



The Effects of Short Term Intracanal Medicaments on the Fracture Resistance of Root in Pulp Revitalization Procedure

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ABSTRACT

Introduction: The objective of this study was to evaluate the effects of short term intracanal medicaments on the fracture resistance of root in simulated necrotic immature teeth in pulp revitalization procedure. **Methods and Materials:** Bovine teeth ($n=180$) were selected, sectioned coronally and apically and then, internally fragilized. Intracanal medicament groups were arranged as follows: “Triple Antibiotic Paste” (TAP) group ($n=60$), “Calcium Hydroxide” (CH) group ($n=60$) and the control group ($n=60$). No medication was used in the control group. Fracture resistance tests were performed after 7, 14, and 21 days. At allocated intervals, 20 teeth from each group had fractured. The Kruskal-Wallis and Mann-Whitney tests were performed to verify the effects of the employed medicaments at each time point. Friedman test and Wilcoxon signed-rank test were also performed to verify the association between time and fracture resistance. The level of significance was set at 0.05. **Results:** After 7 days, there was no statistical difference between groups ($P=0.376$). Intragroup analysis revealed that, after 21 days, the TAP group ($P=0.015$) and the CH group ($P=0.006$) presented a statically significant reduction in fracture resistance comparison with 7 days. Statistical difference was not verified for the control group after 7, 14 and 21 days ($P=0.25$). There was no statistical difference between CH group and the control group after 7, 14 and 21 days ($P>0.05$). The reduction was significant for TAP after 14 and 21 days ($P=0.018$, $P=0.033$ Respectively). **Conclusions:** This *in vitro* study showed that the duration at which TAP and CH remained in the root canal influenced the fracture resistance of bovine teeth with simulated incomplete root formation.

Keywords: Calcium Hydroxide; Endodontics; Regeneration; Triple Antibiotic Paste

Introduction

Endodontic treatment of immature non-vital permanent teeth is one of the most challenging situations in endodontics. Traditionally, apexification therapy has been the most widely used treatment for cases of necrosis of immature teeth through the use of calcium hydroxide (CH) or mineral trioxide aggregate (MTA) [1]. As an alternative, pulp revitalization aims to fill the root canal with pulp-like tissue after the induction of a blood clot inside the root canal system to serve as an anchorage site for cells from the apical region [2]. This technique has an advantage: it allows the thickness of the root to increase and apical closure [2].

The American Association of Endodontists [3] and most of the published research and clinical cases suggest that this procedure be performed over two visits [2, 4]. At the first session of pulp revitalization, passive decontamination of the canal is recommended with associated intracanal medication, which can be CH or a triple antibiotic paste (TAP), composed of ciprofloxacin, metronidazole and minocycline (1:1:1) and the medication must remain for 1-4 weeks. Double antibiotic pastes (DAP) and modified triple antibiotic pastes (MTAP) can also be used to avoid the discoloration induced by TAP [3, 5, 6]. In the second session, the intracanal medicament is removed, apical bleeding is stimulated by inserting a file and foraminal opening, and, after clot formation, a cervical cap with MTA [3] or Biodentine [3, 4] is inserted.

Little is known about the effects of antibiotic pastes on the mechanical properties of dentin. Acids are usually added to some antibiotics to maintain chemical stability, tonicity, and to ensure physiological compatibility. However, the exposure of dental hard tissues to such antibiotics can cause demineralization and negatively affect their mechanical properties [7, 8]. Minocycline has been reported to demineralize hard dental tissues [8, 9]. In addition, exposure of enamel to aqueous solutions of tetracycline has caused a dramatic and continuous reduction in microhardness [10]. Similarly, some authors have demonstrated that CH, because it is highly alkaline, could significantly reduce the fracture resistance of the dental root [7, 11-14]. However, there is no consensus on the time required for these changes to be significant.

Research about pulp revitalization is of great interest to the scientific community. There have been few studies on the effects of antibiotic pastes on the mechanical properties of dentin [7, 15-17] and new studies about CH should be considered.

