

Antimicrobial Effect and Transdental Diffusion of New Intracanal Formulations Containing Nitrofurantoin or Doxycycline

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The aim of this study was to investigate *in vitro* the antimicrobial effect and diffusion against *E. faecalis* of new intracanal medications on the external root surface. The medications tested were a placebo gel (PC); the new formulations with either 3% nitrofurantoin (NIT) or 3% doxycycline hydrochloride (DX) and 2% chlorhexidine (CHX) gel as positive control. The new formulations were tested using the traditional agar diffusion test (ADT) and an adapted agar diffusion method (AADM), where the teeth were filled with the medications and left to diffuse on agar surface seeded with *E. faecalis*. In the ADT, the larger zones of microbial growth inhibition were seen in DX, followed by CHX and NIT. In the AADM test only DX and CHX showed antimicrobial effect. Statistically significant differences between groups were observed by the Kruskal-Wallis test ($\chi^2=47.126$; $p<0.001$). The new intracanal formulations with DX and NIT have demonstrated antimicrobial effect against *E. faecalis*, but only DX was able to diffuse through the dentinal tubules and exert antimicrobial effect outside the roots.

Key Words: Endodontics, *enterococcus faecalis*, doxycycline, nitrofurantoin.

Introduction

Endodontic infections are essentially polymicrobial and in most cases the microorganisms grow in the root canal system as sessile biofilms. A variety of bacterial species may be isolated from primary periapical infections. In the secondary infections, one of the most often isolated species is *Enterococcus faecalis*, which is seen as an opportunistic pathogen and a natural inhabitant of the oral cavity that survives as a mono-infection (1).

Despite the action of the chemomechanical preparation with antimicrobial irrigating solutions, some microorganisms, including *E. faecalis*, may survive in the root canal system. The use of an intracanal dressing that could act through the root canal system would certainly improve the outcome of the endodontic treatment (2).

The intracanal medication should have a broad antimicrobial spectrum, should be able to diffuse through the dentinal tubules and all ramifications of the root canal system including the areas that neither the instruments nor the irrigating solution can reach, and should not be cytotoxic to live tissues (3). Several substances have been tested and improved in order to achieve the ideal results. Calcium hydroxide (Ca(OH)₂) is believed to have most of the properties of an ideal root canal dressing due to its high pH. However, it is not equally effective against all species of microorganism found in the root canal system (4).

Chlorhexidine gluconate has been used in endodontics as an irrigant. Recently, due to its antimicrobial effect

and adsorption to dental hard tissues, it has also been suggested as an intracanal medication (5,6). Authors have demonstrated that chlorhexidine is more effective than Ca(OH)₂ when used against *E. faecalis* (5), and that the use of intracanal medication with chlorhexidine alone or associated with calcium hydroxide reduces the microbial factors responsible for pain and inflammation after endodontic treatment (7).

Antibiotics have been used in endodontics and for dental traumas systemically and locally. Although local application of antibiotics could cause side effects such as discoloration (8), this may be a more effective way for delivering the antibiotics (9) and reducing the bacterial load inside the root canal system, since the antibiotic agents increase significantly the antibacterial properties of endodontic materials (10). This effectiveness is due to the fact that it does not depend on circulation to reach the infected sites with compromised vasculature (11).

Recently, an alternative root canal irrigant – MTAD, a mixture of 3% doxycycline, 4,25% citric acid and 0,5% polysorbate 80 detergent (Tween 80) – was introduced as a final irrigant in the root canal preparation. Several studies concluded that it is a promising irrigating solution due to its biocompatibility (12) and clinical effectiveness against *E. faecalis* (13–15) and other microorganisms (16).

However, regarding the antimicrobial component of MTAD (doxycycline), some authors claim that nitrofurantoin, a synthetic nitrofurantoin commonly used in

urinary tract infections where *E. faecalis* is usually involved (17,18), has demonstrated better efficacy than doxycycline against the same bacteria (18).

