UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Analysis of pH and antimicrobial activity of Ambroxol Hydrochloride and N-Acetylcysteine pastes to be used as root canal medication

Análise do pH e atividade antimicrobiana das pastas de Cloridrato de Ambroxol e N-Acetilcisteína utilizadas como medicação intracanal

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ABSTRACT

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The aim of the present study was to evaluate the pH and antimicrobial activity of N-Acetylcysteine (NAC) and Ambroxol Hydrochloride (AMB) pastes comparing with calcium hydroxide paste (CHP). Experimental pastes made of NAC, AMB and CHP, employing propylene glycol as vehicle with 500 mg / 0,5 mL concentration, were prepared for testing. The pH was measured after 3 hours, 3 and 7 days with a calibrated pHmeter; freshly pastes were inserted into tubes and immersed into flasks containing 10 mL of ultrapure water; the pH of ultrapure water was used as control. For in vitro antimicrobial activity evaluation, biofilm of *Enterococcus faecalis* was induced on 60 blocks of bovine dentin which were divided in 3 experimental and a control (no treatment) groups (n=15). The pastes were prepared and after 7 days of contact the blocks were analyzed under a confocal microscope using the Live/Dead dye; the percentage of live cells was measured using bioImage software. The NAC and AMB presented acidic pH and the CHP presented pH higher than 11 in all periods. There were statistically significant differences between all groups at all periods analyzed ($P<0.05$). The NAC and AMB groups offered a lower number of viable cells than the CHP group, which presented no significant differences in comparison to the CON group. The AMB presented greater antimicrobial action than CHP ($P<0.05$). We can conclude that the N-Acetylcysteine and Ambroxol Hydrochloride pastes presented acid pHs which, against *E. faecalis* biofilm, presented antimicrobial activity, with Ambroxol Hydrochloride being more effective. Further studies should be done in order to suggest its use on the endodontic therapy.

Keywords: *Enterococcus faecalis*; Biofilm; Calcium hydroxide; N-Acetylcysteine; Ambroxol hydrochloride; Antimicrobial action.

RESUMO

RESUMO

O objetivo do presente estudo foi avaliar o pH e a atividade antimicrobiana das pastas N-acetilcisteína (NAC) e Cloridrato de Ambroxol (AMB) em comparação à pasta de Hidróxido de Cálcio (CHP). Pastas experimentais feitas de NAC, AMB e CHP, empregando propilenoglicol como veículo com concentração de 500 mg / 0,5 mL, foram preparadas para teste. O pH foi medido após 3, 72 e 168 horas com um medidor de pH calibrado; pastas frescas foram inseridas em tubos e imersas em frascos contendo 10 mL de água ultrapura; o pH da água ultrapura foi utilizado como controle. Para avaliação da atividade antimicrobiana, o biofilme de *Enterococcus faecalis* foi induzido *in vitro* em 60 blocos de dentina bovina, os quais foram divididos em três grupos experimentais e um controle (sem tratamento) (n = 15). As pastas foram preparadas e após 7 dias de contato os blocos foram analisados sob microscopia confocal utilizando o corante Live / Dead; a porcentagem de células vivas foi mensurada usando o software bioImage. O NAC e AMB apresentaram pH ácido e o CHP apresentou pH superior a 11 em todos os períodos. Houve diferenças estatisticamente significantes entre todos os grupos em todos os períodos analisados (P <0,05). Os grupos NAC e AMB ofereceram um número menor de células viáveis que o grupo CHP, que não apresentou diferença significativa em comparação ao grupo CON. O AMB apresentou maior ação antimicrobiana que o CHP (P <0,05). Coinclui-se que as pastas de N-Acetilcisteína e Cloridrato de Ambroxol apresentaram pH ácido que, contra o biofilme de *E. faecalis*, apresentou atividade antimicrobiana, sendo o Cloridrato de Ambroxol mais eficaz. Outras análises devem ser feitas com ambas as substâncias a fim de sugerir seu uso na terapia endodôntica.

