Histologic evaluation of MTA as pulpotomy and pulp capping agent

치수절단술과 치수복조술에 이용한 MTA의 조직학적 평가

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국문초록

치수절단술과 치수복조술에 이용한 Mineral trioxide aggregate의 조직반응

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치수절단술과 치수복조술은 치수조직의 치유 능력에 의존한다. Mineral trioxide aggregate(MTA)는 훌륭한 봉쇄능, 치수조직에 대한 미약한 염증 반응과 높은 상아질 가교 형성 능력으로 인해 치수절단술과 치수복조술에 많이 사용되고 있다. 본 연구에서는 개의 치아에 MTA를 이용하여 치수절단술과 치수복조술을 시행한 후 치수 조직에 나타나는 조직반응을 광학현미경하에서 관찰하여 향후 임상적 효용성을 평가하고자 한다.

생후 8개월 된 세 마리의 개에서 각각 4개의 치아를 사용하였다. Ketamine(0.4 mg/kg, Ketamine HCL)과 Rumpon(1.5 ml/10kg, Xylazine HCL)을 이용하여 전신 마취시킨 후 고속핸드피스를 이용하여 각 개당 2개 치아의 협측 치경부에 5급 와동을 형성하고 와동 바닥에 직경 0.8mm의 치수노출을 시행하였다. 나머지 2개의 치아에는 치수절단술을 시행하고 생리식염수와 3% 차아염소산나트륨을 교대로 세척하여 노출된 치수를 지혈 시킨 후 각각 MTA를 적용하고 4개 치아모두를 아말감으로 수복하였다. 술 후 1, 2, 4주에 각각 개를 희생하고 치아를 발거하여 10% 포르말린용액으로 조직을 고정하였다. 40일 간 10% 포름산으로 탈회시킨 후 순차적인 농도의 에탄올로 세척 후에 파라핀에 조직을 매몰하여 협설 방향으로 7세 두께로 조직을 절단한 후 haematoxylin-eosin 염색을 시행하였다. 광학현미경하에서 치수괴사, 상아질 가교 형성 유무, 염증반응 등을 관찰하여 다음과 같은 결과를 얻었다.

- 1. 1주 경과한 조직 소견에서 치수절단술을 시행한 경우 염증소견을 보이지 않았고 주변 상아모세포는 건전한 상태로 관찰되었다. 치수복조술을 시행한 경우 경도의 염증반응과 함께 모세혈관의 증식을 보였고 정상의 인접 상아모세포가 관찰되었다.
- 2. 2주 경과한 조직 소견에서 치수절단술을 시행한 부위 하방에 상아질 가교의 형성과 함께 골모세포양세포와 골세포가 관찰되었다. 치수복조술을 시행한 부위에서는 그 보다 작고 부분적인 형태의 수복상아질의 형성이 관찰되었다.
- 3. 4주 경과한 조직 소견에서 치수절단술을 시행한 부위에 상아질 가교의 두께 가 두꺼워졌으며, 더 많은 수의 골모세포양세포와 골세포가 관찰되었다. 치수복조술을 시행한 부위에서는 노출된 치수 부위를 완전히 봉쇄하며, 기존의 상아모세포 층과 연결된 구조의 반응성 상아질의 형성을 보였다.

이상의 결과로, 치수절단술과 치수복조술에 생체친화적인 MTA의 사용은 치수의 생활력을 유지하고 상아질 가교를 형성하는 조직 반응 소견을 보여 임상적 사용이 가능하리라 사료된다.

I. INTRODUCTION

In many clinical situations, during tooth preparation and decayed dentin removal, it is possible to accidentally expose the dental pulp. The exposed vital pulp possesses a potential to heal. Pulp capping is a procedure in which an exposed pulp is covered with a dressing or cement that is placed directly at the site of exposure, by which the pulp is protected from additional injury, permitting healing and repair¹⁾. Pulpotomy is a therapeutic procedure, which consists of the surgical amputation of coronally inflamed pulp and the wounded surface of the radicular pulp is treated with a medicament or dressing agent^{2,3)}.