The purpose of this experiment was to create an intracanal medication based on the formulation of MTAD. The better efficacy of nitrofurantoin to treat some *E. faecalis* infections when compared with doxycycline led the authors to choose it for the new formulation. The purpose of this study was to compare the antimicrobial effect of each new formulation against *E. faecalis* and its ability to diffuse through dentin.

Material and Methods

The antibacterial effect of the new formulations against *E. faecalis* was determined by an agar diffusion test (ADT) and an adapted agar diffusion method (AADM) through the external surface of the roots.

Agar Diffusion Test (ADT)

The methodology for the ADT was adapted from Vianna and Gomes (19). A 24-h culture of *E. faecalis* (ATCC 29203) in BHI agar was used to prepare a bacterial suspension containing 10^8 viable bacterial cells/mL. The suspension was standardized at 0.5 in the spectrophotometer and McFarland scale. A sterile swab was used to place *E. faecalis* suspension on Muller Hinton agar plates. After drying for 10 min at 36 °C, a sterile glass tube was used to create wells (4 mm diameter x 4 mm depth) in the agar. The wells were filled with the substances described below:

- Placebo gel (PC) (50% propylene glycol; 0.5% polysorbate 80; 0.76% anhydrous monobasic sodium phosphate (NaH_2PO_4); 1.94% anhydrous disodium phosphate (Na_2HPO_4), and 41.8 g of 2% hydroxyethylcellulose gel)
- 2% chlorhexidine (CHX) 10 g of 20% chlorhexidine digluconate; 50% propylene glycol; 0.5% polysorbate 80, and 39.5 g of 2% hydroxyethylcellulose gel)
- 3% nitrofurantoin (NIT) (3g of nitrofurantoin; 50% propylene glycol; 0.5% polysorbate 80; 0.76% NaH_2PO_4 ; 1.94% Na_2HPO_4 , and 41.8 g of 2% hydroxyethylcellulose gel)
- 3% doxycycline (DX) (3.33g of doxycycline hydrochloride; 0.1 g of sodium metabisulfite; 50% propylene glycol; 0.5% polysorbate 80; 0.76% NaH_2PO_4 ; 1.94% Na_2HPO_4 , and 43.37 g of 2% hydroxyethylcellulose gel)

After incubation at 36 °C for 24 h, the zones of microbial growth inhibition were measured and recorded. The inhibitory zone was considered the smaller distance between the outer margin of the well and the beginning of the microbial growth. Means and standard deviations were used to compute the differences in efficacy of the tested substances.

Adapted Agar Diffusion Method (AADM)

The methodology used for AADM was a modification

of the one previously described by Gomes et al. 2003 and 2009. Fifty extracted human canine teeth were selected and the single root canal was confirmed radiographically. Curettes were used to remove soft tissues and debris from tooth surfaces and if any kind of anomaly, such as a fracture were detected, the tooth was excluded from the study. Teeth crowns were removed 3 mm above the cemento-enamel junction and the roots were standardized to a length of 15 mm with a water-cooled diamond saw. A size 10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) was introduced into each canal until it could be seen at the apical foramen. The working length was established subtracting 1 mm from this measurement (14 mm). In order to preserve patency, the same K-file was used to recapitulate the canal 1 mm beyond its length between each file. The anatomic diameter of apical foramen was sized using manual K-files, beginning with size 10 files, inserted until the working length was reached. File sizes were progressively increased until an instrument that bound at the working length was obtained. The size of the obtained instrument was recorded for each root. The roots were enlarged with K-files three numbers up to apical file size determined at the working length

The teeth were instrumented using the crown-down principles, combining Gates Glidden drills (Dentsply/Maillefer) with K-files. The cervical two-thirds (10 mm) of the canals were prepared using K-files 25 to 35, followed by a size 2 Gates Glidden bur and then by a size 3, 1 mm shorter. The apical stop was established using files three numbers up to the anatomic diameter file. At each file change the root canals were irrigated with 1 mL of 5,25% NaOCl. After mechanical preparation, the teeth were rinsed with 5 mL of 17% ethylenediamine tetra-acetic acid (EDTA) solution for 3 min, to remove the smear layer, followed by 1 mL of 5,25% NaOCl and finally with 5mL of saline.

