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백서에서 치수절단술에 사용하는 TheraCal LC, MTA 그리고 Formocresol의 비교

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〈Abstract〉

Comparison of TheraCal LC, Mineral trioxide aggregate, and Formocresolas pulpotomy agents in rat molar

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Formocresol (FC)은 치수절단술에 일반적으로 사용되어 온 재료이지만, 재료의 독성 때문에 현재 calcium hydroxide나 mineral trioxide aggregate (MTA)가 치수절단술에 널리 사용되고 있다. 최근 레진계열 calcium silicate 제재인 Theracal LC가 치수 이장재로 개발이 되었으며, 이는 광중합을 통해 경화되기 때문에 사용이 편리해서 MTA를 적용할 수 없는 치아에 사용할 수 있다. 이번 연구의 목적은 FC, MTA 및 Theracal LC를 각각 치수절단술 후에 적용했을 경우 경조직 형성 능력과 치수반응을 비교하는 것이다. Sprague Dawley Rat의 상악 대구치 치수절단술 후 FC, MTA 및 Theracal LC를 적용하였다. 경조직 형성 여부를 확인하기 위해 Skyscan을 사용해 마이크로 컴퓨터 단층촬영(micro CT) 이미지를 획득하고, hematoxylin and eosin (H&E) 염색을 하여 조직학적 반응을 확인하였다. Dentin matrix protein-1 (DMP-1)의 발현을 확인하기 위해 면역형광 염색을 시행하였다. FC를 사용한 시편에서는 경조직 형성이 관찰되지 않았으며, 치수절단술이 시행된 인접면에 염증반응이 관찰되었고 DMP-1발현은 확인되지 않았다. MTA와 Theracal LC를 사용한 시편에서는 경조직 형성이 관찰되었고 DMP-1의 발현이 확인되었다. 결론적으로, MTA나 Theracal LC를 사용한다면 남아있는 치수의 생활력과 기능을 유지시켜 보다 좋은 치료 예후를 기대할 수 있을 것으로 사료된다.

주제어 : formocresol, 면역형광법, MTA, 마이크로 컴퓨터 단층촬영, TheraCal LC

Ⅰ. INTRODUCTION

Pulpotomy is a common therapeutic procedure for not only young permanent teeth with open apex, but also for acutely inflamed primary teeth. Various medicaments or dressing agents are applied on the wounded surface of the pulp to promote healing or to cause fixation of the underlying tissue (Alacam, 1988).

Formocresol (FC), glutaraldehyde, ferric sulfate, zinc oxide eugenol, polycarboxylate cement, calcium hydroxide, and mineral trioxide aggregate (MTA) have generally been recommended as pulpotomy agents (Waterhouse, 1995). More recently, pulpotomy using laser (Liu, 2006) and

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odontogenic protein as a pulpotomy agent were introduced (Sabbarini et al., 2008).

FC, developed by Buckely in 1904, is mainly composed of formaldehyde which is a hazardous substance and is considered to be a potential carcinogenic agent (Yamasaki et al., 1994). FC (Nippon Shika Yakuhin KK, Shimonoseki, Japan) consists of 40% formalin, 40% cresol, and 20% ethanol. In spite of its clinical toxicity, FC has been widely used as a pulpotomy agent because of its great success rate (Fuks, 2002). Many studies reported on its genotoxic effect on humans based on previous animal studies, possible toxic, mutagenic, and carcinogenic risk (Lewis, 1998), necrosis and sloughing of the tissue when in contact with gingiva (Sipes and Binkley, 1986), and relationship between pulpotomy using FC and dentigerous cyst (Asián-González et al., 2007). Because of these side effects, FC must be used with caution.

MTA, which consists of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, oxides of iron and magnesium, and bismuth oxide, has attracted attention in the current endodontic field because of its ideal characteristics as a treatment material such as sealing ability (Nakata et al., 1998), biocompatibility, dentin bridge formation ability (Salako et al., 2003), and cementum and periodontal ligament regeneration (Ford et al., 1996).