The main goals of both of these procedures are to induce dentinal bridge formation by pulp cells and to maintain pulp vitality¹⁻³⁾. The dentinogenic potential can be the result of an inductive biological effect of the capping material on pulp cells or as a part of the wound healing mechanism in the traumatized pulp. Healing of pulp tissue involves odontoblastic activation and dentinal bridge formation at the site of pulp exposure and undifferentiated pulp cells may potentially replace necrotic odontoblasts⁴⁾.

For more than 70 years calcium hydroxide has played a major role in performing direct pulp-capping. This material has been reported to have pulpal protection properties. It has a bactericidal effect because of its high pH. It stimulates various cell enzyme systems involved with fibroblastic proliferation, migration, healing and eventual tissue repair, as either soft or hard tissue replacement^{5,6)}. Nevertheless, it has been shown that the macroscopic dentinal bridge formed by calcium hydroxide does not constitute continuous seal and may allow bacterial leakage through numerous tunnel

defects⁶⁾.

Also, formocresol(FC) is the most commonly used agents for pulpotomized primary molars. But concerns have been expressed about formocresol pulpotomy because of observed: (1) pulpal responses with inflammation an necrosis (2) cytotoxicity (3) systemic disturbances (4) mutagenic and carcinogenic potential and (5) immunologic response. Because of its deleterious effects the use of FC is decreasing considerably worldwide. Thus, different alternatives have been proposed to maintain pulp vitality effectively⁷⁾.

Not only the toxic potential of the material used but also the marginal seal must be considered when the biological properties of restorative materials are evaluated. This concept is in agreement with the definition of biocompatibility as the ability of a material to perform with an appropriate host response in a specific application⁸⁾.

Mineral trioxide aggregate (MTA) has been introduced as a superior material for pulpal therapy. Many favorable features are its excellent sealing ability, biocompatibility, ability to form dentinal bridge and cementum and periodontal ligament regeneration^{9,10)}. Several studies reported better responses in dental pulps treated with MTA than teeth treated with calcium hydroxide or acid-etched dentin bonding^{11,12)}. Furthermore, MTA has demonstrated minimal leakage of dye and bacteria in comparison with other restorative materials^{13,14)}.

The purpose of this in vivo study was to evaluate the presence or absence of necrosis, dentinal bridge formation, inflammation, odontoblast configuration and resorption of dentinal walls in dog teeth when MTA was used as pulpotomy and pulp capping agent according to time periods.

II. MATERIALS AND METHODS

Three 8-month-old dogs were used in this study. They were anaesthetized with Ketamine(0.4 mg/kg, Ketamine HCL) and Rumpon(1.5 ml/10kg, Xylazine HCL) injected intravenously at the beginning of the experimental procedures. In addition, infiltration anesthesia was performed with lidocaine hydrochloride containing 1: 80,000 epinephrine. Intact and easily accessible teeth with healthy periodontium were selected. The periapical radiographs were taken before the procedure (Fig. 1). After the teeth were isolated with cotton rolls, Class V cavities were prepared on the facial surface of each tooth using #330 carbide bur under constant water-spray coolant. The teeth were selected and randomly divided into six groups of two specimens each (Table 1). The pulps were intentionally exposed with a round carbide bur of 0.8mm diameter at the cavity floor. Standardized pulp exposure (0.8 millimeter in diameter) was completed. In pulpotomy groups, a spoon excavator was used for coronal pulp amputation. Bleeding was controlled by repeated and alternative irrigation with 3% solution of sodium hypochloride and saline and application of sterile pellets moistened with saline until physiological hemostasis occurred.

