

EFFECT OF DIFFERENT COMBINATIONS OF PAPAIN-BASED GELS ON DISSOLVING PULP TISSUE

EFEITO DE DIFERENTES COMBINAÇÕES DE GÉIS À BASE DE PAPAÍNA NA DISSOLUÇÃO DO TECIDO PULPAR

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ABSTRACT: The focus of this study was to evaluate the capacity to dissolve pulp tissue of various combinations of papain-based gels and other antimicrobial agents. 105 bovine pulps were used, of standardized sizes, fragmented into 15mm-sized portions and weighed on an analytical balance, divided into 7 groups (n=15): 1 - 0.9% Saline Solution (negative control); 2- 8% Papain gel; 3- 8% Papain gel + 0.5% Chloramine; 4- 0.5% Chloramine gel; 5- 8% Papain gel + 2% Chlorhexidine; 6- 2% Chlorhexidine gel; and 7- 5.25% Sodium Hypochlorite solution (positive control). After initial weighing, the pulp fragments were inserted in test tubes for dissolution for time intervals of 30, 60, 90 and 120 minutes, and then weighed again. The data were analyzed using the Kruskal–Wallis and Mann–Whitney tests ($p<0.05$). In the time interval of 120 minutes the 0.5% chloramine gel demonstrated 64.9% ability to pulp dissolve, followed by 8% papain gel with 61.3%; papain associated with 0.5% chloramine, 58%; and papain associated with 2% chlorhexidine, 55.4%; which showed statistically significant difference with 5.25% Sodium Hypochlorite ($p<0.05$). All the gels that contained papain and the 0.5% chloramine gel promoted pulp tissue dissolution, however on a significantly lower scale than 5.25% sodium hypochlorite. The 2% chlorhexidine demonstrated no capacity to dissolve pulp, as did the control.

KEY WORDS: Papain. Dental pulp. Chlorhexidine. Dissolution.

INTRODUCTION

The success of endodontic treatment is mainly related to microbial control (KAKEHASHI et al., 1965), by the use of chemical substances with antimicrobial activity and the capacity to dissolve organic matter (HEGDE et al., 2016), in addition to have lubricating properties and low cytotoxicity (SAFAVI et al., 1990).

Sodium hypochlorite (NaOCl) is the most used endodontic irrigant at present, although there are researchers (MORRIS et al., 2001; DAMETTO et al., 2005; MOREIRA et al., 2009; BEHRENTS et al., 2012; KERBL et al., 2012; PASCHOALINO et al., 2012; GUNESER et al., 2015) proposing other alternatives such as chloramine, chlorhexidine, papain gel (DUARTE et al., 2001) and Octenidine and QMix 2in1 (ARSLAN et al., 2015). NaOCl is highly cytotoxic, has allergenic potential and harms the bond of resin cements because it causes changes in the collagen fibers of root dentin (MORRIS et al., 2001; MOREIRA et al., 2009), and it is capable of changing the integrity of spongy bone if it passes through the apical foramen (BEHRENTS et al.,

2012; KERBL et al., 2012; PASCHOALINO et al., 2012).

Thus, the use of other irrigant solutions, such as chlorhexidine gluconate has been justified (OKINO et al., 2004; FERRAZ et al., 2007; AKGUN et al., 2013; BOLLA et al., 2013), due to its antimicrobial action, substantivity, less odor and cytotoxicity than NaOCl (DAMETTO et al., 2005). However, chlorhexidine in gel and also in an aqueous solution show no potential to dissolve organic matter (OKINO et al., 2004). Another irrigant that has been suggested is papain gel, because it is a proteolytic enzyme, has the capacity to dissolve pulp tissue, in addition it has bactericidal action, as the chloramines (DUARTE et al., 2001).

Considering that the presence of infected pulp remainders, particularly in the regions where mechanical instrumentation is not effective, it is a factor responsible for endodontic treatment failure, the aim of this study was to evaluate the capacity to dissolve pulp tissue of various combinations of papain-based gels and other antimicrobial agents such as chlorhexidine and chloramine.

MATERIAL AND METHODS

A random selection was made of 105 incisors extracted from bovine mandibles, which were immediately stored in physiological solution. The dental pulps were removed on the same day as the teeth were extracted, by fracturing the incisors in the cervical region, separating the crown from the root in the bucco-lingual direction with the help of a stabilizer of the lathe type on the tooth root, followed by fracturing the crown with a metal device of 4.90 N, under manual pressure, performed in the pathology laboratory of the State University of Southwest Bahia.

