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Antimicrobial Effect of 0.2% Chlorhexidine in Infected Root Canals

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A B S T R A C T

The aim of this study was to identify bacteria from the infected root canals of teeth with chronic apical periodontitis, and to evaluate the antibacterial effect of 0.2% chlorhexidine (CHX), as an irrigant, in reducing the microbial flora 48h after root canal preparation. A total of 44 subjects were randomly divided in the experimental group and the control group. The first bacterial samples from all root canals were obtained in the beginning, before any treatment. During mechanical instrumentation, root canals were irrigated three times, with 0.2% CHX in the experimental group, and with saline solution in the control group. All canals were dried and temporarily sealed with zinc oxide-sulfate cement. After 48h the second samples were obtained. Bacterial samples were subjected to microbiologic processing. The study indicates that 0.2% CHX is significantly effective in reducing the microbial flora, and could be used as an irrigant solution.

Key words: *chlorhexidine, irrigants, microbiology*

Introduction

The aim of the endodontic treatment of infected root canals with pulp necrosis and chronic periapical lesion is the elimination of bacteria and the inactivation of endotoxins as much as possible before filling[1]. It is well known that only mechanical action of endodontic instruments is not enough for removing the majority of bacteria because of the complexity of internal dental anatomy (apical deltas, lateral canals, accessory canals). Therefore, various irrigating solutions have been recommended as an adjunct during mechanical instrumentation to reduce debris and to eliminate microorganisms from the root canal system[2].

CHX, a bisbiguanidine, was introduced in medicine in late 1940s during the search for antimalaric agents. Chlorhexidine has been used in medicine as a surface disinfectant, and during the last 20 years in dentistry, against plaque and gingivitis. Its antimicrobial activity ranges from pH 5.5 to 7.0[3]. It has antimicrobial activity against Gram-positive and Gram-negative organisms, bacterial spores, lipophilic viruses, and it is relatively nontoxic[3]. CHX has also been shown to have long-term antimicrobial properties because of its ability to bind to mucous membrane and dentine (hydroxyapatite)[4]. A prolonged gradual release of this bound CHX creates a bacteriostatic milieu in root canal over a prolonged period of time[5]. It has been demonstrated residual antimicrobial activity of 2% CHX in root canal system for as long as 168h after instrumentation[6]. CHX is a potent antimicrobial agent against *Enterococcus faecalis*, a microorganism which has been implicated endodontic treatment failures[7]. However, it has been pointed out that CHX should be used in a concentration greater than 0.12% to eliminate *E. faecalis*[8]. The potential factor of virulence is its ability to survive inside polymorphonuclear leucocytes (PMN) and macrophage, making it resistant to killing[9]. It has been demonstrated the total elimination of *Staphylococcus aureus, E. faecalis and Escherichia coli* from root canals after irrigation with 0.12 or 2% CHX[10]. Spratt et al.[11] found that *Prevotella intermedia, Porphyromonas gingivalis and Porphyromonas endodontalis* were significantly resistant to all irrigants for up to 15 min. It has been proved great inhibitory effect of chlorhexidine digluconate against *Candida albicans* at all concentrations[12].

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The aim of this study is to identify bacterial species from infected root canals and to evaluate the antimicrobial effect of 0.2% CHX, as an irrigant, in infected root canals *in vivo*.

Materials and Methods

The study was carried out at the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb, and at the Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb. The study was approved by Ethical Committee, University of Zagreb.

Patient selection

The cohort study consists of 44 randomly selected subjects (20 to 63 years old) of both sexes, who were randomly divided in two groups, the experimental group and the control group. The experimental group included 25 subjects (20 female and 5 male) aged between 21 and 60 years old. In the control group there were 19 subjects (11 female and 8 male) aged between 21 and 63 years old. All subjects were informed about the aim of the treatment, as well as the advantages and disadvantages of the treatment, and informed consent was obtained.

Clinical and radiographic examinations were performed before clinical procedures. All single rooted teeth were diagnosed as having pulpal necrosis and chronic apical periodontitis, 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.

