Antibacterial efficacy of several intracanal medicaments for endodontic therapy

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The antibacterial efficacy of intracanal medicaments for 2 and 7 days, in open apex root canals contaminated with *Enterococcus faecalis* biofilms was compared. One hundred and thirty-eight standardized uniradicular human roots were inoculated with *E. faecalis*. Colony forming units (CFU) were recorded before and after medication. Samples were divided in two (2/7 days), and subdivided ($n=8$ /group): triple antibiotic paste (TAP); double antibiotic paste (DAP); dental base paste (DBP), consisting of calcium hydroxide, propylene glycol and calcium carbonate; DBP+0.2% chlorhexidine (CHX); DBP+0.2% cetrimide (CTR); DBP+0.2% CHX+0.2% CTR; 2.5% sodium hypochlorite (NaOCl); and distilled water. At both periods TAP, DAP, DBP+0.2% CHX, DBP+0.2% CTR and DBP+0.2% CHX+0.2% CTR exhibited no growth (NG) of *E. faecalis*. Distilled water and 2.5% NaOCl obtained the lowest CFU reduction. No growth with DBP was reached only at 7 days. CHX and/or CTR pastes were as effective as DAP and TAP. A 2-day period provided an effective disinfection.

Keywords: Regenerative endodontic therapy, Double antibiotic paste, Chlorhexidine, Cetrimide, Propylene glycol

INTRODUCTION

Endodontic infection or trauma of a permanent tooth can lead to pulp necrosis and, in the case of an immature tooth, to the interruption of tooth development, resulting in incompletely formed roots with wide open apices, reduced root length, and thin, fragile dentinal walls¹. Regenerative endodontic therapy (RET) has become a viable alternative in the treatment of immature teeth with pulpal necrosis as long as an adequate disinfection of the root canal system is guaranteed². Most of RET clinical reports have used either triple antibiotic paste (TAP)³⁻⁷ or pure calcium hydroxide ($\text{Ca}(\text{OH})_2$) paste⁸⁻¹¹, as intracanal medicaments for this purpose.

$\text{Ca}(\text{OH})_2$ as unique intracanal medicament has been used in RET with satisfactory results⁸⁻¹¹. However, several authors claim that it can destroy cells with potential to differentiate into new pulp due to its high pH³. Moreover, it may induce an uncontrolled calcification of the canal space that would prevent the ingrowth of soft tissue with an odontogenic potential⁹.

TAP consisting of metronidazole, ciprofloxacin and minocycline, has been successfully used to disinfect the canals in RET, achieving closure of the apex and thickening of the dentinal walls³⁻⁷. A potential disadvantage when using TAP is the possibility of tooth discoloration^{4,12,13} due to minocycline^{4,13}. The double antibiotic paste (DAP), that excludes minocycline, has also been successfully used for RET^{14,15}. Other concerns associated with intracanal use of antibiotics are: fear of promoting antibiotic resistance, risk of allergic reaction¹² and biocompatibility of the medicament¹⁶. An excessive concentration of TAP might affect the host tissue, limiting tissue regeneration¹⁷. Therefore, the

possibility of using chlorhexidine (CHX) or cetrimide (CTR) as intracanal medication in these regenerative procedures could be a safe alternative¹⁸. In fact, it has recently been reported, that a combination of 2% CHX gel with $\text{Ca}(\text{OH})_2$ as intracanal medicament is as effective as TAP¹⁹.

E. faecalis is an opportunistic pathogen associated with different forms of periradicular diseases, including primary^{20,21} and persistent endodontic infections²². This is due to its ability to adhere to dentin and penetrate along dentinal tubules²³ and to colonize the dentin in the form of biofilm²⁴. Its ecologic advantage could allow it to resist the bactericidal effect of $\text{Ca}(\text{OH})_2$ ^{23,24}, and of a wide range of antibiotics²⁵. In contrast, TAP has shown to be effective against this bacteria²⁶⁻²⁸. However, there is a lack of consensus, not only referring to the medication used, but also to the length of the treatment, which ranges in literature from 7 days to several weeks^{3-8,11-14}.

