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ORIGINAL ARTICLE

Do Different Proportions of Antibiotics in the CTZ Paste Interfere with the Antimicrobial Action? In Vitro Study

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Abstract

Objective: To evaluate the antimicrobial action of the CTZ paste in three different proportions by diffusion in agar with the microorganisms: *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. **Material and Methods:** Three different proportions of antibiotics were tested: GROUP A - CTZ paste in the ratio of 33.33% chloramphenicol + 33.33% tetracycline + 33.33% zinc oxide, mixed with 2 drops of eugenol (1:1:1 ratio); GROUP B - CTZ paste in the proportion of 25% chloramphenicol + 25% tetracycline + 50% zinc oxide, mixed with 2 drops of eugenol (1: 1: 2 ratio); GROUP C - CTZ paste with 13% chloramphenicol + 13% tetracycline + 74% Zinc Oxide, mixed with 2 drops of eugenol (1:1:6 ratio); PC GROUP - Positive Control (0.12% Chlorhexidine); and NC GROUP - Negative Control (0.9% Saline solution). Data were analyzed through descriptive statistics (means and standard deviation). The one-way ANOVA and Tukey's test were used, with a significance level of 5%. **Results:** No statistical differences for *Enterococcus faecalis* between groups A, B, and C ($p = 0.1986$) were found. There were statistical differences for *Escherichia coli* between groups B and C ($p = 0.029$), and for *Candida albicans* between groups A and C ($p = 0.006$). Groups A, B, and C had significant differences with both Positive and Negative Controls for all the microorganisms. **Conclusion:** The three different ratios of CTZ paste showed antimicrobial efficacy against *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* microorganisms.

Keywords: Tooth, Deciduous; Endodontics; Pulpectomy; Anti-Bacterial Agents.

Introduction

The endodontic technique using a paste based on chloramphenicol, tetracycline, zinc oxide, and eugenol, referred to as CTZ, was introduced in the pediatric dentistry practice by Cappiello in 1964 [1]. It is an alternative for the endodontic treatment of deciduous teeth, whose technique is simplified and easy to perform, dispensing with the instrumentation of root canal systems, in addition to the possibility of being used in public health [1,2]. The success of the paste is due to its composition, since chloramphenicol and tetracycline are broad-spectrum antibiotics, and zinc oxide and eugenol have antimicrobial action [3].

The root canal system of the deciduous teeth has severe curvatures and many accessory canals. In addition, the deciduous teeth undergo rhizolysis in different directions and proportions [4]. Thus, instrumentation for the elimination of microorganisms is hampered. The use of antimicrobial pulps represents one of the most important aspects of treatment success [3].

In vitro studies have shown that the CTZ paste has higher antimicrobial activity compared to the Guedes-Pinto paste [3,5], zinc oxide and eugenol, calcium hydroxide paste and Vitapex® [5], Calen®, L&C®, zinc oxide, and eugenol and MTA [3]. In clinical practice, the use of CTZ paste in necrotic primary molars showed promising clinical and radiographic results [6]. In most of the studies, CTZ is used in a 1:1:2 ratio, 25% of chloramphenicol, 25% of tetracycline and 50% of zinc oxide, mixed with Eugenol [1,5-12].

Although the American Academy of Pediatric Dentistry (AAPD) [13] reports the increased prevalence of antibiotic-resistant microorganisms, no studies have been found in the literature evaluating the antimicrobial capacity of the CTZ paste with a decrease in the proportion of antibiotic. Thus, this work evaluated the in vitro antimicrobial action of the CTZ paste in three different proportions.

Material and Methods

Study Design

An *in vitro* study was carried out to evaluate the antimicrobial action of the CTZ paste in three different proportions by means of agar diffusion. Three microorganisms were used: *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. All these strains were isolated, identified, cultivated, and maintained viable in the laboratory of the São Leopoldo Mandic Research Center, Campinas, SP, Brazil.

The groups tested were: Group A - CTZ paste in the proportion of 33.33% chloramphenicol + 33.33% tetracycline + 33.33% zinc oxide, mixed with two drops of eugenol (1:1:1 ratio); Group B - CTZ paste in the proportion of 25% chloramphenicol + 25% tetracycline + 50% zinc oxide, mixed with 2 drops of eugenol (1:1:2 ratio); Group C - CTZ paste with 13% chloramphenicol + 13% tetracycline + 74% zinc oxide, mixed with 2 drops of eugenol (1:1:6 ratio); PC Group (Positive Control) - 0.12% Chlorhexidine Gluconate; and NC Group (Negative Control) - 0.9% Sterile saline solution (Table 1). The CTZ capsules were manipulated at the Fórmula & Ação Laboratory (São

Paulo, SP, Brazil), in the amount of nine 200 mg capsules for group. All the manipulated capsules were mixed with 2 drops of Eugenol (Biodinâmica Química e Farmacêutica Ltda, Ibiporã, PR, Brazil).

