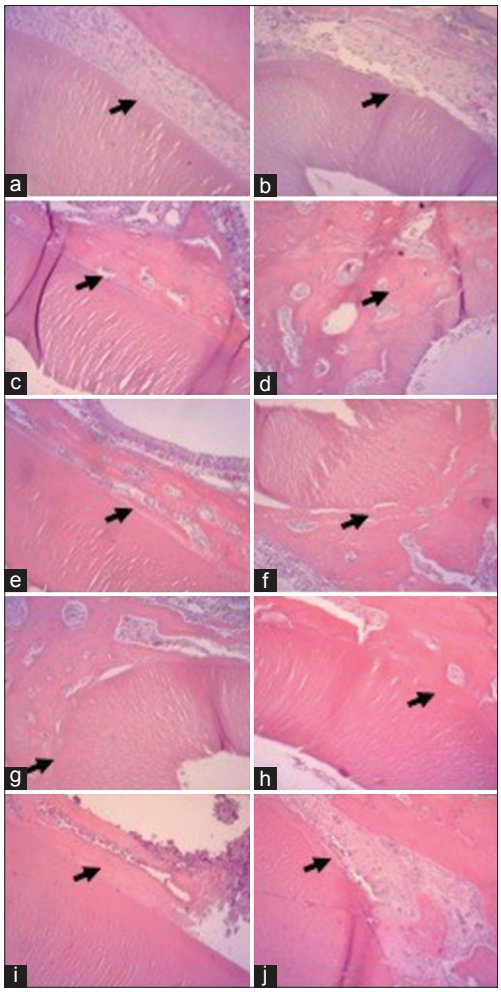

Figure 1: a / b.Images of histological sections, the AL group at 30 and 60 days. Reduced presence of ankylosis 30 days and moderate presence of RRS gaps. Increase 100X, c / d.Imagens of histological sections, the ALHC group at 30 and 60 days. Moderate presence and ankylosis and high presence of RRS gaps. Increase 100X, e / f .Imagens of histological sections, the APAL group at 30 and 60 days. Moderate presence of ankylosis and moderate presence of RRS gaps. Increase 100X, g / h. Images of histological sections, the APSF group at 30 and 60 days. Moderate presence of ankylosis and moderate presence of RRS gaps. Increase 100X, i / j. Images of histological sections, the control group at 30 and 60 days. Moderate presence of ankylosis and moderate presence of RRI gaps. Increase 100X

The RRS percentage for the ALCH group at 60 days [Figure 1d] was significantly greater than that of the control group [Figure 1j] at 60 days (P = 0.01). No statistically significant differences were seen in comparison with the other groups [Table 1].

At 30 days, Group AL showed the fewest specimens of ankylosis [Figure 1a]; the difference between this group and the others was statistically significant [Table 2].

DISCUSSION

The use of acetazolamide for inhibiting tooth resorption was first suggested in 2002,[5] when it was used for treating root surfaces of late reimplanted teeth. Root resorption was seen probably because 20 min of exposure to acetazolamide was not long enough.[5] In this study, acetazolamide was used as an intracanal medication in different combinations and with longer times; these differences may explain why less root resorption was seen in this study than in those studies.

Table 1: Median and standard deviation of the percentage of the perimeter of inflammatory resorption (RRI) and replacement resorption (RRS) at 30 and 60 days

Group	Day	% Perimeter of RRI[A] (Median±SD)	% Perimeter of RRS[B] (Median±SD)
AL	30	0.76±3.98	0.00±5.58
	60	1.06±2.80	1.77±4.45
ALHC	30	1.05±1.11	0.46±1.18
	60	2.23±5.67	11.65±17.76*
APAL	30	0.56±3.40	10.04±10.57
	60	0.00±1.02	0.00±7.65
APSF	30	0.00±1.10	4.86±9.01
	60	2.66±4.33	12.18±11.53
C	30	1.07±6.09	0.00±9.02
	60	0.00±1.44	0.00±0.78*

Note: Kruskal-Wallis test: [A]P=0.53; [B]P=0.01. Dunn Test: (*) asterisks in column indicate statistically significant differences

Table 2: Percentage relative to presence of ankylosis in groups, at 30 and 60 days

Group	Percentage relative to ankylosis
AL 30	30%[a]
ALHC 30	50%[b]
APAL 30	60%[b]
APSF 30	70%[b]
C 30	60%[b]
AL 60	70%[b]
ALHC 60	90%[b]
APAL 60	60%[b]
APSF 60	90%[b]
C 60	50%[b]

Test of difference between two proportions: different letters indicate statistically significant differences

The superiority of acetazolamide (in liquid form) to calcium hydroxide was shown when both were used as an intracanal medication; acetazolamide prevented resorption gaps at 60 days.[6]

In a study with a similar methodology, the effectiveness of an experimental paste of acetazolamide in physiological solution was compared to that of calcium hydroxide paste in physiological solution.[8] Both intracanal substances limited root resorption, but neither of them were capable of inhibiting it.

The low rates of RRI observed here (statistically insignificant differences between groups and times) match those of the previous studies. This confirms that its occurrence is directly related to the combination of bacteria in the canal and damage to root cement[1,4] as stimulatory factors.[12] Thus, it was possible to confirm that the protocol consisting of endodontic treatment, filling with adequate intracanal medication[4,13-15] and systemic antibiotic therapy[15-17] was effective for controlling this type of resorption.

Ankylosis was seen in all the groups. However, at 30 days, Group AL presented a significantly lower percentage of ankylosis than did the other groups. Statistically significant differences for this condition were not seen between the other groups. Because it is the precursor to substitution resorption, the correlation between ankylosis and RRS could be observed from the results for the AL and ALHC groups.

In addition to having the greatest presence of ankylosis, the ALHC group also showed the highest percentage of RRS at 60 days; the group differed significantly ($P < 0.05$) from the control group. A direct relationship could be established between the presence of ankylosis and RRS gaps because extensive areas of RRS were observed in the same group and in the periods observed.

Except with Group ALHC, statistically significant differences were not seen between the groups, including the control. Because of this, it was inferred that the combinations containing acetazolamide were as effective as the control in limiting substitution root resorption. This highlights the action of calcium hydroxide[13,15,16] and acetazolamide[6,8] in limiting resorption.

In this research, at 30 and 60 days, the acetazolamide in Groups AL, APAL, and APSF limited substitution resorption to the same extent as the control group did, but it did not prevent the condition from occurring. However, Group AL showed the lowest percentage (statistically significant) of ankylosis at 30 days, a result which corroborates the findings by Mori and Garcia[5] One reason may be that, unlike the pastes, the liquid form diffused easily through dentinal tubules and tissues, particularly in the first 30 days after application.[7] Nevertheless, the facility of this diffusion and the presence of open apices, inherent to the animal model used, may explain the rapid escape of the medication and the decrease in its action at 60 days. This finding suggests that if acetazolamide is administered in the liquid form, it should be changed at 30 days to prolong the effects of the medication.

The results of this study showed that acetazolamide, except when combined with calcium hydroxide, effectively controlled root resorption, confirming the substance's antiresorptive properties. Further research with acetazolamide is suggested to confirm the differences seen between the other groups.

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Conflicts of interest
There are no conflicts of interest.

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