The pulp was completely removed from the root by traction with hemostatic forceps, with the help of a caliper and a scalpel. The pulp was measured and cut, into standardized 15mm portions from the middle thirds, size corresponding to approximately 60% of the total surface area of the content of the pulp sample, discarding the cervical and apical portions. The fragments were washed for 30 seconds with saline solution and enveloped in transparent plastic PVC film (Royal Pack, Paulista, Brazil), and stored at a temperature of -20°C until they were used. This study was approved by the ethics committee in animal research, CEP:468/2015.

The formulations of the papain-based, chlorhexidine and chloramine gels, used to evaluate the capacity to dissolve pulp tissue, were prepared from the solution of these substances in the general Chemistry Laboratory of the State University of Southeast Bahia. Sodium hypochlorite was formulated in a concentration of 5.25% and adjusted to pH 9.0 with the addition of boric acid. All substances, except sodium hypochlorite, were produced in Aristoflex® gel base - Ammonium Acryloyldimethyltaurate / VP Copolymer (Clariant International Ltd., Muttentz, Switzerland), and remained stored under refrigeration until they were used.

Seven experimental groups (n=15) were established, as follows: Group 1 (Saline solution – 0.9% NaCl, Negative Control), Group 2 (8% Papain gel), Group 3 (8% Papain gel + 0.5% chloramine gel), Group 4 (0.5% chloramine gel), Group 5 (8% Papain gel + 2% chlorhexidine gel), Group 6 (2% chlorhexidine gel) and Group 7 (5.25% Sodium Hypochlorite solution – NaOCl, Positive Control).

To perform the dissolution process, the pulp fragments were removed from the freezer at -20°C and allowed to return to ambient temperature. After this, they were washed for 30 seconds with the saline solution, lightly dried on filter paper for the same period of time and weighed on a precision

analytical balance (Shimadzu AW-220, Tokyo, Japan). After weighing the fragments, they were placed in test tubes with 5mL of the substance to be analyzed. At the end of each time interval of 30, 60, 90 and 120 minutes, the test substances were changed and the pulp fragments were washed again, dried and weighed according to the pre-established criteria.

Statistical procedure

Statistical analyses were performed with the program SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistical analysis including mean and standard deviation were calculated for the groups evaluated. The statistical method was chosen based on the model of distribution and variance of data evaluated by the Kolmogorov–Smirnov and Levene tests, respectively. The mean pulp dissolution values were statistically analyzed using the non-parametric Kruskal–Wallis and Mann–Whitney tests ($p < 0.05$).

RESULTS

The sodium hypochlorite almost completely degraded the pulp fragments (95.7%) at 30 minutes ($p < 0.05$), followed by the agents with lower capacity to dissolve: 8% Papain associated with 2% chlorhexidine (37.6%); 8% Papain associated with 0.5% Chloramine (29%); 8% Papain (27.7%) and 0.5% Chloramine (19.5%) (Figure 1) without statistical significant difference among them ($p > 0.05$) (Table 1).

Over the immersion times of 60, 90 and 120 minutes the pulp dissolution gradually increased. At 60 minutes 5.25% Sodium Hypochlorite demonstrated its greatest potential ability to dissolve 100% of the pulp samples ($p < 0.05$).

At 120 minutes 0.5% chloramine demonstrated 64.9% dissolution ability, and 8% papain in gel dissolved 61.3%; papain with 0.5% chloramine dissolved 58%; papain with 2% chlorhexidine dissolved 55.4%. There was statistically significant difference of these groups with 5.25% Sodium Hypochlorite ($p < 0.05$).

The 2% chlorhexidine and the negative control groups demonstrated no capacity to dissolve pulp ($p > 0.05$) throughout the experiment (Table 1).

The groups of 8% Papain, 8% Papain + 0.5% Chloramine, 0.5% Chloramine, 8% Papain + 2% Chlorhexidine, 2% Chlorhexidine and 5.25% NaOCl demonstrated statistically significant difference between the initial period with the periods of 60 minutes, 90 minutes and 120 minutes ($p > 0.05$).