To our knowledge, there are no microbiological studies comparing *in situ* the efficacy of TAP, DAP and $\text{Ca}(\text{OH})_2$ paste alone or in combination with CHX and/or CTR at different periods of time. Therefore, the aim of this study was to compare the antimicrobial activity of different intracanal medications for 2 and 7 days, in simulated open apex root canals contaminated with *E. faecalis* biofilms.

MATERIALS AND METHODS

The Ethics Committee of the institution where the experiment was performed previously approved the protocol (UGR-438). The experimental method used in this study was a modification of the model used by Haapasalo and Orstavik²⁹. One hundred and thirty-

Table 1 Log₁₀ reduction of colony formed units of *Enterococcus faecalis* in both follow-up periods: 2 and 7 days

Groups	2 days post-treatment	7 days post-treatment	<i>p</i> *
	Log ₁₀ reduction	Log ₁₀ reduction	
TAP	3.72 (0.26) ^a (NG)	3.67 (0.18) ^a (NG)	0.715
DAP	3.71 (0.13) ^a (NG)	3.66 (0.14) ^a (NG)	0.491
DBP+0.2% CHX	3.51 (0.25) ^a (NG)	3.59 (0.26) ^a (NG)	0.549
DBP+0.2% CTR	3.46 (0.21) ^a (NG)	3.60 (0.15) ^a (NG)	0.146
DBP+0.2% CHX+0.2% CTR	3.44 (0.36) ^a (NG)	3.40 (0.22) ^a (NG)	0.775
DBP	3.05 (0.80) ^{a,b}	3.58 (0.21) ^a (NG)	0.105
Irrigation with 2.5% NaOCl	2.20 (1.37) ^{b,c}	0.76 (1.11) ^b	0.038
Irrigation with distilled water	1.09 (1.11) ^c	0.48 (0.73) ^b	0.215

Comparison between groups and follow-up periods. Mean (SD)

TAP: triple antibiotic paste, DAP: double antibiotic paste, DBP: dental base paste, CHX: chlorhexidine; CTR: cetrimide; NaOCl: sodium hypochlorite; NG: No growth.

The same superscript letter shows differences that were not statistically significant determined by the Tukey's test once that ANOVA test for 2 and 7 days showed statistically significant differences ($p < 0.001$ in both cases).

* Comparison by Student's *t*-test ($p < 0.05$).

RESULTS

Mean values obtained at baseline in Log₁₀ ranged between 3.72 (TAP) and 3.39 (distilled water) for specimens incubated for 2 days without statistical significant differences among the experimental groups evaluated. In the same way, no significant differences for baseline results were recorded among the specimens incubated for 7 days, regardless the treatment applied. In this case, mean values ranged from 3.67 (TAP) to 3.4 (distilled water and DBP+0.2% CHX+0.2% CTR).

The results are shown in Table 1. At 2 days post-treatment, TAP, DAP, DBP+0.2% CHX paste, DBP+0.2% CTR paste and DBP+0.2% CHX+0.2% CTR paste exhibited NG of *E. faecalis*. Without achieving NG, DBP and 2.5% NaOCl, obtained statistically the same efficacy. The worst results were achieved with distilled water (1.09 Log₁₀ reduction).

At 7 days post-treatment NaOCl and distilled water achieved similar Log₁₀ reduction (0.76 and 0.48, respectively) that was significantly lower than the obtained with the other 6 medications, which showed NG.

Overall, there were no statistical differences between both follow-up time periods (2 and 7 days), except for 2.5% NaOCl treatment that showed a higher Log₁₀ reduction at 2 days.

