The effect of MTAD as a final root canal irrigants on the coronal bacterial leakage of obturated root canals

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ABSTRACT

The purpose of this study was to evaluate the effects of MTAD, EDTA and sodium hypochlorite(NaOCl) as final irrigants on coronal leakage resistance to *Enterococcus faecalis*. Forty extracted human maxillary molars were used in this experiment. The teeth were randomly divided into positive control group (Group 1; n = 5), negative control group (Group 2; n = 5) and three experimental groups (n = 30). In Group 3 (n = 10), the root canals were irrigated with sodium hypochlorite. In Group 4 (n = 10) and 5 (n = 10), the root canals were irrigated with sodium hypochlorite and rinsed with EDTA and MTAD, respectively. The teeth in each group were cleaned and shaped to #40 profile with .04 taper, and obturated with gutta-percha and AH-26 root canal sealer. The coronal portion of each tooth was placed in contact with inoculum of *Enterococcus faecalis* in Brain Heart Infusion (BHI) culture media. Each root tip was placed in a vial containing sterile culture media. The vials were placed in anaerobic chamber and observed everyday for turbidity for 180 days. Statistical analysis was performed using Fisher's Exact Test. After 180 days, Group 3, 4, and 5 showed 7, 4 and 5 leaking samples respectively. The differences in leakage resistance were not statistically significant among Group 3, 4 and 5. (J Kor Acad Cons Dent 33(4):397-404, 2008)

Key words : MTAD, Irrigation, Bacterial leakage, *Enterococcus faecalis*

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I. INTRODUCTION

The major goal of root canal treatment is to remove irritants from the root canal system, to obturate the cleaned and shaped root canals, and to prevent recontamination.

Ray and Trope[1] proposed that coronal microleakage may be a major cause of endodontic failure. Many previous studies have examined that coronal leakage might occur, even in well obturated root canals[2-4].

One of the most important concerns in root canal cleaning and shaping is how to remove the smear layer that is formed during root canal preparation.

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The smear layer is created during instrumentation from the cutting action of the microinstruments and the compression of the resulting crumbs against the root canal walls. It is composed of inorganic particles of calcified tissue and organic elements such as pulp tissue debris, odontoblastic processes, microorganisms, and blood cells[5,6]. It delays the action of endodontic disinfectants[7] and acts as a physical barrier which deteriorates adhesion and penetration of sealers into dentinal tubules[8,9]. Thus, it can decrease the sealing ability of root canal filling material. Some investigators suggested that the removal of smear layer might significantly improve the sealability especially in the coronal area[10-12].

A new solution, MTAD (Biopure MTAD, Tulsa, OK, USA), was recently developed as root canal cleanser. MTAD contains a mixture of a tetracycline isomer, an acid, and a detergent. Recent studies on MTAD demonstrated that MTAD has antimicrobial effect and is able to effectively remove smear layer. Torabinejad *et al.* have shown that MTAD is able to safely remove the smear layer and is effective against *Enterococcus faecalis*, a microorganism resistant to the action of other antimicrobial medications such as calcium hydroxide[13-19]. Portenier *et al.* demonstrated that the full concentration (100%) MTAD and 0.2% chlorhexidine were both effectively killed *Enterococcus faecalis*[20].

The smear layer removing ability of MTAD was also investigated by recent studies. Tay *et al.* demonstrated that root canal instrumentation produced 2-5 ㎛ thick smear layer which could be effectively removed by EDTA and MTAD[21]. They reported that canal irrigation with EDTA and MTAD eroded dentin and created a zone of demineralized collagen matrices, which can form dentin hybridization by infiltration of hydrophilic adhesive and sealer. Ghoddusi *et al.* also reported that the use of EDTA or MTAD produced more resistance to bacterial leakage than sodium hypochlorite[22] due to their superior smear layer removing ability. However Tay *et al.* pointed out the potential risk of nanoleakage between hydrophobic root canal sealers and collapsed collagen matrices, which was confirmed in another study of them[23].

However, there have been very few studies comparing the leakage resistance of the root canals irrigated with MTAD (Biopure MTAD, Tulsa, OK, USA), EDTA and sodium hypochlorite. The purpose of this study was to evaluate the effect of a new root canal cleanser, MTAD, in comparison with EDTA and sodium hypochlorite, on the coronal leakage of obturated root canals using a bacterial leakage test model.