Palavras-chave: *Enterococcus faecalis*; Biofilme; Hidróxido de Cálcio; N-Acetilcisteína; Cloridrato de Ambroxol; Atividade antimicrobiana

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The endodontic infection is a true challenge for clinicians, even after the biomechanical preparation, due to the number of bacteria inside the root canal system and the anatomical complexity of each case (Vera *et*. al 2012).

The use of intracanal medication between sessions before the obturation, optimizes the success rate of the endodontic therapy, especially the Calcium Hydroxide paste in cases of endodontic failure (Zancan *et*. al 2016; 2019). However, *E. faecalis*, one of the most frequent bacteria in those cases, some strains of this bacteria have shown resistance to the alkaline action of the Calcium Hydroxide (Evans *et*. al 2002; Nakajo *et*. al 2006; Chávez de Paz *et*. al 2007).

Considering the importance of the antisepsis of the root canal system, and aiming a direct action on the biofilm, the mucolytic agent N-Acetylcysteine has been showing favorable results in this scenario (Choi *et*. al 2018; Costa *et*. al 2017). Moreover, this composite has shown in previous studies, some antimicrobial action against *E. faecalis* (Ulusoy *et*. al 2016; Quah *et*. al 2012).

Another substance used to help to disaggregate and solve mucus, is the Ambroxol Hydrochloride, often used in pulmonary infections (Cataldi *et*. al 2014). This one acts in the composition of mucus, lowering its viscosity and thickness, what could be favorable to pass through the polysaccharides of the biofilm and reach the bacterial cells (Li *et*. al 2017; Hull *et*. al 2018).

Considering the resistance of the *E. faecalis* to the Calcium Hydroxide, one of the most used intracanal medication in endodontic therapy, the aim of the present study was to evaluate the pH and antimicrobial activity of the N-Acetylcysteine and Ambroxol Hydrochloride based pastes, comparing with Calcium Hydroxide paste, against an *E. faecalis* biofilm.

2 ARTICLE

2 ARTICLE

The article presented in this Dissertation was formatted according to the Journal of Endodontics instructions and guidelines for article submission.

Analysis of pH and antimicrobial activity of Ambroxol Hydrochloride and N-Acetylcysteine pastes to be used as root canal medication

ABSTRACT

The aim of the present study was to evaluate the pH and antimicrobial activity of N-Acetylcysteine (NAC) and Ambroxol Hydrochloride (AMB) pastes comparing with calcium hydroxide paste (CHP). Experimental pastes made of NAC, AMB and CHP, employing propylene glycol as vehicle with 500 mg / 0,5 mL concentration, were prepared for testing. The pH was measured after 3 hours, 3 and 7 days with a calibrated pHmeter; freshly pastes were inserted into tubes and immersed into flasks containing 10 mL of ultrapure water; the pH of ultrapure water was used as control. For in vitro antimicrobial activity evaluation, biofilm of *Enterococcus faecalis* was induced on 60 blocks of bovine dentin which were divided in 3 experimental and a control (no treatment) groups (n=15). The pastes were prepared and after 7 days of contact the blocks were analyzed under a confocal microscope using the Live/Dead dye; the percentage of live cells was measured using bioImage software. The NAC and AMB presented acidic pH and the CHP presented pH higher than 11 in all periods. There were statistical significant differences between all groups at all periods analyzed ($P<0.05$). The NAC and AMB groups offered a lower number of viable cells than the CHP group, which presented no significant differences in comparison to the CON group. The AMB presented greater antimicrobial action than CHP ($P<0.05$). We can conclude that the N-Acetylcysteine and Ambroxol Hydrochloride pastes presented acid pHs which, against *E. faecalis* biofilm, presented antimicrobial activity, with Ambroxol Hydrochloride being more effective. Further studies should be done in order to suggest its use on the endodontic therapy.