Further in-depth discussion of these topics may help establish more coherent and scientifically based protocols. Therefore, the aim of this study was to evaluate the effects of intracanal medicaments, used in pulp revitalization protocol, on the fracture resistance of simulated immature teeth in short period. The null hypothesis tested was that there was no reduction in fracture resistance after using intracanal medication at different intervals.

Materials and Methods

This was an *in vitro* study on freshly extracted bovine teeth.

Specimen selection and preparation

Freshly extracted bovine incisors ($n=180$) of similar size and shape were selected by measuring the buccolingual and mesiodistal widths in millimeters, allowing a maximum deviation of 10% from the average. A digital caliper (Mestra, Sondika Bilbao, Spain) was used at this stage. All teeth were analyzed under an operating microscope (Alliance, São José dos Campos, SP, Brazil) at 20× magnification, and those with caries, cracks, or fractures were excluded from the study. The specimens were cleaned in water and stored in 0.1% thymol solution until the beginning of the experiment.

An experimental model was established to simulate teeth with incomplete root formation from bovine teeth, described by Bortoluzzi *et al.* [18]. The crowns of all teeth were coronally and apically sectioned (8mm above and 12mm below the cemento-enamel junction, respectively) using a slow-speed diamond precision saw with a water coolant (Isomet 1000; Buehler, Lake Bluff, IL, USA), providing specimens of 20mm [18].

The remaining pulp tissue was removed using Hedstroem files (Dentsply, Bailagues, Switzerland), and the fragility of the dentinal walls was achieved using number 703 bur (ISO: 500-104-168-007-021, JET carbide burs, Beavers Dental Products Ltd., Morrisburg, Canada). The radicular thickness was standardized at 2 ± 0.2 mm in the cervical third and 1.0 ± 0.2 mm in the apical third, measured using a digital caliper (Teknikel, Istanbul, Turkey).

The bovine teeth were then subjected to passive decontamination of the root canal, as recommended for pulp revitalization in clinical practice [3]. The canals were irrigated with 20mL of 1.5% sodium hypochlorite then irrigated with 20mL of saline. Concomitant with irrigation, aspiration was performed to simulate clinical practice. Subsequently, the root canals were dried with sterile absorbent paper tips (Dentsply, Bailagues, Switzerland) and randomly divided into experimental groups, according to the intracanal medicament used: TAP group ($n=60$), CH group ($n=60$), and control group (no intracanal medication, $n=60$).

TAP, in its classical composition, contains 250mg of ciprofloxacin, 400mg of metronidazole and 50mg of minocycline in equal proportions in polyethylene glycol (liquid/powder ratio, 3:1) as described in the literature [4]. CH paste (Biodinâmica, Ibioporã, PR, Brazil) in polyethylene glycol has a liquid/powder ratio of 1:2. In all groups, medicaments were applied using a lentulo spiral (Dentsply, Bailagues, Switzerland) with a slow-speed handpiece. To avoid extravasation of the medication, apical and coronal plugs were made with 2mm of Coltosol (Coltene, Rio de Janeiro, RJ, Brazil). The teeth in three groups were kept in normal saline at 37°C for 7, 14, and 21 days. The solution was changed every week.

Periodontal ligament simulation

Before performing the fracture resistance tests, periodontal ligaments were simulated according to a previous study [19]. The teeth were immersed in melted wax (Duradent, Odonto Com. Imp. Ltda, São Paulo, SP, Brazil) up to 2mm from the cemento-enamel junction in a way that a 0.3-mm layer of wax coated the root. Then, the teeth were placed in PVC tubes (Tigre do Brasil, Osasco, SP, Brazil) _with 20 mm in diameter and 25mm in length_ containing newly manipulated self-cure acrylic resin (Clássico, Rio de Janeiro, RJ, Brazil) according to manufacturer's recommendations. To maintain the same insertion axis for all specimens, a surveyor (Bioart, São Carlos, SP, Brazil) was used.

After polymerization of the resin, the teeth were withdrawn from the PVC tubes and the wax was removed with heated water. The tubes containing acrylic resin were filled with polyether-based molding material (Impregum F-ESPE, Seefeld, Germany). The teeth were reinserted into the PVC

tubes and the excess molding material was removed with no. 12 scalpel blades. The purpose of this step was to allow the molding material to fill the space previously occupied by the wax, creating an area with resilience similar to the periodontal ligament, approximately 0.3 mm thick.