DISCUSSION

Various studies have also used *E. faecalis* to evaluate the antibacterial effectiveness of different intracanal medications, as this strain is highly resistant against different antibacterial agents^{26,28,32} and it is recovered

from root canals of teeth with persistent periapical lesions. Thus, the NG of *Enterococcus* during root canal treatment is essential²¹, although it must be considered that endodontic infection is polymicrobial.

In general, for all experimental groups, baseline CFUs values were lower, between 10³ and 10⁴, than those reported previously for other authors^{28,33}. This could be due to methodological differences, as a lower concentration of the inoculum and/or a shorter root length³³. These baseline values should be taken into account, since a low microbial load could explain that no growth of *Enterococcus* was determined for experimental groups in which antibiotics or antiseptics in paste were applied, either at 2 or 7 days post-treatment. Moreover, although sampling at baseline may have some repercussion on the bacterial reduction, as it recovers a small part although representative of the total load, this procedure was similar for all experimental groups and thereby the produced effect.

In the present study DBP, that contained Ca(OH)₂, did not show NG capacity of *E. faecalis* when compared with the other medicaments at 2 days post-treatment. However, this difference disappeared after 7 days, attaining as the others a 100% of NG capacity. This is consistent with the study by Madhubala *et al.*²⁶ in which Ca(OH)₂ also showed a gradual increase in its antibacterial activity, although they reported a maximum of 59.4% on day 7. Also, Shokraneh *et al.*²⁸, did not find significant differences between TAP and Ca(OH)₂, alone or in combination with CHX, in the deep dentin; although TAP showed a significantly higher antibacterial activity in surface dentin. The good results of Ca(OH)₂ in this study could be due to its combination with propylene glycol which is a biologically well-

accepted vehicle with significant antibacterial properties that allows a sustained release of hydroxyl ions and enhances the diffusivity of calcium hydroxide into the dentinal tubules³⁴.

No differences between NaOCl and distilled water experimental groups were detected at 2 and 7 days post-treatment, which may be a surprising result a priori. NaOCl is a strong antimicrobial but with no residual activity³⁵. It loses its activity just after its application, therefore no interference with bacterial regrowth would be expected. This fact could explain that at 2 days post-treatment the difference with the Log₁₀ reduction obtained by distilled water was 1 unit and at 7 days differences were even lower (0.28).

TAP has shown to be very effective in eliminating endodontic pathogens *in vitro*^{27,36}, *in situ*^{26,28} and *in vivo*³⁷. In agreement, the application of TAP produced a 100% reduction of *E. faecalis* at 2 and 7 days post-treatment. This antibacterial efficacy against *E. faecalis* was also reported by Madhubala *et al.*²⁶, although in their study, TAP required 7 days to show its higher antibacterial effect (98.46%).

According to our results, DAP was as effective as TAP against *E. faecalis* in the root canal space, agreeing with Sabrah *et al.*²⁷, who obtained no significant difference between TAP and DAP antimicrobial activity against this bacterium when compared *in vitro*. This also corroborates the good clinical outcomes of DAP in the cases reports published by Iwaya *et al.*¹⁴ and by Hargreaves *et al.*¹⁵. Therefore, the elimination of minocycline could allow obtaining the same clinical results avoiding the risk of tooth discoloration, even so, it still remains the risk of promoting antibiotic resistance, of allergic reaction or toxicity of the medicament.

Despite the satisfactory results of TAP and DAP for RET, it should be borne in mind that medicaments at concentrations currently used could adversely affect stem cells survival^{2,38}. According to this, Ruparel *et al.*² showed that TAP and DAP significantly reduced the survival of stem cells of the apical papilla (SCAPs) in a concentration dependent fashion, since lower concentrations of 0.01–0.10 mg/mL of TAP and DAP did not have an effect on SCAPs, while Chuensombat *et al.*¹⁷ determined that 0.39 mg/mL TAP was a safe dose, although it was unable to eradicate all bacteria. Recent recommendations suggest doses no higher than 1 mg/mL seeking for a balance between safety and antimicrobial effectivity^{38,39}.