TheraCal LC (Bisco Inc., Schamburg, IL, USA) used in this study is a new light-cured, resin-modified calcium silicate-filled base/liner material containing approximately 45% type III Portland cement, 10% radiopaque component, 5% fumed silica (acts as a hydrophilic thickening agent), and 45% resin component. TheraCal LC as a material for direct/indirect pulp capping is reported to have the ability to release Ca (Reston and de Souza Costa, 2009; Gandolfi et al., 2012), induce formation of apatite(Hebling et al., 2009), and it is biocompatible (Shayegan et al., 2008; Lee et al., 2017). Moreover, this material showed lesser water solubility and higher release of Ca compared to MTA and calcium hydroxide-based materials (Gandolfi et al., 2012).

Previous reports have evaluated the physical characteristics and biocompatibility of TheraCal LC, and they have reported similar or better results for TheraCal LC compared to previous calcium hydroxide-based materials and MTA. However, there is a lack of research on its ability to form hard tissue in vivo, especially for pulpotomy (Hebling et al., 2009; Reston and de Souza Costa, 2009; Gandolfi et al., 2011; Gandolfi et al., 2012).

Therefore, the aim of this in vivo study was to evaluate the capacity of hard tissue formation and pulpal response after pulpotomy with TheraCal LC, MTA, and FC.

II. MATERIALS AND METHODS

1. Animals and Surgical Procedures

All animal experiments were conducted in accordance with the ethical standards formulated by the Animal Care and Use Committee of Chonnam National University (CNU IACUC-H-2016-1).

Twenty-two male Sprague-Dawley (SD rats, 9 weeks old, weighing 200-250g, SAMTACO co. LTD, Osan, Korea) were randomly divided into 3 experimental groups according to the pulpotomy agents; FC (n=6), MTA (n=6), dental) and TheraCal LC (n=6) groups, and a control group (n=4). We used same agent in each rat. The rats were anesthetized with an intraperitoneal injection of 50 mg/kg of Zoletil 50 (Virbac, Carros, France) and 15 mg/kg of Rompun (Bayer, Leuverkeusen, Germany). Twenty-two upper first molar pulps were exposed by drilling with a 1/4 round diamond bur. Coronal pulp was removed with a spoon excavator up to the canal entrance level, and hemostasis was achieved with sterile cotton application and abundant sterile saline irrigation of the pulp chamber.

In group I, sterile cotton pellets moistened with FC were placed on the pulp stump. In group II, exposed pulps were capped with a 2-mm thick layer of ProRoot MTA (Dentsply, Johnson City, TN, USA) according to the manufacturer's recommendations. In group III, 2-mm thick layer of TheraCal LC was dispensed via a syringe directly onto the pulp stump and light activated with LED curing units (Dr's light; GoodDrs, Incheon, Korea) for 20 seconds. Materials used in this study are described in Table 1. The exposed site of all experimental groups was acid-etched (ETCH-37, Bisco Inc.), a dentine adhesive (Xeno V, Dentsply DeTrey GmbH, Constanz, Germany) was applied, and it was covered with flowable resin (Gaenial Universal Flo, GC Corporation, Tokyo, Japan). Three out of 6 rats in each group were sacrificed after 2 weeks and the remaining rats were sacrificed after 4 weeks. All animals were anesthetized and sacrificed by intracardiac perfusion with 4% paraformaldehyde buffered with sodium cacodylate 0.1 M at pH 7.2-7.4.

2. Micro CT Imaging

Maxillary alveolar regions containing the first molar were surgically isolated and immersion-fixed in 4% paraformaldehyde solution (pH 7.4) overnight. After fixation, micro-CT scans were performed with a SkyScan 1172 scanner (SkyScan, Kontich, Belgium). The scans of the teeth were obtained at 13.5 um resolution using 55 kV voltage, 181 uA current, 10 W power, and a 0.5 mm Aluminum filter. The resulting slices were reconstructed with NRecon 1.6.2.0 (SkyScan). Following reconstruction, the images were visualized with DataViewer 1.4.3 (SkyScan).

