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Original article

COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF PHOTOACTIVATED DISINFECTION WITH FOTOSAN AND STANDARD ENDODONTIC TREATMENT

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ABSTRACT

Introduction: The purpose of endodontic treatment is to eliminate the bacterial infection in the root canal system and allow healing of apical periodontitis. Sometimes the anatomical complexity of the root canal system makes complete removal of bacteria almost impossible even if the conventional methods of chemo-mechanical debridement are performed strictly according to the protocol. So additional methods of root canal disinfection can be applied such as photoactivated disinfection (PAD).

Purpose: The aim of our study was to compare the antibacterial activity of PAD with FotoSan, and conventional endodontic therapy in the treatment of infected root canals.

Materials and methods: The study involved 36 teeth of patients who are diagnosed with pulp necrosis or with the periapical chronic periodontitis and require endodontic treatment and divided into two groups of 18 teeth each. The first microbiological sample was taken by placing a sterile paper point in the root canal, after endodontic access cavity preparation.

All root canals are prepared by Protaper Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). In the first group, the disinfection of the root canals was made with photoactivated disinfection with FotoSan. In the second group was performed the endodontic treatment with the standard protocol of irrigation - 2.5% sodium hypochlorite solution and 17% EDTA. After that, the root canals are dried with sterile points, and a second microbiological sample is taken.

Results: In all compared pairs, there was no significantly different effect regarding the number of microorganisms.

Conclusions; The disinfection with NaOCl has the strongest antimicrobial effect in this study. Second place is occupied by PAD with FotoSan. We believe that the established antimicrobial effect of PAD make the method appropriate as complementary to routine endodontic treatment.

Keywords: Endodontic treatment, root canal disinfection, photoactivated disinfection,

INTRODUCTION

The purpose of endodontic treatment is to eliminate the bacterial infection in the root canal system and allow healing of apical periodontitis. Primary root canal therapy is a highly predictable procedure; however, the inability to sufficiently disinfect the root canal system may lead to failure or persistent apical pathosis [1]. While mechanical debridement combined with chemical irrigation removes the bulk of infecting microorganisms, residual bacteria are readily detectable in approximately one-half of teeth just prior to obturation [2]. Certain operative problems such as inadequate instrumentation, a missed canal, or an inadequate restoration may lead to complications after the endodontic treatment. [3]. In addition, the anatomical complexity of the root canal system makes complete debridement of bacteria almost impossible even if conventional methods of chemo-mechanical debridement are performed strictly according to the protocol [3, 4, 5].

Photodynamic therapy (PDT) is a technique that utilizes reactive oxygen species (ROS) produced by a nontoxic dye or photosensitizer (PS) molecule in the presence of low intensity visible light to kill mammalian or microbial cells. The photochemical process involves exciting the PS molecule with visible light of the appropriate wavelength matched to an absorption band. The PS molecule goes to the first excited singlet state which may then undergo intersystem crossing to slightly lower energy but longer-lived triplet state. The long-lived triplet state further reacts via type I or/and type II photochemical pathways to produce superoxide, hydroxyl radicals [6, 7], and singlet oxygen [8] respectively, leading to cytotoxicity.

Though PDT was discovered in the field of microbiology over 100 years ago, it has been mostly applied for cancer treatment and ophthalmology. Its application in the field of infectious diseases underwent a major setback with the discovery of antibiotics in the 1940s. But with the recent rise in antibiotic resistance throughout the world, there has been renewed interest in alternative antimicrobial therapies. Antimicrobial PDT also known as photoactivated disinfection (PAD) represents an alterna-

tive treatment for drug resistant pathogens and has made a comeback as a possible approach to treat multidrug resistant infections. [9, 10, 11].

The aim of our study was to compare the antibacterial activity of PAD with FotoSan, and conventional endodontic therapy in the treatment of infected root canals.

MATERIALS AND METHODS

The study involved 36 teeth of patients who are diagnosed with pulp necrosis or with chronic periapical periodontitis and require endodontic treatment.