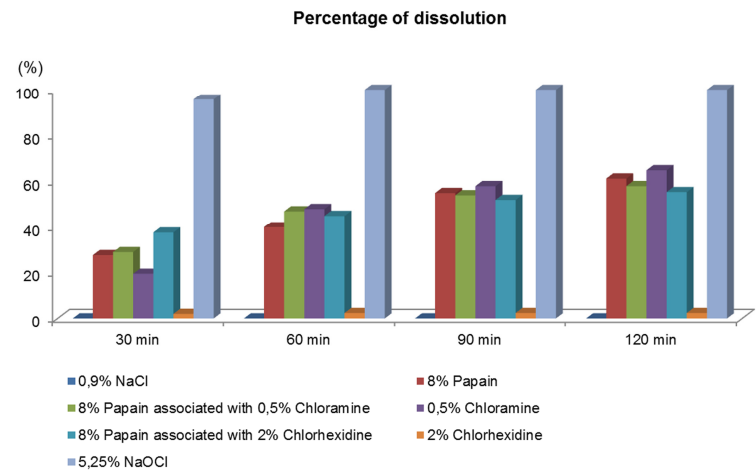


Figure 1: Percentage of the dissolving of pulp tissue at different times

Table 1: Mean weight in grams of the pulp fragments initially and after times of immersion in the test solutions.

Groups		Times of immersion									
		Initial		30 min		60 min		90 min		120 min	
		Mean (SD)	St*	Mean (SD)	St*	Mean (SD)	St*	Mean (SD)	St*	Mean (SD)	St*
1	0.9% NaCl [†]	0.0103 (0.0003)	Aa	0.0104 (0.0003)	Ab	0.0103 (0.0002)	Aa	0.0103 (0.0005)	Aa	0.0103 (0.0003)	Aa
2	8% Papain	0.0101 (0.0004)	Aa	0.0073 (0.0004)	Bab	0.0061 (0.0003)	Bbc	0.0044 (0.0003)	Bcd	0.0039 (0.0002)	Bd
3	8% Papain + 0.5% Chloramine	0.0100 (0.0004)	Aa	0.0071 (0.0003)	Bab	0.0053 (0.0005)	Bbc	0.0046 (0.0004)	Bcd	0.0042 (0.0003)	Bd
4	0.5% Chloramine	0.0097 (0.0005)	Aa	0.0078 (0.0002)	Bab	0.0051 (0.0002)	Bbc	0.0040 (0.0004)	Bcd	0.0034 (0.0004)	Bd
5	8% Papain + 2% Chlorhexidine	0.0101 (0.0003)	Aa	0.0063 (0.0005)	Bab	0.0055 (0.0003)	Cbc	0.0047 (0.0005)	Ccd	0.0045 (0.0003)	Cd
6	2% Chlorhexidine	0.0103 (0.0003)	Aa	0.0101 (0.0004)	Aa	0.0100 (0.0004)	Ab	0.0100 (0.0002)	Ab	0.0100 (0.0003)	Ab
7	5.25% NaOCl [#]	0.0094 (0.0007)	Aa	0.0004 (0.0000)	Ba	0.0000 (0.0000)	Cb	0.0000 (0.0000)	Cb	0.0000 (0.0000)	Cb

[†]Negative Control. [#]Positive Control. *St: Statistically, Kruskal-Wallis and Mann-Whitney Test. Capital letters- Different letters express statistical significance between the groups for each time (p<0.05). Lowercase Letters - Different letters express statistical significance between the times for each group (p<0.05). SD: Standard Deviation.

DISCUSSION

In medicine, papain is widely used and demonstrated no significant allergenic properties in humans (DA SILVA et al., 2010). In dentistry, papain gel associated with 0.5% chloramine is used in the chemical-mechanical removal of carious dental tissues (CARRILLO et al., 2008; GIANINI et al., 2010; AMARAL et al., 2011), and it has been indicated as an irrigant solution for teeth requiring endodontic treatment (DUARTE et al., 2001). According to MIYAGI et al. (2006) papain up to the concentration of 8% associated with 0.5% chloramine has shown no cytotoxicity, and no mutagenic potential (LOPES et al., 2008; DA SILVA et al., 2010).

In this study, the test tubes were static because substances with different viscosities were being compared (liquid and gel). Agitation could influence of the potential to dissolve pulp tissue (NIEWIEROWSKI et al., 2015), because of variation in the amount of solution that would contact with the fragments and the mechanical action performed by movement of the fluids.

It was noted that NaOCl 5.25% solution showed the greatest potential to dissolve pulp tissue. After 30 minutes the NaOCl had degraded almost the entire fragment, (95.7%), with great effectiveness in the dissolution of organic matter, which corroborates with the findings of other studies (IRALA et al., 2010; STOJICIC et al., 2010; GUNESER et al., 2015; HEGDE et al., 2016). Over the same period, it has shown that 8% papain associated with 2% chlorhexidine, promoted a faster dissolution compared with pure 8% papain, and papain with 0.5% chloramine. Suggests that, the ionic combination between free radicals present in chlorhexidine/papain increase the papain proteolytic capacity on the organic matter, in the initial period of 30 minutes. Whereas in the time intervals of 60 and 90 minutes these rates were more even, with a slight predominance for chloramine, but without statistical difference.

