DOI: 10.15386/cjmed-750

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ENDODONTIC INTRACANAL MEDICATIONS

ANDRADA TONEA[1], MANDRA BADEA[2], LIVIU OANA[3], SORINA SAVA[1], DAN VODNAR[4]

[1]Department of Prosthodontics and Dental Materials, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Prevention in Dentistry, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Department of Anesthesiology and Surgical Propedeutics, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[4]Department of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Abstract

Background and aims. *The sterilization of the entire root canal system represents the main goal of every endodontist, given the fact that the control of the microbial flora is the key point of every root canal treatment. The diversity of microorganisms found inside the root canal and also the resistance of some bacterial species to intracanal medications led to a continuous development of new endodontic products. The present study focuses on the comparison of the antibacterial and antifungal properties of different endodontic products, two commercially available, one experimental plant based extract, and two control substances.*

Methods. *The disc diffusion assay was used to determine the antibacterial and antifungal properties of chlorhexidine, calcium hydroxide, a mix extract between Arctium lappa root powder and Aloe barbadensis Miller gel, Amoxicillin with clavulanic acid and Fluconazole (as control substances). Two of the most common microorganisms found in endodontic infections were chosen: Enterococcus faecalis (ATCC 29212) and Candida albicans ATCC(10231).*

Results. *All tested substances showed inhibition zones around the discs, for Enterococcus faecalis and Candida albicans, including the experimental mix extract of Arctium lappa root powder with Aloe vera gel.*

Conclusion. *The experimental mix extract of Arctium lappa root powder and Aloe vera gel is able to inhibit very resistant microorganisms, like Enterococcus faecalis and Candida albicans.*

Keywords: disc diffusion, antimicrobial, antifungal, endodontic, chlorhexidine, calcium hydroxide, *Arctium lappa, Aloe vera*

Background and aims

The sterilization of the entire root canal system represents a therapeutic key step in every case of endodontic infection. An insufficient biomechanical instrumentation, a poor adapted root canal filling or the reinfection trough a leakage process can lead to the survival of resistant endodontic microorganisms [1,2].

Microbiological cultures studied across time showed that *Enterococcus faecalis* plays a very important role in the etiology of endodontic reinfection. *Enterococcus faecalis* is a Gram positive, facultative anaerobic bacteria. It can resist to alcohol or surfactants and can survive in severe conditions, like extreme high pH, high temperatures or absence of nutrients. Its prevalence in endodontic infections is 40% and varies from 24% to 77% in endodontic

Manuscript received: 13.12.2016
Received in revised form: 25.04.2017
Accepted: 24.05.2017
Address for correspondence: andrada.tonea@umfcluj.ro

reinfections [3,4].

Although the main preoccupation of researchers was the study of endodontic bacteria, yeasts play a very important role in the occurrence of root canal infection and reinfection. The genus *Candida* and in particular *Candida albicans* is the most often recovered yeast from the root canals of teeth with primary endodontic infections [5].

Candida albicans is exceptional isolated alone, in pure cultures, but can be commonly found together with Gram positive and Gram negative microorganisms. It has the capacity to adhere and invade dentinal tubules and to survive to severe environmental conditions [6].

The interest of researchers for intracanal irrigants and antiseptic substances has increased over the years, as they became more aware of the importance of the removal of microorganisms from the endodontic system. Different antiseptic and antibacterial substances were developed, including plant based products, with various actions and therapeutic properties [7].

The aim of this study is to compare the antibacterial and antifungal properties of some intracanal medications and therewith to introduce an experimental antiseptic substance, based on a mix extract between *Arctium lappa* root powder and *Aloe vera* gel.

Materials and method

Bacterial strains, culture conditions and tested substances

For the testing of the antibacterial and antifungal activity the following microorganisms and culture conditions were used: two bacterial strains, a facultative aerobic bacteria, *Enterococcus faecalis* (ATCC 29212) and one yeast, *Candida albicans* (ATCC 10231), purchased from Microbiologics, Saint Cloud, Minnesota, USA. The tested microorganisms were obtained from the American Type Culture Collection. The bacteria were cultured on Columbia agar for *Enterococcus faecalis* and on Sabouraud agar for *Candida albicans* and cultures were stored at 4°C and subcultured once a month. Culture media were purchased from Biomerieux, Lyon, France.