Keywords: *Enterococcus faecalis*; Biofilm; Calcium hydroxide; N-Acetylcysteine; Ambroxol hydrochloride; Antimicrobial action

INTRODUCTION

During biomechanical preparation of the root canal system, the total number of viable bacteria is lowered by the physical action of the instruments associated with the irrigating solutions (1). Nevertheless, a considerable number of pathogens remain viable, mainly in areas of anatomical complexity and inside the dentinal tubules (2).

In treatment of teeth with pulp necrosis, the intracanal medications is to achieve more effective antisepsis of the root canal system (2-4). The proposal to use the calcium hydroxide paste in Endodontics has been established in the practice for decades, due to its antimicrobial activity and stimulation of tissue repair (5, 6). However, some pathogens such as *E. faecalis* have shown resistance to it (7), especially when in the form of biofilm (4, 8).

The biofilm consists in the aggregate of microorganisms embedded in interlaced fibers of polysaccharides that favor greater adhesion to the contact surfaces and maintain a complex nutrition network among them, thereby cooperating to provide a high rate of cellular viability. The formation of the polysaccharide layer also acts as protection, hindering the action of medications against the biofilm (9-11), even when associated with biologically active vehicles such as PMCC (4).

N-Acetylcysteine (NAC) is a compound used mainly on pulmonary diseases, known as a potent antioxidating agent capable of modulating proinflammatory cytokines (12). NAC has also shown favorable action on disorganizing bacterial biofilms, inhibiting bacterial adhesiveness, reducing extracellular polysaccharide production and reducing cell viability. Thus, because of the action of NAC on biofilm, its use isolate or associated with intracanal medications may increase the bacterial susceptibility to the antimicrobial agent (13).

The Ambroxol Hydrochloride (AMB) is another substance used in pneumology that acts directly on the composition of mucus, helping to eliminate it and reduce its viscosity (14), nonetheless there is no report in the literature about its effect on bacterial biofilm. Based on one of the prevailing factors affecting the success of endodontic treatment - namely microbial control - the use of a substance that has mucolytic action could favor the dissolution of polysaccharides, and consequently the action of this substance on the biofilm.

Therefore, considering the importance of biofilm control in Endodontics, the aim of the present study was to evaluate the pH and antimicrobial activity of NAC and AMB based pastes, comparing with calcium hydroxide paste, against an *Enterococcus faecalis* biofilm. The null hypothesis tested was that none of the two substances would not produce higher pH or greater antimicrobial activity than the calcium hydroxide paste.

MATERIAL AND METHODS

Pastes Preparation

Pastes of N-Acetylcysteine / propylene glycol (NAC), Ambroxol Hydrochloride / propylene glycol (AMB) and Calcium Hydroxide/propylene glycol (CHP) were prepared in a concentration of 500 mg/ 0,5 mL. Each composite was weighed in a digital analytical balance. The 0,5 mL of propylene glycol were added with the help of a volumetric pippete. Moreover, the manipulation process of each paste was conducted inside a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil).

pH Analysis

For pH analysis, the pastes were manipulated as described and inserted into plastic tubes (n = 10) with 1 mm of diameter and one aperture; they were immediately immersed into 10 mL of ultrapure water and agitated. Sequentially, the pH of the solutions was measured with a previous calibrated (4, 7, and 12) pHmeter (model 371; Micronal, São Paulo, SP, Brazil) at the period of 3, 72 and 168 hours. After each period, the tubes were immersed into new flasks containing newly ultrapure water; pH of the ultrapure water was used as control. The analysis was performed by a blinded experienced operator.