The groups of gels that contained papain in the formulation and the 0.5% chloramine group achieved pulp dissolution of 55.4% to 64.9% at the end of 120 minutes, which was statistically significant in comparison with 0.9% NaCl (negative control) and 2% chlorhexidine gel. Thus a gradual degradation of the fragments was observed, however, at a much slower speed compared with NaOCl.

The 2% chlorhexidine group underwent a small loss of weight during the process, however it was not statistically significant compared to the

control group, which is in agreement with other studies (MARLEY et al., 2001; OKINO et al., 2004), and demonstrated its inability to dissolve organic materials (MARLEY et al., 2001).

Among the morphological changes observed, 5.25% NaOCl promoted rapid dissolution with characteristics of tissue dehydration. This was also observed with 0.5% chloramine, however, with slower dissolution. When exposed to 2% chlorhexidine dehydration followed by tissue hardening was observed, but without dissolution. The papain-containing gels demonstrated that the pulps underwent a slow dissolution, without the characteristic of dehydration, and remained softened. In saline solution, the pulp fragments maintained the same characteristics of initial tissue.

In the comparison between pulp degradation times for each gel, 8% Papain, 8% Papain + 0.5% Chloramine, 0.5% Chloramine, 8% Papain + 2% Chlorhexidine, 2% Chlorhexidine and 5.25% NaOCl did not show statistically significant difference between the initial period and 30 minutes, although after 30 minutes the 5.25% NaOCl has demonstrated great pulp dissolution capacity. Considering that the NaOCl solution has some undesirable properties, such as tissue irritation and allergenic potential (CALISKAN et al., 1994; DUARTE et al. 2001; GUNESER et al., 2015), it encourages the search for new substances with better characteristics. In this context, 2% chlorhexidine appears to be a promising material, nevertheless, studies (TULUNOGLU et al. 1998; WACHLAROWICZ et al., 2007; MOREIRA et al., 2009) have shown evidence that it may affect the bond between certain adhesive systems and dentin when used as an intracanal irrigant (SIMSEK et al. 2013).

The results obtained in this study for 8% papain and its associations and/or 0.5% chloramine, demonstrated promising results as an alternative material for use in clinical practice. However, further studies are necessary to evaluate whether if 8% papain and/or 0.5% chloramine are capable of affecting the bond between adhesive systems and dentin, after being used as an intracanal irrigant.

CONCLUSIONS

All the gels that contained papain, and the 0.5% chloramine gel promoted pulp tissue dissolution, however on a significantly lower scale than 5.25% sodium hypochlorite.

The 2% chlorhexidine demonstrated no capacity to dissolve pulp, as did the control.

RESUMO: O foco deste estudo foi avaliar a capacidade de dissolução do tecido pulpar de várias combinações de géis à base de papaína e outros agentes antimicrobianos. Foram utilizadas 105 polpas bovinas, de tamanhos padronizados, fragmentadas em porções de 15mm e pesadas em balança analítica, divididas em 7 grupos (n = 15): 1 - 0,9% de Solução Salina (controle negativo); 2- 8% de gel de papaína; 3- 8% de gel de papaína + 0,5% de cloramina; 4- 0,5% de gel de cloramina; 5- 8% de gel de papaína + 2% de clorhexidina; 6- 2% de gel de clorexidina e 7- 5,25% de solução de hipoclorito de sódio (controle positivo). Após pesagem inicial, os fragmentos de polpa foram inseridos em tubos de ensaio para dissolução durante intervalos de tempo de 30, 60, 90 e 120 minutos e depois pesados novamente. Os dados foram analisados pelos testes de Kruskal-Wallis e Mann-Whitney ($p < 0,05$). No intervalo de tempo de 120 minutos o gel de cloramina a 0,5% demonstrou 64,9% de capacidade de dissolver polpa, seguido pelo gel de papaína a 8% com 61,3%; papaína associada a 0,5% de cloramina, 58%; e papaína associada a clorexidina a 2%, 55,4%; que apresentaram diferença estatisticamente significativa com hipoclorito de sódio a 5,25% ($p < 0,05$). Todos os géis que continham papaína e o gel de cloramina a 0,5% promoveram a dissolução do tecido de polpa, contudo em uma escala significativamente inferior a 5,25% de hipoclorito de sódio. A clorexidina a 2% não demonstrou capacidade para dissolver a polpa, assim como o controle.

PALAVRAS-CHAVE: Papaína. Polpa dentária. Clorexidina. Dissolução.

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