The Antimicrobial Effect of Calasept, Superlux Calcium Hydroxide Liner and Gutta-percha with Calcium Hydroxide

Danijela Vrček[1]
Goranka Prpić-Mehičić[2]
Arjana Tambić-Andrašević[3]
Renata Poljak-Guberina[4]
Nada Galić[2]
Marina Katunarić[2]

[1]Private dental practice, Varaždin
[2]Department of Dental Pathology
School of Dental Medicine
University of Zagreb
[3]Klinika za infektivne bolesti "Dr Fran Mihaljević", Zagreb
[4]Private dental practice, Zagreb

Summary

Microorganisms are the main cause of disease in root canals and periapically. Facultative bacteria and fungi have been identified in persistent endodontic infection. The objective of this study was to determine in vitro *the efficacy of: Calasept (Speiko, Darmstadt, Germany), Superlux calcium hydroxide liner (Dental Material Gesellschaft, Hamburg, Germany) and gutta-percha with calcium hydroxide (Roeko, Langenau, Germany) in direct contact with microorganisms. The microorganisms used were:* Enterococcus faecalis *(ATCC 29212) and* Candida albicans *(NCTC 3123). An overnight mixed broth culture of microorganisms (10^8 CFU/ml) was prepared. Paper points and gutta-percha points were immersed in suspension for 5 min. The paper points were then covered with Calasept and Superlux calcium hydroxide liner. At intervals of 0; 6; 12; 24; 48 and 72 h at 37°C, aerobically. The antimicrobial effect of Calasept and Superlux calcium hydroxide liner apparently occured after 6h on* Enterococcus faecalis *and 12 h on* Candida albicans*. With gutta-percha, this effect did not occur for either* Enterococcus faecalis *or* Candida albicans *after 72 h.*

Key words: Candida albicans, Enterococcus faecalis*, Calasept, Superlux calcium hidroxide liner, gutta-percha with calcium hydroxide.*

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Address for correspondence:

Goranka Prpić-Mehičić
Zavod za bolesti zuba
Stomatološki faklultet
Gundulićeva 5, 10000 Zagreb
Croatia

Introduction

Microorganisms and their by-products are the main cause of pathological changes in dental pulp and periradicular tissues and therefore the success of endodontic treatment depends on elimination of bacteria and their substrate from the root canal (1). This is normally accomplished by mechanical instrumentation, supported by various irrigating solutions, and antibacterial dressing of the canal between appointments (2, 3).

However, some cases are resistant to routine therapy and the infection may persist for months or even years despite treatment (4).

Facultative bacteria and fungi have been identified in persistent endodontic infection (5).

Calcium hydroxide containing materials have been widely used in endodontic therapy to stimulate apexification, to repair perforations, to promote healing by hard tissue formation in root fractures, and to control external and internal inflammatory root resorption. Calcium hydroxide is also the main component in root canal sealers and in several pastes that are used as intracanal dressings in cases of periapical lesions (6, 7). Its excellent bactericidal action as well as induction of mineralization are due to high pH (alkaline) and dissociation into OH^- and Ca^{2+} ions (8). Different commercially available calcium hydroxide containing pastes have various chemical composition, and therefore the alkalizing potential of each compound may be different (5, 9).

Over 300 microbial species or types inhabit the mouth. Some have important roles in the development of oral and dental diseases (10, 11). *Candida albicans* is the most common yeast isolated from the oral cavity of either healthy or medically compromised adults. *Candida albicans* have been isolated from dental plaque, dental caries, subgingival flora and root canals. It can cause disease in the presence of predisposing factors such as poor oral hygiene, diabetes, malignancy, HIV infection, immunosuppressing therapy and others (12).

Many reports showed that *Enterococcus faecalis* is a common isolate from infected root canals and in cases of therapy, resistant apical lesions. It has been also used in several studies of antibacterial properties of materials because of its relative resistance (13).

The aim of our study was to determine *in vitro* the antimicrobial properties of three calcium hydroxide containing materials, which are usually used as intracanal dressing. The antimicrobial efficiency of investigated matherials was tested in direct contact with microorganisms. Following calcium hydroxide containing materials were used: Calasept (SPEIKO - Dr. Speir GmbH, Germany), Superlux calciumhydroxide - Liner (DMG - Dental - Material Gesellschaft, Germany) and Calcium hydroxide cones (ROEKO Langenau, Germany; is originally gutta percha coated with calcium hydroxide).

Materials and methods

Three different calcium-hydroxide containing materials were tested *in vitro* to determine its antimicrobial efficacy. Calasept (Speico, Germany), Superlux calcium hydroxide-Liner (Dental Material Gesellschaft, Germany) and Calcium hydroxide cones (ROEKO Langenau, Germany) were used in direct contact with microorganisms. These three materials were chosen for our study because they are widly used by dentists as intracanal dressings between two appointments.

The microorganisms used were: *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (NCTC 3123).

An overnight mixed broth culture of microorganisms (10^8 CFU/ml) was prepared. A total of 36 absorbed sterile paper cones N 50 (ROEKO Langenau, Germany) and 18 gutta-percha cones covered with calcium hydroxide were immersed in this suspension of microorganisms for 5 min. Cones were then placed on Petri dishes (7). The paper points were then covered with Calasept and/or Superlux calcium hydroxide liner, respectively. At intervals of 0; 6; 12; 24; 48 and 72 h, three points of each specimen were removed from contact with calcium hydroxide pastes. They were immersed into tryptose soya broth and incubated for 72 h at 37°C, aerobically. After that period the results of antimicrobial efficasy were read out (Stereomycroscopically).

