Dental Traumatology

Dental Traumatology 2012; **28**: 474–477; doi: 10.1111/j.1600-9657.2012.01119.x

The effect of various concentrations of iodine potassium iodide on the antimicrobial properties of mineral trioxide aggregate – a pilot study

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Key words: antimicrobial; *Enterococcus faecalis*; iodine potassium iodide; mineral trioxide aggregate

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Accepted 7 January, 2012

Abstract – *Background*: Mineral trioxide aggregate (MTA) is a multi-purpose dental material with various uses in dentistry. Iodine potassium iodide (IKI) is the most commonly used iodine compound in endodontics. We aimed to assess the antimicrobial activity of tooth-colored ProRoot MTA combined with IKI. *Materials and methods*: The antimicrobial activity of IKI was assessed at three concentrations (1%, 2%, and 4%) as the mixing agents combined with MTA against *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. For each microorganism, three plates were inoculated with 100 μl of a microbial suspension (McFarland 0.5). Four wells were prepared in each plate. MTA (70 mg) was mixed with any of the three concentrations of IKI (25 μl) or sterile distilled water (25 μl) and placed in each well. The plates were incubated for 24 h at 37°C. Zones of inhibition (ZOI) were measured in millimeters by a blinded observer. Data were analyzed using analysis of variance and the Dunnett *t*-test. *Results*: All MTA mixtures with water or IKI solutions showed inhibitory zones. The mean ZOI of each MTA/IKI mixture was not significantly different from MTA/water mixture ($P > 0.05$). MTA/1% IKI had smaller ZOI than MTA/water against *E. coli, E. faecalis,* and *C. albicans*. MTA/2% IKI showed larger ZOI only against *P. aeruginosa*. MTA/4% IKI showed larger ZOI against *P. aeruginosa* and *E. coli* ($P < 0.05$). *Conclusions*: Substitution of IKI solutions (1%, 2%, and 4%) for water did not significantly increase the antimicrobial activity of MTA.

Mineral trioxide aggregate (MTA) is a multi-purpose dental material used for pulp capping (1), apexification (2), pulpotomy of primary teeth (3), repair of perforations (4), root-end fillings (5), and as orifice plugs (6). It has been reported to display a higher clinical and radiographic success rate compared with other commonly used materials. This success is attributed to the ideal properties of MTA such as biocompatibility, osteoinductivity, and high sealing ability (7–9). As MTA can be used in infected areas, its antimicrobial properties should be carefully evaluated. A number of studies have reported conflicting results regarding the antimicrobial activity of MTA (10–14).

Evidence suggests that the incorporation of silver-zeolite may enhance the antimicrobial activity of MTA (15). Also, some studies have shown that replacing water with chlorhexidine (CHX) improves the antibacterial and antifungal properties of MTA (16, 17). This change, however, may have some adverse effects such as decreased compressive strength (17), increased setting time (18), and increased cell apoptosis (19).

Iodine compounds display significant antimicrobial properties. Iodine interferes with the function of respiratory chain enzymes, dislocates protein synthesis, and alters the lipid membrane function. Some patients, however, exhibit allergic reactions to iodine compounds (20). Iodine potassium iodide (IKI) is the most commonly used iodine compound as an endodontic irrigant. It has rapid antimicrobial activity against *Enteroccocus faecalis*, *Streptococcus sanguis* (21), *Fusobacterium*, *Pseudomonas aeruginosa* (22), *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* (23), and *Candida albicans* (24). The minimum active concentration of IKI is 1% and it is used in concentrations ranging from 1% to 5% (25). 2% IKI has shown less toxicity and tissue irritation than formocresol, camphorated monoparachlorophenol, CHX, and sodium hypochlorite (26, 27). It is also well tolerated by human gingival fibroblasts (28). Combination of IKI with calcium hydroxide (29) and gutta-percha

(30) has been suggested to improve their antimicrobial effects.

There is no study on the antimicrobial activity of MTA and IKI combination. The aim of this study was to assess whether tooth-colored ProRoot MTA has antimicrobial activity and whether substitution of sterile distilled water with IKI at three concentrations of 1%, 2%, and 4% as mixing agents would enhance its antimicrobial action.

