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The effect of different root canal medicaments on the elimination of *Enterococcus faecalis ex vivo*

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ABSTRACT

Objective: The aim of this study was to evaluate the antimicrobial effect of chlorhexidine gel (CHX-G) 2%, chlorhexidine powder (CHX-P) 1%, povidone-iodine (PVP-I), polyhexanide and camphorated-and-mentholated chlorophenol (ChKM) *ex vivo*. **Materials and Methods:** For every medicament group 10 root segments (15 mm long) of extracted human teeth were prepared to ISO-size 45 and sterilized ($n = 50$). The root segments were then inoculated with *Enterococcus faecalis* and aerobically incubated at 37°C. After 1 week, ten root canals were filled with one of the medicaments, respectively and aerobically incubated at 37°C for another week. Ten teeth served as positive controls and were filled with sterile saline solution. After 7 days, the medicaments were inactivated and all root canals were instrumented to ISO-size 50. The obtained dentin samples were dispersed in Ringer solution followed by the preparation of serial dilutions. 10 µl per sample were applied to an agar plate and incubated at 37°C for 48 h. The colony forming units were counted and the reduction factors (RFs) were calculated and statistically analyzed. **Results:** Compared with the positive controls all medicaments exhibited an antibacterial effect against *E. faecalis*. The RFs for CHX-G, CHX-P and ChKM were significantly higher compared to PVP-I and polyhexanide ($P < 0.05$). In contrast to PVP-I and polyhexanide, CHX-G, CHX-P and ChKM were able to eliminate *E. faecalis* from all dentin samples. **Conclusions:** Within the limitations of this *ex vivo* investigation, 2% CHX-G and CHX-P were as effective as ChKM against *E. faecalis*. Thus, when choosing a root canal medicament the better biocompatibility of CHX compared with ChKM should be taken in consideration.

Key words: Camphorated-and-mentholated chlorophenol, chlorhexidine, polyhexanide, povidone-iodine, root canal medication

INTRODUCTION

Enterococcus faecalis

In endodontics, *E. faecalis* is classified as one of the most resistant pathogenic organism, which can be detected in infected root canals.[1] In teeth with periapical periodontitis *E. faecalis* was found in 71% of the cases.[2] *E. faecalis* invades dentinal tubules faster than other endo-pathogenic microorganisms and is able to survive there for the longest time.[3] *E. faecalis* is virulent also in extreme environmental conditions and may survive in obturated root canals without nutrition and is able to be reproduced in contact with human serum.[4] The removal of *E. faecalis* from the root canal system is difficult, e.g., the widely-used and popular root canal medication calcium hydroxide is ineffective in eliminating *E. faecalis* from the dentinal tubules.[5] Hence, for the evaluation of different antiseptic medications root canal dentin was infected with *E. faecalis* in the present study to simulate clinical conditions.

Chlorhexidine digluconate gel

For many decades, calcium hydroxide was the gold standard as root canal medication, but it has also some

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disadvantages, e.g., it has been shown that calcium hydroxide has nearly no effect on *E. faecalis*. In contrast, chlorhexidine digluconate (CHX) was found to be much more effective against *E. faecalis* than calcium hydroxide. Thus, several studies demonstrated that a chlorhexidine gel (CHX-G) 2% is significantly superior in the elimination of *E. faecalis* from the root canal system compared with an aqueous calcium hydroxide preparation.[6-8] Already, a 0.2% CHX solution as root canal medication is able to impede a reinfection with *E. faecalis* for a time period of up to 3 weeks.[6] Despite its strong antiseptic effects CHX is only a slightly toxic. Undesirable side-effects are rare.[7,9] Hence, CHX-G 2% can be recommended as an alternative to calcium hydroxide.[6]

Chlorhexidine digluconate powder

Beside a 2% CHX-G a chlorhexidine powder (CHX-P) mixed with isotone sterile saline solution was tested in this study. This CHX-P is composed of adipic acid, talcum, zinc oxide, silicon dioxide, paraffin, vaseline, wool wax and CHX (1%). To evaluate if this CHX-P mixture with saline solution may be an alternative to CHX-G was one aim of this study. To the best of our knowledge, there are no scientific data concerning the use of CHX-P as root canal medication available. An advantage of CHX-P may be the admixture of adipic acid to obtain pH-independent release of CHX. Furthermore, CHX-P mixture with saline solution has a creamy consistence comparable to an aqueous calcium hydroxide preparation and thus may be easier in handling and more familiar to dentists compared with a CHX-G.