Six groups of the sample were made, they respond to periods of 0; 6; 12; 24; 48 and 72 hours (nine samples in each group). The control group consists of samples taken at 0 hours, (paper cones as well as gutta-perch cones infected with microorganisms) two cones for each tested microorganism.

Results

Significant findings follow. The antimicrobial effect of Calasept and Superlux calcium hydroxide liner in direct contact with microorganisms accured after 6 hrs on *Enterococcus faecalis* and after 12 hrs on *Candida albicans*. However, the antimicrobial effect of Guttapercha with calcium hydroxide was not notified either for *Enterococcus faecalis* or

for *Candida albicans* for the period of 72 hrs (Table 1)

Discussion

The main goal of successful root canal therapy is to eliminate microorganisms from root canals and to prevent subsequent reinfection . It can be done by cleaning, shaping and irrigating the root canals. After instrumentation of the root canal system, microorganisms are still detected and increase rapidly in the empty canals between two appointments (13). So the use of intracanal medicaments has been advocated as an adjunct therapy to cleaning and shaping, because many in vitro and in vivo studies have shown the antimicrobial effect of calcium hydroxide(4) .

It has been observed that many bacteria commonly present in the necrotic pulp tissue are rapidly killed when exposed to a saturated solution of calcium hydroxide, in vitro terms, during the first six minutes (14). However, when calcium hydroxide was applied for 10 minutes *in vivo*, it was ineffective in destroying bacteria which had persisted following biomechanical instrumentation. The inefficiency of calcium hydroxide in such a short period of application is because hydroxil ions do not readily diffuse through dentine due to the buffering capacity of hydroxyapatite (2, 15).

The destruction of bacteria *in vitro* was facilitated by an extensive surface area accessible to hydroxil ions. This is unlikely to be the case in the clinically instrumented canal(14).

Sjögren et al.(16) studying the antimicrobial effect of calcium hydroxide showed that bacteria that had survived biomechanical preparation were eliminated in 7 days, but the process was ineffective after 10 minutes.

Calcium hydroxide had the capacity to hydrolyze the lipid portion of the bacterial lipopolysaccharide, promoting its degradation, and could also alter the biological properties of the endotoxin (17). Kontakiotis et al. (18) showed *in vitro* that calcium hydroxide, by absorbing carbon dioxide, can help the indirect action of antimicrobials on anaerobic and facultative anaerobic bacteria. Esterela et al. (19) raised the hypothesis that calcium hydroxide produced reversible and irreversible bacterial enzymatic inactivation. This can be observed in extreme conditions of pH over a long period, during which there is a total loss of biological activity of the cytoplasmic membrane. When the ideal pH is achieved, enzymatic function reversibility occurs. Lehninger (20) reports that extreme pH causes the uncoiling of many proteins with subsequent loss of biological activity. For many years, the process of denaturation was thought to be irreversible. However, if pH returns to normal, there is a return of native structure and lost biological activity.

Polymorphonuclear leucocytes and osteoclasts favor an acidic pH leading to disintegration of dental hard tissues and subsequently to external root resorption (2). The alcaline pH around the dental hard tissues during endodontic treatment, which can be achieved by the use of calcium hydroxide, is therefore desirable.

Different calcium hydroxide dressings present various chemical compositions, so it is important to realize that the alkalizing potential of each compound may be different. In the present study three different calcium hydroxide containing materials were tested to evaluate its antimicrobial efficiency.

It has been reported that *Enterococcus faecalis* is present in higher number of final than initial root canal samples (16). Measures that are found to reduce the number of bacterial cells in root canal (mechanical instrumentation, the use of antibacterial irrigants and ultrasonic filling) are apparently insufficient. The finding that this bacteria is present more frequently in samples from vital teeth and teeth with extensive coronal destruction suggests that it may enter the root canal from carious lesions, and from gingival sulcus, which appear to be normal oral habitats of these bacteria (2, 13).

Candida albicans is a pleomorphic microorganisam demonstrating different growth forms such as germ tubes, yeasts, pseudo- and true hyphae, and chlamydospores. Each form of growth depends on the environmental conditions, such as pH, temperature and nutritional source (10). The occurrence of yeasts in persistent infections may be a result of contamination during treatment. It is also possible that differences in susceptibility to local root canal dressings among fungi and bacteria may explain the occurrence of yeasts in root canal infections. The

antifungal effect of routinely used root canal medications is not known (2).

Waltimo et al. (21) studied the antimicrobial effect of various endodontic irrigants *in vitro* and showed that *C. Albicans* is highly resistant to calcium hydroxide during a period of 24 hours, and that combining Ca-hydroxide with sodium hypochlorite or chlorhexidine may provide a wide spectrum antimicrobial preparation with a long lasting effect.

Results of our study showed that Calasept and Superlux calcium hydroxide liner are efficient to *Enterococcus faecalis*, which is a bacteria which is quite therapy resistant, within a period of 6 hrs, and to *Candida albicans* within a period of 12 hrs. Guthapercha with calcium hydroxide showed no efficacy to both microorganisms within a period of 72 hrs. The reason for these results may be the *in vitro* experiment, because there are several studies which indicate the difference between calcium hydroxide activity *in vitro* and *in vivo. In vitro* exist direct contact between the microorganism and calcium hydroxide, while *in vivo* includes numerous of conditions that effect the activity of hydroxile ions.

Conclusion

In conclusion our study showed that Calasept and Superlux calcium hydroxide liner are a suggestible as intracanal dressing between two appointments. However, further investigations made *in vivo* are necessary for better understanding of the efficiency of these calcium hydroxide containing materials as an intracanal dressing.