Antimicrobial Activity

Dentin Blocks Preparation

A total of 10 bovine incisors with complete roots were previously selected. A trephine drill (4x4 mm) coupled to a manual motor positioned perpendicular to these roots was used to penetrate from the mesial to the distal portion of the middle and cervical thirds, under abundant irrigation, to obtain 4 blocks of dentin per tooth. Afterwards, the dentin surfaces were polished on a polishing machine (Fortel Indústria

e Comércio Ltda., São Paulo, SP, Brazil). The blocks were then immersed in 1% Sodium Hypochlorite (Rioquímica Ltda., São José do Rio Preto, SP, Brazil) for 15 minutes and then treated with 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil), for 5 minutes to remove any debris resulting from sectioning. Finally, they were placed in Eppendorfs and autoclaved at 121°C.

Biofilm Growth

To reactivate the strain of *Enterococcus faecalis*, 15 µL of the standard strain (American Type Culture Collection [ATCC] 4083) was placed in 3 mL of sterile Brain Heart Infusion (BHI; Oxoid, Basingstoke, UK) at 36°C and bacterial growth was allowed to occur overnight. After this time, the bacterial density was adjusted to 109 cells/mL by means of a spectrophotometer (UV-VISIBLI; Shimadzu, Kyoto, Japan) at an optical density of 1 to 600 nm according to the MacFarland standard of 0.5.

Then the dentin blocks were contaminated. One block of dentin + 100 µL of E. faecalis + 1900 µL of BHI were placed in each well of a 24-well plate and incubated at 37°C aerobically for a period of 21 days for biofilm maturation, with BHI being renewed every 48 hours (15).

Analysis of Antimicrobial Activity against *E. faecalis* Biofilm

After biofilm formation on dentin blocks unbound cells were removed by means of abundant irrigation with distilled water. The pastes were manipulated as previously described and gently putted in direct contact with the biofilm formed on the surface of the blocks where they remained for 7 days. Microbiological procedures were conducted aseptically in a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil) by a blinded experienced operator.

For analysis, under the inverted confocal microscope (Leica, Mannheim, Germany), the dentin disks were washed with 100 µL of PBS (Marca) to remove any medication residues, and then stained with 50 µL of a SYTO8 and propidium iodide (Live and Dead; Marca) solution for 10 minutes. SYTO8 is a fluorescent (green), selective nucleic acid dye, indicated for staining living and dead cells (general dye). In

this case, the function of propidium iodide (red) was to identify the population with damaged cell membrane, or dead cells. Upon penetrating the cell, the red fluorescence overlaps with the fluorescence of SYTO8, revealing red or yellowish dead cells.

In the biofilm in question, 4 points were chosen, in which images at 400x magnification were captured. The parameters evaluated were the total biofilm mass and the percentage of live cells. The images obtained were analyzed by using BioImage software by an experienced and blinded examiner.

Statistical analysis

The data obtained were analyzed for normality by the Shapiro-Wilks test, and non-normal data distribution was observed. The Kruskal-Wallis non-parametric test was used for the overall comparison with the Dunn test as post hoc test. The level of significance was stablished as 5%.

RESULTS

pH Analysis

Table 1 presents the median and standard deviation of the pH evaluated during the experimental periods. The N-Acetylcysteine and Ambroxol Hydrochloride presented pH below 6 in all periods evaluated while calcium hydroxide presented pH higher than 11, There were statistical significant differences between all groups at all analyzed periods.

Antimicrobial activity

Table 2 shows the median, minimum and maximum values of the percentage of live bacterial in the biofilm. Both experimental pastes applied over E. faecalis biofilm surface seems effective with significant difference between the percentages of live cells in comparison with the control ($P < 0,05$); the CHP group was not different from the Control ($P > 0.05$). The best results were offered by Ambroxol Hydrochloride paste

which showed significant difference in comparison with the Calcium Hydroxide paste and the control ($P < 0.05$).

The figure 1 contains the confocal representative image of the biofilms after the contact by 7 days of the pastes with biofilm and the control group.