Polyhexanide

Polyhexanide is a biguanide (polyhexamethylen biguanid) with good antibacterial properties. The antibacterial effect is based on an increase of the permeability of the bacterial cell membrane, which leads to an osmotic imbalance with an outpouring of cytoplasm. Polyhexanide possess a broad antibacterial spectrum, mainly against *Staphylococcus aureus*, *E. faecalis*, *Bacillus subtilis*, *Enterobacter cloacae* and *Streptococcus lactis*. The cytotoxicity of polyhexanide is low and thus the tissue compatibility is high. Compared to other disinfectants such as iodine, hydrogen peroxide or CHX, the local tolerability of polyhexanide is superior.[10-15]

Whereas polyhexanide was introduced to medicine about 15 years ago, mainly as local and wound antiseptic,[11,16] the use in dentistry is seldom, e.g., Rosin *et al*.[17] showed that polyhexanide may significantly impede plaque formation on tooth surfaces. Polyhexanide has a broad antibacterial spectrum, is inodorous and uncolored, does not cause wound burning sensation, does not impair wound healing, is biocompatible and systemic adverse effects are seldom.[16] Antibacterial effects against *E. faecalis* was described *ex vivo*,[10-13] but until now nothing is known about the effect of polyhexanide against *E. faecalis* in root canals. In the present study, a sterile ready-for-use-dissolution of polyhexanide (Prontosan; Braun Meslungen, Melsungen, Germany) was used. Prontosan contains 0.1% polyaminopropyl biguanide (polyhexanide), 0.1% undecylenamidopropyl betain and distilled water. Betain is used to reduce the surface tension and to improve the dispersion of the polyhexanide.

Povidone iodine

In medicine iodine is mainly used as PVP-I, which is a water-soluble complex from polyvinylpyrrolidone (PVP) and iodine. PVP itself has no antibacterial effects, but is a synthetic polymer and serves only as carrier or mediator molecule to store the iodine. It has a high affinity to the bacteria membrane and allows an easy transport of the free iodine to the bacteria surface. Iodine reacts there with free hydroxyl and thiol groups of amino acids and polyunsaturated fatty acids followed by cell death. PVP-iodine is bactericide against gram-negative and positive germs as well as fungicide, virucidal and sporicidal.[18,19] Furthermore, PVP-iodine is able to suppress the activity and expression of bacterial toxins.[20] PVP-iodine has a good biocompatibility, even superior to CHX.[19] In dentistry, PVP-iodine is manly used in the therapy of gingivitis and periodontitis as well as mycosis.[17] In endodontics, PVP-iodine was also described as a root canal irrigant because it shows good penetration into the dentinal tubules.[3,5] A sound antibacterial effect of PVP-iodine against *E. faecalis* was described *in vitro*.[12,21] The PVP-iodine used in this study is available as a mouth antiseptic (Betaisodona). 100 ml contains 10 g PVP-iodine as well as ethanol (96%), levomenthol, methyl salicylate, glycerol, saccharin-sodium, disodiumhydrogenphosphate, citric acid, sodium hydroxide and purified water.