Ⅱ. MATERIALS AND METHODS

Forty, extracted, noncarious, human, three-rooted maxillary molars with closed apices were used in this study. Other factors, such as age and sex of the patient at the time of extraction, were not considered. The teeth were immersed in 2.6 % sodium hypochlorite (NaOCl) for approximately 1 hour and the root surface was carefully debrided using curettes. Then, the teeth were stored in physiologic saline during the experiment. The teeth were randomly divided into 3 experimental groups of 10 teeth each and 2 control groups of 5 teeth each. Both buccal roots were removed at the furcation. The orifice of each canal was enlarged with a #330 bur (Komet Carbide FG 330, Lemgo, Germany) and was sealed with glass ionomer (GC Fuji Ⅱ LC, Tokyo, Japan). Only the palatal root was used in this experiment.

After preparing a conventional access for each tooth, #10 K-type file (Maillefer, Zurch, Switzerland) was inserted to determine the working length by penetrating the apical foramen under the microscope (OPMI pico, Zeiss, Germany) and subtracting 1 ㎜. The root canal of each tooth was cleaned and shaped to a size 40 master apical file using 0.06 Taper ProFlie NiTi rotary systems (Maillefer Profile, Zurich, Switzerland) in a crown-down manner. The root canals in groups 1 to 4 were irrigated with 2 ㎖ of 5.25 % sodium hypochlorite between instrumentations. The irrigant was delivered into each canal with a 28 gauge needle (Max-i-Probe irrig-

ant system, KerrHawe, Switzland). The tip of the irrigating needle penetrated within 1 to 2 ㎜ short of working length in each canal.

In group 1 (n = 5, positive control) and 2 (n = 5, negative control), the canals were irrigated with 2 ㎖ of 5.25 % sodium hypochlorite between instrumentations.

In group 3 (n = 10), the canals were irrigated in the same manner as previous groups. After canal shaping, the canals were irrigated with 5 ㎖ of 5.25 % sodium hypochlorite and left without a dry for 5 minutes.

In group 4 (n=10), the canals were irrigated in the same manner as previous groups. After canal shaping, the root canals were irrigated with 5 ㎖ of 17 % EDTA (Roth International, Chicago, USA) and soaked for 5 minutes to remove the smear layer and rinsed with 5 ㎖ of 5.25 % sodium hypochlorite, following the method used in previous study of Ghoddusi *et al.* [22].

In group 5 (n = 10), the canals were irrigated using MTAD (Biopure MTAD, Tulsa, OK, USA) according to the manufacturer's instructions. The canals in this group were irrigated with 1.3 % NaOCl. After shaping, the root canals were soaked with 1 ㎖ of MTAD for 5 min and rinsed with 4 ㎖ MTAD. MTAD was freshly prepared before each usage according to Park's study [24].

The canals were dried with paper points (Diadent, Seoul, Korea) and obturated as follows: all of the root canals were obturated using continuous wave of condensation technique. In group 1 (positive control group), canals were obturated with gutta-percha (Diadent, Seoul, Korea) without sealer. The canals in groups 2 to 5 were obturated with gutta-percha and AH 26 (Dentsply, York, PA, USA). All of the specimens were stored in 100 % humidity for 1 day. To standardize the length of obturation, the coronal filling was removed with a System B (Analytic Endodontics, Orange, CA, USA) and 10 ㎜ of gutta percha filling was remained in the root canal. After confirming the length of obturation radiographically, all of the access openings were sealed temporarily with Caviton (GC, Tokyo, Japan) and the specimens were stored in an incubator with 100 % humidity at 37℃ for 30 days. Then, all of the specimens were sterilized with ethylene oxide (EO) gas. The external root surface of each tooth in group 1, 3, 4, and 5 was coated with triple layers of nail varnish excluding the apical foramen. The access opening of each tooth in negative controls (group 2) was filled with sticky wax, and all of the external surfaces were completely sealed with triple layers of nail varnish

A dual chamber anaerobic bacteria model was assembled using a 5 ㎖ irrigation syringe as the upper chamber and 20 ㎖ scintillation vial (Iwaki, Tokyo, Japan) as the lower chamber. The syringe was secured via a hole drilled through the cap of the 20 ㎖ scintillation vial. The syringe tip was placed into the chamber. The tooth was attached with sticky wax to the tip of the syringe to complete the upper chamber and the joint sealed with three coats of nail varnish. A cap to cover the tube opening of the upper chamber was made with putty. The assemblies of leakage models were placed in envelopes and sterilized in EO gas.