Materials and methods

Microorganisms

We used laboratory standard strains of *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *C. albicans* (ATCC 10231) (IROST, Tehran, Iran). All bacterial strains were inoculated in 5-ml samples of LB Broth (Merck, Darmstadt, Germany) and incubated aerobically for 24 h at 37°C. *C. albicans* was grown in Sabouraud's dextrose agar (Merck, Darmstadt, Germany) for 24 h at 37°C in aerobic conditions.

Preparation of test materials

First, 4% IKI was prepared by mixing 4 g of iodine (Merck, Darmstadt, Germany) in 8 g of potassium iodide (Merck, Darmstadt, Germany) and adding distilled water to a 100-ml volume. Then 2% IKI and 1% IKI was obtained by diluting 4% IKI in a ratio of 1/2 and 1/4 with sterile distilled water. Mixtures of MTA were prepared by mixing 70 mg of tooth-colored ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) with 25 μl of each IKI concentration (1%, 2%, and 4%) or sterile distilled water.

Antimicrobial assay

Three experimental and a control plate were prepared for each microorganism. Each plate had a 5-mm thickness of Mueller-Hinton broth (Merck, Darmstadt, Germany) for bacterial strains and Sabouraud's dextrose agar for *C. albicans*. The plates were inoculated with 100 μl of a microbial suspension (standardized to McFarland 0.5). Four wells measuring 6 mm in diameter and 2 mm in depth were made with a sterile pasture pipette in the agar of each plate. Using a new sterile plastic amalgam carrier, each tested MTA mixture was placed into a well of the experimental plates. Twenty-five microlitre of each liquid was poured into a well of the control plates. All plates were preincubated for 2 h at room temperature, followed by incubation for 24 h at 37°C. Zones of inhibition (ZOI) around the MTA mixtures and controls were measured to the nearest millimeter by a blinded observer.

Statistical analysis

The data were analyzed using SPSS software, version 13 (SPSS Inc, Chicago, IL, USA). Analysis of variance and the Dunnett *t*-test were used to determine significant differences between the ZOI of each MTA/IKI mixture and MTA/water. Statistical significance was defined at $P < 0.05$.

Results

The ZOI of the experimental and control groups are presented in Tables 1 and 2. All MTA mixtures and IKI solutions showed inhibitory effects against tested microorganisms. The mean ZOI of MTA mixtures expressed in millimeter were as follows: MTA/water (11.46 ± 3.62); MTA/1% IKI (9.86 ± 3.13); MTA/2% IKI (11.60 ± 3.56); MTA/4% IKI (13.46 ± 3.29). There was no significant difference between the mean ZOI of each MTA/IKI mixture and the mean ZOI of MTA/water ($P > 0.05$). In comparison with MTA/water, MTA/1% IKI had smaller ZOI against *E. coli* ($P = 0.014$), *E. faecalis* ($P = 0.028$), and *C. albicans* ($P < 0.001$); MTA/2% IKI showed larger ZOI only against *P. aeruginosa* ($P = 0.009$); MTA/4% IKI had larger ZOI against *P. aeruginosa* ($P < 0.001$) and *E. coli* ($P = 0.001$).

Discussion

In this study, predominant isolated microorganisms from failed endodontic therapy cases were chosen. *E. faecalis* is a gram-positive facultative anaerobic coccus, which is the most frequent strain in treatment-resistant cases (31). Although it consists of a small proportion of the root canal flora in initial endodontic infections, it can enter and remain in the root canal system during or after treatment. Some factors involved in this resistance are high pH tolerance (32), surviving without other microbial supports (21), intracanal drug resistance, and good dentinal penetration ability (33). *C. albicans* is a fungus that has been occasionally found in untreated cases. But it is common in endodontic failures (31). Both *C. albicans* and *E. faecalis* are calcium hydroxide-resistant

Table 1. The mean of zones of inhibition (mm) in the experimental groups (MTA mixtures with sterile water and IKI solutions)

Microorganism	Water	1% IKI	2% IKI	4% IKI
Escherichia coli	10.33	7.00	10.33	16.00
Staphylococcus aureus	9.33	8.33	9.00	10.67
Candida albicans	18.33	15.00	18.00	17.67
Enterococcus faecalis	9.00	7.67	8.67	9.67
Pseudomonas aeruginosa	10.33	11.33	12.00	13.33

Table 2. Zones of inhibition (mm) in the control groups (sterile water and IKI solutions)

Microorganism	Water	1% IKI	2% IKI	4% IKI
Escherichia coli	0	13	15	27
Staphylococcus aureus	0	18	26	38
Candida albicans	0	30	33	40
Enterococcus faecalis	0	16	20	30
Pseudomonas aeruginosa	0	11	14	25

microorganisms and can survive in food-deprivation conditions. *E. coli*, a gram-negative facultative anaerobic rod, *P. aeruginosa*, a gram-negative obligatory aerobic rod and *S. aureus*, a gram-positive facultative anaerobic coccus, are also isolated from root-filled canals with apical periodontitis (34).