Camphorated-and-mentholated chlorophenol

ChKM is a mixture of 27% 4-chlorophenole, 71% racemic camphor and 1.6% levomenthol and is market in Germany as "Walkoff`s ChKM solution" (Haupt-Dental, Würzburg, Germany). ChKM is comparable to international common camphorated parachlorophenol (CMPC) products and was

introduced to dentistry in 1905. Chlorophenols like ChKM are active antiseptics and good disinfectants for root canals. They have a higher antibacterial, antiseptic and disinfectant potential compared to other disinfectants or phenol.[22,23] CMPC products were shown to be highly effective against *E. faecalis*.[3,5] The often described antibacterial effect of CMPC products is based on its ability to destroy the bacteria membrane by binding on its proteins and lipids.[24,25]

Thus, ChKM and other CMPC products are still in use today even though in literature, the use of ChKM and other CMPC solution is controversial because of possible toxicity of the ingredients like 4-chlorophenol and other chlorophenols.[22,26]

Aim of this study

The aim of this study was to compare the antimicrobial effect of the different medications concerning against *E. faecalis* in the root canal of extracted human teeth *ex vivo*. Following questions should be answered:

- Are PVP-I and polyhexanide an alternative as root canal medication in the elimination of *E. faecalis*?
- Is CHX-P as effective as CHX-G in the elimination of *E. faecalis* from the root canal system?
- Are CHX preparations as effective as ChKM in the elimination of *E. faecalis* from the root canal system?

MATERIALS AND METHODS

A total of 65 caries-free extracted single-rooted human incisors were used for this study and stored in ethanol (90%). The handling of all human samples followed strictly the "Declaration of Helsinki". After trimming all root segment consistently to a length of 15 mm, the root canals were prepared with Hedström files up to International Standard Organization (ISO) 45 staying 1 mm short of the apex. During the preparation, the root canals were irrigated with NaOCl (3%). The smear layer was removed with citric acid (10%) and the root canals irrigated with saline solution (0.9%) for neutralization. Finally, the root segments were sterilized (Varioklav-Dampfsterilisator; H+ P-Labortechnik, Oberschleißheim, Germany). After sterilization, the root segments were inoculated with *E. faecalis* of human oral origin (*E. faecalis*, DSM2570, German Collection of Microorganisms and Cell Cultures, Brunswick, Germany) in yeast-extract-glucose-solution McFarland 0.5 (10 µl per root). Then, the root segments were aerobically incubated at 37°C and 100% humidity (Laboratory Heating and Drying Cabinet, Heraeus, Hanau, Germany) for 1 week. After 3 and 6 days, the bacterial suspension was renewed. Furthermore, at these days (3 d, 6 d and 1 week) samples were taken to control the growth of *E. faecalis* in pure culture. After 1 week, the root canals were dried with sterile paper points. Ten root segments were filled with one of the tested medications, respectively:

- CHX-G 2% (prepared by the pharmacy of the University Medical Centre)
- CHX-P 1% (Chlorhexidin-Heilpuder; Riemser, Greifswald, Germany)
- PVP-I 10% (Betaisodona; Mundipharma, Limburg/Lahn, Germany)
- Polyhexanide 0.1% (Prontosan; Braun Melsungen, Melsungen, Germany)
- and ChKM (Walkhoff´s ChKM-Lösung, Haupt-Dental, Würzburg, Germany).

Ten teeth served as a positive control and were filled with sterile saline solution (0.9%).

The CHX-P was mixed with sterile saline solution (0.9%) in a ration 1:1 and filled into the root canals with a Lentulo spiral (ISO 30). The other medications were liquid or gel-like and thus injected into the root canals. All root segments were aerobically incubated at 37°C and 100% humidity (Laboratory Heating and Drying Cabinet, Heraeus, Hanau, Germany) for 1 week.

After 1 week, the medications were removed from the root canal by irrigation with 2 ml of a sterile saline solution (0.9%) and drying with sterile paper points. The remaining medications were inhibited by irrigation with a sterile inactivation solution. The inactivation solution contained 3% tween 80, 3% saponine, 0.1% histidine, 0.1% cysteine, 0.1% tryptone and 0.85% NaCl. For inactivation, all root canal were filled twice with this solution for 15 min and dried with sterile paper points. To remove the inactivation solution root canals were finally irrigated with sterile saline solution (0.9%) and drying with sterile paper points. The inactivation solution was tested in a previous pilot test. It was proven that the inactivation solution clearly inhibited all tested medication without having a positive or negative effect on the growing of the *E. faecalis*.