The teeth were sterilized in individual tubes containing 5 mL of Brain Heart Infusion Broth (BHI) and the process confirmed by overnight incubation of the specimens at 36 °C. Then, they were divided in 5 groups of 10 teeth each, according to the substance used as intracanal dressing (20), which were the same described in the agar diffusion test (ADT): a) Teeth sealed without medication (WM - negative control); b) CHX - positive control, c) PC; d) NIT and e) DX.

Immediately after filling the root canals, the foramen and the pulp chamber were sealed with wax. The Muller Hinton agar plates were first seeded by a swab with the *E. faecalis* 10^8 cells/mL suspension and the teeth were then placed inside. The plates were kept for 2 h at room temperature to allow the diffusion of the agents through the dentinal tubules and the agar, and then incubated at 36 °C for 48 h. After this period the zones of microbial growth inhibition were measured, considering the shortest distance

from the outer margin of the tooth to the initial growth.

The non-parametric Kruskal-Wallis test was used to verify any significant difference between groups. In the post-hoc procedures the Tamhane test was used for multiple group comparisons, allowing the assumption that variances are not equal and adjustment for type I errors.

Results

Agar Diffusion Test (ADT)

Figure 1 and Table 1 show that, with the exception of the PC, all tested formulations had potential to eliminate *E. faecalis*. DX was the one that exhibited the largest inhibition zone followed by NIT and CHX.

Adapted Agar Diffusion Method (AADM)

Figure 2 shows the area of microbial inhibition of the different intracanal medications through the external

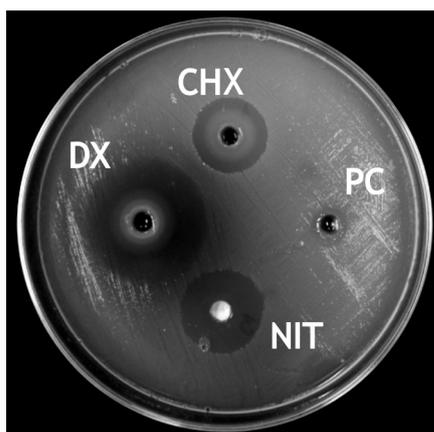


Figure 1. Zones of microbial growth inhibition (mm) in ADT of the intracanal dressings against *E. faecalis* (CHX: 2% chlorhexidine; PC: Placebo; NIT: 3% nitrofurantoin and DX: 3% doxycycline).

root surface against *E. faecalis*. Only CHX and DX showed inhibition zones, measuring 11.17 (± 2.17) mm and 5.52 (± 2.9) mm respectively, while PC and NIT did not inhibit *E. faecalis* growth as well as the negative control (WM). The Kruskal-Wallis test ($\chi^2=47.126$; $p<0.001$) revealed statistical differences between the groups (Fig. 3). In a multiple comparisons *post-hoc* procedure it was possible to observe statistically significant differences between CHX and DX and the other groups.

Discussion

In both tests (ADT and AADM) DX presented the largest zones of microbial growth inhibition, demonstrating a better antibacterial effect and/or a better diffusion ability compared with NIT and CHX. NIT was not able to exert any antimicrobial action on the external root surface (AADM) during the incubation period. With the methodology used in this study, when the mean area of microbial inhibition is equal to zero, it means either the medication did not present any antibacterial effect or the substances were not able to diffuse through the agar or through the dentinal tubules. When ADT was applied, DX, NIT and CHX were effective in inhibiting *E. faecalis* growth, as also shown

Table 1. Mean and standard deviation of inhibition zone (mm) of *E. faecalis* growth produced by the intracanal medication used in the experimental groups

Intracanal medication	Inhibition zones (mm)
2% chlorhexidine	20.70 (± 0.30)
Placebo gel	0 (± 0.0)
3% nitrofurantoin	24.33 (± 0.10)
3% doxycycline hydrochloride	36.27 (± 0.24)