3. Histologic Measurement

After the micro CT measurements, the specimens were decalcified in Calciclear (National Diagnostics, Atlanta, GA, USA) for 8 weeks, dehydrated in a graded series of ethanol, and then embedded in paraffin. Sagittal sections were cut at 5-㎛ thickness. Specimens were stained with hematoxylin and eosin (H-E) to evaluate dentin bridge formation and pulpal inflammation. Specimens were scanned with Panoramic MIDI scanner (3D HISTECH, Budapest, Hungary), and digital image analysis was performed with Panoramic Viewer software (3D HISTECH).

4. Immunofluorescence

Immunofluorescence staining was performed using TSA ™kit (Invitrogen, Carlsbad, CA, USA). The specimens were washed with PBS, and fixed in 4% paraformaldehyde for 20 min at room temperature. The mouse polyclonal primary antibody for DMP-1 and rabbit antibody were used as primary and secondary antibodies, respectively. The nucleus was stained with 4`,6-diamidino-2-phenylindole. Sections were reacted with the primary antibody overnight, and subsequently with the biotinylated secondary antibody for 1 hour. Finally, they were incubated in Tyramide working solution for 10 minutes. After washing again with PBS for 5 minutes, endogenous peroxidase activity was blocked using 3% H_2O_2 for 1 hour. The samples were examined using an LSM confocal microscope (Carl Zeiss, Standort Gottingen, Vertrieb, Germany).

Ⅲ. RESULTS

1. Micro CT Analysis

Specimens were scanned by micro CT to assess the degree of hard tissue formation. The micro CT images after 2 weeks of pulpotomy showed evidence for formation of hard tissue (green color layer) in the MTA and TheraCal LC groups (Figure 1). Figure 2 shows specimens obtained from SD rats, which were sacrificed at 4 weeks after pulpotomy. Hard tissue formation was not observed under

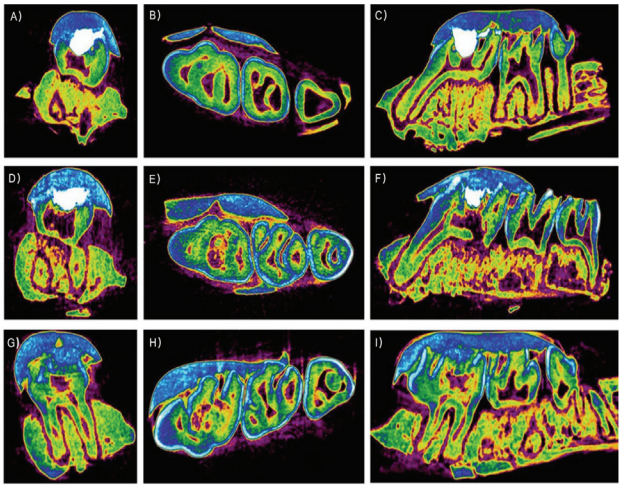

Figure 1. Micro CT images after 2 weeks of pulpotomy. (A-C) Pulpotomy with FC, (D-F) Pulpotomy with MTA, (G-I) Pulpotomy with TheraCal LC. (A, D, G) B-L sagittal view, (B, E, H) coronal view, (C, F, I) M-D sagittal view. Blue and white (pulpotomy agent or restorative materials), purple (pulp space), green (dentin).