DISCUSSION

The purpose of using intracanal medications in the treatment of teeth with pulp necrosis is to achieve greater antisepsis of the root canal system (1, 14). The use of calcium hydroxide paste in Endodontics has been established in the practice for decades, due to its biological action (16-19) and because it forms a physical barrier that prevents canal recontamination (14). However, the difficulty of calcium hydroxide penetrating into the dentinal tubules may affect its antimicrobial action (19, 20), as same as some bacterial resistance factors (4, 5).

In relation to the pH, N-Acetylcysteine and Ambroxol Hydrochloride presented acid pH, with values between 2.5 and 3.5 and between 5 and 6, respectively. The pH of the Ambroxol Hydrochloride is next of the neutral. Although the pH of the N-Acetylcysteine is acid and the calcium hydroxide is basic, the antimicrobial action of both substances were lowest than Ambroxol Hydrochloride. This fact suggest that the pH alkaline or acid do not interfere in the antimicrobial action of the biofilm of Enterococcus faecalis. The Ambroxol Hydrochloride has a minor size of the particle and can present an action of dissolution greater and these facts could favor a higher penetration in the biofilm matrix and greater antimicrobial action. When organized in biofilm, microorganisms are capable of producing an extracellular polysaccharide matrix that acts by favoring bacterial viability and development, and also serves as a barrier to the action of drugs (5, 21-23).

This fact was also confirmed in the present study since no statistical difference between the Control group and the group treated with the CHP for 7 days. This was possibly also due to mechanisms that some microorganisms, such as *E. faecalis,* presents when induced to stress, to maintain internal homeostasis, either by passive means such as low membrane permeability and cytoplasm buffering, or by active means such as transport of ions across the membrane (proton pump) (4, 5). *E. faecalis,* a gram-positive anaerobic facultative coccus, is one of the most prevalent and difficult

pathogen to deal with in persistent infections after root canal treatment, due to the factors cited before (4-6).

However, using means of penetrating the lipoprotein membrane, the exopolysaccharide layer and disorganizing the biofilm are necessary. The use of mucolytic agents such as Ambroxol Hydrochloride and N-Acetylcysteine as intracanal dressings was the alternative tested in the present study, trying to find a way to make direct contact with the bacterial cells. The null hypothesis tested was rejected since the use of Ambroxol Hydrochloride and NAC were effective against *E. faecalis* biofilm, with statistical difference between them and the Control group.

N-Acetylcysteine is a drug with properties that favor the decrease in adhesion capacity of bacteria to a substrate (24, 25). It also reduces the production of extracellular polysaccharides and has antimicrobial action (8), including against *E. faecalis* in Endodontics (7, 10, 26). It also could have good use as intracanal dressing, because of its effects as an antioxidating agent, and also modulating the inflammatory process (27), however, until the present no study evaluated it usage as intra-canal dressing. In the same way, Ambroxol Hydrochloride is widely used in medicine for the treatment of pulmonary diseases (11, 28, 29), thus, in Endodontics, the use of this drug could shown innovative and promising results in different stages of endodontic treatment.

Groups treated with Ambroxol Hydrochloride demonstrated satisfactory effects, in agreement with the literature that demonstrated its anti-biofilm action (11, 12). This drug acts on different stages of biofilm formation, such as its aggregation and maturation, which tended to favor the effect of the Calcium Hydroxide against *E. faecalis* (11). The group treated with Ambroxol Hydrochloride offered the best results than the obtained with N-Acetylcysteine, with no statistical difference between them. Continuous growth of the bacteria in contact with calcium hydroxide could be related to the *E. faecalis* resistance to the alkaline medium (6) and its strong adherence to dentin when in the form of biofilm (22, 23, 30). Ambroxol Hydrochloride favored both a higher level of antisepsis and action against the exopolysaccharide layer (28, 29).

Considering the methods applied, this study showed that the use of N-Acetylcysteine and Ambroxol Hydrochloride as intracanal dressings showed effective activity against *E. faecalis* biofilm. The use of the two substances showed better antimicrobial action than the compounds conventionally used as intracanal dressings

and may be a new alternative in the complement of endodontic therapy, principally in cases of persistent infection where the calcium hydroxide is not resolving. But futures studies are necessary, principally to investigate the cytotoxicity of these substances.

