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Research Article

Antimicrobial Efficacy and the Ability to Dissolve Pulp Tissues of Bio pure MTAD and Sodium Hypochlorite

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Abstract

The Aim: of this study is to compare the antimicrobial efficacy and the ability to dissolve pulp tissues of Bio pure MTAD and sodium hypochlorite as endodontic irrigants.

Methods: A total of 36 patients were divided into three groups of 12 teeth each according to the three different irrigating solutions used (Bio pure MTAD, 5.25% and 2.5% sodium hypochlorite). Cleaning and shaping were completed for every root canal using the corresponding irrigant and the root canal for every case was ready for obturation. The swabs were transferred aseptically into Eppendorf tube. Microbial growth of *Enterococcus faecalis* was verified by blackening of Bile Esculin Azide agar. Furthermore, thirty six sterile Wassermann tubes were divided into three groups according to the tested materials. A piece of immediately extirpated vital human pulp (2mm x 1mm) was placed in each tube. All tubes were incubated for 24 hours at 37 °C. Conditions of the pulp tissue were examined every hour by means of a magnifying lens. At the end of 24 hours the remaining tissues of any pulp specimen were examined under the Stereomicroscope.

Results: Bio pure MTAD completely inhibited the growth of *Enterococcus faecalis*, however, sodium hypochlorite had poor antimicrobial efficiency against *Enterococcus faecalis*. Sodium hypochlorite dissolved extirpated pulp tissues completely; while Bio pure MTAD did not.

Conclusions: combination of using Sodium hypochlorite during cleaning and shaping and Bio pure MTAD as a final reins is better.

Keywords: Bio pure MTAD; *Enterococcus faecalis*; Antimicrobial Efficiency; Sodium Hypochlorite

Introduction

The most common causative factor of pulpal disease is microorganisms. This is because the body defending cells and molecules from periradicular tissues do not function in such tissue [1]. When the pulp is necrotic, only a confined number of species can colonize in the pulp space. Some groups of oral microbial species have been conveyed to be associated with particular forms of periradicular diseases [2]. In general, species more frequently found in primary root canal infections usually belong to the genera *Bacteroides, Fusobacterium, Prevotella, Porphyromonas, Treponema, Peptostrep-*

tococcus, Eubacterium, Actinomyces, and *Streptococcus* [3]. *Enterococcus faecalis* is Gram-positive, nonspore-forming cocci that occur singly, in pairs, and in short chains. It is a facultative anaerobe and is a normal commensal adapted to the ecologically complicated environments of the oral cavity and gastrointestinal and vaginal tracts. *Enterococcus faecalis* has been detected in primary root canal infections [4]. It has essential resistance to a variety of antibiotics such as the beta-lactamas, aminoglycosides, vancomycin, and trimethoprim-sulfamethoxazole. Searches reported that *Enterococcus faecalis* can be found in several cases of persistent infections [5,6]. Generally, the disinfecting process is critical for successful root-canal treatment, and irrigation of the root canals to remove microorganisms and cleanse pulpal debris is an important step. There is a great importance of using antimicrobial irrigants during the chemo-mechanical preparations. Effective endodontic irrigants require a broad spectrum of antimicrobial activity, as well as lack of toxicity to periapical and oral mucosal tissues. Root canal irrigation is designed to remove debris, dentin chips, bacteria, toxic products, and substrates essential for bacterial growth [7]. Sodium hypochlorite has been the irrigant for nonsurgical endodontic procedures. Sodium hypochlorite dissolves necrotic and vital tissue, has antimicrobial activity, and used as a lubricant in the canal. It also has less toxicity when kept inside the canals. Yet, it is highly toxic to the periapical tissues if it was extruded outside the apex [8]. A new root canal irrigant, Bio pure MTAD was found to be efficient as a final rinse, to remove the smear layer with less erosive changes and has the ability to disinfect contaminated root canal. The suggested regimen of Bio pure MTAD is initial irrigation of the canals by (1.3%) sodium hypochlorite through instrumentation and final 5 minute irrigation with Bio pure MTAD [9].