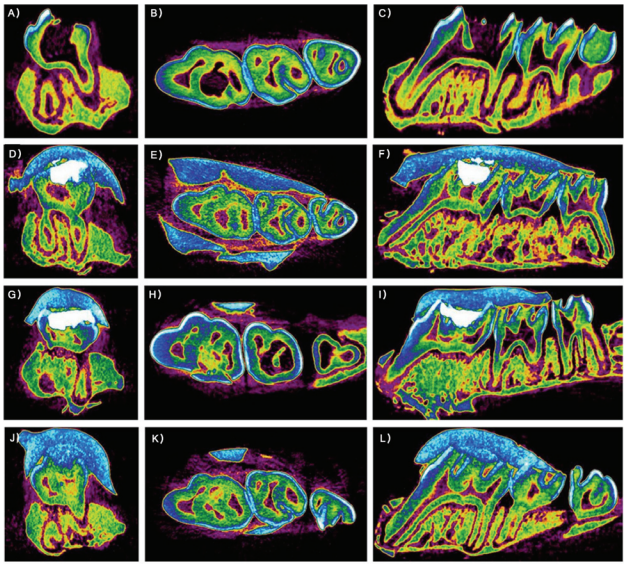

Figure 2. Micro CT images after 4 weeks of pulpotomy. (A-C) Specimen in the control group (no treatment), (D-F) Pulpotomy with FC, (G-I) pulpotomy with MTA, (J-L) Pulpotomy with TheraCal LC. (A, D, G, J) B-L sagittal view, (B, E, H, K) coronal view, (C, F, I, L) M-D sagittal view. Blue and white (pulpotomy agent or restorative materials), purple (pulp space), green (dentin).

FC (Figure 2D and E). Compared to Figure 1, hard tissue formation was clearly observed in the MTA and TheraCal LC groups (Figure 2G-I and J-L).

2. Histologic analysis (H&E stain)

Light microscopic examination of molar in SD rats showed normal dentin, secondary dentin, and pulp tissue (Figure 3A). After 2 weeks of pulpotomy, the tissue surrounding all experimental groups showed an acute inflammatory reaction (Figure 3B). In the FC group, an area of inflammatory infiltrate just beyond the pulpotomy site (I) was seen and calcific deposition was partially observed (Figure 3Ba). A shallow dentinal bridge beneath the MTA was observed. During this period, calcific tissue formation was observed along the root canal wall (Figure 3Bb). In the TheraCal LC group, pulp necrosis was observed in the coronal site, whereas the pulp was vital in the middle and apical sites. Also, there was formation of tertiary dentin close to the pulpotomy area (Figure 3Bc). After 4 weeks of pulpotomy, necrotic area just beyond the pulpotomy site followed by an area of inflammatory infiltrate was observed in the FC group. At the interface between the FC and the pulp, hard tissue formation was not observed, but formation of tertiary dentin was observed in the lower part of necrotic area (Figure 3Ca). In the MTA group, the dentin bridge became thicker in the 4- week sample, and normal pulp cells were observed under the dentin bridge (Figure 3Cb). Dentin bridge could be observed under TheraCal LC, and the pulp remained vital despite the partial presence of inflammatory cells under the dentin bridge. In addition, odontoblasts were normally arranged in the pulp tissue near the dentin area, and lacunae were formed inside the newly formed dentin bridge (Figure 3Cc and d).

3. Immunofluorescence Analysis

Expression of DMP-1 with regard to formation of mineralized tissue was assessed by immunofluorescence assay. DMP-1 expression was not observed in the FC group (Figure 4A). However, DMP-1 expression in the pulpotomy site was clearly observed in the TheraCal LC group (Figure 4B).

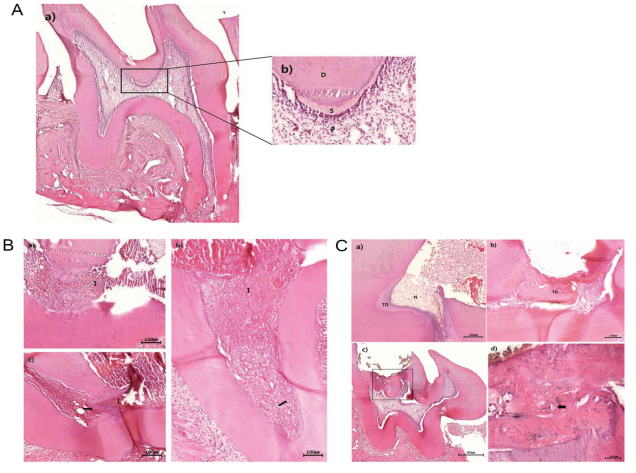

Figure 3. Histological findings of pulpotomy. (A) Normal tissue of maxillary first molar of SD rats (D: dentin, S: secondary dentin, P: pulp), (B) Pulpotomy with a) FC, b) MTA, c) TheraCal LC after 2 weeks (I: Inflammatory tissue, Black arrow: Tertiary dentin formation), (C) Pulpotomy with a) FC, b) MTA, c) TheraCal LC after 4 weeks, d) high magnified view of black box in c) (N: Necrotic area, TD: Tertiary dentin, black arrow: lacunae).