Table 1. CTZ composition of groups A, B, and C, and substances used as positive (PC) and negative controls (NC).

Groups	Chloramphenicol	Tetracycline	Zinc Oxide	Eugenol	Ratio
A	33.33%	33.33%	33.33%	2 drops	1 : 1 : 1
B	25%	25%	50%	2 drops	1 : 1 : 2
C	13%	13%	74%	2 drops	1 : 1 : 6
PC	0.12% Chlorhexidine Gluconate				
NC	0.9% Saline Solution				

Agar Preparation

Nine Petri dishes were used, six of them containing 20 ml of the Brain Heart Infusion Agar medium (BHIA; Difco Laboratories, Detroit, MI, USA, lot 0000237071) for *E. faecalis* and *E. coli*, and the three remaining ones with the Sabouraud Dextrose Agar culture medium (Neogen Corporation, Lansing, MI, USA, lot 107876B) for *C. albicans*. After being autoclaved (at 121°C with 1 ATM during 20 minutes), the plates were cooled, with the culture medium presenting 5mm thickness. The microbial mixture was incubated for 24 hours at 37°C.

After this period, the cells were suspended in sterile saline solution (0.9%) (Dinâmica Química Contemporânea Ltda., Diadema, SP, Brazil, lot 68837). The suspension of each microbial strain was adjusted to match the tube of 1 McFarland turbidity standard. In the preparation of the microbial mixture, an inoculum of 200 µl of *Enterococcus faecalis*, of 400 µl of *Escherichia coli* and of 300 µl of *Candida albicans* was removed from each suspension and transferred to each sterile test tube, obtaining the test mixtures, which were used immediately after preparation. The plates were inoculated with the microbial suspension using disposable and sterile swabs (Absorve, CRAL Artigos para Laboratório LTDA, São Paulo, SP, Brazil). Three wells of 5 mm in diameter and 5 mm deep were used to insert the CTZ of groups A, B and C and an absorbent paper disc of the same diameter for the positive and negative controls in each Petri dish (Prolab, José dos Pinhais, PR, Brazil).

The capsules, previously encoded per group, were shaken and opened. For manipulation, the powder was dispensed into a sterile glass plate, and then two drops of Eugenol were added, with the glass in a vertical position. They were spatulated with a no. 24 spatula (SSWhite Duflex, Juiz de Fora, MG, Brazil) sterile, in the consistency of toothpaste for the same operator.

Soon after the paste was manipulated, it was placed in one of the three 5mm diameter wells of each Petri dish. After the wells were completely filled with the CTZ paste and the control groups, the plates were transferred to a bacteriological oven and kept in the incubator at 37 °C for 24 hours.

Evaluation of Samples

Measurements of the diameters of the zones of inhibition of microbial growth were performed using a millimeter pachymeter (Mitutoyo Sul Americana Ltda., Suzano, SP, Brazil). The

positive control was verified with the use of 0.12% chlorhexidine gluconate substance (Periogard, Colgate-Palmolive Indústria Ltda, São Bernardo do Campo, SP, Brazil) and the negative control with 0.9% sterile saline solution. During all phases of the experiment, the aseptic technique was observed, and the tests were conducted by the same operator. The evaluator was blind.

Data Analysis

For the analysis of the data, means and standard deviation of the inhibition zone size were used. One-way ANOVA and Tukey test were used with a significance level of 5%. IBM SPSS Statistics for Windows Software, version 25 (IBM, Armonk, NY, USA).

