

ORIGINAL ARTICLE/ARTICOLO ORIGINALE

Histomorphometric analysis of pulp submitted to the action of bromelain associated with irrigation solutions

Analisi istomorfometrica della polpa sottoposta all'azione di bromelina associata a soluzioni irriganti

KEYWORDS

Bromelains,
Endodontics,
Root Canal Irrigants,
Sodium Hypochlorite.

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Bromelina,
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Abstract

Aim: The objective of this study was to evaluate the tissue disgregation capacity of pulp in different solutions associated or not with bromelain.

Methodology: Fifty bovine mandibular incisors were used, divided into five groups: C: Control, H: sodium hypochlorite 2.5%, B: bromelain, BL: bromelain + L-cysteine, BH: bromelain + sodium hypochlorite 2.5%. The pulp fragments were stored at -20 °C, washed with saline solution and moved to 25 °C in eppendorf tubes with 1 ml of each substance. For the histomorphometric evaluation, the pulp fragments were prepared as histological routine, observed with optical microscopy and evaluated using ImageJ 1.49 software.

Results: The histomorphometric results showed that, in C: there was no alteration per tissue; H: there was a peripheral and central tissue disintegration; B: there was a protein and cellular degradation pattern, from the peripheral to the central area; BL: there was a central degradation. In BH, showed an effect enhanced by bromelain, when related to sodium hypochlorite, showing greater tissue degradation.

Conclusions: The association of bromelain with sodium hypochlorite potentiated the disintegration action of the pulp, opening possibilities for several clinical applications.

Obiettivi: L'obiettivo di questo studio era di valutare la capacità di disaggregazione del tessuto pulpare in diverse soluzioni associate o meno alla bromelina.

Materiali e Metodi: Sono stati utilizzati cinquanta incisivi mandibolari bovini suddivisi in cinque gruppi: C: Controllo, H: ipoclorito di sodio 2,5%, B: bromelina, BL: bromelina + L-cisteina, BH: bromelina + ipoclorito di sodio 2,5%. I frammenti pulpari sono stati conservati a una temperatura di -20 °C, lavati con soluzione fisiologica e portati a 25 °C in provette eppendorf con 1 ml di ciascuna sostanza. Per la valutazione istomorfometrica i frammenti di polpa sono stati preparati come da routine istologica, poi sono stati osservati con microscopia ottica e valutati con software ImageJ 1.49.

Risultati: L'analisi istomorfometrica evidenzia che: il gruppo C non evidenzia alterazione del tessuto; il gruppo H presenta una disintegrazione del tessuto periferico e centrale; il gruppo B mostra un pattern di degradazione cellulare e proteica, dalla periferia alla zona centrale; il gruppo BL presenta una zona di degradazione centrale; il gruppo BH ha presentato un effetto potenziato dalla bromelina quando correlata all'ipoclorito di sodio, presentando una maggiore degradazione del tessuto.

Conclusioni: L'associazione di bromelina con ipoclorito di sodio ha potenziato l'azione di disintegrazione della polpa, aprendo le possibilità per diverse applicazioni cliniche.

Introduction

One of the causes of endodontic treatment failure may be due to residual organic tissue or infected tissue left in the root canal after instrumentation (1). That may occur because of contamination and microlleakage during and after

treatment (2). Thus, there is recommendation for the use of instruments that produce mechanical action associated to chemical substances with antimicrobial activity, organic matter dissolution capacity, lubricating properties and low cytotoxicity (3). One of the main auxiliary chemical substances used in this procedure is the sodium hypochlorite (NaOCl).

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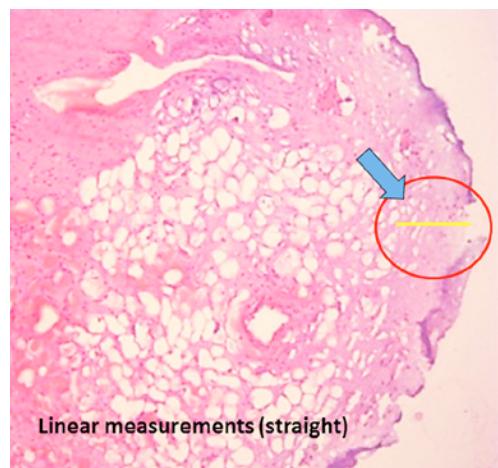
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Figure 1
Linear measures of pulp: measurements were made of the periphery of the pulp tissue using the tool "Straight" (ellipse), following the peripheral delimitation of the pulp tissue (arrows).