The following substances were tested: chlorhexidine gel 2% (Cerkamed), calcium hydroxide (Calxyd-Spofa) and a plant extract. The plant extract was obtained as follows: 100 mg of burdock powder was suspended in 1 ml distilled water, mixed for 8 hours and filtered by 0.45 µm Millipore filter. The obtained burdock root extract was mixed with 100 ml *Aloe vera* gel. As control substances, specific antibiotics and antifungals were used as follows: Amoxicillin with clavulanic acid for *Enterococcus faecalis* and *Fluconazole* for *Candida albicans.*

Disc diffusion assay

The primary screening test was carried out by disc diffusion, using 100 µl of suspension containing 10^8 CFU/mL of *Enterococcus faecalis* and 10^6 CFU/mL of *Candida albicans*, spread evenly on the surface of the Columbia agar and Sabouraud agar. Sterile 6 mm discs were used and processed, in triplicates, to contain 20 µl chlorhexidine 2%, 20 µl calcium hydroxide and 20 µl of the plant based extract. These discs were then placed on the inoculated agar. The control substances were also placed on the inoculated plates.

For *Enterococcus faecalis*, the inoculated plates were incubated for 48 h at 37°C in gas bags (GenBag Co2, Biomerieux, Lyon, France) and for *Candida albicans* the inoculated plates were incubated for 2-3 days at 30°C. Inhibition zones around discs indicated the presence of antimicrobial and antifungal activity. For optimal fidelity of results, each assay was repeated three times.

Data analysis

Data analysis was performed by applying the ANOVA one way test, to investigate the statistical significance of the data between test groups.

Results

Antimicrobial efficacy test

Enterococcus faecalis and *Candida albicans* were efficiently inactivated by some of the tested substances (Figure 1). The average inhibition zone of *Enterococcus faecalis* for chlorhexidine 2% was 14.15 mm, for calcium hydroxide 0.04 mm, while the plant mix extract showed an average inhibition diameter of 5.69 mm. The control substances (Amoxicillin and clavulanic acid) exhibited an average inhibition diameter of 13.66 mm (Table I).

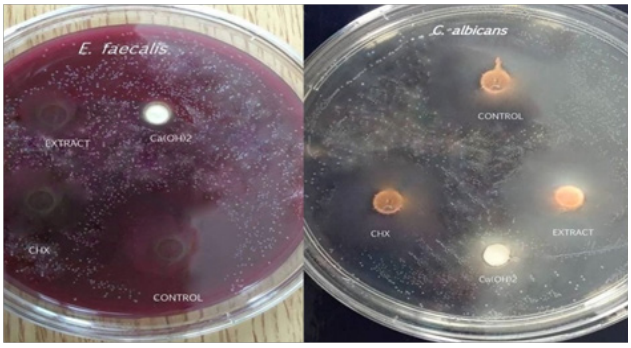

Figure 1. Antimicrobial efficacy test for *Enterococcus faecalis* showing inhibition zones around chlorhexidine 2%, calcium hydroxide, Amoxicilin and clavulanic acid, experimental mix extract. Antimicrobial efficacy test for *Candida albicans* showing inhibition zones around chlorhexidine 2%, calcium hydroxide, Fluconazole and experimental mix extract.

Table I. Antimicrobial efficacy test: diameters of inhibition zones (mean and standard deviation) for *Enterococcus faecalis* ATCC-29212 around the tested substances.

Group	Count	Inhibition zone (mm)	
		Mean	Standard deviation (+/-)
Control	3	13.66	0.030
CHX 2%	3	14.15	0.020
$Ca(OH)_2$	3	0.04	0.020
Extract	3	5.69	0.010

The average inhibition diameter of *Candida albicans* for chlorhexidine 2% was 12.18 mm, followed by the average inhibition diameter of the plant mix extract, 11.62 mm. The average inhibition diameter for calcium hydroxide was 0.09 mm. The control substance (Fluconazole) exhibited an average inhibition diameter of 12.68 mm (Table II).

All the test groups showed a statistically significant difference when compared to the control group ($p<0.05$).

Table II- Antimicrobial efficacy test: diameters of inhibition zones (mean and standard deviation) for *Candida albicans* ATCC-10231 around the tested substances.