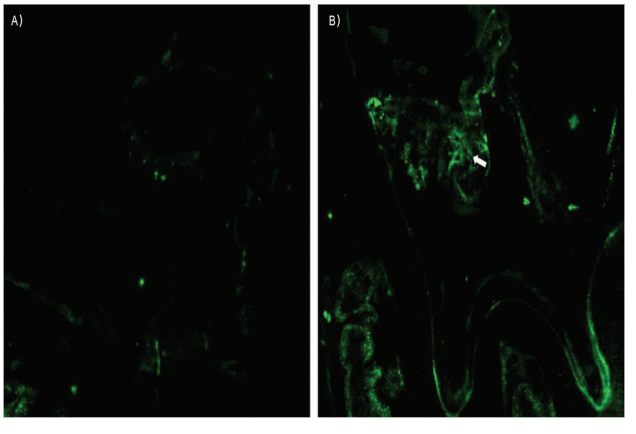

Figure 4. Representative images for DMP-1 immunofluorescence analysis of pulpotomy with A) FC, B) TheraCal LC after 4 weeks

Ⅳ. DISCUSSION

Pulpotomy is a therapeutic procedure to maintain total or partial vitality and function of the remaining radicular pulp by removing infected or decayed coronal pulp of primary or immature permanent teeth. Pulpotomy agents should maintain radicular pulp tissue, prevent bacterial microleakage, and should be biocompatible. Also, pulpotomy agents should be able to promote pulpal healing (Shayegan et al., 2008). FC is known to fix the pulp in contact by mummification, and histological reaction of the pulp depends on the concentration and application time. Pulp tissue in contact with FC is generally necrotized. Inflammatory infiltration occurs in tissues near the fixed tissue and the tissue is back to normal over time. There was replacement of inflammatory and necrotic tissue with bone or osteodentin after the application of FC (Salako et al., 2003). Therefore, the pulp maintains a metastable state, and FC pulpotomy could be considered as a clinically successful treatment as the symptoms disappear. However, in case of pulpotomy with FC, root canal obliteration, internal resorption, and pulp necrosis have been reported as frequent complications (Waterhouse et al., 2000). In the present study, when FC was applied, necrosis and inflammatory infiltration of the pulp tissue were observed without hard tissue formation. In addition, hard tissue formation was not observed on micro CT and expression of DMP-1 was not confirmed in the immunofluorescence test.

In this study, when TheraCal LC and MTA were used as pulpotomy agents, hard tissue formation was clearly observed, unlike in the sample with FC application, and the pulp remained vital. This was considered to be because of constant calcium release from TheraCal LC and MTA. In a study which compared calcium releasing ability of TheraCal LC, ProRoot MTA, and Dycal, TheraCal LC showed the highest calcium release (Gandolfi et al., 2012) . Calcium ions accelerate the expression of bone associated

Table 1. The materials for this investigation

Agents (lot number)	Composition	Type	Light curing time
Dental Formocresol (W26)	Formalin, Cresol, Ethanol	Liquid	No
ProRoot MTA (144796)	Portland cement, Tricalcium silicate, Bismuth oxide, Dicalcium silicate, Tricalcium aluminate, Tetra calcium aluminoferrite, Calcium sulfate dehydrate or gypsum	Power/ Water	No
TheraCal LC (1600001760)	Portland cement, Polyethylene glycol, dimethacrylate, MEHQ, AeroSil 200, Bis-GMA, camphorquinone, bariumsulfate, EDMAB	Single Syringe	20 seconds