Sodium hypochlorite is still most widely used irrigation solution by dentists (4). However, many studies have proposed other alternatives, considering that NaOCl is cytotoxic, has allergenic potential and compromises the binding of resin cement and alteration in the peripheral dentin matrix (5, 6). In addition, the undesirable effects such as pain, edema hyperemia, inhibits neutrophil migration and damages endothelial and fibroblast cells that can occur in case of extravasation of hypochlorite (7, 8, 9, 10).

This suggests the necessity of studies that seek to develop laboratory tests with the objective of reaching an irrigating solution that best performs its function without damaging the periapical tissues (11) or reducing the volume and frequency of sodium hypochlorite solution in patients. Extreme caution should be exercised in the application of NaOCl to root canals to avoid increasing occurrence of harmful consequences caused by the spreading of this highly alkaline and cytotoxic material into tissues outside the root canals (12).

Bromelain is one of the agents that has been widely used as an anti-inflammatory drug in the field of medicine and dentistry, as well as its antibacterial efficacy (13). It is a crude aqueous extract from stems and immature fruits of pineapples, configuring a not very common and complex mixture that differ in thiol-endopeptidases and other components not yet fully clarified, such as phosphatases,

glycosidases, peroxidases, cellulases, glycoproteins and carbohydrates, as well as others (14).

In light of this, that the association of bromelain with sodium hypochlorite may potentiate the removal of pulp tissue, this research was conducted by adding L-cysteine, which can catalyze the hydrolysis of peptide bonds by deprotonation (15). For that, an *in vitro* study was designed to evaluate the dissolution power and tissue denaturation of these products on the root pulps of bovine teeth.

Materials and Methods

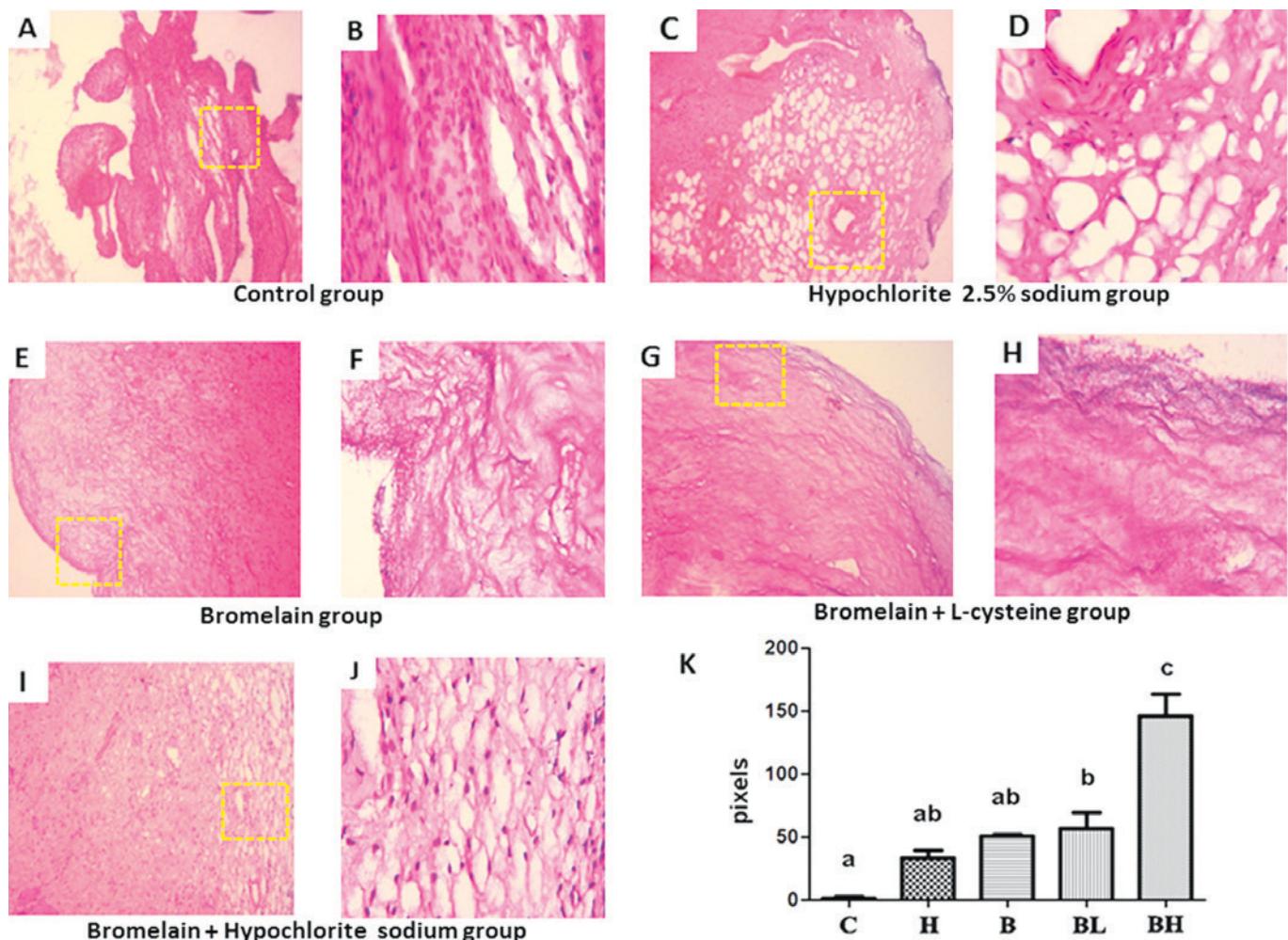
Samples. A total of 50 bovine mandibular incisors were extracted and randomly stored -20 °C in physiological solution during transport to the Laboratory, until further analysis. For this study, no permission from the animal ethics committee was necessary, once that the teeth of animals were collected after slaughtering for meat consumption.