The MTA (ProRoot[®], Dentsply, Tulsa, OK, USA) were prepared according to the manufacture's directions with MTA powders and sterile saline in a 3:1 ratio to provide sandy and putty mixture. The MTA was placed at the exposed site with a MTA Endo Carrier and tubes and light pressure was applied with a wet cotton pellet resulting in cramming of material into pulp space. The cavities were immediately restored with amalgam. Light pressure was applied to the amalgam during condensation (Fig. 2).

TABLE 1. Tooth distribution by groups and experimental periods

Group		Description	Time period	total
Group	A-1	Pulpotomy + MTA + Am	1 week	2
	B-1	Pulp capping + MTA + Am	1 week	2
Group	A-2	Pulpotomy + MTA + Am	2 week	2
	B-2	Pulp capping + MTA + Am	2 week	2
Group	A-4	Pulpotomy + MTA + Am	4 week	2
	B-4	Pulp capping +MTA + Am	4 week	2

MTA: Mineral trioxide aggregate, Am: Amalgam.

Group A: pulpotomy, Group B: pulp capping.

At 1, 2 and 4 weeks after operation, the dogs were sacrificed by intravenous injection of 10% Nembutal. The mandible and maxilla of each animal were immediately dissected free. The teeth were immediately removed from the jaws with diamond disk. To fix the pulp, the apical thirds of each tooth root was sectioned to allow penetration of 10% neural buffered formalin solution (pH 7.2). The teeth were kept in formalin for 4 days and subsequently demineralized in 10% formic acid for 40 days. After washing and dehydration in ascending series of ethanol, the teeth were cleaned in xylene. They were then vaccum-infiltrated with paraffin wax and finally embedded in paraffin for 12hs.

Serial sections 7μ m thickness were cut facio-lingually through the

exposure site and stained with haematoxylin-eosin. The amalgam was gently removed before sectioning.

All of the samples were analyzed for necrosis, calcified bridge formation, inflammation (acute or chronic), odontoblast configuration and resorption under light microscope (Ziess, Gottingen, Germany). All specimens were seen by an oral pathologist who was not aware of used materials and time intervals.

II. RESULTS

In all post operative periods, the light microscopic analysis showed histological changes in the superficial reaction zone close to the MTA-pulp interface. The underlying pulp tissue was consistently found to be a normal structure without any sign of inflammation or tissue degeneration.

The reaction zone was of variable width and composed of pulpal cells, remnants of blood, areas of tissue coagulation, traces of capping material, dentinal fragments and post operatively formed hard tissue. A few sattered inflammatory cells (predominantly macrophages and lymphocytes) were also seen in the examined specimens of early observation period. The analysis was further focused at the reaction zone along the wound surface and the MTA-pulp interface at each experimental period between pulpotomy and pulp capping procedure.

1. Pulpotomy after 1 week

The pulp tissue was consistently found to be a normal structure without any sign of inflammation or tissue degeneration (Fig. 3A). Adjacent Odontoblast were observed normally (Fig. 3B).

2. Pulp capping after 1 week

A small particle of calcified bridge combined with dentin fragment could be seen under the exposure site. Osteoblasts like cells were observed around dentin chip. Mild inflammation as a few macrophages and lymphocyte could be seen. Adjacent odontoblasts could be seen normally (Fig. 4).

3. Pulpotomy after 2 weeks

The dentinal bridge which charactered by osteodentin was found (Fig. 5A). Osteoblast-like cell and osteocytes embedded in calcified matrix were seen in this group (Fig. 5B).

4. Pulp capping after 2 weeks

The numbers of inflammatory cells were less than in the 1-week specimen. The osteodentin barriers surrounded by osteoblasts were found to be atubular in form (Fig. 6).

5. Pulpotomy after 4 weeks

The thickness of the bridge had increased. Similar to the 2-week specimens, osteoblast and osteocyte were seen around and in the osteodentin (Fig. 7).