Case selection

Patients complete medical history was taken. Those who have systemic diseases or have taken antibiotics for the last three months are excluded from the study. Each patient signs an informed consent. In each case (tooth), a preliminary X-ray is taken to detect the presence of periapical changes and get an idea of the morphology, length and number of root canals.

The teeth are isolated with a rubber dam. Then they are cleaned with 2% chlorhexidine solution. Sterile burs are used for the preparation of the endodontic cavity and access. Old obturations and carious lesions are carefully removed. Microbiological samples are taken by placing a sterile paper point in the root canal for 60 seconds. After removing the paper point from the root, it is immediately placed in a sterile transport environment and then transported to a microbiological laboratory. **This is the initial microbiological sample.**

The treated teeth are divided into **two groups** depending on the method used for root canal disinfection:

I group - 18 cases - root canals are prepared by Protaper Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). Root canal irrigation is performed with sterile saline solution, after working with each instrument.

Once the root canals have been prepared, they are dried with sterile paper points. **In this group, the disinfection is made with photoactivated disinfection with FotoSan.** A diode laser with a wavelength that matches the absorption spectrum of the photosensitizer FotoSan is used. A photosensitizer - 0.5 ml is introduced into the root canals.For radiation, a "Lacsta-Milon" laser with a wavelength of 665 nm, 1 minute with the following parameters: F = 0 (continuous emission), PR = 4, E = 999, P = 200, power of 200 mW / cm² to activate the photodynamic process. Irradiation is performed with circular movements in the apical-coronary direction for 1 minute with a fiber-optic fiber with a thickness of 200 μm at 1 mm from the working length. Root canals are irrigated with saline to remove the photosensitizer, dried with sterile paper points, and a microbiological sample is taken.

II group - 18 cases - root canals are prepared by Protaper Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). After working with each canal instrument, the root canals are irrigated with 2.5% sodium hypochlorite solution and 17% EDTA. Root canals are dried with sterile paper points, and a second microbiological sample is taken.

RESULTS

In all compared pairs, there was no significantly different effect regarding the number of microorganisms (MO) (Table 1, 2, 3, 4).

Table 1. Antibacterial activity of PAD with FotoSan in infected root canals

Sample number	Isolated microorganisms prior to treatment	Amount of microorganisms /cfu/ml	Isolated microorganisms after disinfection with PAD	Amount of microorganisms /cfu/ml
1	*Streptococcus gordonii*	5.100 000	No	No
	Neisseria polysacharea	2.10 000	No	No
2	*Enterococcus .faecalis*	8.100 000	No	No
	Bacillus brevis	6.100 000	Bacillus brevis	4.1 000
3	*Streptococcus pyogenes*	3.100 000	No	No
4	*Klebsiella pneumoniae*	7.1000 000	K. pneumoniae	2.10 000
5	*Streptococcus mutans*	3.100 000	Str.mutans	3.1 000
	Actinomyces.viscosus	6.10 000	No	No
6	*E. faecalis*	2.10 000 000	E.faecalis	3.1000
	Lactobacillus. acidophillus	5.10 000		
7	*Staphylococcus aureus*	8.10 000	No	No
	Streptococcus mitis	2.100 000	No	No
8	*E.faecalis*	4.100 000	No	No
9	*S.aureus*	1.100 000	No	No
	Neisseria. flavescens	2.100 000	No	No

10	*Streptococcus sanguis* *Moraxella. catarrhalis*	3.100 000 2.100 000	No No	No No
11	*E. faecalis*	3.100 000	No	No
12	*Stapylococcus.haemolyticus* *E. faecalis*	8.1000 000 4.10 000	Staph.haemolyticus	3.100
13	*S.mutans* *Cor. xerosis*	2.100 000 5.10 000	No No	No No
14	*S. aureus* *Enterobacter. cloaceae*	4.100 000 5.1000 000	No Ent. cloaceae	No 2.10 000
15	*Streptococcus intermedius* *Actinomyces neuii*	2.10 000 9.10 000	No No	No No
16	*S.mutans* *S. mitis*	3.100 000 1.100 000	No No	No No No
17	*E.faecalis*	1.100 000	No	No
18	*E.faecalis* *Enterobacter. aerogenes*	8.10 000 9.100 000	No E. aerogenes	No 3.1 000

