Determination of the antibacterial activity of MTA, and pulpotec filling materials against selected microorganisms (in vitro study)

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ABSTRACT

Background: This *in vitro* study concerned with the assessment of the antibacterial effects of two materials used as a pulpotomy medicament, mineral trioxide aggregate (MTA) and a new filling material Pulpotec (PD) on various species of microorganisms, using agar diffusion test.

Materials and methods: Abase layer of petri plates was made using Muller-Hinton agar. After solidification, two cavities were made in agar and filled with fresh mixed materials. *Staphylococcus aureus, Enterococcus Faecalis*, and *Escherichia coli* were seeded by pour plate. The plates were preincubated for 2 hours at room temperature followed by incubation at 37°C. The inhibition zone diameters were measured in (mm) at 24, 48 and 72 hours. Data were analyzed statistically using descriptive and analytic statistics, the descriptive including mean and standard deviation, and the analytic statistics in which t-test was run for multiple comparisons (between materials, and microorganisms).

Results: The highest mean diameters of growth inhibition zones were observed around PD against all tested microorganisms over 72 hours. According to t-test, there was a high significant difference between PD in comparison with MTA ($P< 0.01$). The difference was highly significance between the tested microorganisms.

Conclusion: The *in vitro* antibacterial activity of pulpotec offers additional advantage over MTA.

Key words: antibacterial agent, mineral trioxide aggregate, pulpotec, pulpotomy. (J Bagh Coll Dentistry 2010;22(4):115-118).

INTRODUCTION

While various chemical and physical irritants can cause irritation and even necrosis of the pulp, the most common causes for pulpal inflammation (pulpitis) are bacteria and/or their products entering the pulp through a deep caries lesion or a leaking filling, e.g. an inflammatory reaction in the pulp starts long before bacteria invade the pulp tissue. The inflammatory reaction is first initiated by bacterial antigens interacting with the local immune system [1–3]. Therefore, apart from appropriate methods of carious dentin removal or precise clinical evaluation of dentin, also application of the materials having high antibacterial properties seems to be indispensable.

Numerous studies have been performed to assess the antibacterial activity of different materials used in dental treatment, the agar diffusion test (ADT) is the most commonly used technique for evaluating this property of dental materials [4]. Mineral trioxide aggregate (MTA), which was introduced in 1993 [5], has been examined since 1995 as a potential antibacterial material [6]. According to recent studies, (MTA) is a biocompatbile dental material and it was suggested that these biological properties may be due to its excellent sealing ability [7], high alkalinity [8], induction of hard tissue formation [9], antibacterial effect [6], and stimulation of healing in the pulpal tissue [10,11].

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Dentinal bridge formation was the usual occurrence when MTA was used as a pulp-capping agent in dogs [11], monkeys [10], and as a pulpotomy material in human mature teeth [12,13]. Pulpotec (PD) is a radiopaque, non resorbable filling paste used for rapid and long term treatment of pulpitis by pulpotomy in vital molars, both permanent and deciduous. PD composed of powder (polyoxymethylene, iodoform, and zinc) and liquid (dexamethasone acetate, formaldehyde, phenol, guaiacol, and subsidiary substances). The addition of pharmacological constituents ensures an aseptic treatment, induces cicatrization of the pulpal stump at the chamber-canal interface, whilst maintaining the structure of the underlying pulp. It also avoids the numerous failures that have been noted with total pulectomy. The efficiency and the properties of (PD) pulpotec are substantiated by a radiographic file compiled on the basis of results of over 300 pulpotomies performed with pulpotec and monitored for periods of 3 to 13 years [14].

True endodontic pathogens or those associated with therapy-resistant cases [15] were selected as test bacteria for this experiment. Although aerobic and facultative bacteria are usually minor constituents of primary infections, they have been found with higher frequency in cases of treatment failure [16]. An attempt was made to select representative gram-negative/ positive and cocci/ bacilli bacteria commonly isolated from oral infection.

The aim of this *in vitro* study was to evaluate and compare the antibacterial effectiveness of

MTA and PD over different time periods using the agar diffusion method.

MATERIALS AND METHODS

The *in vitro* antibacterial activity of two materials, ProRoot MTA (DentsplyTula Dental, Tulsa, OK, USA) and pulpotec (PD, Switzerland) against three reference strains of bacteria was evaluated by the agar diffusion test (agar-well technique).