Group	Count	Inhibition zone (mm)	
		Mean	Standard deviation (+/-)
Control	3	12.68	0.026
CHX 2%	3	12.18	0.017
$Ca(OH)_2$	3	0.09	0.026
Extract	3	11.62	0.020

Discussion

Previous research evaluated the effectiveness of different antiseptic products, as root canal therapy agents. Several studies proved the antimicrobial effect of chlorhexidine. From its use as an irrigant solution [8] to the use in form of gel, directly inside the root canal [9], chlorhexidine is known as a reliable antimicrobial agent.

Calcium hydroxide is mostly used for its antiseptic properties. However, the mechanisms of this substance are still being studied. Regarding its role against endodontic biofilms, studies showed that calcium hydroxide is not as effective as other substances used as intracanal medications [10].

In the continuous pursuit of developing the antimicrobial endodontic treatment, a number of plants were studied. *Propolis*, *Morinda citrifolia* (noni fruit), *Marticaria recutitia* (chamomile), *Melaleuca alternifolia* (tea tree), *Aloe vera* and *Arctium lappa* (burdock) are some examples, whose pharmaceutical characteristics have been recognized along the time [11].

Arctium lappa, also known as burdock, is a plant commonly found in Europe [12] and acknowledged for its healing effects. In the traditional medicine, the root of burdock was known for its antioxidant and antidiabetic properties. Antimicrobial effect of *Arctuim lappa* in dentistry, against oral microorganisms and also against microorganisms associated with endodontic infections, were investigated and proven during several studies [13,14].

Antimicrobial, antifungal and anti-inflammatory properties of *Aloe vera* are highly recognized all over the world. The plant is used in different areas of general medicine, but also of dental medicine. From the use as an oral dentifrice [15] or as a local drug in periodontal pockets [16] in periodontology, to the use of *Aloe vera* gel against microbial strains in endodontic infections, a great variety of dentistry domains were covered by the properties of this plant. Regarding the endodontic treatment, the antimicrobial properties of *Aloe vera* were previously investigated and compared with other plants and antiseptic substances [17]. The efficiency of *Aloe vera* was also studied in case of the root canals of primary teeth, exhibiting a higher antimicrobial activity compared to medications based on eugenol [18].

The huge diversity of endodontic microorganisms that populate the root canal system and also their different metabolisms influence the response to the intracanal dressings applied during the treatment.

Candida albicans is the predominant yeast identified directly from the infected root canals [19]. It is able to diminish the host defense system with the help of various characteristics such as adhesion to different types of surfaces, production of enzymes, morphologic transition or formation of the biofilm [20]. *Candida albicans* is present in a high percentage in the previously treated root canals [21].

Enterococcus faecalis is known for its resistance to antiseptic root canal medications. It is frequently present in primary endodontic infections, but also in the case of reinfection of previously filled root canals. *Enterococcus faecalis* can be found in biofilms, together with other microorganisms and rarely alone, as a single bacteria [22,23,24].

Conclusions

Based on this study, chlorhexidine 2% exhibits the most efficient antimicrobial activity against *Enterococcus faecalis* and *Candida albicans*.

Calcium hydroxide demonstrates a very low efficiency against both microorganisms and should not be used as an antimicrobial substance on its own, but rather for its other biological and chemical properties.

The experimental mix extract of *Arctium lappa* root powder and *Aloe vera* gel is very efficient against *Candida albicans*, but exhibits a smaller diameter of inhibition for *Enterococcus faecalis*. Considering these results, further studies are necessary in order to evaluate the antibacterial effect of this product.

Given the fact that endodontic bacteria are organized in biofilms, further investigations must be conducted, to determine the behavior of the experimental product, not only against single microorganisms, but also against complex bacterial communities.

References

1. Hwang JH, Chung J, Na HS, Park E, Kwak S, Kim HC. Comparison of bacterial leakage resistance of various root canal filling materials and methods: Confocal laser-scanning microscope study. Scanning. 2015;37(6):422–428.

