

Antimicrobial Activity of 2% Chlorhexidine

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Introduction: A great interest regarding 2% chlorhexidine solution is over its efficiency on gram-negative bacteria, but chlorhexidine sensitive microorganisms include gram-positive bacteria, gram-negative bacteria and fungi. The aim of the study was to analyze the antimicrobial and antifungal action of 2% chlorhexidine solution against the microorganisms isolated from infected root canals before and after irrigation.

Material and method: Clinical material. We took samples from 20 incisors of 20 patients. Sampling procedure. We followed all the rules necessary for sampling under sterile conditions. Three microorganisms: *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* were identified from infected root canals, based on culture, and biochemical characteristics and pathogenicity tests. The statistical analysis was performed using a statistical analysis program (SPSS Statistics 16.0). A log₁₀ transformation of the CFU (colony forming units) values was performed to normalize the data.

Results: The frequency of the isolated microorganisms before irrigation with 2% chlorhexidine solution were: *Enterococcus faecalis* – 3.18±1.84 CFU, *Staphylococcus aureus* – 1.92±0.79 CFU, *Candida albicans* – 2.12±1.10 CFU and after irrigation were: *Enterococcus faecalis* – 0.67±0.20 CFU, *Staphylococcus aureus* – 0.95±0.26 CFU, *Candida albicans* – 1.02±0.35 CFU. The difference between the means of isolated germs' CFU before and after irrigation with 2% chlorhexidine solution is statistically significant (p<0.05).

Conclusions: The use of 2% chlorhexidine solution as endodontic irrigant reduces the number of the studied microorganisms' colony forming units.

Keywords: chlorhexidine, endodontic irrigant, microorganisms

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Introduction

Endodontic irrigation is essential to accomplish a complete cleaning of the root canal. Nowadays, a root canal mechanic treatment is not conceivable without the use of irrigation solutions and proper medication. It is very important for the used irrigation solutions to have a broad antimicrobial and antifungal spectrum, to be stable and effective against necrotic residues [1], to be easily inserted and removed from the inner root canal, and to not injure the pulp and periapical tissues [2]. There is a great interest regarding 2% chlorhexidine solution regarding its efficiency on gram-negative bacteria, but chlorhexidine sensitive microorganisms include gram-positive bacteria, gram-negative bacteria and fungi.

The aim of our study was to analyze the antimicrobial and antifungal action of 2% chlorhexidine solution against the microorganisms isolated from infected root canals before and after irrigation.

Material and methods

Clinical material

We took samples from 20 incisors of 20 patients. The following features were recorded for each patient: clinical symptoms, caries, swelling of periodontal tissues, mobility, periodontal status of the tooth, status of the root canal. All single rooted teeth were diagnosed as having simple and complicated gangrene, but without clinical symptoms

(pain), without periodontal pockets deeper than 4 mm, not mobile and without root fracture. The patients did not report the use of antibiotics for at least a month before treatment.

Sampling procedure

We followed all the rules necessary for sampling under sterile conditions. Before access cavity preparation, the teeth were individually isolated from the oral cavity with a rubber dam. Carious tissue was removed with a sterile high-speed diamond drill bur under water cooling, to present the orifice of the root canal. The working length of the root canal was determined using an electronic apex locator and periapical radiographs (1 mm from the radiographic apex).

We collected two sets of microbiological samples. The first set was collected before irrigation with 2% chlorhexidine solution, by introducing sterile paper cons into the root canal for 60 seconds. After withdrawal the paper cons were inserted in sterile tubes with 0.9% saline solution. The root canals were instrumented using K-type files and Hedström files until the limit established by working length, with "Step-back" technique, which ended after the use of three files larger than the last file used for apical instrumentation. The second set of microbiological samples, collected after irrigation with 2% chlorhexidine solution, was obtained by using sterile cons inserted into the full length of the root canal and kept in place for 60 seconds, which afterwards were placed in sterile tubes with 0.9% saline solution. The inoculations were carried out using the 0.9% saline solution suspensions.

Microbial isolation

The culture media used for inoculation were:

- ▶ Agar diffusion – 5% sheep blood for the isolation of *Enterococcus* spp., *Staphylococcus* spp. and other gram-positive bacteria;
- ▶ Bile esculin agar medium for *Enterococcus* spp.;
- ▶ Pyruvate broth for *Enterococcus faecalis*;
- ▶ Chapman medium for *Staphylococcus aureus*;
- ▶ Sabouraud medium for *Candida* spp.;
- ▶ CandiSelect chromogenic medium for *Candida albicans*.

