IN VITRO STUDY OF THE EFFECTIVENESS OF INTRACANAL IRRIGANTS ON CANDIDA ALBICANS

Elka Radeva, B. Indjov, R. Vacheva*
Department of Conservative Dentistry,
Faculty of Dental medicine, Medical University, Sofia.
**Department of Microbiology and Virology,*
Military Medical Academy, Sofia.

SUMMARY

Introduction. Frequently isolated pathogens from teeth with necrotic pulp include various enteric bacteria (Klebsiella, Enterobacter), fungi (especially Candida spp.) and enterococci (Enterococcus faecalis). Candida albicans is the most often isolated species. Fungi can be found both in primarily infected teeth and in teeth where the endodontic treatment has failed.

The aim of the current study has been to evaluate the in vitro effectiveness of some of the intracanal irrigants used in eliminating Candida albicans.

Materials and Methods. The study used clinical isolates of Candida albicans from infected root canals.

The effectiveness of the following endodontic irrigants were studied: 17% EDTA, 2% CHX (chlorhexidine), 6% NaOCl (sodium hypochlorite), 3% NaOCl 8 3% H_2O_2 (hydrogen peroxide).

The study used an agar-diffusion method: cavities were made in the agar and then filled with the studied medicaments.

The data were input and processed using the statistical package SPSS 15.0.1. The level of significance for rejecting the null hypothesis was fixed at $p<0,05$.

Results. The largest observed zone of inhibition of bacterial growth was achieved with 6% NaOCl – 25.88mm and 17% EDTA – 24.50mm.

The 2% chlorhexidine solution came third in effectiveness compared to the other solutions – the zone of inhibition of the bacterial growth was 17.75mm.

3% NaOCl was less effective than 6%NaOCl, with an inhibition zone of 14.00mm.

The lowest effectiveness was registered in the case of 3% H_2O_2 – 5.38mm.

Conclusions. In infected root canals there are some microorganisms (Candida albicans, E. Faecalis) that are more resistant to chemical and mechanical procedures and this must be taken into account when using various endodontic irrigants and intracanal medicaments. With the reduction of concentration, the effectiveness of NaOCl against Candida albicans is reduced significantly. The 2% chlorhexidine solution is more effective than 3% NaOCl in eliminating Candida albicans.

Keywords: Candida albicans, intracanal irrigants, infected root canal

INTRODUCTION

The following pathogens are often isolated in teeth with necrotic pulp: enteric bacteria (Klebsiella, Enterobacter), fungi (especially Candida spp.) and enterococci (Enterococcus faecalis).

Candida spp. are gram-positive yeast-like fungi; various studies have reported isolating them from primary endodontic infections. These fungi are oval or round in shape. They develop optimally at a temperature of 37єC and in a slightly acidic medium pH - 6-6,5.

Candida albicans is the most frequently isolated variety. Other isolates include: C. glabrata, C. guilliermondii, C. inconspicua, C. krusei, C. parapsilosis, C. tropicalis, C. crusei (11,10,). They are also part of the normal oral microflora. It is possible to isolate them from plaque, caries, subgingival microflora (6) and active periodontal cavity (1). They enter the root canal from the oral cavity because of being in proximity to the former - in cases of pathologically open endodontium, mistakes in isolating the field of surgery, or when the sealing of the endodontium gives way between separate visits to the dentist during lengthy treatments.

Baumgarthner et al. (5) find Candida albicans in 21% of the samples taken from infected root canals in using the PCR (polymerase chain reaction) method. Kubo et al. report the presence of Candida albicans in 11.36%, while Waltimo et al. have isolated 48 types of fungi from infected root canals with apical periodontitis. In a previous study, we isolated Candida albicans in association with other micro-organisms in 37.5% of cases of chronic infectious periapical periodontitis and an open endodont (2).

Fingi can also be found in teeth that have undergone unsuccessful endodontic treatment. Siqueira et al. have

isolated Candida albicans in 2 out of 24 canals where the endodontic treatment failed. Molander et al. have found Candida albicans in 3 out of 68 samples of teeth having undergone unsuccessful treatment and with chronic periapic lesions.

These reports show that the fungi are among the microorganisms that are most resistant to medicinal treatment. This fact is also demonstrated by Candida albicans' resistance to certain medicaments with standard usage in endodontics, including calcium hydroxide mixed with an inert vehicle.

The multiple experimental studies show that there is constant striving to find more effective medicaments and combinations to eliminate this fungus.

AIM

The aim of this study is to assess the in vitro effectiveness of some intracanal irrigants used for eliminating Candida albicans.

MATERIAL AND METHODOLOGY

The study used clinical isolates of Candida albicans from infected root canals (Periodontitis acuta sicca, Periodontitis chronica gr. diff.). See Fig. 1.