Materials and Methods

The following irrigating solutions were used in the study:

1. Bio pure MTAD (DENTSPLY/ Tulsa Dental, Tulsa, OK) a mixture of tetracycline isomer, acid and detergent. Bio pure MTAD composed of two parts: a powder (bottle) and liquid (syringe) that are mixed at the time of use. It is available in two dose sizes: a single-canal dose (5ml), and a multi-canal dose (20ml).
2. 5.25% sodium hypochlorite (Sigma, Pool, UK.): Full strength form and diluted form 2.5% sodium hypochlorite can be prepared by adding sterile distilled water.

Clinical study

Selection of patients

A total of 36 permanent teeth with necrotic pulps were chosen from healthy patients referred to the clinic of endodontics department, at the Faculty of Dentistry, Suez Canal University. All 36 teeth were single-rooted, with primary root canal infection who did not have any previous endodontic treatment. The age of these patients ranged between12 and 35 years. A legal consent was obtained from all patients prior to the study.

Clinical sampling

After cleaning the marked tooth with paste, a rubber dam was placed to isolate the crown. The operative field and the surrounding areas were disinfected with 0.2% chlorhexidine. A sterile Endo Z bur was used for opening the access cavity. Freshly prepared sterile trypticase soy broth was distributed in sterilized Eppendorf tubes (approximately 72 tubes) and each tube contained 1 ml of the broth for culturing of the swabs. Afterwards, two to three sterile paper points were put into the root canal for 1 min. If the root canals were found to be dry, sterile trypticase soy broth was put into the canal using a sterile syringe without overfilling the canal prior to insertion of the paper points. Paper points were then removed from the canals and moved aseptically into its markedly corresponding Eppendorf tube for each case forming the first swabs. The cases were divided into three groups of 12 teeth each corresponding to the three different irrigating solutions used (Bio pure MTAD, 5.25% and 2.5% sodium hypochlorite). For standardization of root canal diameter, Gates-Glidden drills sizes 4, 3 and 2 were used to prepare the root canal orifices. The apical thirds were prepared with K-files sizes 25, 30, 35 and 40 using the balanced force technique. Cleaning and shaping were completed for every root canal using the corresponding irrigant and the root canal for every case was ready for obturation. The canals were dried with sterile paper points. Sterile trypticase soy broth was put into the canal using a sterile syringe without overfilling the canal prior to insertion of the paper points that were retained in the canal for 1 min. The paper points for each swab were transferred aseptically into its markedly corresponding Eppendorf tube for each case forming the second swabs. All the marked Eppendorf tubes were incubated at 37 ºC for 48 hours. Microbial growth of *Enterococcus faecalis* was verified by turbidity of the broth. Turbid tubes were recorded positive (+ve) and the clear ones were recorded negative (-ve). The data obtained were statistically analyzed. Freshly prepared sterile Bile Esculin Azide

agar (Sparks, MD 21152 USA. 38800 Le Pont de Claix, France), a selective medium, was used for confirming the presence of *Enterococcus faecalis*. So it was distributed in sterilized Eppendorf tubes (approximately 72 tubes) and each tube contained 1 ml of the agar slop. After incubation and recording of the culturing results of the swabs, 100 µl was taken aseptically from each tube and transferred to culturing on Bile Esculin Azide agar slop in a corresponding marked tube. The tubes were then incubated for 48 hours at 37°C. Microbial growth of *Enterococcus faecalis* was verified by blackening of the broth before and after irrigation of the root canals. Black agar was recorded positive (+ve) and the clear ones were recorded negative (-ve). The data obtained were statistically analyzed.