The vials were placed in an anaerobic chamber (Sheldon Manufacturing, Inc., Cornelius, OR) containing 85 % N_2, 5 % CO_2, and 10 % H_2 for 48 hours. This was done to eliminate any oxygen in the system, reduce the media before inoculation, and check for sterility of the system. Brain Heart Infusion (BHI) broth supplemented with yeast extract (5 g/L), hemin (5 mg/L), and menadione (10 mg/L) was aseptically placed into the lower chamber until exposed root apex immersed completely in the BHI. Two millimeters of BHI broth turbid with *Enterococcus faecalis (ATCC 29212)* was pipetted into the upper chamber syringe reservoirs. The tube cap was replaced to prevent evaporation. Fresh BHI inoculated with *Enterococcus faecalis* was added to the upper chamber every 3 to 4 days. The vials were observed everyday for turbidity of the broth in the lower chamber, indicating bacterial growth from penetration of the bacteria past the root apex. The day of turbidity was recorded. The experiment was conducted for six months. Statistical analysis was performed using Fisher's Exact Test. (SAS 9.1, SAS Institute Inc., Cary, NC, USA)

Ⅲ. RESULTS

The positive controls showed turbidity at 1 or 2 days after inoculation. The negative controls did not leak for the entire experimental period. 1 and 2 samples were lost in group 3 and 4, respectively due to contamination during experiment. The number of leaking samples per group was described in Table 1.

On the 50th day of experiment, 3, 1, and 0 samples leaked in Group 3, 4, and 5, respectively. On the 80th day of experiment, 5, 3, and 1 samples leaked in group 3, 4, and 5 respectively. On the 180th day of experiment, 7, 4, and 5 samples leaked in group 3, 4, and 5, respectively. On the 180th day of experiment, there was no statistical difference among three experimental groups ($p > 0.05$).

Ⅳ. DISCUSSION

So far, to our knowledge, there have been very few studies regarding the effect of final rinsing with MTAD on bacterial leakage. Ghoddusi *et al.*[22] compared the bacterial leakage of root canals which were rinsed with either MTAD or EDTA. There was no significant difference between MTAD and EDTA treated groups in bacterial leakage. However, in their study, they used *streptococcus mutans*, which could be easily destroyed by root canal irrigation with sodium hypochlorite or calcium hydroxide. In this experiment, we used *Enterococcus faecalis* which are known to be resistant to sodium hypochlorite and calcium hydroxide. This rendered our study to simulate the clinical situations more closely than previous studies.

The observation period of our study was 180 days, which is relatively longer that those of other leakage studies that used *Enterococcus faecalis*. Fathi *et al.*[25] and Yucel *et al.*[26] observed bacterial *(Enterococcus faecalis)* penetration for 60 days in their studies. In their studies, all the samples leaked after 60 days. But in our study, there were samples which didn't leak until 180 days, which indicated the superior ability of MTAD and EDTA to NaOCl as a smear layer removing agent.

Sundqvist reported that *Enterococcus faecalis* is important microorganism in endodontic failure[27]. In the study of Fabricius *et al.*, *Enterococci* have been shown to have an ability to survive in root canals as single organisms without the support of other bacteria[28]. In most of recent bacterial leakage test, single bacterial inoculation with *Enterococcus faecalis* was used[25,26,29]. That is the

Table 1. The number of leaking samples per group

Days	n	The number of leaking samples		
		Group		
		50	80	180
1	5	5	5	5
2	5	0	0	0
3	9	3(33%)	5(56%)	7(78%)[a]
4	8	1(13%)	3(38%)	4(50%)[a]
5	10	0(0%)	1(10%)	5(50%)[a]

* Same letter indicate no significant difference between groups ($P > 0.05$)
(Group 3 vs 4 ; $p = 0.4775$, Group 4 vs 5 ; $p = 0.0519$, Group 3 vs 4 ; $p = 1.0$)

reason why we used *Enterococcus faecalis* for this study.

In group 5, MTAD was used with 1.3% sodium hypochlorite because it is known to be more effective than MTAD only. In a previous study[16], sodium hypochlorite was used as an irrigant to assist MTAD to remove the smear layer and there were no significant differences among 1.3 %, 2.6 %, and 5.25 % sodium hypochlorite in the ability as root canal irrigants when MTAD was used as a final rinse to remove the smear layer. Therefore, in our study, 1.3 % sodium hypochlorite was used because this is less toxic[16]. However, there still might be a possibility that the difference in the concentrations of sodium hypochlorite had an effect on the results of this study.

The leakage test model used in this study was modified from that of Ghoddusi *et al.*[22]. There are two major differences between the method of us and Ghoddusi *et al.* Firstly, they used *streptococcus mutans* and we used *Enterococcus faecalis.* Secondly, they didn't use NaOCl as final irrigant as we did in experimental group 3.