The pulps were removed by means of a cross-sectional cutting in the cervical region, separating the crown from the root. The root pulp was completely removed with Hedstroem (Denstply) type. The pulp fragments were washed during 30 seconds in a physiological solution and, immediately after, they were stored in eppendorf tubes at -20 °C until they were used. Sodium hypochlorite was formulated at a concentration of 2.5% and adjusted to a pH of 9.0, irrigation solution concentration commonly used in endodontic treatment.

Experimental Design. Five experimental groups were established with 10 samples for each group ($n = 10$) as follows

- Group 1, C: control (saline solution)
- Group 2, H: 2.5% sodium hypochlorite diluted in saline solution
- Group 3, B: bromelain 5 mg for each 10 ml saline solution
- Group 4, BL: bromelain 5 mg for each 10 ml saline solution and L-Cysteine, 3 mM
- Group 5, BH: bromelain 5 mg and 2.5% sodium hypochlorite

The pulp fragments in the process of dissolution were moved from -20 °C to 25 °C, washed with saline solution for 30 seconds,



A) In control group there was no tissue change in any of the samples (150x) **B)** In control group there was no tissue change in any of the samples (600x) **C)** Hypochlorite 2.5% sodium group (150x) **D)** In hypochlorite 2.5% sodium group there was a disintegration of the tissue both peripherally and in its central region (600x) **E)** Bromelain group (150x) **F)** Bromelain group, protein and cell degradation pattern occurred, from the peripheral to the central region (600x) **G)** Bromelain associated with L-cysteine group (150x) **H)** When the bromelain is associated with L-cysteine group we observed a central degradation of the tissue **I)** The bromelain is associated with 2.5% sodium hypochlorite group (150x) **J)** The bromelain associated with 2.5% sodium hypochlorite group demonstrated a potentiating effect (600x) **K)** Measurements of alteration in the pulp from groups.

Different letters represent statistical difference $p<0.05$. C, Control group; H, Hypochlorite 2.5% sodium group; B- bromelain group; BL- bromelain + L-cysteine; BH- bromelain + hypochlorite. Hematoxylin and eosin stain.

dried with filter paper for another 5 seconds. They were put in eppendorf tubes with 1 mL of the solution for a 30 minute time.

Histomorphometric analysis. The pulp tissues were fixed in 10% buffered formaldehyde in 7.0 pH (Dinamica, Diadema, Brazil), dehydrated in increasing concentrations of alcohol: 30, 50, 70, 90 and 100%, diaphanized with xylol, included in paraffin and shaped in blocks for the microtomy with sections of 6 μm of thickness.