The study was conducted on double-layered plates, in which the base layer was made of 10 ml of sterilized MH agar (Muller-Hinton agar; Difco, USA) poured into 2×10 cm sterilized petri plates. After solidification two uniform cavities (5mm diameter, one for each test material) were punched at equidistant points in agar by means of sterile copper coil. The cavities were filled by materials immediately after being mixed according to the manufacture instructions.

The following bacterial strains obtained from the department of Microbiology Collage of Medicine / Al –Mustansiria University were used as indicator microorganisms in the study: *Staphylococcus aureus* (gram -positive coccus), *Enterococcus faecalis* (gram-positive coccus), *Escherichia coli* (gram-negative bacillus). After activation from stock culture, microorganisms were maintained in MH broth (Muller-Hinton broth; Difco, USA) until use. Overnight culture of the microorganisms were done. All the microbial strains were grown at 37°C for 24 hours in MH broth and then seeded into15 ml of the MH agar, to produce a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 10^8 colony forming unit ml^{-1}. The seeded agar was added over the plates immediately after the insertion of freshly mixed test materials. The plates were kept at room temperature for 2 h for pre-diffusion of the materials and then incubated at 37°C for 24, 48, and72 hours.

A total of 31 plates were used, plates were divided into three test groups with ten plates each. So microorganisms were tested ten times. Negative control was prepared, maintaining a plate without inoculums, for the same period and under identical incubation conditions. All assays were carried out under aseptic conditions. The diameter of the zones of bacterial growth inhibition formed around the wells was measured with a millimeter ruler.

Statistical analysis was performed using descriptive statics represented by mean and standard deviation. Student's t-test was performed to find any statistically significant difference between materials, and bacteria.

RESULTS

The antibacterial activities of test materials determined by the means and standard deviation of inhibition growth zones in millimeters on all test microorganisms after 24, 48, and 72 hours are shown in Table 1. Results of 24 h incubation revealed that the antibacterial effect of PD on all test microorganisms was superior to that of MTA. Decreasing order of inhibition zones produced by PD and MTA on all microorganisms ranged from 9.91 to 20.00, and 2.37 to 5.12mm at 24 h, respectively. The results for 48 and 72 h were similar to that for 24 h with all test materials.

The largest mean diameters of inhibition zones of bacterial growth were founded around PD with the *S. aureus*, followed in descending order by *E. faecalis*, and *E. coli*, while the largest mean diameters were founded around MTA with *E. faecalis*, *S. aureus*, and then *E. coli*. There was a highly significant difference significant between the antibacterial of PD and MTA ($P < 0.01$) for all tested microorganisms and for all the experiment time (Table 2).

Table 1: The antibacterial activity of test materials represented by means (mm), and standard deviation against different bacteria at all time intervals

Bacteria	Time	Material			
		PD		MTA	
		Mean	SD	mean	SD
S .aureus	24 h	20.00	0.90	3.56	0.41
	48 h	19.33	0.51	3.31	0.53
	72 h	19.00	0.89	3.18	0.45
E. Faecalis	24 h	13.00	1.10	5.12	0.35
	48 h	12.33	1.50	4.41	0.32
	72 h	12.33	1.50	4.41	0.32
E. coli	24 h	9.91	0.28	2.37	1.02
	48 h	8.66	0.49	1.87	0.99
	72 h	8.00	0.85	1.68	1.06

Table 2: Comparison between antibacterial effects of pulpotec and MTA after 3 time period

Bacteria	Time	t-test	P-value	Sig
S. aureus	24 h	37.56	0.000	H.S
	48 h	48.02	0.000	H.S
	72 h	48.48	0.000	H.S
E. faecalis	24 h	23.28	0.000	H.S
	48 h	23.76	0.000	H.S
	72 h	23.76	0.000	H.S
E. coli	24 h	23.75	0.000	H.S
	48 h	20.84	0.000	H.S
	72 h	21.06	0.000	H.S

H.S : Highly Significant at level P < 0.01

Additionally, (Table 3) indicated highly significant difference in the effects of PD, and MTA between different bacteria during the period of the experiment.