Ability to dissolve pulp tissues

Thirty six sterile Wassermann tubes were divided into three groups for the three tested solutions, 12 tubes for each solution. In the first group, each test tube contained 1ml of freshly prepared Bio pure MTAD. In the second group, each test tube contained 1ml (5.25%) sodium hypochlorite (full strength) and in the third group, each Wassermann tube contained 1ml (2.5%) sodium hypochlorite (diluted form). A piece of immediately extirpated vital human pulp (2mm x 1mm) was placed in the solution of each Wassermann tube. All Wassermann tubes were then closed with cotton stops, and incubated for 24 hours at 37 °C. Conditions of the pulp tissue were examined thoroughly every hour by means of a magnifying lens and the surface changes were recorded for each tube. At the end of 24 hours the remaining tissues of any pulp specimen were examined under the Stereomicroscope (Olympus Zoom Stereomicroscope, Sz 40 - 45, Japan).

Results

Clinical study

In group one, where each root canal was irrigated with freshly prepared Bio pure MTAD all the tubes were clear. After culturing on Bile Esculin Azide agar slop in a corresponding marked tube, all the tubes were recorded negative (-ve) for *Enterococcus faecalis* growth. Therefore, the Chi-Square ratios were 100% negative and 0% positive, as shown in (Table 1) and (Figure 1). In group two, where each root canal was irrigated with 5ml full strength (5.25%) sodium hypochlorite, seven tubes were recorded clear and negative (-ve) to *Enterococcus faecalis* growth in Bile Esculin Azide agar slop. Five tubes were recorded turbid, positive (+ve) and turned to black color due to *Enterococcus faecalis* growth in Bile Esculin Azide agar slop. The Chi-Square ratios were 58.30% negative and 41.70% positive, as shown in (Table 1) and (Figure 2). In group three, where each root canal was irrigated with 5ml diluted (2.5%) sodium hypochlorite two tubes were recorded clear and negative (-ve) to *Enterococcus faecalis* growth in Bile Esculin Azide agar slop. Ten tubes were recorded turbid, positive (+ve) and turned to black color due to *Enterococcus faecalis* growth in Bile Esculin Azide agar slop. The Chi-Square ratios were 16.70% negative and 83.30% positive, as shown in (Table 1) and (Figure 2).

Figure 1: Clinical study records for group one (Biopure MTAD),

a) Control Bile Esculin Azide agar slop.

b) Clear Trypticase soy broth containing swab.

c) Negative record of Bile Esculin Azide agar slop.

Figure 2: Clinical study records for groups 2 and 3 (5.25% and 2.5% sodium hypochlorite).

a) Control Bile Esculin Azide agar slop.

b) Turbid Trypticase soy broth containing swab.

c) Positive record of Bile Esculin Azide agar slop.

No.	Group	Negative	Positive
1	Biopure MTAD,	100%	0%
2	5.25% sodium hypochlorite (full strength)	58.30%	41.70%
3	2.5% sodium hypochlorite (diluted)	16.70%	83.30%

Table 1: Chi-Square values of antimicrobial activity test in the clinical study.

Chi-Square value = 17.61; P < 0.0001

Ability to dissolve pulp tissue

In the first group (Bio pure MTAD) no changes were recorded in the extirpated vital human pulp tissues. No dissolving ability was recorded with Bio pure MTAD as shown in (Figure 3). In the second group (5.25% sodium hypochlorite) all pieces of extirpated vital human pulp tissues dissolved within 7 to 10 minutes. Full strength (5.25%) sodium hypochlorite exhibited high dissolving ability, as shown in (Figure 4). In the third group (2.5% sodium hypochlorite) all pieces of extirpated vital human pulp tissues dissolved within 10 to 15 minutes. Diluted (2.5%) sodium hypochlorite exhibited good dissolving ability.

Figure 3: Extirpated pulp tissue after immersion in Biopure MTAD for 24 hours. No changes were recorded in all fragments of extirpated pulp tissues, (Stereomicroscope 40X).

Figure 4: a) Extirpated pulp tissue immersion in 5.25% sodium hypochlorite. b) Complete dissolving of the extirpated pulp tissue after 10 minutes.