To obtain dentin samples each root canal was prepared with respectively one sterile Hedström file ISO size 50 (VDW, Munich, Germany) up to 1 mm short of the apex. To standardize the volume of the excised dentin the root canal was prepared with one circumferential filing. All collected dentin chips

were placed together with the particular Hedström file in a test tube filled with 5 ml Ringer´s solution (Merck, Darmstadt, Germany). Every test tube was sonicated (Ultrasonic Cleaner, Branson Ultraschall, Dietzenbach, Germany) and vortexted for 10 s. Then a serial dilution 1:20 and 1:400 was performed to allow an exact enumeration of *E. faecalis* colonies. In the positive control group without medication an additional dilution 1:8000 was performed because a higher bacteria density was expected. From the original undiluted samples as well as from the diluted samples, 10 µL were removed and exposed to agar plates (trypticase soy agar with 5% sheep blood). The agar plates were aerobically incubated at 37°C and 100% humidity (Laboratory Heating and Drying Cabinet, Heraeus, Hanau, Germany) for 2 days. After 48 h from every serial dilution of every tooth, a suitable agar plated was selected for counting the colony forming units (CFU). The CFU were counted at 8 fold magnification using a stereomicroscope (Stemi 2000; Carl Zeiss, Oberkochen, Germany), then multiplied with the dilution factor and converted to CFU/ml, subsequently. Afterward, all values were transformed to common logarithm to approach normal distribution and to determine the median of every single group for the calculation of the reduction factors (RFs).

The RF is a measurement for the antiseptically effect of a medication, is displayed in logarithmical degrees and can be calculated as following:

RF = log CFU (control group) median – log CFU (experimental group) median.

In those experimental groups where a complete growth inhibition occurred the CFU was "0". Mathematically, the common logarithm of "0" is not defined. Hence, it was decided that in those cases the RF is identically to the log CFU of the control group because the according medication could eliminate all bacteria from the root canal of its corresponding control group.

Every single step of the experimental process was performed under sterile conditions.

Finally, the results were statistically evaluated using the Chi-square test to analyze the correlation between bacterial growth and the used medication. To compare every experimental group with the positive control group a non-parametric Mann-Whitney-U-test was performed. The level of significance was set at 5%.

RESULTS

The RFs for CHX-G, CHX-P and ChKM were significantly higher compared to PVP-I and polyhexanide ($P < 0.05$) [Table 1]. In contrast to PVP-I and polyhexanide, CHX-G, CHX-P and ChKM were able to eliminate *E. faecalis* from all dentin samples [Table 2]. Table 3 shows the asymptotic statistical significances between the medicaments and the positive control group. Hence, compared to the positive control group, all medicaments exhibited an antibacterial effect against *E. faecalis*. For CHX-G 2%, CHX-P 1% and ChKM these differences were statistically significant ($P < 0.05$).

Table 1: Effectiveness against *E. faecalis* of the medicaments. Log RFs of all medicaments. The RF is a unit for the antiseptically effectiveness of the medicaments

Root canal medicament	ISO 50
CHX gel 2%	3.24*
CHX powder 1%	3.24*
Polyhexanide	0.31
Povidone-iodine	0.59
ChKM	3.49*

*Reduction factors are statistically significantly different compared to polyhexanide and povidone-iodine ($P<0.05$; Mann-Whitney-U test). ISO: International Standard Organization, CHX: Chlorhexidine, ChKM: Camphorated-and-mentholated chlorophenol, *E. faecalis: Enterococcus faecalis*, RFs: Reduction factors

Table 2: Percentage of *E. faecalis*-free dentin specimens after the application of the medicaments in the root canal segments