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Table 1. Mean and standard evaluation of the pH proportioned by the pastes at the analyzed periods. Different lowercase letters indicate significant differences (P<0.05) between the pastes in each period; Different uppercase letters indicate significant differences (P<0.05) between periods in the same paste.

	3hs	72hs	168hs
N-Acetylcisteine	3.12 +/- 0.10^{c,A}	3.02 +/-0.10^{c,A}	2.88 +/-0.07^{c,B}
Ambroxol Hydrochloride	5.52 +/-0.13^{b,A}	4.75 +/-0.23^{b,B}	5.77 +/-0.13^{b,C}
Calcium hydroxide	11.45 +/-0.2^{a,A}	11.51 +/-0.25^{a,A}	11.61 +/-0.28^{a,A}

Table 2. Median (Med) and Minimum and Maximum (Min-Max) Values of the Percentage of Live Cells after Contact with the Experimental Medicaments for a week. Different lower letters indicate statistically significant difference between the groups (P<0.05)

	CH + P	**AMB + P**	**NAC + P**	**Control**
% Live Cells	81,79 [ac] (23,33 – 99,42)	20,05 [b] (0 – 94,35)	59,74 [ab] (2,76 – 98,19)	93,31 [c] (76,53 – 99,87)

CH + P, Calcium hydroxide + propylene glycol;

AMB + P, Ambroxol hydrochloride + propylene glycol;

NAC + P, N-Acetylcysteine + propylene glycol.