Microbiological sampling and clinical procedure

The teeth were isolated with a rubber dam (Dental Dam, Coltene Whaledent, NY, US), and the operative field was disinfected with 10% proviodon-iodine solution (Betadine, Alkaloid, Skopje, Macedonia) of active iodine[13]. Carious tissue was removed with a sterile high-speed diamond drill under water cooling to present the orifice of the root canal. The operative field was disinfected again with a 10% proviodon-iodine solution of active iodine[10]. All subsequent procedures were performed aseptically. The first microbiological samples (pre-clinical procedure) were collected by introducing a sterile paper point (Johnson & Johnson, New Jersey, US) into root canal for 60 seconds. After withdrawal the samples were placed in transport media for anaerobes (BBL) and sent immediately to the Department of Clinical Microbiology. After that, the working length of the root canal was determined using an electronic apex locator (Endometer ES-02, Artronic, Zagreb, Croatia) and periapical radiographs (1mm from the radiographic apex). Canals were then instrumented using K-type files and Hedström files (Dentsply Maillefer, Ballaigues, Switzerland), 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. During mechanical instrumentation canals were irrigated three times. In the experimental group the root canals were irrigated with a syringe (27-gauge needle) containing 2 mL of 0.2% CHX solution (Plivasept Glukonat, Pliva, Zagreb, Croatia) for 30s. In the control group the root canals were irrigated with a syringe (27-gauge needle) containing 2 mL of saline solution (0.9% NaCl, Pliva, Zagreb, Croatia) for 30s. In both groups, the irrigating solution was agitated with a sterile master apical file size file to working length to make sure that all parts of canal were treated. Canals were then dried with paper points, sterile cotton pellet was placed at the canal orifice and the cavity was temporarily sealed with zinc oxide-sulfate cement (Cavit G, 3M ESPE, Seefeld, Germany) for the next 48 hours[13]. At the next appointment (after 48h), all teeth were aseptically reopened under rubber dam isolation with a sterile high-speed diamond drill under water cooling. The second microbiological samples were obtained in the same manner mentioned for the first appointment, and sent to the laboratory. All root canals were irrigated with 5.25% sodium hypochlorite, dried, and obturated with gutta-percha points (DiaDent, Seoul, Korea) and Diaket sealer (3M ESPE, Seefeld, Germany) using the lateral condensation technique. The master point gutta-percha was ISO#30 for all root canals. Finally, access cavities were sealed with glass-ionomer cement (Fuji IX GP, GC Inc., Tokyo, Japan), used as a base, and a light-cure composite resin (Tetric EvoCeram, Ivoclar Vivadent, Schaan, Liechtenstein), after proper etching, priming and bonding.

Microbiological procedures

Microbiological processing included transport of the samples to the laboratory, cultivation and identification of bacteria. The samples were transported to the laboratory in an anaerobic transport within 3 hours. Samples were spiral-plated on nonselective and selective agar plates. Nonselective agar plates supplemented with 6% sheep blood were used for isolation of facultative anaerobes and yeasts (Blood agar, Columbia agar, Becton Dickinson, Sparks, USA). For cultivation of anaerobes several selective media were used (BHI agar, brucella blood agar, KVLB agar), according to Isenberg[14]. Plates were visually inspected every 48h up to 7 days. Identification was done by macro and micromorphology, and by commercial tests for identification (Api 20A, BioMerieux, Marcy l'Etoile, France).

Statistical analyses

The results were analyzed by the descriptive statistical method, showing the occurrece of particular bacteria species in each group. χ^2-test was used in order to determine statistically relevant differences in the number of subjects with bacteria in root canals, and those without, before and after the treatment, in the experimental and control group.

Results

The distribution of all isolated microorganisms from root canals in the experimental and control group, before and after the treatment, was shown in Table 1.

Figure 1 showed the comparison in the number of isolated microorganisms between the experimental group and the control group, before and after the treatment. In the experimental group there was statistically significant reduction of microorganisms (65.46%) after irrigation with 0.2% CHX (p=0.008). In the control group there was insignificant. reduction of microorganisms (27.45%) after irrigation with saline solution (p=0.146). The results of Hi-quadrat test showed significant differences in the reduction of number of isolated microorganisms between the experimental and the control group, after the treatment (p<0.02).