Table 2. Antibacterial activity of 2,5% NaOCl and 17% EDTA in infected root canals

Sample number	**Isolated microorganisms prior to treatment**	**Amount of micro-organisms /cfu/ml**	**Isolated microorganisms after disinfection with 2,5% NaOCl and 17% EDTA**	**Amount of micro-organisms /cfu/ml**
1	*E.faecalis* *A.viscosus*	5x100 000 3x10 000	No No	No No
2	*Streptococcus parasanguis* *Kingella denitrificans*	4x100 000 7x10 000	No No	No No
3	*Klebsiella.pneumoniae*	9x100 000	K.pneumoniae	1x100
4	*Candidaalbicans*	3x 10 000	No	No
5	*S.mitis*	2x 10 000	No	No
6	*E.faecalis* *E. cloaceae*	3x100 000 2x10 000	No No	No No
7	*Arcanobacterium haemolyticum* *E. faecalis* *Bacillus brevis*	8x100 000 5x10 000 6x10 000	No No No	No No No
8	*S.sanguis* *Corynebacteriumulcerans*	4x1000 000 1x10 000	Str.sanguis No	2x1000 No
9	*E. faecalis* *Eikenella.corrodens*	8x100 000 9x100 000	No No	No No
10	*Str.pyogenes* *Lactobacillus fermentum*	6x 100 000 6x10 000	No No	No No
11	*S.gordonnii* *Kingella kingae* *Neisseria polysaccharea*	6x 100 000 5x100 000 2x10 000	No No No No No	No No No
12	*S.aureus* *E. faecalis* *Streptococcus.constelatus*	6x10 000 8x10 000 3x 100 000	No No No Str.constelatus	No No 9x100

13	*Gemella morbillorum*	8x100 000	No	No
	S.aureus	2x10 000	No	No
14	*E.faecalis*	7x100 000	No	No
	C.matruchotii	5x10 000	No	No
15	*E.faecalis*	6x10 000	No	No
	Actinomyces neuii	7x100 000	No	No
16	*Morganella morganii*	8x100 000	M.morganii	3x10
	Staph.aureus	9x10 000	No	No
17	*E.faecalis*	4x1 000 000	No	No
	Streptococcus.anginosus	6x10 000	No	No
	Neisseria mucosa	5x 10 000	No	No
18	*E.coli*	2x100 000	No	No
	Candida albicans	3x 1 000	No	No

Table 3. Numbers of microorganisms before and after treatment

Method	Before and after treatment	N	Amount of microorganisms /cfu/ml				p
			Mean	Median	Min	Max	
NaOCl/EDTA	Before treatment	18	1 035 722,2	755 000,0	20 000,0	4 110 000,0	<0.001
	After treatment	18	168,3	0,0	0,0	2 000,0	
FAD FotoSan	Before treatment	18	2571666,6	400 000,0	100 000,0	20 050 000,0	<0.001
	After treatment	18	2238,8	0,0	0,0	2 000,0	

In all two methods, significant differences in the amounts of microorganisms before and after treatment ($p < 0.001$) have been observed (Table 4).

Table 4. Numbers of microorganisms before and after treatment

Before and after treatment	Method	Number of samples	Amount of microorganisms				p
			Mean	Median	Min	Max	
Before treatment	NaOCl/EDTA	18	1 035 722,2	755 000,0	20 000,0	4 110 000,0	0,569
	PAD Fotosan	18	2571666,6	400 000,0	100 000,0	20 050 000,0	
After treatment	NaOCl/EDTA	18	168,3	0,0	0,0	2 000,0	0,130
	PAD Fotosan	18	2238,8	0,0	0,0	2 000,0	

NaOCl has the most strong antimicrobial activity in vivo. The remaining causative agents, after the irrigation and disinfection of root canals, are in a small amount – only 10%.

After in vivo PAD with FotoSan, approximately 23% of the isolates remained with 3 lg less than the initial isolation in smaller amount 1000-10,000 cfu / ml again at the expense of the enterobacteriaceae from the group KES, Enterobacter spp., Klebsiella spp.