Root canal medicament	ISO 50
CHX gel 2%	100*
CHX powder 1%	100*
Polyhexanide	20
Povidone-iodine	30
ChKM	100*

*Statistically significant difference compared to polyhexanide and povidone-iodine ($P<0.05$; Chi-square test). ISO: International Standard Organization, CHX: Chlorhexidine, ChKM: Camphorated-and-mentholated chlorophenol, *E. faecalis: Enterococcus faecalis*

Table 3: Asymptotic statistical significances of the medicaments compared to the positive control group

Root canal medicament	ISO 50
CHX gel 2%	0.002*
CHX powder 1%	0.002*
Polyhexanide	0.622
Povidone-iodine	0.055
ChKM	0*

*Significant difference compared to the positive control group ($P<0.05$; Mann-Whitney-U test. ISO: International Standard Organization, CHX: Chlorhexidine, ChKM: Camphorated-and-mentholatedchlorophenol

DISCUSSION

The aim of this study was to compare different medications regarding their effect against *E. faecalis* in root canals *ex vivo* in a previously proved experimental setup.[27,28]

Chlorhexidine digluconate gel

As in previous studies[6-8] CHX was used as a 2% gel. CHX-G 2% was chosen as a kind of reference because it is well-known from *in vitro* studies that CHX is highly effective against *E. faecalis*[6-8,27] and other microorganisms[28] in root canals. This effect may last up to 15 d.[8] Thus, these results are in fully accordance with the current literature. The concentration of CHX 2% is non-hazardous because of the relatively low cytotoxicity of CHX and thus in comparison to other root canal medications CHX displays a better tissue tolerance.[7,9] However, a disadvantage of CHX is that the antimicrobial effect is related to the environmental pH value. CHX is most effective in tissues showing a neutral physiological pH value. Hence, the antimicrobial effect of CHX might be reduced in the presence of alkaline calcium hydroxide.[9,29] Thus, for root canal medications a pH-independent release of CHX is desirable.

Chlorhexidine digluconate powder

In addition, CHX was tested as a powder mixture with saline solution resulting in an aqueous suspension comparable in its consistence to calcium hydroxide preparations. The 1% CHX-P was as effective as the 2% CHX-G. Because of the admixture of adipic acid to obtain pH-independent release of CHX, the CHX-P may be favorable compared to CHX-G, e.g., as a balanced mixture with calcium hydroxide. However, these speculations warrant further studies. Another advantage of the CHX slurry may be that the handling is comparable to an aqueous suspension of calcium hydroxide. Most dentists are familiar with the handling of such slurries. However, a drawback of this CHX mixture is that the powder is hydrophobic and mixing with saline solution is comparatively time consuming. Clinically, it might be problematic to completely remove the slurry from the root canal system, which may lead to remnants of the medication as know from calcium hydroxide.[29] The other components in the powder such as talcum, zinc oxide, silicon dioxide, paraffin, vaseline and wool wax may have no influence on the elimination of microorganisms from the root canal, but may impair a tight sealing of the root canal when obturating the canal using sealer and gutta-percha. Hence, this may also impair apical healing. Further research is needed regarding the afore mentioned aspects, but CHX-P may be an interesting alternative as root canal medication being as effective as CHX-G.

Polyhexanide

Polyhexanide is more and more often used in medicine in the treatment of infected wounds because of its good antimicrobial effects in combination with a high biocompatibility.[10-16] Thus, to the best of our knowledge this study for the 1st time assessed polyhexanide as an alternative to established root canal medications. However, polyhexanide was not able to eliminate *E. faecalis* from all dentin samples, which may be related to the quite low concentration of 0.1% of this ready to use a mixture. The effects of polyhexanide decrease with the reduction of the concentration. Hence, it may be speculated that polyhexanide in a higher concentration may have been more effective. In a further study, the most effective concentration of polyhexanide against *E. faecalis* should be determined.