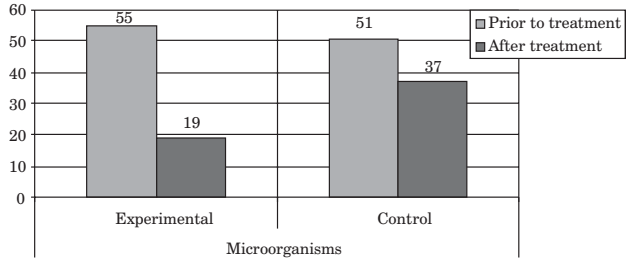

Fig. 1. Comparision between the total number of microorganisms among the experimental and the control group, before and after the treatment, p=0.008, p=0.146.

The pulp space of 2 teeth in the experimental group were sterile in the beginning, before any treatment, while all other contained bacteria. At the second appointment, 48h after root canal preparation and irrigation had been complete, the experimental group yielded 65% positive subjects (with isolated microorganisms from root canals) as compared with 89% positive subjects in the control group.

Discussion and Conclusion

Endodontic microbiota and their byproducts are responsible for the accumulation of inflammatory and immunologic cells in the periapical region causing pulpal and periapical pathosis[15]. Today, special attention has been dedicated to the investigation and discovering of microorganisms which are responsible for periapical pathosis[16]. Since endodontic infections are polimicrobe, it is difficult to determine microorganisms that are responsible for the development of periapical pathosis. In other words, many opportunistic species can be found in infected root canals and many of them are just the result of gangrene and don't cause periradicular damages. Particular species have been determined as a cause of different periapical pathosis[17]. In the study of Siqueira et al.[15] *Porphyromonas endodontalis* (42.6%), *Porphyromonas gingivalis* (27.8%), *Prevotella nigrescens* (7.6%) and *Streptococcus anginosus* (16.7%) were demonstrated as the most represented bacteria. The same author detected

TABLE 1
DEMONSTRATION OF ALL THE ISOLATED MICROORGANISMS FROM ROOT CANALS AMONG THE GROUPS, BEFORE AND AFTER THE TREATMENT

Microorganisms	Experimental group		Control group	
	Before the treatment	After the treatment	Before the treatment	After the treatment
Prevotella oralis	28%	8%	32%	21%
Prevotella intermedia	24%	4%	32%	16%
Prevotella melaninigenica	12%	8%	16%	16%
Prevotella buccae	4%	0%	11%	5%
Prevotella ruminicola brevis	16%	4%	26%	16%
Fusobacterium nucleatum	12%	4%	11%	5%
Veillonela parvula	8%	0%	5%	0%
Porphyromonas gingivalis	20%	8%	16%	16%
Porphyromonas asacharoliticus	12%	4%	11%	11%
Propionibacterium acnes	24%	8%	26%	26%
Eubacterium lentum	4%	0%	5%	5%
Actinomyces odontolitycus	8%	4%	11%	5%
Bacteroides buccalis	8%	4%	11%	5%
Bacteroides uniformis	8%	8%	11%	0%
Bacteroides fragilis	4%	0%	5%	5%
Lactobacillus Jenseni	4%	0%	5%	11%
Peptostreptococcus magnus	8%	4%	11%	16%
Peptostreptococcus indolicus	12%	0%	16%	11%
Streptococcus spp.	4%	8%	11%	5%

fungi in 2% of cases. The present study demonstrated *Prevotella oralis* (28% in the experimental group, 32% in the control group), *Prevotella intermedia* (24% in the experimental group, 32% in the control group), *Prevotella ruminicola brevis* (26% in the control group) and *Propionibacterium acnes* (24% in the experimental group, 26% in the control group) as the most frequently isolated species in infected root canals. Similar results were obtained by Peters et al.[18] who found also *Prevotella intermedia* and *Propionibacterium acnes* as the most represented bacteria, and *Actinomyces odontolyticus* and *Capnocytophaga spp*. as well. This study was based on the identification of anaerobes and facultative anaerobes, while in the study of Peters et al. growth of anaerobes, facultative anaerobes and aerobes were analysed. Rocas et al.[19] found »red complex« which has been connected with the occurrence of periapical pathosis (*Treponema denticola, Porphyromonas gingivalis, Bacteroides forsythus*). The reasons for these different results are probably the variety of microorganisms in the root canal system, different cultivation and identification methods, different processes of sample collection and transport[20]. In addition, it has been pointed out the importance of microorganisms inter-relationship providing an environment in which bacteria multiplies, causing different types of periapical pathosis. Host resistance is another factor that may influence expression of each microorganism and their combinations. Development of modern identification methods, including molecular techniques, gives the opportunity for the detection of previously unidentified species in infected root canals[21,20].