Fracture resistance

All samples were subjected to a compression load at a speed of 0.5mm/min in a hydraulic test machine (Instron 3365, Instron Brasil Equipamentos Científicos Ltda, São José dos Pinhais, PR, Brazil) until a fracture or crack was detected [18, 19]. A 5000N load cell was used for this experiment. A metallic cylindrical device was used to fix the specimen in the test apparatus at a 45° angle, so that the load could be applied 135° in relation to the longitudinal axis of the tooth. It simulated a traumatic blow to the middle third of the dental crown from a buccal direction. The tests were performed 7, 14, and 21 days after the insertion of intracanal medicaments. In each group, 20 teeth were subjected to fracture resistance at each time interval.

Statistical analysis

The effects of the type of used medication and the residence time on the fracture resistance of dentine were analyzed by means of specific tests, according to the distribution of normality in the groups. Distribution of the data was tested using the Shapiro-Wilk test. The effects of the type of intracanal medicament on the fracture resistance of dentine and the time that the medicament remained in the root canal were examined using Kruskal-Wallis and Mann-Whitney tests. To assess the relation between time and fracture resistance (Intragroup analysis) Friedman test and Wilcoxon signed ranks test were used. A 5% level of statistical significance was applied for the analyses.

Results

The median values of fracture resistance in different groups are shown in Table 1. After 7 days, no statistical difference was found between the groups ($P=0.376$). After 14 and 21 days, the

TAP group presented significantly lower values of fracture resistance than that of the control group ($P=0.018$ and 0.033 , respectively). There was no statistical difference between the CH group and the control group after 7, 14 and 21 days ($P>0.05$).

Intragroup analysis revealed that, after 21 days, the TAP group ($P=0.015$) and the CH group ($P=0.006$) presented a statically significant reduction in comparison with 7 days. Statistical difference was not verified for the control group after 7, 14 and 21 days ($P=0.25$).

Discussion

TAP and CH are the most commonly intracanal medicaments used in pulp revitalization [3]. With regard to CH, the results of this research revealed that there was no statistical difference between CH group and the control group after 7, 14 and 21 days. Other studies have shown that after exposure to CH for 30 days or longer, significant changes in the mechanical properties of dentine are observed [14, 19, 20]. Kawamoto *et al.* [21] affirmed that the alkalinity of CH might lead to the denaturing of dentine collagen fibrils, leaving the radicular dentine more prone to fracture [21]. These fibrils are encapsulated by the inorganic hydroxyapatite crystals and are not readily accessible to CH. Therefore, time would be required for CH to penetrate into and denature the collagen fibrils resulting in a dentine that is more brittle and susceptible to fracture. In a systematic review, Yassen and Platt [22] verified that most *in vitro* studies showed a reduction in the mechanical properties of dentine after exposure to CH for 5 weeks or longer [22]; however, there is no consensus on the exact time because of the heterogeneity of the studies. Variations in dimensions, size, type of teeth, and direction of applied forces make comparisons difficult between studies. In this study, we attempted to simulate the pulp revitalization protocol, which was not performed in other studies.

Although literature presents conflicting results regarding the residence time of CH and its influence on the mechanical properties of dentine, several authors agree that CH decreases resistance to fractures [11-13, 19, 20, 23]. In this study, in the

Table 1. Median (Interquartile range) of load at fracture (Newton) for bovine teeth treated with pulp revitalization medications and untreated control group after 7, 14 and 21 days

Group	7 days (Newton) Median (IR)	14 days (Newton) Median (IR)	21 days (Newton) Median (IR)	P-value**
Control	1058.26 (230.27) ^{Aa}	1097.77 (158.98) ^{Aa}	1049.14 (247.45) ^{Aa}	0.25
TAP	994.84 (246.97) ^{Aa}	973.20 (134.16) ^{Bab}	882.63 (193.83) ^{Bb}	0.015
CH	1097.19 (242.60) ^{Aa}	1056.75 (209.82) ^{ABab}	870.86 (121.19) ^{ABb}	0.006
P-value*	0.376	0.018	0.033	-