The anti-microbial activity of the following endodontic irrigants was studied:

1. 17% EDTA
2. 2% CHX (chlorhexidine)
3. 6% NaOCl – (sodium hypochlorite), Vista Dental, USA
4. 3% NaOCl (Vista Dental, USA)
5. 3% H_2O_2 (hydrogen peroxide)

The study used an agar-diffusion method; little cavities were dug in the agar and filled with the studied medicaments.

The agar used was Mueller-Hinton, with 5% defibrinated sheep blood (Bul Bio, National Center of Infectious and Parasitic Diseases – Sofia). The Petri dishes were inoculated with a suspension from the respective strain of Candida albicans, prepared in a sterile physiological solution of 0,5 McFarland density.

In each Petri dish, three cavities were filled with medicament – the quantity used for each cavity was 5 µL.

The Petri dishes were preincubated for 30 minutes at room temperature and then incubated at 37єC for 24 and 48 hours.

For reading the results at 24 and 48 hours and determining the antimicrobial activity of the studied substances, the zones of inhibition around the medicaments in the cavities were measured in millimeters.

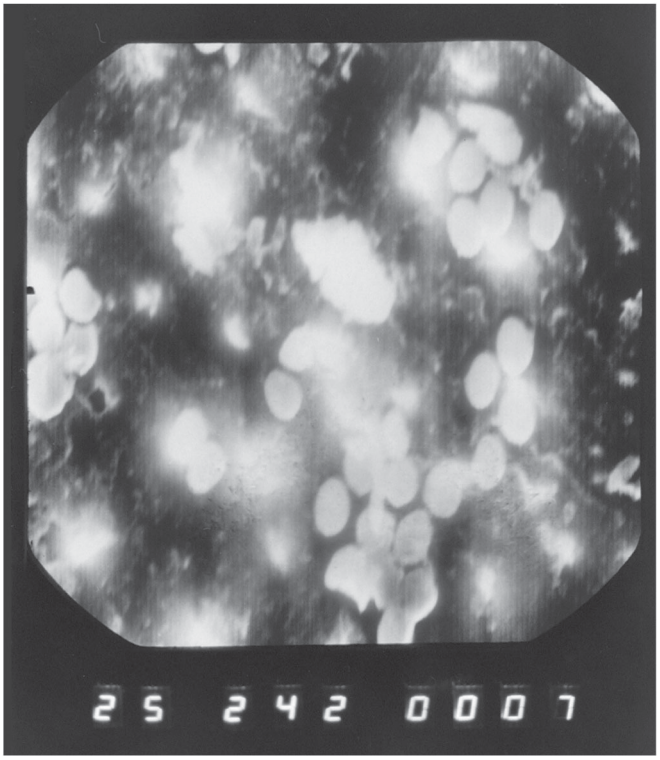

Fig. 1. Dentine in a root canal infected with Candida albicans (magnification x 2420)

Fig. 1. shows colonies of C. albicans with few cells, which we have observed on an SEM.

The data were input and processed using the statistical software package SPSS 15.0.1. The level of significance for rejecting the null hypothesis was fixed at $p<0,05$.

The following methods were applied:

1.Descriptive analysis – the frequency distribution of the considered characteristics according to groups of study were presented in a table.

2.Analysis of variance – calculating the estimates for the central tendency and the dispersion.

3.Graphical analysis – for visualizing the results obtained.

4.Shapiro-Wilk test – to check the normality distribution.

5.Nonparametric test of Kruskal-Wallis – for checking hypotheses of difference among several independent samples.

6.Student's parametric test – for checking hypotheses of difference between two independent samples.

7.Mann-Whitney nonparametric test – for checking hypotheses of difference between two independent samples.

RESULTS

Table 1 shows a comparative analysis of the diameter of the zones of activity in applying the endodontic irrigants used in the current study. The largest zone of inhibition was achieved by the 6% NaOCl solution – 25.88 mm and the 17% EDTA -- 24.50 mm.

The 2% chlorhexidine solution came third in effectiveness among the used medicaments – the zone of inhibition of bacterial growth was 17,75 mm.

The 3% NaOCl solution was less effective than the 6% NaOCl, with a inhibition zone of 14.00 mm. Fig.2

The least impact was achieved by the 3% H_2O_2 – 5.38 mm. Fig.3

The difference between 17% EDTA and 6% NaOCl is statistically correct with regard to the other three irrigants, which also differ significantly from one another (Table 1 and Fig. 4).