Since mechanical instrumentation does not eliminate and neutralize all microbiota and their toxins from root canal, additional antimicrobial agents become necessary during endodontic treatment[22]. Many studies analysing antimicrobial effects of different endodontic irrigants are preceded by *in vitro* conditions of a particular isolated microorganism. However, an *in vitro* environment, created on an agar plate, cannot provide authentic complex conditions present in infected root canals. And it has been proven that a disinfectant against one particular microorganism is not necessarily efficient in mixed infections that are characterized by many bacterial interactions providing survival to each other in different conditions. Therefore, *in vivo* studies are more desirable when antimicrobial effects of endodontic irrigants are analysed.

Leonardo et al.[23] showed in the *in vivo* study 100% reduction of *Streptococcus mutans,* and 77.78% reduction of anaerobic microorganisms from infected root canals 48h after root canal preparation with additional irrigation with 2% CHX solution. Similar results were obtained in this study where antibacterial effects of 0.2% CHX, in reducing the intracanal microbial flora, had been evaluated. The present study showed significant reduction of all isolated bacteria at the second appointment, 48h after root canal preparation with additional irrigation with 0.2% CHX. CHX was significantly more effective than the saline control in providing a bacteria-free root canal. The most significant reduction, although not total, was for *Prevotella intermedia* (83.33%), *Prevotella oralis* (71.42%), *Proprionibacterium acnes* (60%). These bacteria were also the most represented before the treatment. Also, this study showed 100% reduction of *Peptostreptococcus indolicus, Prevotella buccae, Veillonela parvula, Eubacterium lentum, Bacteroides fragilis, Lactobacillus Jenseni.* These results tally with the results of the *in vivo* study of Zamany et al.[24] where additional rinse with 2% CHX provided more significant reduction of intracanal bacteria compared to the saline control. However, total disinfection of the root canal system wasn't achieved.

Root canal infections are mixed infections and endodontic irrigants permeate throughout dentinal tubules, but their effectiveness is dependent on the type of bacteria found within the tubules[25]. Furthermore, smear layer produced by instrumentation and the inability of CHX to dissolve organic material may prevent the irrigant from achieving contact with all the bacteria within the dentinal tubules[26,27]. CHX has been demonstrated to have no significant effect against bacterial lipopolysaccharide[28]. The study of Tanomaru et al.[29] indicated that biomechanical preparation with 2% CHX did not inactivate the effect of endotoxin (*E. coli* LPS).

The results of this study clearly prove the antimicrobial effect of 0.2% CHX during endodontic treatment. 0.2% CHX may be used as an irrigating solution in root canals due to its intracanal antimicrobial activity, although, total elimination of microorganisms shouldn't be expected.

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ANTIBAKTERIJSKI UČINAK 0,2% KLORHEKSIDINA U INFICIRANIM KORIJENSKIM KANALIMA

S A Ž E T A K

Svrha ovog rada bila je identifikacija pojedinih bakterijskih vrsta u inficiranim korijenskim kanalima zuba s kroničnim apikalnim parodontitisom, te procjena antibakterijske učinkovitosti 0,2% klorheksidina (CHX), primjenjenog kao otopina za ispiranje kanala, na prisutne bakterije, 48 sati nakon preparacije kanala. 44 ispitanika s dijagnozom kroničnog apikalnog parodontitisa su slučajnom metodom razvrstani u ispitnu i kontrolnu skupinu. Prvi mikrobiološki uzorci iz korijenskih kanala uzeti su na početku, prije preparacije i ispiranja kanala testiranim otopinama. Korijenski kanali su tijekom mehaničke instrumentacije po tri puta ispirani; 0,2% CHX-om u ispitnoj skupini i fiziološkom otopinom u kontrolnoj skupini. Korijenski kanali su zatim osušeni te privremeno zatvoreni cink oksid-sulfatnim cementom. Nakon 48 sati prikupljeni su drugi mikrobiološki uzorci iz kanala. Svi uzorci su mikrobiološki obrađeni. Ova studija ukazuje na statistički značajnu učinkovitost 0,2% CHX u smanjenju mikroorganizama u inficiranim korijenskim kanalima te se preporuča njegova upotreba kao ispirućeg sredstva tijekom endodontske terapije.