*Intergroup analysis: Kruskal-wallis/Mann-Whitney tests ($P\leq 0.05$); **Intragroup analysis: Friedman/Wilcoxon signed rank tests ($P\leq 0.05$); Different upper case letter indicates significant difference between different groups within the same time-point and different lower case letter indicates significant difference between the three time-points within the same group

intragroup analysis, it was observed that the longer CH remained in the canal, the lower the fracture resistance had become. The decrease in the fracture resistance, that was noted after the root canals were filled with CH, can be explained by the fact that dentine is a mineralized connective tissue and 22% of its content is organic materials [11, 24]. This organic matrix contains phosphate and carboxylate groups, which work like bonding agents between hydroxyapatite and collagen fibers [11, 24]. The use of an alkaline chemical, for instance CH, as an intracanal medication can dissolve, denature, or neutralize these acidic organic components in the dentinal tissue, resulting in the weakening of dentine and making the tooth more susceptible to fractures [11, 19, 24].

The TAP group presented significantly lower values for fracture resistance in comparison with the control group after 14 and 21 days. Furthermore, fracture resistance in the TAP group was more reduced after 21 days when compared to 7 days. In another study, there was a significance reduction in fracture resistance only after 3 months [7]. However, these authors used 5-mm root cylinders prepared from teeth with complete root formation and the force applied was vertical and simulation of the periodontal ligament was not performed. In addition to fracture resistance, the effect of TAP on microhardness has also been investigated [17, 25]. Although fracture resistance and microhardness are different mechanical properties, such works reinforce the idea of mechanical compromise influenced by the TAP.

The performance of an antibiotic paste lies in the fact that acids are commonly added to some antibiotics to maintain chemical stability, control tonicity, or to ensure physiological compatibility [7]. However, long-term exposure of dental hard tissues to acidic antibiotics might cause demineralization and negatively affect their mechanical properties. Minocycline, a component in TAP, has been found to chelate calcium and demineralize dental hard tissues [7-10]. The results of another study suggested superficial collagen degradation and demineralization of radicular dentine caused by CH and antibiotic paste, respectively, after 1, 2, and 4 weeks of exposure [26]. Besides the mechanical and aesthetic criteria, TAP has biological disadvantages, such as cytotoxicity (even at low concentrations), sensitivity, and difficulty with removal [27-29].

The "American Association of Endodontists" [3] recommend that an intracanal medication for revascularization should remain inside the tooth for 1-4 weeks. However, Nagata et al. [30] observed that the largest bacterial reduction occurred after passive decontamination. After 21 days with an intracanal medicament (CH and TAP), the authors noted an increase in the

bacteriological level, suggesting that a prolonged residence of the medications may allow bacterial selection or coronary infiltration of new microorganisms. The actual relationship between antimicrobial activity and significant interference in mechanical properties have not yet been fully elucidated and deserves attention.

The major limitation of this study was that the force applied during dental trauma could not be reproduced accurately in the laboratory. In this *in vitro* study, the force was applied progressively at a constant rate until tooth fracture. Clinically, any force that may lead to the fracture of a tooth root is likely to be a sudden impact force rather than a continuously applied force. In addition, it is likely to be applied at varying angles rather than only at 45° [20, 31, 32].

In this *in vitro* study, the results indicated that TAP and CH could reduce the fracture resistance of dentin in a short-time interval. This research added to the existing knowledge to strengthen the clinical protocol for pulp revitalization in a more practical, safe, and targeted way. Discovering the balance between the time needed for effective decontamination of the root canal system whilst maintaining dentine fracture resistance is essential to achieve treatments with more predictable clinical success.

Conclusion

The null hypothesis, which tested that TAP and CH used in pulp revitalization had no significant effect on root fracture resistance at all-time points, was rejected. We concluded that the time, at which TAP and CH remained in the canal, affected the fracture resistance of bovine teeth with simulated incomplete root formation. Also, the reduction was significant for TAP after 14 and 21 days.

Conflict of Interest: 'None declared'.

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