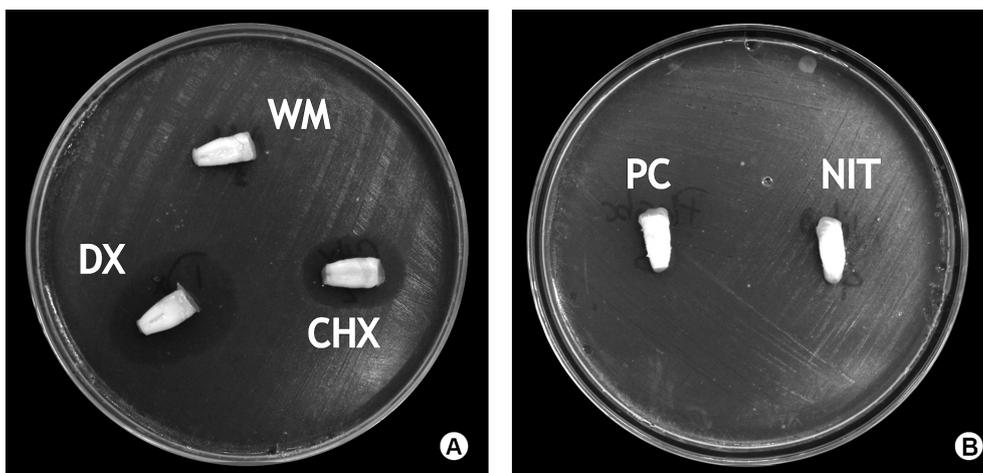


Figure 2. Zones of microbial growth inhibition (mm) formed by the studied intracanal medications against *E. faecalis* after diffusion through dentinal tubules. a) WM - without medication; CLX: 2% chlorhexidine and DX: 3% doxycycline; b) PC: placebo and NIT: 3% nitrofurantoin.

by other authors (17).

Although the size of the inhibition zones would indicate activity against the microorganisms, their effectiveness depends on many factors, like the molecular size, solubility and diffusion of the materials through the agar medium, sensitivity of the drug, bacterial source, the number of inoculated bacteria, pH of the substrate in plates, agar viscosity, storage conditions of the agar plates, time of incubation and the metabolic activity against the microorganisms (4,21).

In the AADM test the cementum layer could affect the diffusion of substances to the agar. However, some authors observed that presence or absence of cementum layer does not interfere significantly in the inhibition zones (20).

The NIT formulation presented a higher viscosity than DX. Although it demonstrated antimicrobial effect against *E. faecalis* in the ADT, this factor could have prevented it from reaching the external root surface, explaining the lack of diffusion observed in AADM. Therefore, it was understood that the methods applied in the present study could be used to analyze and verify the existence of antibacterial activity and diffusion ability through dentinal tubules, but further studies are required.

The detergent used in intracanal formulations, Tween 80, reduces the surface tension allowing an increased wetting effect, and consequently a better penetration of

intracanal medication into dentinal tubules and irregular spaces with difficult access (22). Since NIT presented still a high viscosity, the authors believe that it may be beneficial to increase the detergent weight percentage in the medication to observe better antimicrobial results outside the roots.

Since the low pH of MTAD induces dentin demineralization (23), in this study a phosphate buffer was used to induce a neutral pH, with the goal of keeping the new formulations inside the root canals for longer periods.

The main purpose of this study was to create an intracanal medication based on MTAD with good activity against *E. faecalis* (13,14), respecting the requirements of an ideal medication.

Chlorhexidine 2% is used as the gold standard for in-house assay due to its broad spectrum and effectiveness against *E. faecalis* (24). For the CHX group, concentration of 2% was chosen because its effectiveness is well established. In the new formulations containing DX or NIT, concentration of 3% was used since they were based on MTAD solution.

The use of antibiotics in irrigant solutions made it possible creating new formulations of intracanal dressings using these products.

In the present study, the formulation with DX was the most effective against *E. faecalis*, showing larger inhibition zones than NIT. Nitrofurantoin is effective against many

Gram positive and negative bacteria, including *E. faecalis* (17,18) and doxycycline is a tetracycline broad-spectrum antibiotic, effective against a wide range of microorganisms (22). In some studies, DX showed low activity against most strains of *E. faecalis* (25). These results could also be explained by the use of a strain more susceptible to doxycycline than nitrofurantoin and this characteristic deserves further investigation.