Discussion

The, Bio pure MTAD completely disinfected the roots and the teeth by inhibiting the growth of *Enterococcus faecalis.* Shabahang and Torabinejad [10] found that the grouping of 1.3% NaOCl as a root canal irrigant and Bio pure MTAD as a final rinse was significantly more efficient against *Enterococcus faecalis*. Mohammadi and Shahriari [11] also stated that the Bio pure MTAD group and sodium hypochlorite group exhibited the lowest and highest numbers of colony-forming units (CFUs). Therefore, the antimicrobial efficiency of Bio pure MTAD was significantly greater than that of sodium hypochlorite. Mohammadi [12] also investigated the antimicrobial efficiency of three concentrations (100%, 10%, 1%) of Bio pure MTAD and stated that the fully concentrated Bio pure MTDA revealed the greatest antimicrobial efficiency against *Enterococcus faecalis.* Two to five minutes exposure of *Enterococcus faecalis* to Bio pure MTAD was effective in killing the organism up to 200 dilutions. Moreover, physical removal of cells of *Enterococcus faecalis* through debridement of the root canal are important, since remnants may maintain the inflammation [13]. The result of the present study was in disagreement with Malkhassian., *et al.* [14] evaluate the antibacterial efficiency of a final rinse with Bio pure MTAD and intra-canal medication with 2% chlorhexidine gel (CHX) in teeth with apical periodontitis. They found that the final rinse with Bio pure MTAD and medication with CHX did not reduce bacterial counts within levels attained by canal preparation with sodium hypochlorite. Sodium hypochlorite recorded low or no antimicrobial efficiency against *Enterococcus faecalis*. Neglia., *et al*. [15] and Estrela., *et al.* [16] recorded that the sodium hypochlorite has low antimicrobial efficiency against *Enterococcus faecalis*. Moreover, Tirali., *et al.* [17] added that increasing sodium hypochlorite concentration increases its antimicrobial action. The results of the present study were in disagreement with other researchers [18-20] who recorded that sodium hypochlorite has a higher antimicrobial effect than the other tested materials. Giardino., *et al.* [21] evaluated the antimicrobial efficacy of 5.25% sodium hypochlorite, Bio pure MTAD and Tetraclean against *Enterococcus faecalis.* The 5.25% NaOCl only can remove the biofilm. However, treatment with Tetraclean disaggregated the biofilm at a higher degree in a considered time intervals but the Bio pure MTAD did not. Shenoi., *et al*. [22] and Charlie., *et al.* [23] also investigated Bio pure MTAD had a significantly higher mean inhibition zone against *E faecalis* than the other NaOCl. Concerning the ability to dissolve pulp tissue, Bio pure MTAD exhibited no dissolving ability and no changes were recorded in the extirpated vital human pulp tissues. Beltz., *et al.* [24] recorded also that there was no solubilizing effect of Bio pure MTAD on pulp and dentin. Concerning the ability to dissolve pulp tissue, (5.25%) and (2.5%) sodium hypochlorite recorded a high ability to dissolve the extirpated vital human pulp tissues. Morgan., *et al.* [25], Yang., *et al.* [26] and Naenni., *et al.* [27] were in agree-

ment with our results. Moreover, Spanó., *et al.* [28] and Okino., *et al.* [29] stated that the dissolution speed of sodium hypochlorite was directly proportional to the concentration of the solution. The major advantage of NaOCl is its capability to dissolve necrotic tissues. The use of EDTA and NaOCl is suggested for smear layer removal and has been shown to be more efficient than NaOCl only. Bio pure MTAD has 45% solubilizing effect on organic tissue like pulp where as 5.25% NaOCl has been more than 90% effective in dissolving organic content of the pulp [28-30].

Conclusions

Bio pure MTAD has great efficiency against *Enterococcus faecalis* more than sodium hypochlorite. While, sodium hypochlorite dissolved extirpated pulp tissues completely; on the other hand Bio pure MTAD did not. Coupling the advantage of Bio pure MTAD and sodium hypochlorite as irrigating solutions during cleaning and shaping of endodontic treated teeth by using sodium hypochlorite during cleaning and shaping and Bio pure MTAD as a final rinse is beneficial.

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