2. Siqueira JF Jr, Rôças IN, Ricucci D, Hülsmann M. Causes and management of post-treatment apical periodontitis. Br Dent J. 2014;216(6):305-312.
3. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93–98.
4. Sedgley C, Buck G, Appelbe O. Prevalence of Enterococcus faecalis at multiple oral sites in endodontic patients using culture and PCR. J Endod. 2006;32(2):104–109.
5. Shah N, Madhu KS, Murthy BS, Hemanth B, Mathew S, Nagaraj S. Identification of presence of Candida albicans in primary root canal infections: An in vitro study. Endodontology. 2016;28(2):109-113.
6. O'Donnell LE, Millhouse E, Sherry L, Kean R, Malcolm J, Nile CJ, Ramage G. Polymicrobial Candida biofilms: friends and foe in the oral cavity. FEMS Yeast Res. 2015 Nov;15(7). doi: 10.1093/femsyr/fov077.
7. Mohan S, Gurtu A, Singhal A, Vinayak V. Naturopathy and Endodontics-A Synergistic approach. JDSOR. 2012 Oct-Dec:27-29.
8. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299-303.
9. Sinha N, Patil S, Dodwad PK, Patil AC, Singh B. Evaluation of antimicrobial efficacy of calcium hydroxide paste, chlorhexidine gel, and a combination of both as intracanal medicament: An in vivo comparative study. J Conserv Dent. 2013;16(1):65-70.
10. Zancan RF, Vivan RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, Duarte MA. Antimicrobial Activity and Physicochemical Properties of Calcium Hydroxide Pastes Used as Intracanal Medication. J Endod. 2016;42(12):1822-1828.
11. Pujar M, Makandar S. Herbal usage in endodontics-A review. Int J Contemp Dent 2011;2:34-37.
12. Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA. Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae). Vol. 4. Cambridge University Press. 2006; p. 215.
13. Gentil M, Pereira JV, Sousa YT, Pietro R, Neto MD, Vansan LP, de Castro França S. In vitro evaluation of the antibacterial activity of Arctium lappa as a phytotherapeutic agent used in intracanal dressings. Phytother Res. 2006;20(3):184-186.
14. Groppo FC, Bergamaschi Cde C, Cogo K, Franz-Montan M, Motta RH, de Andrade ED. Use of phytotherapy in dentistry. Phytother Res. 2008;22(8):993-998.
15. Pradeep AR, Agarwal E, Naik SB, Clinical and microbiologic effects of commercially available dentifrice containing aloe vera: a randomized controlled clinical trial. J Periodontol. 2012;83(6):797-804.
16. Bhat G, Kudva P, Dodwad V. Aloe vera: Nature's soothing healer to periodontal disease. J Indian Soc Periodontol. 2011;15(3):205-209.
17. Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against Enterococcus faecalis: An in vitro study. J Conserv Dent. 2012;15(3):293-297.
18. Kriplani R, Thosar N, Baliga MS, Kulkarni P, Shah N, Yeluri R. Comparative evaluation of antimicrobial efficacy of various root canal filling materials along with aloevera used in primary teeth: a microbiological study. J Clin Pediatr Dent. 2013;37(3):257-262.
19. Waltimo TM, Haapasalo M, Zehnder M, Meyer J. Clinical aspects related to endodontic yeast infections. Endod Topics. 2004;9:66–78.
20. Waltimo TM, Haapasalo M, Zehnder M, Meyer J. Clinical aspects related to endodontic yeast infections. Endod Topics. 2004;9:66–78.
21. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(5):632-641.
22. Kumar J, Sharma R, Sharma M, Prabhavathi V, Paul J, Chowdary CD. Presence of Candida albicans in root canals of teeth with apical periodontitis and evaluation of their possible role in failure of endodontic treatment. J Int Oral Health. 2015;7(2):42-45.
23. Benbelaïd F, Khadir A, Abdoune MA, Bendahou M, Muselli A, Costa J. Antimicrobial activity of some essential oils against oral multidrug–resistant Enterococcus faecalis in both planktonic and biofilm state. Asian Pac J Trop Biomed. 2014;4(6):463-472.
24. Vivacqua-Gomes N, Gurgel-Filho ED, Gomes BP, Ferraz CC, Zaia AA, Souza-Filho FJ. Recovery of Enterococcus faecalis after single-or multiple-visit root canal treatments carried out in infected teeth ex vivo. Int Endod J. 2005;38(10):697-704.
25. Rôças IN, Siqueira JF Jr. Characterization of microbiota of root canal-treated teeth with posttreatment disease. J Clin Microbiol. 2012;50(5):1721-1724.