protein which is controlled by calcium channel (Jung et al., 2010) , activate ATP which plays an important role in the mineralization process (Torneck et al., 1983) and stimulate osteoblast differentiation (Clapham, 1995). As a result, calcium ions play a key role in the biological cellular event which is involved in formation of mineralized hard tissues. In addition, calcium ions are essential for differentiation and mineralization of pulp cells (Schröder, 1985) and they also control the level of osteopontin and bone morphogenetic protein-2 during calcification process of the pulp (Rashid et al., 2003). Also, the activity of pyrophosphatase increases as the release of calcium ions increases, and mineralization and dentin bridge formation improves consequently (Estrela and Holland, 2003). In addition, the constant breakdown of calcium hydroxide into calcium ions and hydroxyl ions in TheraCal LC and Proroot MTA makes it alkaline by increasing pH of the surrounding fluid. The release of hydroxyl ions into surrounding tissues affects survival and proliferation of bacteria and creates an antibacterial environment. Such antibacterial properties decrease the risk of bacterial re-infection in the dentin/restoration interface and improve pulp viability by reducing secondary caries formation. Alkalinity causes an inflammatory reaction with tertiary dentin formation, but according to Gandolifi et al., release of hydroxyl ions decreases for 7-14 days and the physiological pH is reached again, which creates a favorable environment for pulp cell viability (Gandolfi et al., 2012).

DMP-1 is one of the noncollagenous proteins which includes extracellular matrix of bone and dentin, and contains acidic, phosphorylated domains as seen in the cDNA sequence of DMP-1. Such domains play an important role in regulation of matrix mineralization (George et al., 1993). In addition, DMP-1 also regulates mineralization of bone and dentin by high binding affinity between nucleation of hydroxyapatite crystals and collagen fibrils (He et al., 2003; He and George, 2004). In this study, immunofluorescence was performed in order to examine the expression of DMP-1 with regard to formation of mineralized tissue when FC, MTA, and TheraCal LC were used as pulpotomy agents. As a result, DMP-1 was not expressed in the FC group, whereas DMP-1 expression was observed in the TheraCal LC group. According to previous studies, DMP-1 is expressed by reacting with a direct pulp capping agent in the inflammation area (Chaussain et al., 2009). Therefore, DMP-1 expression played an important role in mineralized tissue formation by reacting with MTA and TheraCal LC in the tissue adjacent to the pulpotomy area.

MTA and various calcium hydroxide-based materials are clinically used in pulpotomy. Teeth treated with these

materials have a good prognosis, but dissolution of materials into the tissue fluid tends to occur over time because of high solubility of calcium hydroxide-based materials. Therefore, the sealing ability that prevents bacterial invasion reduces. In addition, MTA causes discoloration of treated teeth, and has disadvantages such as difficult handling and long setting time. TheraCal LC is easier to handle than MTA as it is dispensed from a syringe and has lower solubility than MTA and traditional calcium hydroxide-based materials (Gandolfi et al., 2012).

V. CONCLUSION

In conclusion, although TheraCal LC was initially developed as a direct / indirect pulp capping material, its use as a pulpotomy agent can be expected to facilitate mineralized tissue formation and maintain pulp vitality compared to previously used materials.

VI. ACKNOWLEDGEMENT

The authors deny any conflicts of interest related to this study.

Bin-Na Lee and Young-Sang Song contributed equally to this work as first authors.

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ABSTRACT

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TheraCal LC, a new light-cured, resin-modified calcium silicate-filled base/liner material, has been introduced as a pulpotomy agent. The aim of this study was to evaluate the capacity of hard tissue formation and pulpal response after pulpotomy with TheraCal LC. Twenty-two 9-week-old male rats were anesthetized, cavities were prepared in maxillary first molars and pulps were capped with formocresol (FC), mineral trioxide aggregate (MTA), and TheraCal LC. Specimens obtained from rats were scanned using a high-resolution micro CT system. The specimens were prepared and evaluated histologically, and immunofluorescence assay was performed to assess the dentin matrix protein-1 (DMP-1) expression. On micro CT analysis, the MTA and TheraCal LC groups showed thicker hard tissue formation than the FC group. On hematoxylin and eosin (H&E) staining, MTA and TheraCal LC groups showed dentine bridge formation with vital pulp beneath the materials. On immunofluorescence analysis, DMP-1 was highly expressed in the TheraCal LC group compared to the FC group. TheraCal LC showed similar capacity to form hard tissue as MTA when it was used as a pulpotomy agent. Because of its good manipulation and faster setting time compared to MTA, TheraCal LC could be considered as a good alternative to MTA.

Key words: Formocresol, Immunofluorescence, MTA, micro CT, TheraCal LC