6. Pulp capping after 4 weeks

The hard tissue barriers in contact with vital pulp completely bridging the pulpal wound surfaces were found. The barriers consisted of a coronal irregular layer of osteodentin and were continuous with normal dentin and predentin. This seemed to be not a reparative dentin but a reactionary dentin which formed by primary odontoblasts (Fig. 8).

IV. DISCUSSION

The main purpose of this study was to evaluate and compare the pulpal responses to MTA as pulpotomy and pulp capping agent. The healthy pulp has good healing potential when bacterial microleakage can be controlled 14,15). Cox and associates 160 have shown that pulp healing is more dependent on the capacity of the capping material to prevent bacterial microleakage rather than the specific properties of the material itself. Therefore, it seems that mechanically exposed pulp possess an inherent healing capacity for cellular reorganization and dentin bridge formation when there is a proper biological seal⁸⁾. In this study, there was a continuous reparative dentin bridge formation at 2 weeks after treatment with MTA without inflammation and tissue necrosis in both pulp capping and pulpotomy group. This result is similar to several previous studies 13,17,18). The cells in pulp capping group showed typical odontoblast characteristics, while the cells of reparative dentin in pulpotomy group were round in shape, organized as a sheet of cells and trapped in osteodentin-like mineralized tissue. In group of pulp capping with MTA after 4 weeks the lower layer of the calcified bridge contained predentin and dentinal tubule-like structures as normal dentin. On the other hand, there was an osteodentin formation in pulpotomy group. This result reflected that two different types of reparative dentin formation, dentin-like and bone-like dentin, maybe dependent, partly at least, on the type and extent of the injury and the effect of the associated defense reaction on the structural and functional integrity at the dentin-pulp border⁶. In addition, it could suppose that a dentin chip pushed inside during operation could play a role with reactionary dentin formation under

some communication with odontoblast.

Following these results, MTA indicate the material's biocompatibility and regeneration ability. These findings are similar to several previous in vivo studies with MTA^{13,14,18)}. Furthermore, Koh et al¹⁷⁾ believe that MTA stimulates the release of cytokine that, in turn, promotes hard tissue genesis.

Dental pulp repair, characterized by the formation of a dentinal bridge, has been favorably reported following pulp capping of exposed pulps with calcium hydroxide materials. Some of the previous studies have observed that there were tunnel defects or incomplete dentinal bridge formation that could be a source of bacterial invasion after pulp capping used calcium hydroxide and finally it failed to provide a long-term seal against microleakage⁷. Relatively new material, MTA has better sealing ability. So it is widely used for pulp capping, root-end filling and repair of root perforations ^{13,15,16}. Several previous studies have shown that the pulp responds favorably to the protection by a MTA layer⁹⁻¹⁸ and furthermore the reparative dentin was consistently thicker and more uniform under MTA compared with calcium hydroxide^{10,15}. Once set, MTA has a compressive strength that equals some of the fortified ZOE bases. Because MTA sets hard and has negligible solubility, it should prevent recontamination of dental pulp, too¹⁴.

In addition, when a practitioner caps and fills the cavity with MTA, it is necessary to use gentle pressure to pack the MTA and minimize any harmful effect on pulp tissues which would have been injured during cavity preparation. In this study, using MTA carrier and tube can help easy and appropriate application of MTA on exposed pulp. Considering its creamy character, cramming with some pressure inside the pulp is better to achieve complete sealing. When amalgam is used as a final restoration just after pulp capping, more pressure will be needed to condense it and may dislodge

MTA, which is not yet set at that time.

It should be emphasized that these procedures were done under ideal conditions; ie, all teeth were free of caries and pulpal inflammation, which may impair the healing. Therefore, studies under such compromised conditions would be of great value¹⁶⁾. No serious inflammatory reactions were observed in this study. When teeth were injured by disease or carious progression prior to capping greater inflammatory reactions would be more likely to occur. Therefore, the clinicians should concern that these results are different from clinical situation.

