Antimikrobni učinak Apexita in vitro

Pezelj-Ribarić, Sonja; Brekalo, Ivana; Miletić, Ivana; Abram, Maja; Karlović, Zoran; Anić, Ivica

Antibacterial Activity of Calcium Hydroxide Root Canal Sealer (Apexit) - *in vitro* Study

Sonja Pezelj-Ribarić[1]
Ivana Brekalo[1]
Ivana Miletić[2]
Maja Abram[3]
Zoran Karlović[2]
Ivica Anić[2]

[1]Department of Oral Pathology
Department of Stomatology
Medical School
University of Rijeka
[2]Department of Dental Pathology
School of Dental Medicine
University of Zagreb
[3]Department of Microbiology
Medical School
University of Rijeka

Summary

The aim of this study was to determine the in vitro *antimicrobial activity of calcium hydroxide root canal sealer (Apexit) in direct contact with* Pseudomonas aeruginosa, Escherichia coli, Serratia marcescens *and* Staphylococcus aureus.

The direct contact test (DCT) was performed. The sealer was mixed and placed on the side wall of microtiter plate wells and 10 µl of bacterial suspension (10^6 bacteria) was placed onto its surface. Bacteria were in direct contact with the sealer for 1 hour at 37°C. BHI broth (250 µl) was then added and the growth of each strain was measured after 1 hour, 6 hours, 20 hours and 24 hours.

After 1 hour the number of E. coli *and* P. aeruginosa *decreased to 7-9 x 10^3 and* S. marcescens *to 7 x 10^2 bacteria. The number of* S. aureus *was 1.4 x 10^5 bacteria. Six hour samples showed that the number of* P. aeruginosa *decreased to 10^1 and gram-positive* S. aureus *to 7.5 x 10^4. After 20 hours only* S. aureus *(10^1 bacteria) survived after prior contact with Apexit.*

The 24-hour samples showed complete bacterial growth inhibition of all the bacterial strains tested in DCT with Apexit.

Key words: *antibacterial effect, Direct contact test, Apexit.*

Acta Stomat Croat
2001; 475-477

ORIGINAL SCIENTIFIC PAPER
Received: March 19, 2001

Address for correspondence:

Sonja Pezelj-Ribarić, DDS, PhD
Department of Oral Pathology
Department of Stomatology
Medical School
Braće Branchetta 20
51000 Rijeka
Croatia
tel: +385 51 21 28 65
fax: +385 51 65 11 77

Introduction

It has been established that bacteria and their products are the major etiological agents of endodontic disease (1-3). Instrumentation, irrigation with solution of sodium hypochlorite and intracanal medication are common procedures used to disinfect the root canal. However, it has been shown that after biomechanical preparation of the root canal system bacteria can still be detected and increased rapidly

in the empty canals between appointments (4, 5). Root canal filling materials that have antibacterial activity may play an important role in killing any remaining root canal bacteria. Antibacterial properties of the endodontic sealer can improve the success rate of endodontic therapy (6, 7).

Calcium hydroxide has been used extensively in dentistry (8, 9) and has also been added to several endodontic sealers to improve their biological properties and to enhance antibacterial activity (10). The beneficial antibacterial effect of $Ca(OH)_2$ is attributable to its ionic constituents calcium and hydroxyl ions and to the hydroxyl alkalinising properties during its diffusion through dentine (11).

In several studies, the antibacterial activity of $Ca(OH)_2$ containing sealers, using qualitative Agar diffusion test (ADT), was evaluated (12-14). The limitation of this technique is its dependence on diffusion making it more suitable for testing soluble materials or vapours. To overcome the disadvantages of ADT, a quantitative Direct contact test (DCT) based on measuring the kinetics of bacterial growth during close and direct contact with the tested materials was used (15). The aim of this study was to determine *in vitro* antimicrobial activity of the calcium hydroxide root canal sealer (Apexit) in direct contact with the gram negative fermenters: *Escherichia coli* and *Serratia marrcescens*; gram-negative bacteria: *Pseudomonas aeruginosa*, and gram-positive *Staphylococcus aureus*.

Materials and methods

A commercially available root canal sealer Apexit based on calcium hydroxide (Vivadent, Schaan, Lienchenstein) was used.

Bacterial strains used in this study were *Escherichia coli*, *Serratia marcescens, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. All strains were isolated from the oral cavity and identified by standardised biochemical systems (API E; API NE; API Staph, bioMerieux, Marcy-l'Etoile, France) at the Department of Microbiology, Medical faculty, University of Rijeka, Croatia. The dose of viable organisms was estimated to $1x10^6$/ml according to the McFarlands Standard (bioMerieux, Marcy-l'Etoile, France) and subsequently was measured directly by counting the number of colonies formed CFU on blood agar plates after an overnight incubation at 37°C.