The sections were stained with hematoxylin and eosin and prepared with Canada balsam (Dinamica, Diadema, Brazil).

Afterwards, the analysis of the image capture was made with the ImageJ v1.49 Software (US National Institutes of Health, Bethesda, MD.) on the pulp linear measure as shown in figure 1.

The area the disintegration of the tissue was measured according presented in fig-



ure 1. Seven images per group were analyzed, totaling 35 images in magnification of 150x and 600x using the light microscope (figure 2).

Statistical Analysis. Results are expressed as median \pm standard error from the parameters obtained. Kolmogorov-Smirnov test was used to check the normality of the data. Differences between the groups were examined with the ANOVA one-way test followed by Newman-Keuls test. For the analysis of the data Graph Pad Prism5® was used, considering the value of $p<0.05$ as statistically significant.

Results

The histologic evaluation showed that, in the linear measurements of the tissues evaluated, the control group presented no tissue change in any of the samples, as seen in the figures 2A, 2B. In the 2.5% sodium hypochlorite group there was a significant disintegration of the tissue both peripherally and in its central region ($P <0.05$) (figures 2C, 2D). In the bromelain group, there was a protein and cell degradation pattern from the peripheral to the central region (figures 2E, 2F). The bromelain associated with L-cysteine group, there was a central degradation of the tissue (figures 2G, 2H). In the bromelain associated with 2.5% sodium hypochlorite group, a potentiating effect can be observed, showing greater tissue degradation of the pulp than in the other groups when tested without combination (figures 2I, 2J). The histomorphometric results are demonstrated in figure 2K.

Discussion

In the group where bromelain was used associated with 2.5% sodium hypochlorite a potentiating effect could be observed, showing greater tissue degradation of the pulp than the others when tested. The use of a cysteine protease activator was used, but did not cause a change in the proteolytic activity of bromelain.

During the chemical-mechanical preparation of endodontic treatment, there may be parts of the root canal that remain un-

touched, which may contain necrotic bacteria and tissue substrates leading to microleakage. This coronary microleakage of restorations has the potential to dissolve the sealing cement, compromising the prognosis of endodontic treatment and resulting in failure of the therapy (16, 17). Endodontic irrigants should have low systemic toxicity and yet allow an optimal disinfection of the root-canal system. Because of the complex anatomy of root canals, approximately 50% of canal walls remain uninstrumented during preparation, which results in an insufficient debridement (18). The use of NaOCl as a root canal irrigator can cause serious clinical problems when extruded into the periradicular tissues, confirming known NaOCl toxicity to the soft tissues after inadvertent extrusion (19).

Bromelain acts by direct proteolytic action, hydrolyzing peptide bonds (20). This proteolytic action occurs on soluble proteins, as well as on membrane structural proteins, mainly on receptors and surface markers (21). It is one of the extensively investigated proteolytic enzymes due to its astonishing applications in various industries (22).

Studies demonstrate the efficacy of bromelain, an enzyme found in the juice and stem of *Ananas comosus* (pineapple), which has therapeutic potential in fibrinolytic, antiedematous, antibacterial agent, antithrombotic and anti-inflammatory activities both in vitro and in vivo (13, 23, 24). Cellular removal with bromelain was also erratic similar to the other groups tested, with papain, ficin and trypsin where, the distribution with wedge-shaped areas had the highest cell removal. Residual cells were predominantly fibroblasts with adjacent vascular and neural elements intact (25).

In addition, many authors have pointed out that the bromelain reduce edema and inflammation (26, 27). Bromelain has a particularly intense and effective action in edema without acting on the fibrinogen, thus not interfering with the coagulation mechanism (28). It also inhibits lysosomal proteases and reduces platelet aggregation values (27). The properties cited above demonstrate that the bromelain can be



used alone or associated with sodium hypochlorite improving several processes involved in endodontic instrumentation.

Conclusions

The association of bromelain with sodium hypochlorite potentiated the disintegration action of the pulp, opening possibilities for several dental applications.

Clinical Relevance

The bromelain properties can be useful in endodontic instrumentation, because the bromelain presented a potent capacity of

disintegrate the pulp tissues, when used alone or associated with sodium hypochlorite, desirable feature in endodontic instrumentation.

Conflict of Interest

The author declares there is no conflict of interests.

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