In this study, the resistances to bacterial leakage were not significantly different in group 3, 4 and 5. These results are different from the observations of Ghoddusi *et al.*[22]. They proposed that use of MTAD or EDTA as root canal irrigant had reduced bacterial leakage compared with sodium hypochlorite. The difference of the results of this study and those of Ghoddusi *et al.* might be explained from the experimental design. In this study, in group 3, the root canals were soaked with sodium hypochlorite for 5 minutes. This rendered the same experimental conditions as group 4 and 5, in which the root canals were soaked with MTAD and EDTA for 5 minutes. On the contrary, Ghoddusi *et al.* used the sodium hypochlorite as irrigant only between instrumentation and the root canals were not soaked with sodium hypochlorite. The 5 minutes soaking with sodium hypochlorite might have resolved possible tissue remnant, which might have caused a better seal.

On the other hand, the results of this experiment showed that the effect of MTAD and EDTA as root canal irrigant had a similar effect on bacterial leakage. These results are consistent with the findings of Park *et al.*, who reported the similar bacterial leakage resistance between root canals irrigated with MTAD and EDTA[24].

Doxycycline is a major component of MTAD. It is strongly adsorbed to tooth surfaces which makes tooth retain its antimicrobial activity[30,31]. The results of previous study showed that a short-term exposure of dental hard tissues to tetracycline may result in a long-lasting antibacterial capacity.

However, tetracycline is well-known cause of tooth discoloration when prescribed during tooth development[32]. Moreover, in recent studies[33,34], tooth discoloration was observed when it was used as intracanal medicament. In this study, tooth discoloration was observed during observation period. This phenomenon was due to the photo-oxidation reaction. This photo-oxidation process was probably triggered by the use of sodium hypochlorite as an oxidizing agent[34].

In contemporary endodontics, using both EDTA and NaOCl is an effective method for removal of smear layer. However, 17% EDTA solution may have an erosive effect on dentin structure. Thus, it should not be remained in the root canal more than 1 min during endodontic treatment[35]. But, MTAD does not have an erosive effect and is less destructive to the root dentin structure compared with EDTA when used as a root canal cleanser[16,17].

The observation periods of this study was longer than any other leakage studies that compared efficiency of MTAD, EDTA and sodium hypochlorite. Moreover, *Enterococcus faecalis* was used in this experiment to test the leakage of bacterial species that are most resistant to endodontic disinfection. This study demonstrated that MTAD and EDTA showed similar effect in reducing bacterial leakage when used as root canal irrigants.

Considering the results of this experiment, use of MTAD as a final irrgant might be as effective as EDTA. And, the use of MTAD might be more effective than NaOCl in resistance to bacterial leakage, although there was no statistical difference.

V. CONCLUSION

The results of this study showed that MTAD was as effective as EDTA, and might be more effective than NaOCl in resistance to bacterial leakage when used with AH-26 sealer and gutta-percha.

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국문초록

최종 근관세척제로서의 MTAD 근관세척제가 치관부 세균미세누출에 미치는 영향

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본 연구의 목적은 최종 근관세척제로서의 MTAD, EDTA 그리고 차아염소산 나트륨 용액이 충전된 근관의 세균 (*Enterococcus faecalis*) 미세누출에 미치는 영향을 평가하기 위한 것이다. 40개의 발치된 사람의 대구치를 양성대조군 (1군 : n = 5), 음성대조군 (2군 : n = 5), 그리고 실험군 (3, 4, 5 군 : n = 10)으로 각각 무작위 배정하였다. 3군에서는 근관형성시 차아염소산 나트륨만 사용하여 근관세척을 시행하였으며, 4군 및 5군에서는 차아염소산 나트륨과 함께 EDTA와 MTAD를 각각 최종 근관세척제로 사용하였다. 실험군 및 대조군의 치아들은 .06 taper를 가지는 40번 profile을 master apical file로 근관형성을 시행하였으며 gutta-percha와 AH-26 근관봉함제로 근관충전하였다. 각 치아의 치관부는 *Enterococcus faecalis*를 함유한 BHI 배지와 접촉되도록 하였고 치근 끝부분은 멸균된 배양액에 위치되도록 하였다. 각각의 치아는 anaerobic chamber내에 위치되었으며 180일 동안 혼탁도를 관찰하였다. 통계처리는 95% 신뢰수준에서 Fiher's exact test를 사용하였다. 180일의 관찰기간이 경과된 후, 3, 4, 5 군은 각각 7, 4, 5 개의 치아에서 미세누출이 일어났으며 이 차이는 통계적으로 유의성이 없었다.

주요어: MTAD, 근관세척, 세균미세누출, *Enterococcus faecalis*