To evaluate the antimicrobial activity of MTA, we used the agar diffusion method, which has been the most commonly used technique for the evaluation of antimicrobial activities of MTA. This method, however, is technique-sensitive and has some limitations. The outcome measure (i.e., zone of inhibition) is dependent on diffusibility and solubility of tested materials through agar media (10).

In our study, all samples of MTA/water mixture showed inhibitory effects against the tested microorganisms. Our results are consistent with those of Eldeniz et al. (10), Al-Hezaimi et al. (11), and Sipert et al. (12), who reported that MTA has antimicrobial effect against *E. faecalis*. However, our results do not coincide with those of Estrela et al. (13) and Torabinejad et al. (14) who did not find any inhibitory effect for MTA against *E. faecalis* and *S. aureus*. Differences in the results might be attributed to the various species of the microorganisms, the methods of the investigations, the source of prepared material (35), and also the type and the concentration of MTA (11, 36) used in these studies.

Replacement of distilled water with CHX has been shown to cause significant elevation in antimicrobial activity of MTA (16, 17). Stowe et al. (16) compared the antimicrobial activity of MTA mixtures with 0.12% CHX and distilled water against *Actinomyces odontolyticus*, *Fusobacterium nucleatum*, *S. sanguis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*. MTA/CHX mixture showed significantly more antimicrobial activity than MTA/water among all species except *P. aeruginosa*. In addition, Holt et al. (17) reported the higher antimicrobial activity of MTA/2% CHX mixture compared with MTA/water mixture against *E. faecalis*. However, setting time of MTA mixtures with both the liquid (up to 72 h) and the gel forms (at least 1 week) of CHX was increased adversely (17, 18). This may account for the larger inhibitory zone of MTA/CHX mixture compared with MTA/water mixture as the former combination sets later and can diffuse more through agar media.

In the control plates, all concentrations of IKI solutions (1%, 2%, and 4%) exhibited inhibitory zones against the tested microorganisms. In the experimental plates, combinations of MTA with three concentrations of IKI showed different results. In comparison with MTA/water, MTA/1% IKI mixture not only did not show increased inhibitory effect against *S. aureus* and *P. aeruginosa* but also it showed decreased inhibitory effect against *C. albicans, E. faecalis,* and *E. coli*. Although very little is known about the chemical reactions between MTA components and IKI, some antagonist reactions may adversely affect the antimicrobial effects of both materials. Nevertheless, we observed an increase in the antimicrobial effects of MTA mixtures with higher concentrations of IKI. MTA/2% IKI mixtures showed greater inhibitory zones only against *P. aeruginosa* compared with MTA/water. MTA/4% IKI mixtures were more effective only against *E. coli* and *P. aeruginosa* than MTA/water. Combinations of MTA with 2% and 4% IKI were more effective on gram-negative bacteria than gram-positive bacteria, which may be related to the differences in the cell walls and cell envelopes among gram-positive and gram-negative bacteria.

In the present study, we observed that MTA/IKI mixtures set faster than MTA/water. This might be another reason accounting for the less inhibitory zone around MTA/IKI mixtures as the mixture sets faster and can diffuse less through agar media. We also observed that 1% and 2% IKI did not have a notable effect on the color of MTA, and MTA/IKI mixtures were easier to handle than MTA/water mixture.

Although agar diffusion method has been the most commonly used technique for the assessment of antimicrobial activities of MTA, we suggested tube dilution method to overcome the limitations of this method. In order to fully assess the viability of using IKI with MTA, it is also suggested that pH, sealing ability, allergenicity, and physical properties, specifically the setting time of MTA/IKI mixtures be evaluated.

Conclusion

Within the limitations of our study, we can conclude that replacing distilled water with IKI to mix with MTA might not improve the antimicrobial properties of MTA.

Acknowledgements

This study was supported by Isfahan University of Medical Sciences (grant no. 389219). We would like to thank Dr. Bahram Soleimani for his statistical consultation. The authors deny any conflicts of interest.

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