The Petri dishes were incubated at 37°C for 24 hours. Three microorganisms: *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* were identified from the infected root canals, based on culture and biochemical characteristics and pathogenicity tests. *Staphylococcus* spp. was identified based on pathogenicity factors: coagulase, pigment and hemolysins. *Enterococcus* spp. was identified based on hemolysis on sheep blood agar, colony pigmentation and catalase reaction. *Enterococcus faecalis* was identified based on Gram stains (gram-positive cocci), colony morphology (colonies are punctiform, convex with an entire margin) and negative catalase reaction. By using bile esculin agar medium, the presence of *Enterococcus faecalis* was determined by the blackening of the medium.

We also observed a positive pyruvate-fermentation by the development of a yellow color on pyruvate broth. *Candida* spp. was identified based on morphology, pigmentation of the colonies and odor of the culture. *Candida albicans* was identified based on the detection of hexosaminidase enzymatic activity and purple colored colonies.

Statistical analysis was performed using SPSS Statistics 16.0. A log10 transformation of the colony forming unit (CFU) values was performed to normalize the data. We used the student t test for paired data to analyze the differences between means. A p level <0.05 was considered to be statistically significant.

Results

The frequency of the isolated microorganisms from the infected root canals before and after irrigation with 2% chlorhexidine solution, is shown in Table I and Table II, respectively.

The difference between the means of isolated germs' CFU before and after irrigation with 2% chlorhexidine solution is statistically significant (p<0.05).

Discussions

The microorganisms interested in this study were relevant because they are part of the endodontic flora [3,4].

Enterococcus faecalis is part of the untreated infected root canal flora. Because it is a persistent microorganism, it plays an important role in the etiology of periradicular lesions, that remain following root canal treatment [5]. In patients who received endodontic treatment and retreat-

Table I. The frequency of the isolated microorganisms before irrigation with 2% chlorhexidine solution

Microorganisms	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Colony forming units	3.18±1.84	1.92±0.79	2.12±1.10

Table II. The frequency of the isolated microorganisms after irrigation with 2% chlorhexidine solution

Microorganisms	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Colony forming units	0.67±0.20	0.95±0.26	1.02±0.35

ment, an increased prevalence of *Enterococcus faecalis* was observed, compared with patients with no endodontic treatment history [6].

The current study showed a significant reduction in the number of *Enterococcus faecalis* colony forming units. Similar results were found in other studies. Wang et al. [7] evaluated in their study the efficiency of 2% chlorhexidine gel used as endodontic irrigation solution on microorganisms that are found in the root canals. The results showed a significant decrease in the number of colony forming units of *Enterococcus faecalis* after the use of 2% chlorhexidine gel during mechanical root canal treatment. Vijaykumar et al. [8] evaluated the action of several endodontic irrigation solutions on *Enterococcus faecalis*, showing that the use of an even lower concentration of chlorhexidine (0.2%) also resulted in a significant decrease in the number of colony forming units.

It is well known that *Staphylococcus aureus*, *Candida albicans* and *Enterococcus faecalis* have the ability to neutralize the immunoglobulins and produce the lysis of cells and tissues, leading to a rapid invasion of human tissue [9]. For these reasons they are considered extremely pathogenic and virulent [10], and especially *Staphylococcus aureus* is shown to be multiresistant in a study conducted by Szekely et al. [11]. An experimental study compared the antimicrobial activity of 2% chlorhexidine gluconate, paramonochlorophenol combined with furacin and 1% sodium hypochlorite against *Staphylococcus aureus*, *Candida albicans*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The microorganisms' strains were obtained from a microbiology department and 40 blood agar dishes were used. The results showed that 2% chlorhexidine yielded the largest inhibition zones, and the difference between its inhibition zones and the ones given by 1% sodium hypochlorite was statistically significant [12]. The results of the study conducted by Al-Nazhan et al. [13] showed a total inhibition of the *Candida albicans* growth, when the culture medium was exposed to 2% chlorhexidine solution.

In the present study it has been shown that 2% chlorhexidine solution was efficient in decreasing the microorganisms count from the root canals, results that are consistent with those found in other similar studies. In *in vivo* studies the results regarding the efficiency of the

endodontic irrigation solutions are lower in comparison with in vitro results, because it has been shown in another study that the components of dentin and the contents of the root canal system affect the performance of irrigation solutions [14]. An *in vivo* study showed a 77.78% reduction in the number of anaerobic microorganisms from the infected root canals after irrigation with 2% chlorhexidine solution [15].

Conclusions

The use of 2% chlorhexidine solution as an endodontic irrigant during mechanic root canal treatment reduces significantly the number of the *Staphylococcus aureus*, *Candida albicans* and *Enterococcus faecalis* colony forming units.

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