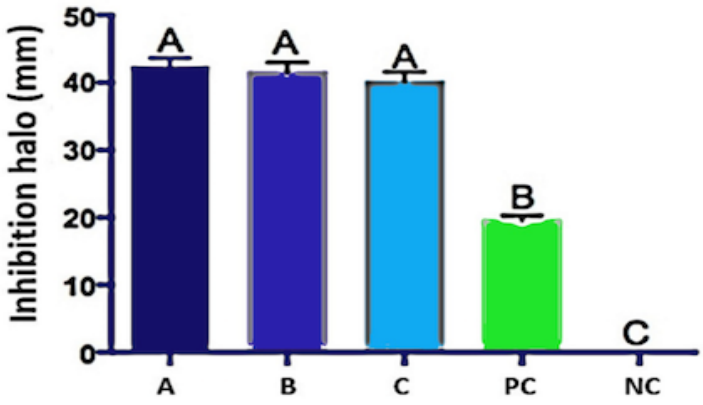
Results

The results of the halo diameters, in millimeters, after 24 hours of inhibition of bacterial growth induced by different concentrations of the CTZ paste in different proportions, that is, Group A (1: 1: 1), Group B (1: 1: 2), Group C (1: 1: 6), PC Group: 0.12% Chlorhexidine gluconate; NC Group: 0.9% Saline solution, are summarized in Table 2 and Figures 1 to 3, according to the microorganisms *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans*, respectively.

Table 2. Measurement of the inhibition halo (mm) in groups.

Groups	*E. faecalis* Mean (SD)	*E. coli* Mean (SD)	*C. albicans* Mean (SD)
A	42.41 ± 1.17	40.16 ± 1.38	32.24 ± 1.12
B	41.55 ± 1.43	41.03 ± 0.41	29.94 ± 0.88
C	40.19 ± 1.36	38.38 ± 0.59	28.61 ± 0.49
PC	19.61 ± 0.64	20.42 ± 0.26	7.62 ± 2.19
NC	0	0	0

*Groups A = CTZ 1:1:1 ratio; B= CTZ 1:1:2 ratio, C= CTZ 1:1:6 ratio, PC = 0.12% Chlorhexidine gluconate; NC = 0.9% saline solution.

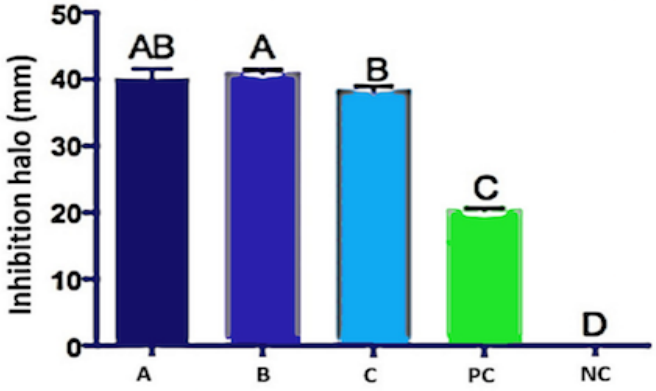


*Different letters indicate statistical differences between groups (p<0.05).

Figure 1. Mean and standard deviation of the inhibition halos of the *Enterococcus faecalis* samples.

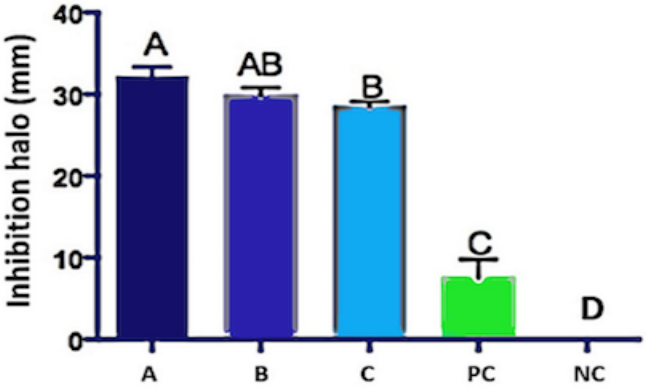
The one-way analysis of variance (ANOVA) showed a statistical difference (p<0.05) in the groups tested regarding the size of the inhibition halo. The Tukey test showed that for the *Enterococcus faecalis* bacterium there was no statistical difference between groups A (42.41 ± 1.17), B

(41.55 ± 1.43) and C (40.19 ± 1.36) ($p<0.05$) (Figure 1). For all microorganisms, there were statistical differences between all groups tested and the PC and NC, presenting a greater inhibition halo (Figures 1, 2 and 3).



*Different letters indicate statistical differences between groups ($p<0.05$).

Figure 2. Mean and standard deviation of the inhibition halos of the *Escherichia coli* samples.



*Different letters indicate statistical differences between groups ($p<0.05$).

Figure 3. Mean and standard deviation of the inhibition halos of the *Candida albicans* samples.