Table 1: Comparative analysis of the diameter of the zones of inhibition of bacterial growth according to the irrigants used

Irrigants	N	$\overline{X}$ *	SD	Min	Max
17%EDTA	8	24,50[a]	2,45	22,00	30,00
2%CHX	8	17,75[b]	1,75	14,00	20,00
6%NaOCl	8	25,88[a]	2,17	23,00	28,00
3%NaOCl	8	14,00[c]	0,76	13,00	15,00
3% H_2O_2	8	5,38[d]	0,74	4,00	6,00

* - Similar letters show the lack of significant difference and different letters show there is such difference. ($p < 0,05$).

N – Number of microbial strains

X – Arithmetic mean of the diameter of the zone of activity

SD – Standard deviation

Min – Minimal diameter of the zones in millimeters

Max – Maximum diameter of the zones in millimeters

Fig. 2. Zones of suppression of bacterial growth

1-Above: 17% EDTA
2-Left: 2% CHX
3-Right: 6% NaOCl

Fig. 3. Zones of suppression of bacterial growth

4-Above: 3% NaOCl
5-Below: 3% H_2O_2

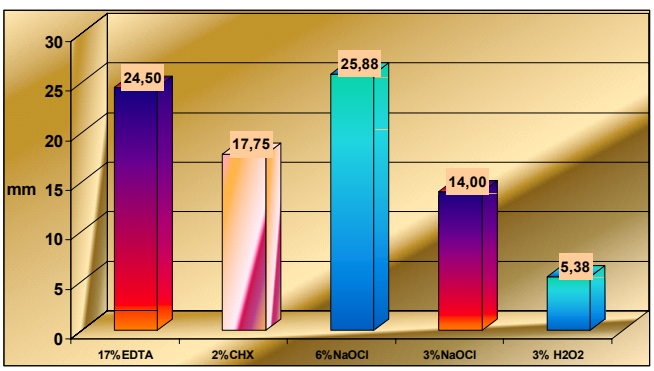

Fig. 4: Arithmetic mean values of the diameter of zone of activity according to the irrigant used

DISCUSSION

Most studies related to the infection of the root canal system focus on the strict anaerobes, since they prevail in non-treated teeth with necrotic pulp. Their presence is related to the ecological condition in the root canal, the feeding, the low oxygenation-reduction potential, pH, temperature, and bacterial interactions that are important for the bacterial growth. Various studies (13,6) show that the obligate anaerobes are easier to eliminate through endodontic procedures than facultative anaerobes (isolated in 19% to 22% of cases); the latter prove more resistant to chemical-mechanical procedures. Microorganisms that survive chemical-mechanical procedures and the application

of root canal medicaments are the enterococci, streptococci, lactobacilli, and some fungi such as Candida albicans (6).

Siqueira study the colonization of root dentine by five types of fungi: Candida albicans, C. glabrata, C. guilliermondii, C. parapsilosis and Saccharomyces cerevisiae. SEM analysis shows that Candida albicans builds colonies along the dentine in the root canal and penetrates the dentine tubules; this defines it as a dentinophile microorganism compared to other species (10). This accounts for the fact that Candida albicans is isolated more often from infected root canals.

The endodontic infection is polymicrobial and not all medicaments are equally effective on all microorganisms in the infected root canal.

The effectiveness of NaOCl has been confirmed by various studies (13, 8, 9). Sodium hypochlorite is a major endodontic irrigant. It is used in treating the root canal; the area is washed with NaOCl between the application of different instruments. This irrigant dissolves the organic tissue and the organic components of the infected layer. It enters into a reaction with the proteins of organic tissue to give chloramine, which is an important anti-bacterial agent.

Sodium hypochlorite destroys the pathogenic microorganisms attached in the biofilm and in the dentine tubules. It has the pH of an alkaloid (11-12). In the current study we have used 3% and 6% NaOCl; in the case of 6% NaOCl the zone of inhibition of Candida albicans bacterial growth – 25.88 mm – was most pronounced compared to the other tested solutions. In the case of 3% NaOCl it was 14.00mm. The results show that a decrease in concentration significantly reduces the effectiveness of NaOCl against Candida albicans.

Chlorhexidine is a strong antiseptic. It displays affinity to dentine, forms depots, and has longer anti-microbial activity.

It is used as a final irrigant but cannot be used as a main irrigant because it cannot dissolve the necrotic tissue. The antimicrobial activity of CHX is pH-dependent, with an optimum between 5.5 and 7.0. As the pH increases, the antimicrobial activity of the substance is significantly reduced. Usually the 2% solution of chlorhexidine is used in endodontics. This concentration was used in our study as well. The solution ranks third in effectiveness compared to the other irrigants, with 17.75 mm zone of inhibition of bacterial growth. Chlorhexidine demonstrated higher activity than the 3% NaOCl – 14.00 mm. When combined with $Ca(OH)_2$, its activity decreases to 13.63 mm, which is still higher than the activity of $Ca(OH)_2$ used on its own – 11.00 mm. Other researchers report similar results(7).