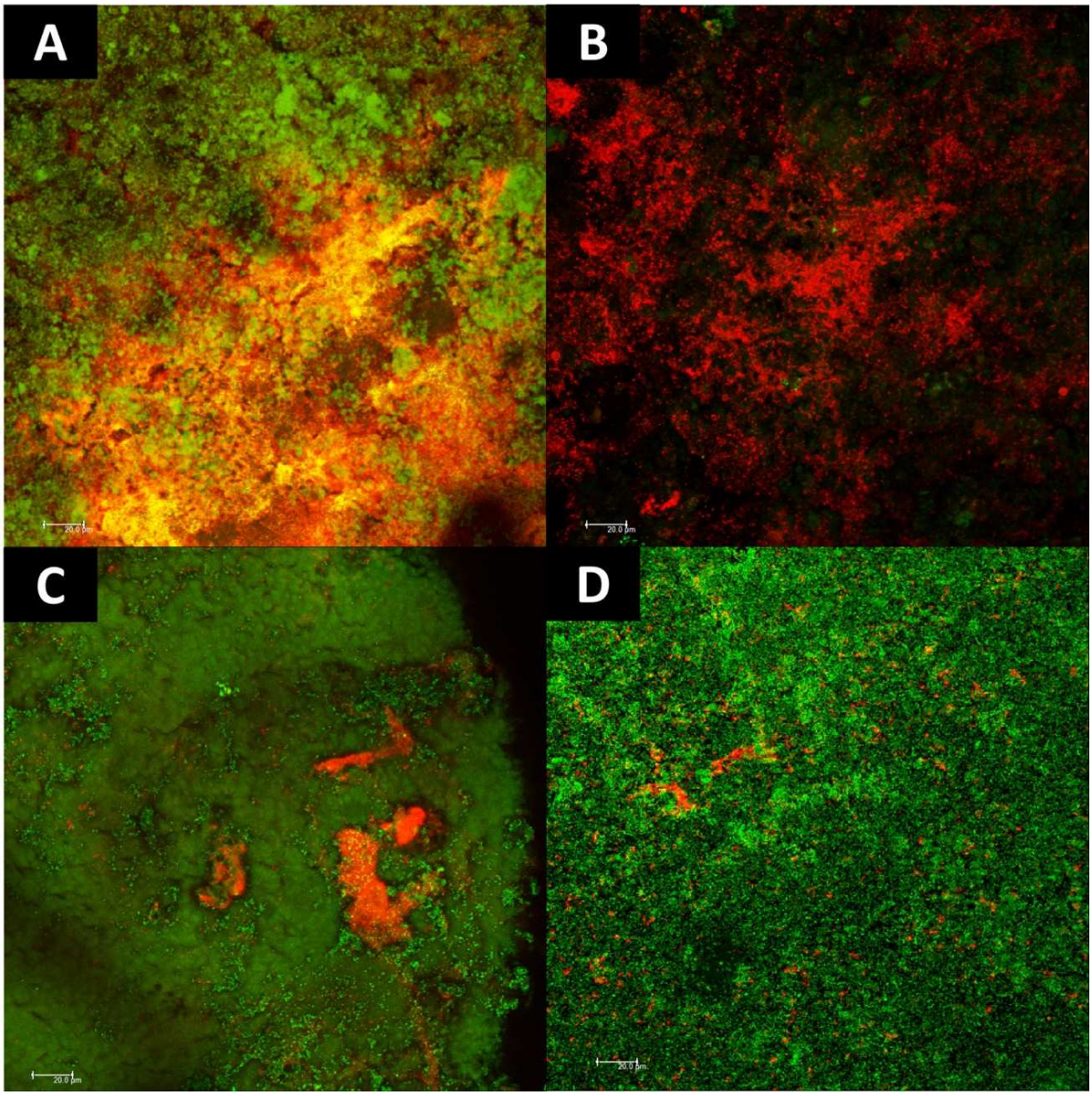

Figure 1 – Representative confocal images of antimicrobial action of the studied pastes: A – N-Acetylcysteine; B – Ambroxol Hydrochoride; C – Calcium Hydroxide; D – Control.

3 DISCUSSION

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The purpose of using intracanal medications in the treatment of teeth with pulp necrosis is to achieve greater antisepsis of the root canal system (Zehnder *et*. al 2006; Hull *et*. al 2018). The use of calcium hydroxide paste in Endodontics has been the established practice for decades, due to its biological action (Siqueira Jr *et*. al 1999; Peters *et*. al 2005; Byström *et*. al 1985) and because it forms a physical barrier that prevents canal recontamination (Hull *et*. al 2018). However, the difficulty of calcium hydroxide penetrating into the dentinal tubules may affect its antimicrobial action (Wang *et*. al 1988; Nerwich *et*. al 1993), as same as some bacterial resistance factors (Zancan *et*. al 2016; Estrela *et*. al 2005).

In relation to the pH, N-Acetylcysteine and Ambroxol Hydrochloride presented acid pH, with values between 2.5 and 3.5 and between 5 and 6, respectively. The pH of the Ambroxol Hydrochloride is next of the neutral. Although the pH of the N-Acetylcysteine is acid and the calcium hydroxide is basic, the antimicrobial action is lowest than Ambroxol Hydrochloride. This fact shows that the acidy pH is not reason of the antimicrobial action. The Ambroxol Hydrochloride has a minor size of the particle and this fact could favor a higher penetration in the biofilm matrix and greater antimicrobial action. When organized in biofilm, microorganisms are capable of producing an extracellular polysaccharide matrix that acts by favoring bacterial viability and development, and also serves as a barrier to the action of drugs (Estrela *et*. al 2005; Chávez de Paz *et*. al 2007; Nakajo *et*. al 2006; Perez-Giraldo *et*. al 1997).

This fact was also confirmed in the present study since no statistical difference between the Control group and the group treated with the CHP for 7 days. This was possibly also due to mechanisms that some microorganisms, such as *E. faecalis,* presents when induced to stress, to maintain internal homeostasis, either by passive means such as low membrane permeability and cytoplasm buffering, or by active means such as transport of ions across the membrane (proton pump) (Zancan *et*. al 2016; Estrela *et*. al 2005). *E. faecalis,* a gram-positive anaerobic facultative coccus, is one of the most prevalent and difficult pathogen to deal with in persistent infections

after root canal treatment, due to the factors cited before (Zancan *et*. al 2016; Estrela *et*. al 2005; Holland *et*. al 2017).