On the other hand, this detrimental effect on SCAPs survival has not been evidenced for Ca(OH)₂ regardless of the concentration tested²; even promoting SCAP survival and proliferation^{2,38}. Therefore, medicaments used in RET should be carefully selected and used at adequate concentrations to have antibacterial efficacy without evoking toxicity to the host stem cells². The current clinical recommendation of the American Association of Endodontists⁴⁰ suggests the use of Ca(OH)₂ or 0.1 mg/mL of TAP. However, a recent study showed that 0.125 mg/mL of DAP and TAP did not have cytotoxic effect on dental pulp stem cells, but it was not

enough to completely eradicate established biofilm of *E. faecalis*, although it showed a significant antibacterial effect³². Nevertheless, the exact concentration used clinically is not usually precisely reported^{4,5,12,39}.

CHX and CTR have proven to be effective against *E. faecalis*, alone⁴¹ or in combination⁴² when used as irrigating solutions, which is in line with the results of this study, where DBP+0.2% CHX paste, DBP+0.2% CTR paste and DBP+0.2% CHX+0.2% CTR paste exhibited NG of *E. faecalis* at both experimental periods. In clinical situations, Nagata *et al.*¹⁹ have reported that a paste composed of Ca(OH)₂+2% CHX gel is as effective as TAP. According to this, Soares *et al.*¹⁸ presented a new proposal for pulp revascularization with mechanical decontamination and intracanal medication of Ca(OH)₂+2% CHX gel, obtaining a satisfactory root development. However, Ordinola-Zapata *et al.*⁴³ reported that TAP was more effective than 2% CHX gel and Ca(OH)₂ at killing bacteria in the biofilms of an intraorally infected dentin model. Another study evaluated the viability of *E. faecalis* in human radicular dentin after a 14-day exposure to Ca(OH)₂, TAP, 2% CHX gel and 1% CTR solution. Both TAP and 1% CTR significantly reduced the viability of *E. faecalis* in comparison with 2% CHX gel and Ca(OH)₂⁴⁴. This could be due to the presentation in gel of the CHX, as according to the results of a pilot study of our research group, in which 0.2% CHX gel and 0.2% CTR gel, alone or in combination, were much less effective than the paste presentation (data not shown). Further investigation should be carried out to clarify the influence of the mode of presentation of these medicaments on their effectiveness.

Regarding CHX biocompatibility, 2% CHX solution showed a good biocompatibility when injected subcutaneously in rats, while 2% CHX gel presented a moderate inflammatory response at 14 days, which decreased at 30 days⁴⁵. However, in other studies, CHX showed to be cytotoxic on osteoblastic, endothelial and fibroblastic cell lines⁴⁶ and particularly on SCAPs⁴⁷. For this reason, in this study 0.2% CHX was used instead of 2%. Referring to CTR, its biocompatibility has hardly been investigated. Öncü *et al.*⁴⁸ compared the antibacterial properties and toxicity of various root canal irrigants: 5.25% sodium hypochlorite (NaOCl), 2% CHX and 0.2% CHX+0.2% CTR (Cetrexidin®). Cetrexidin® and 2% CHX were more effective and had more residual antibacterial effects and lower toxicity than 5.25% NaOCl.

CONCLUSIONS

On the basis of the findings reported in this study, it can be concluded that CHX and/or CTR pastes were as effective as DAP and TAP as short-term intracanal medicaments in RET and CTR could be an alternative once its biocompatibility is proven.

Moreover, long periods of intracanal medication would not be required to achieve an effective disinfection of the root canals. However, research is warranted

to compare the efficacy and biocompatibility of these medicaments and to test them in a mixed species biofilm as well as in a clinical scenario to obtain predictable outcomes and to establish a standardized treatment protocol for necrotic immature permanent teeth.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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