Table 3: Comparison of the antibacterial effects of Pulpotec and MTA between bacteria at 3 time period

Bacteria	Time	Pulpotec			MTA		
		t-test	P-value	Sig	t-test	P-value	Sig
S. aureus* & *E. faecalis	24 h	13.31	0.000	H.S	4.56	0.0003	H.S
	48 h	15.83	0.000	H.S	4.43	0.0004	H.S
	72 h	15.21	0.000	H.S	4.84	0.0002	H.S
S. aureus* & *E. coli	24 h	20.51	0.000	H.S	5.81	0.000	H.S
	48 h	31.98	0.000	H.S	4.95	0.0002	H.S
	72 h	36.59	0.000	H.S	5.20	0.0002	H.S
E. faecalis* & *E. coli	24 h	9.16	0.000	H.S	9.32	0.000	H.S
	48 h	13.34	0.000	H.S	8.01	0.000	H.S
	72 h	16.49	0.000	H.S	8.32	0.000	H.S

DISCUSSION

The current study was performed for a few reasons. Firstly, antibacterial properties of dental materials are essential, especially in the case of those materials which get in a direct contact with the pulp, periodontium or alveolar bone. Secondly, Pulpotec is a new material and no previous study in Iraq has been done to detect its antibacterial action. Agar diffusion test was used, which is the most widely used *in vitro* method for the evaluation of antibacterial activity, that identifies the materials more likely to have an antimicrobial effect via direct comparisons between them [17,18].

The selection of microorganisms for the present study was based on the fact that some microorganisms have been often related to endodontic infections, such as *S. aureus* [17,19], and *E. faecalis* [20]. According to Sunde et al. [19], these microorganisms are present in 75% of periapical lesions.

In this study, freshly mixed materials were immediately transferred into agar plates. Because of various transitory or permanent products, the material should be tested immediately after mixing and also after it is assumed to reach its final chemical structure. PD and MTA are inserted into the tooth in a freshly mixed, incompletely set stage, and thus its likely that during a period after clinical application of the material, local responses are provoked by components with no or partial reaction. After setting, the release of active ingredients from the materials is still possible. The difference in antibacterial patterns of various materials may also be related to the degree of setting [4].

The antimicrobial activity of MTA was reported by Torabinejad et al. [6], who detected its efficiency against some facultative bacteria; however, no activity was found against *E. faecalis, S. aureus, B. subtailis* and *E. coli* or against anaerobic bacteria. Estrela et al.[21], demonstrated that MTA did not reveal any antimicrobial activity against *S. aureus, E. faecalis, P. aeruginosa, B. subtilis, or C. albicans.* In this study, the same poor results are obtained with MTA, with a highly significant difference. Under the condition of the current work, the most susceptible microorganism for MTA was *Enterococcus faecalis* and the weakest antibacterial action was observed against *Escherichia coli* ,these results are in disagreement with those of Cepowicz et al.[22], who assessed the antimicrobial properties of MTA and found that *Escherichia coli* was the most susceptible microorganism.

Pulpotec has been indicated for treatment of pulpitis in both primary and permanent vital molars [14]. In the present investigation pulpotec showed antibacterial activity against *S. aureus, E. faecalis,* and *E. coli,* this result is probably due to the toxicogenic components in its composition (iodoform and formaldehyde). The antimicrobial activity of iodoform (active agent: iodine) has been discovered early for the use in medicine. It can rapidly penetrate the cell walls of microorganism. The mechanism of action by which iodine kills, is the damaging of microbial proteins through oxidation of amino acids and the reaction with the carbon-carbon double bond of unsaturated fatty acids [23]. Negam & Kataia [24], evaluated the antibacterial activity SPAD (root canal obturating paste containing formaldehyde) and Pulpdent (calcium hydroxide apexification paste) against Streptococci group D , isolated from infected root canals using agar diffusion test, and found that the largest zones of bacterial growth inhibition zones formed around SPAD, denoting its strong antibacterial effect, on the contrary, calcium hydroxide was unable to cause inhibition of bacterial growth throughout the period of the study.

Under the conditions of this *in vitro*, it was concluded that the favorable results of pulpotec in comparison with MTA, indicate potentiality of PD as an antibacterial agent. However, it is necessary to investigate other properties of this new material.

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