Despite the limitations of this study, such as the fact that *in vitro* models present different conditions from *in vivo* infections and the fact that a mono-infection assay (*E. faecalis*) was used to test the intracanal medication, the results are promising and indicate the efficacy of a new intracanal formulation proposed by the authors. Further studies are required to corroborate the present findings and expand the efficacy of these formulations.

The findings of this study suggest that both the new formulations with DX or NIT presented antimicrobial effect against *E. faecalis*. However, only DX was able to reach the dentinal tubules. These new formulations may be promising as intracanal dressings, increasing the outcome of

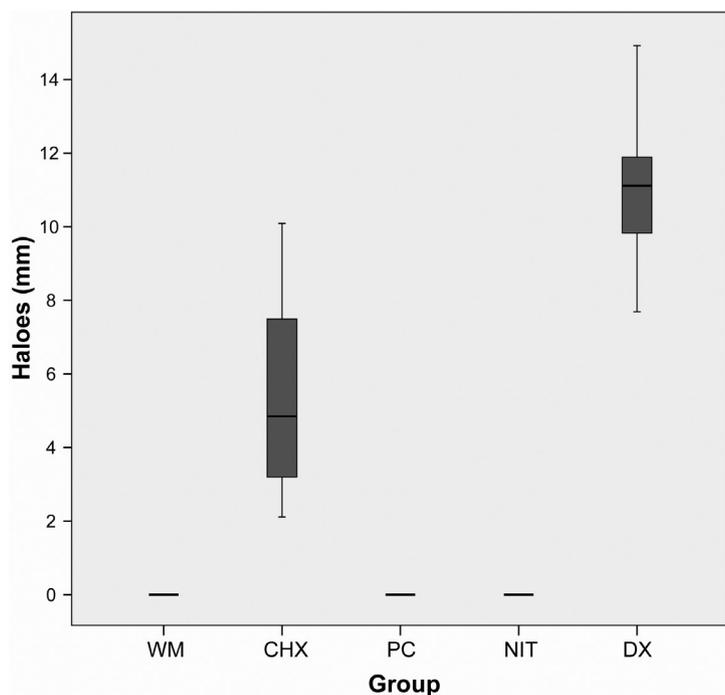


Figure 3. Box-plot graphic of the zones of microbial growth inhibition (mm) formed by the different intracanal medications after 24 h of diffusion through canal dental system. Kruskal-Wallis test ($\chi^2=47.126$; $p<0.001$). WM: without medication; CLX: 2% chlorhexidine; PC: Placebo; NIT: 3% nitrofurantoin and DX: 3% doxycycline.

the endodontic treatment in persistent infections

Resumo

O objetivo deste estudo foi investigar, *in vitro*, o efeito antibacteriano e a difusão frente ao *E. faecalis*, de novas medicações intracanal na superfície externa da raiz. As medicações testadas foram um gel placebo (PC), as novas formulações quer com nitrofurantoina a 3% (NIT) ou hidrócloridrato de doxiciclina a 3%(DX) e um gel de clorexidina a 2% (CHX) como controle positivo. As novas formulações foram testadas usando o tradicional teste de difusão em agar (ADT) e um método de difusão adaptado (AADM), onde os dentes foram preenchidos com as medicações e deixados a difundir numa superfície de agar semeada com *E. faecalis*. No ADT, a maior área de inibição foi registrada para DX, seguida por CHX e NIT. No teste AADM, apenas DX e CHX demonstraram ação antimicrobiana. Foram observadas diferenças significativas entre os grupos através do teste Kruskal-Wallis ($\chi^2=47.126$; $p<0.001$). As novas formulações intracanaís contendo DX e NIT demonstraram ação antimicrobiana quando em contacto com *E. faecalis*, mas apenas DX teve capacidade de difundir através dos túbulos dentinários e exercer ação antimicrobiana fora das raízes.

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