However, using means of penetrating the lipoprotein membrane, the exopolysaccharide layer and disorganizing the biofilm are necessary. The use of mucolytic agents such as Ambroxol Hydrochloride and N-Acetylcysteine as intracanal dressings was the alternative tested in the present study, trying to find a way to make direct contact with the bacterial cells. The null hypothesis tested was rejected since the use of Ambroxol Hydrochloride and NAC were effective against *E. faecalis* biofilm, with statistical difference between them and the Control group.

N-Acetylcysteine is a drug with properties that favor the decrease in adhesion capacity of bacteria to a substrate (Riise *et*. al 2000; Costa *et*. al 2017). It also reduces the production of extracellular polysaccharides and has antimicrobial action (Ordinola-Zapata *et*. al 2013), including against *E. faecalis* in Endodontics (Evans *et*. al 2002; Siqueira *et*. al 2010; Quah *et*. al 2012). It also could have good use as intracanal dressing, because of its effects as an antioxidating agent, and also modulating the inflammatory process (Choi *et*. al 2018), however, until the present no study evaluated it usage as intra-canal dressing. In the same way, Ambroxol Hydrochloride is widely used in medicine for the treatment of pulmonary diseases (Jhajharia *et*. al 2015; Moon *et*. al 2016; Cataldi *et*. al 2014), thus, in Endodontics, the use of this drug could shown innovative and promising results in different stages of endodontic treatment.

Groups treated with Ambroxol Hydrochloride demonstrated satisfactory effects, in agreement with the literature that demonstrated its anti-biofilm action (Jhajharia *et*. al 2015; Toker *et*. al 2009). This drug acts on different stages of biofilm formation, such as its aggregation and maturation, which tended to favor the effect of the Calcium Hydroxide against *E. faecalis* (Jhajharia *et*. al 2015). The group treated with Ambroxol Hydrochloride offered the best results than the obtained with N-Acetylcysteine, with no statistical difference between them. Continuous growth of the bacteria in contact with calcium hydroxide could be related to the *E. faecalis* resistance to the alkaline medium (Holland *et*. al 2017) and its strong adherence to dentin when in the form of biofilm (Nakajo *et*. al 2006; Perez-Giraldo *et*. al 1997; Cheng *et*. al 2015). Ambroxol Hydrochloride favored both a higher level of antisepsis and action against the exopolysaccharide layer (Moon *et*. al 2016; Cataldi *et*. al 2014).

Considering the methods applied, this study showed that the use of N-Acetylcysteine and Ambroxol Hydrochloride as intracanal dressings showed effective activity against *E. faecalis* biofilm. The use of the two substances showed better antimicrobial action than the compounds conventionally used as intracanal dressings and may be a new alternative in the complement of endodontic therapy.

4 CONCLUSIONS

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The N-Acetylcysteine and Ambroxol Hydrochloride pastes presented acid pHs which, against E. faecalis biofilm, presented antimicrobial activity, with Ambroxol Hydrochloride being more effective. The use of both substances should be further studied in order to suggest its use on the endodontic therapy.

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ANNEX

DECLARAÇÃO DE USO EXCLUSIVO DE ARTIGO EM DISSERTAÇÃO

Declaramos estar cientes de que o trabalho **Analysis of pH and antimicrobial activity of Ambroxol Hydrochloride and N-Acetylcysteine pastes to be used as root canal medication** será apresentado na dissertação do aluno Pedro Henrique Souza Calefi e que não foi e nem será utilizado em outra dissertação/tese dos Programas de Pós-graduação da FOB-USP.

Bauru, 03 de Abril de 2020.

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