DISCUSSION

From the two tested patient groups with 18 samples in each group, predominantly polymicrobial associations and rarely monoinfection with a predominance of Gram-positive species were isolated.

It is noteworthy that the most strong antimicrobial activity in vivo has NaOCl (only 10% of the initially isolated microorganisms remain after the action of this disinfection method - the effect is 90%) and PAD with FotoSan (only 23% of the initially isolated microorganisms remain after the impact of this disinfection method, i.e. the effect is 77%). Microorganisms (MO) remaining after treatment are Gram-positive cocci - oral streptococci that are likely to reinfect the dental canals despite the successful removal of the other causative agents from the original association which they were isolated from. Other MO that remain after treatment are enterobacteriaceae of the KES group, *Enterobacter spp., Klebsiella spp*. These species form extremely rigid biofilms due to the overproduction of substances in capsule form and many other ad-

hesion molecules on the surface of their cell wall as outer membrane proteins, lipopolysaccharides and adhesive piles. They are also polyresistant to many antimicrobial agents and are the cause of problematic in-hospital infections. Our results coincide with data from other studies showing a very good antibacterial effect of PAD [4, 12]. Some authors' panels - Fonseca et al. (2008) [5] reported a 99.9% reduction in MO after PAD application. The difference in the results obtained by us, and these researchers may be due to differences in the characteristics of light sources, concentration and amount of photosensitizers, etc.[4].

The results of this clinical study show a very good antibacterial effect of PAD with FotoSan. This is a prerequisite for the usage of the method as additional in endodontic treatment for root canal (RC) disinfection, especially in cases where it is necessary to work with lower concentrations of the rinsing solutions.

The conducted clinical study allows for the reduction of MO in RC after treatment applying the two methods. The used microbiological method permits discovering the MO only in RC lumen, i.e. those MOs, which adhere to the paper point while taking the second microbiological sample. It does not give an idea of the MOs that have stuck to the canal walls and that have entered the dentinal tubules and micro-canals of the apical delta. They are the cause of root canal system re-infection after filling the canal and the appearance of periodontitis after the treatment of infected RC or the failure to treat existing periodontitis. However, the microbiological method used makes it possible to make a comparative assessment of the effectiveness of the two methods. The weaker effect of PAD compared to the conventional method of rinsing solutions in our study was obtained using the specific conditions of the experiment with the particular the photosensitizers FotoSan (concentration, amount, irradiation time, duration of exposure, type of MO, standardized conditions for taking the microbiological sample). But FotoSan is just one of the many photosensitizers and probably not the best. Preclinical studies with Zn-phthalocyanine showed that it has a better effect compared to FotoSan [12]. New future clinical studies after the certification of Zn-phthalocyanine will show their effectiveness as photosensitizers in PAD in root canal treatment.

CONCLUSION

The disinfection of root canals with NaOCl has the strongest antimicrobial effect in clinical studies (90% against all microbial isolates). Second place is occupied by PAD with FotoSan (with slightly lower antimicrobial effect against polyresistant klebsiella and enterobacter) having 77% antibacterial effect against all microbial isolates.

In the clinical application of the two methods, we found that there was no evidence of staining soft and hard tooth tissues for hypersensitivity or toxicity reactions. We believe that the lack of an irritant effect of light or and the established antimicrobial effect of PAD make the method appropriate both as complementary to routine ones and as methods of choice in situations severely impeding the conventional method of rinsing with antiseptic solutions.

The microbiological studies were conducted at Bulgarian Academy of Sciences, Microbiology Institute "Stefan Angelov" (associated with the Institute "Pasteur" in Paris and the Department of Microbiology at the Medical Faculty of Medical University - Sofia). They were carried out by Assoc. Prof. R. Gergova (Department of Medical Microbiology, Medical Faculty, Medical University of Sofia).

For quantitative evaluation of microbial isolates was used the quantitative method of strikes for inoculation [IMAB 2013]. The biochemical identification of endodontic pathogens was conducted using the identification systems suach as Crystal GP, Crystal NH and Crystal Ana BD (Becton Dickinson, Germany)

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