In vitro polyhexanide was able to inhibit *E. faecalis*[12] and after 5 min the microorganisms were completely eliminated from the culture medium.[11] Thus, the effectiveness against *E. faecalis* especially in acute and chronically infected wounds has been proven. Furthermore, it is known that proteins such as mucin, albumin and blood may decrease the effectivity of polyhexanide.[10] Possibly, dentin as well may impair the antibacterial effects of polyhexanide, which may explain the disappointing results in the present study. Furthermore, this point should be clarified in further studies.

Povidone iodine

PVP-I is a biocompatible medication, which is used in medicine and dentistry, e.g., for wound lavage[30] and may be an alternative as root canal medication.[31]

Studies, in which PVP-I was used as a root canal medication showed a fast onset and a good antibacterial effect against *E. faecalis*.[32,33] However, already after 10 min the effect decreased.[32] In contrast to the present study Abdullah *et al*.[21] showed that the use of a 10% PVP-I solution resulted in 100% bacteria reduction after 30 min in an *E. faecalis* biofilm model. This difference may be explained by the fact that in the *E. faecalis* biofilm model used by Abdullah *et al*.[21] the microorganisms grew on sterile cellulose nitrate membrane filters without any contact to dentin. The presence of dentin and its components are responsible for different inhibitory patterns on the activity of the iodine solution.[19] Thus, the antibacterial effect of PVP-I in root canals is quite

low, which is contributed to the inhibiting effect of dentin to PVP-I. This inhibiting effect is correlated to the contact time with the dentin and the concentration of the medication.[19,34] Therefore, due to the inhibition by dentin PVP-I cannot be recommended as root canal medication. Further disadvantages of PVP-I are the well-known allergic reaction to iodine compounds and their potential to induce hyperthyroidism due to excessive incorporation of iodine in the thyroid gland.[19]

Camphorated-and-mentholated chlorophenol

It is well-known that CMPC and ChKM used in root canals possess a strong disinfection activity,[24,25] especially against *E. faecalis*,[3,5,26] which can be confirmed by the results of the present study. On the other hand, the antibacterial effect is only of short duration.[25] Furthermore, CMPC and ChKM are discussed controversially because of their questionable biocompatibility.[24,25,35-40]

Because of its fast diffusion through the dentinal tubules the severe adverse cytotoxic reactions of CMPC and ChKM solution used as an intracanal dressing were found in the periodontium and inflammation in the periapical region was induced.[24,25,36,37,39] An intradermal injection of chlorophenol camphorated menthol led to an edematous tissue alteration with cellular infiltration.[35] The admixture of menthol resulted in local anesthesia and a certain anti-inflammatory effect, whereas the admixture of camphor is claimed to reduce the toxic effects of ChKM by a reduction of the water solubility of the phenol.[26] In fact, it has been shown that in rat pulp tissue camphor increased the cytotoxic effects of chlorophenol; hence, camphor itself has a cytotoxic impact and thus increased the cytotoxic effects of phenol.[37] Such root canal medications display a negative effect on the immune defense because of delimiting the adherent capacity of macrophages.[38] CMPC has a negative influence on the proliferation and viability of periodontal ligament cells and thus, has the potential to cause severe damage of the periodontium.[39] ChKM solutions significantly reduced cell viability in human gingival fibroblasts and induced DNA double-strand breaks in human oral cells; thus, ChKM has a genotoxic capacity.[40] In rat molar teeth, the use of ChKM as root canal medication caused an increase of the apical alteration.[36] It can be summarized that ChKM was 100% effective in the elimination of *E. faecalis* from the root canal system because of its strong antibacterial effect. Unfortunately, for CMPC and ChKM products a markedly minor biocompatibility has been shown in several studies. Thus, the clinical use of CMPC and ChKM is questionable.

CONCLUSIONS

CHX-G 2% and CHX-P 1% were as effective as ChKM against *E. faecalis*. Thus, when choosing a root canal medicament the established better biocompatibility of CHX compared to CMPC and ChKM should be taken into consideration. The use of polyhexanide and PVP-I as root canal medication in the present concentrations cannot be recommended.

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