Discussion

The root canal system of necrotic primary teeth has a polymicrobial nature, which is similar to that of permanent teeth, with a predominance of anaerobic microorganisms [14-17]. The present study tested Gram-negative (*E. coli*), Gram-positive (*E. faecallis*), both anaerobic, and fungus *C. albicans.*

The antimicrobial activity of the root canal system of the deciduous tooth with pulp necrosis is a characteristic of fundamental importance for the success of endodontic treatment [5,18]. Some studies reported a greater success of the CTZ paste in relation to the antimicrobial activity when compared to the other materials tested [3,5], proving to be an appropriate methodology to measure the antimicrobial potential of endodontic materials. However, there are no studies in the literature that have compared different proportions of antibiotics in the CTZ paste, showing that this is an

unprecedented result, which makes it impossible to make more specific comparisons with our study. Despite this, all the proportions of the CTZ paste showed antimicrobial efficacy against the *E. coli*, *E. faecalli*, and *C. albicans* microorganisms.

For *Enterococcus faecalis*, the three paste proportions did not present significant differences, for *Escherichia coli*. There was no significant difference between Groups A and C and for *Candida albicans*. There was no significant difference between Groups B and C. All paste proportions presented an inhibition halo, which may suggest efficacy in the antimicrobial action. The mixture of zinc oxide and Eugenol has neutral pH, so it does not interfere with the action of the antibiotics tetracycline and chloramphenicol present in the CTZ paste. Group C, even with reduced amounts of antibiotics, showed sufficient and effective antimicrobial properties.

It is essential to know the pathogenic microbiota present in the root canal system to develop an effective endodontic therapy strategy. *Enterococcus faecalis* is found to be highly prevalent in root canals of endodontically treated teeth with persistent infection or secondary infections. It plays a crucial role in endodontic treatment failure due to its resistance and its ability to survive treatment. In addition, it can colonize areas inaccessible to disinfection mechanisms [19,20]. There was no significant difference between the groups A, B, and the experimental paste (Group C) in relation to the size of the inhibition halo against *E. faecalis*.

The rational use of antibiotics can be defined as the practice of prescription, which results in optimal indication, dosage, route of administration, and duration of a therapeutic or prophylactic regimen. It provides clinical success with minimal toxicity and reduces the impact on microbial resistance [21]. The indiscriminate use of antibiotics is considered by the World Health Organization (WHO) to be a global problem and, as a consequence, has led to the growth of bacterial resistance [22] and the emergence of superbugs [23]. The evaluation of the CTZ paste with a reduced concentration of chloramphenicol and tetracycline (Group C) aimed to promote the rational use of medications, justified by the fact that children's organs and tissues are developing and are more susceptible to the adverse effects of medication. And the response to medications is conditioned by factors such as age, size, body weight, stage of development, nutritional status, concomitant administration of other drugs, time of administration and pre-existing disease [24].

The results obtained *in vitro* by the experimental paste (Group C), with a reduction of the concentration of chloramphenicol and tetracycline, suggest that the dose of antibiotic of CTZ paste proposed by Cappiello in 1964 [1], is greater than that required for the endodontic treatment of bacteria present in the root canal system of deciduous teeth.

One of the issues related to the endodontic treatment of deciduous teeth is a color change. Thus, the disadvantage of the CTZ paste is the pigmentation of the dental crown caused by its components [8]. This fact generates resistance of pediatric dentists in the use of the CTZ. As the CTZ paste used in the experimental group (Group C) has a lower concentration of tetracycline, the main cause of color change, one possibility is that there is less color change. However, studies that evaluate this in the new proportion need to be performed.

The lack of scientific evidence on the biocompatibility of the CTZ paste and its influence on tooth enamel formation of the permanent successor germ has made its use not recommended in some countries. Some authors [25] observed different staining in 11.7% of the permanent successors of primary teeth treated with CTZ paste at 1:1:1 concentration (Group A). Due to the fact that staining was also observed in permanent teeth whose primary teeth had been treated with Formocresol, they suggested that this color change might be related to local periapical inflammation rather than the components of the formulas. Depending on the degree and severity of infection of the deciduous tooth, the permanent successor may exhibit structural abnormalities with alteration in enamel matrix formation (hypoplasia) or a deficiency in mineralization, altering enamel color (hypocalcification) [26].

In vitro results are very interesting and promising, and it is important to evaluate other properties, such as clinical efficacy and color change of tooth crown treated with CTZ paste in the experimental ratio of chloramphenicol and tetracycline.

Conclusion

The lower proportion of antibiotics in the CTZ paste did not interfere with the antimicrobial activity. The three different ratios of CTZ paste showed antimicrobial efficacy against *E. coli*, *E. faecallis*, and *C. albicans*.

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