These two endodontic irrigants cannot influence the inorganic components of the infected layer. EDTA (pH -7.3 ethylene diamine tetraacetic acid) and citric acid are used as means of demineralization (chelators).

The chelators have the ability to attach to themselves Ca-ions from the dentine and thus to demineralize the surface layers of the root-canal dentine. Thus more effective use of medication becomes possible and better adhesion between the root-canal fillers and the dentine is achieved (3,4). One major shortfall of EDTA is the significant erosion of inter- and pericanalicular dentine. Should saliva enter the once opened dentine canals, it may cause re-infection. In this study, the 17% solution of EDTA demonstrated a zone of activity of 24.50 mm, thus ranking second in effectiveness.

When used in succession, the two solutions NaOCl and EDTA are more effective in reducing the bacterial flora in the root-canal system than NaOCl on its own (13).

The antiseptic action of Hydrogen peroxide (H_2O_2) is due to the atomic oxygen that is released in case of contact with the tissues. Its use also helps the removal of dentine splinters/flakes and tissue residues. Candida albicans was the least influenced by 3% H_2O_2 – 5.38 mm.

Mechanical treatment the root canal, as well as irrigations with different antiseptic solutions during and after it cannot eliminate all microbes; this is why inserts with various medicaments are placed in the root canal between the appointments.

CONCLUSIONS

When using different endodontic irrigants and intra-canal medicaments one has to keep in mind that in the infected root canal there are microorganisms that are quite resistant to the chemical and mechanical procedures (Candida albicans, E. faecalis).

Among the solutions used for irrigating the root canals, 6% NaOCl was the most effective, followed by the 17% EDTA.

Decrease of the concentration significantly reduces the effectiveness of NaOCl against Candida albicans.

The 2% chlorhexidine is more effective than the 3% NaOCl in eliminating Candida albicans.

BIBLIOGRAPHY

1. Dzhemileva T, Chorbadjiiska L, Kisselova A. "Mikrobni izolati ot aktivni parodontalni djobove". *Aktualna stomatologia*, 1997, Vol.1, 3, 7-10 [in Bulgaria]

2. Radeva E, Vacheva R. Comparative Characteristics of Bacterial Isolates From Teeth With Open and Closed Pulp Chamber in Exacerbated Chronic Periodontitis. *Zubolekarski pregled*, 86, 2004, №1, 18-22 [in Bulgaria]

3. Tomov, G., S. Dermendzhieva, S. Vladimirov. Chelators in Endodontics – Essence and Clinical Application. *Zubolekarski pregled*, 86, 2004, 2, 150-157 [in Bulgaria]

4. Topalova-Pirinska, S. Critical Survey of Medicaments Used in Modern Endodontic Practice, Part 2. Properties and biocompatibility of Canal Irrigants. *Zubolekarski pregled*, 87, 2005, 1, 51-57 [in Bulgaria]

5. Baumgarthner J. C., Watts C.M., Xia T. Occurrence of Candida albicans in infections of endodontic origin. J. Endod., 26, 12, 2000, 695-698

6. Ferrari P. H. P., S. Cai, A. C. Bombana. Effect of endodontic procedures on enterococci, enteric bacteria and yeasts in primary endodontic infections. Int. Endod. J., 38, 2005, 372-380

7. Haenni S. et al. Chemical and antimicrobial properties of calcium hydroxide mixed with irrigating solutions. Int. Endod. J., 36, 2003, 100-105

8. Menezes M. M. et al. In vitro evaluation of the effectiveness of irrigants and intracanal medicaments on microorganisms within root canals.Int. Endod. J., 37, 2004, 311-319

9. Radcliffe C. E. et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces Israeli, A. naeslundii, Candida albicans and Enterococcus faecalis. Int. Endod. J., 37, 2004, 438-446

10. Siqueira J. F. et al. Fungal infection of the radicular dentin. J. Endod., 28, 12, 2002, 770-773

11. Siqueira J. F. et al. Elimination of Candida albicans Infection of the Radicular Dentin by Intracanal Medications. JOE, 29, 8, 2003, 501-504

12. Siqueira J. F., Sen B. H. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Radiol Endod, 97, 5, 2004, 632-641

13. Zehnder M. Root canal irrigants. J. Endod., 32, 5, 2006, 389-397

Address for correspondence:
Dr. Elka Radeva
Department of Conservative Dentistry
Faculty of Dental Medicine, Medical University - Sofia
1, Georgy Sofiyski Blvd., 1431 Sofia, Bulgaria
e-mail: eliradeva@abv.bg
Tel. (+ 359) 888 319 813