For the investigation Direct contact test (DCT) was used. The sealer was mixed according to the manufacturer's instruction and used within 20 min. from mixing and put on the sidewall of a set of four wells in 96-well microtiter plates. Each bacterial suspension (10^6 bacteria) was then placed in one well on the surface of the root filling material. After incubation for 1 hour in a humid atmosphere, evaporation of the suspension was evident and ensured direct contact between the bacteria and the tested material. 250 µl of BHI broth was then added to the wells and gently vortexed for 2 minutes. After mixing a serial ten-fold dilution was made with BHI broth and placed on blood agar plates. CFU was counted after 1 hour, 6 hours, 20 hours, and 24 hours. Uncoated wells using an identical size inoculum served as a positive control, while wells coated with the tested material but without the bacterial inoculum served as a negative control. All tests were performed in duplicate and repeated three times.

The results of the viable bacteria are expressed as $\log_{10}$ of the mean colony forming units (CFU) per millilitre. The results were compared, using the Student's t-test for independent samples.

Results

The DCT results showed that freshly mixed Apexit exhibited strong antibacterial activity on gram negative enterobacteria, gram negative enterobacteria *S. marcescens*. One hour after direct contact with the sealer, the number of *S. marcescens* decreased from the initial inoculum of 10^6 CFU, to between 6.9 and 7.1 x 10^2 CFU. Six hours later, the number further decreased ($p \leq 0.001$) and *Serratia* was recovered in a very low number (5 and 10 colonies) in only two out of six samples. After 20 hours no more viable bacteria of this species could be isolated (Figure 1).

E. coli was also sensitive to Apexit (Figure 2), but less than *S. marcescens* according to the higher number of viable bacteria obtained after one-hour contact with the sealer (6.8 - 7.2 x 10^3 CFU; $p \leq 0.001$). However, the difference was not significant at the time points analysed later.

Nonfermentative *P. aeruginosa* exhibited higher resistance to the antibacterial effect of Apexit than the tested fermentative gram negatives (Figrue 3). One hour after incubation with the sealer bacterial growth was 3.9 x 10^3 bacteria ($p \leq 0.001$). From the first to the sixth hour of direct contact with the sealer a rapid reduction ($P \leq 0.001$) of *P. aeruginosa* $p \leq 0.001$ was observed, continuing in a similar manner till the 20th hour ($p \leq 0.001$) when only 5 CFU bacteria were isolated from one sample of the six.

The sensitivity of *S. aureus* was generally low during the first six hours (Figure 4). One hour after direct contact with Apexit, there were between 1 and 1.3 x 10^5 viable bacteria, while after six hours their number had decreased to 6.8 - 7.2 x 10^4 ($p \leq 0.001$). A further significant reduction of bacteria was noticed from six to 20 hours of incubation with the sealer (10-60 CFU; $p \leq 0.001$). After 24 hours only five colonies were isolated in two samples ($P \leq 0,001$).

Discussion

One important characteristic of root filling material is antibacterial activity on microorganisms in the endodontic space. In recent years calcium hydroxide has emerged as a popular choice of intracanal medication (17). The efficiency of $Ca(OH)_2$ is due to its late antimicrobial effect (9, 18) anti-inflammatory property (19), low toxicity (20) and osteogeneic repair potential (16).

In this study antibacterial activity of Apexit based on calcium hydroxide was tested on four bacterial strains isolated from the oral cavity. The direct contact test was used to avoid the major disadvantage of the widely used agar diffusion test, which is dependent on physical properties of the tested materials and is therefore more suitable for soluble materials or vapours (13, 14).

Freshly mixed Apexit destroyed all of the bacteria within 24 hours. This is in accordance with the results of Estrele et al. (18) who showed antibacterial effect of Apexit on anaerobic and aerobic bacteria in vitro. This study also used DCT test and bacteria were: *Staphylococcus aureus*, *Fusobacterium nucleatum*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results showed complete antibacterial effectivnes of Apexit after 72 hours. Antibacterial effect of Apexit was also demonstrated by Sjögren et al. (19) who put intracanal medication of calcium hydroxide in root canals of teeth with periapical pathology. They proved the total removal of Actinobacillus actinomycetemcomitans.

In the literature there have been reports of the immediate antimicrobial potential of $Ca(OH)_2$. Fuss et al. (20) showed that a freshly mixed calcium hydroxide based sealer had the capacity of inhibiting the growth of *Enterococcus faecalis* within 4 hours, while Heling & Chandler (21) in an experimental model of contaminated dentinal tubules showed that application of the same filling material to the pulp canal for four hours did not eliminate *E. faecalis* from the infected teeth.

In this study the bacteria gram positive *S. aureus* showed the greatest resistance. The reduction in the number of its colonies was gradual and slow. The optimal pH for growth of *S. aureus* is between 7-7.5 with tolerance of 4.2-9.3. (22). It is therefore unlikely that the alkalinity of calcium-hydroxide inhibits growth of *S. aureus*. It is possible that other components of the material exhibit antibacterial properties as only a few colonies were isolated after 24 hours in contact with Apexit.

Freshly mixed Apexit showed stronger antibacterial activity on gram negative fermentative enterobacteria especially *S. marcescens*. The number of CFU *Serratie* sharply dropped 1 hour after inoculation. At the same time Apexit showed weaker inhibition of *E. coli* and *P. aeruginosa*. After six hours the difference was not statistically significant.

Conclusion

The results of this study showed that Apexit inhibits the growth of tested bacteria species through evaluation by DCT test.