As recommended by Torabinejad and Chivian, use of MTA as a dressing material could help pulp to recover promptly. However, it would be more suitable dressing in cavities, which have no or little occlusal stress such as class V cavities²⁰⁾. In this study, all teeth treated were well preserved until the end of experimental period.

Following a result of mild trauma to the pulp tissue, the primary odontoblasts are stimulated to produce reactionary dentin. In case of severe insult to the pulp, the primary odontoblasts are usually eliminated. Induction of undifferentiated pulp cells with proper stimulus may result in a new generation of odontoblasts, which can produce reactionary dentin and create a barrier to protect the pulp from further insult¹⁷. In this study, although MTA injured some of pulp cells, it could induce a slight increase in proliferation of odontoblast-like cells and reationary dentin formation. Therefore mild stimulus with bacteriometic seal seem to develope not reparative dentin but reactionary dentin. Further experimental data of immunohistochemistry are needed to determine the character of two different types of reparative dentin.

V. CONCLUSION

This study evaluated histologic pulp response after pulpotomy and pulp capping using mineral trioxide aggregate. Eight teeth of 3 dogs were treated with MTA. Class V cavities were prepared on the facial surface of each tooth and the pulps were intentionally exposed. After bleeding control with repeated and alternative irrigation with 3% NaOCl and saline physiological hemostasis occurred. The MTA applicated on the exposed pulp and wet cotton pellet applied with light pressure. The cavities were immediately restored with amalgam.

At 1, 2 and 4 weeks after operation, the dogs were sacrificed and the teeth were removed. The teeth were fixed in 4% paraformaldehyde and subsequently demineralized in 10% formic acid for 40 days. They were embedded in paraffin and cut facio-lingually in 7μ m thickness and stained with haematoxylin-eosin.

All of the samples were analyzed for necrosis, calcified bridge formation, inflammation(acute or chronic), odontoblast configuration and resorption under light microscope.

The results were as follows:

1. One-week group

In case of pulpotomy, the pulp tissue was consistently found to be a normal structure without any sign of inflammation or tissue degeneration. In case of pulp capping, mild inflammation as a few macrophages and lymphocyte could be seen. In both group normal odontoblasts could be seen at adjacent exposed pulp.

2. Two-weeks group

There were continuous reparative dentin bridge formation at 2 weeks after treatment with MTA without inflammation and tissue necrosis in both pulp capping and pulpotomy group.

3. Four-weeks group

In both pulpotomy and pulp capping group the thickness of the bridge had increased. In case of pulp capping the lower layer of barrier was continuous with normal dentin and predentin. This seemed to be not a reparative dentin but a reactionary dentin which formed by primary odontoblasts and was similar with normal dentin.

These results suggest that two different types of reparative dentin formation, dentin-like and bone-like dentin, maybe dependent, partly at least, on the type and extent of the injury and the effect of the associated defense reaction on the structural and functional integrity at the dentin-pulp border. MTA performs ideally as pulpotomy and pulp capping agent, causing dentin bridge formation and simultaneously maintaining normal pulpal histology during the early wound healing process.

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FIGURE LEGENDS

- Fig. 1. Standard radiograph before treatment
- Fig. 2. Standard radiograph after treatment
- Fig. 3. Pulpotomy with MTA after 1 week: **A.** Absence of inflammation and no dentin bridge has formed (H&E, x40).
 - **B.** Normal odontoblasts near the exposure site(arrows) (H&E, x400)
- Fig. 4. Pulp capping with MTA after 1 week. The pulp is inflamed slightly. a small dentin chip pushed inside the cavity is seen (H&E, x40).
- Fig. 5. Pulpotomy with MTA after 2 week: **A.** Complete dentin bridge(arrow) has formed under MTA (H&E, x40).
 - **B.** Higher magnification of inset of Fig. 5A. Regularly arranged osteoblastic layer(arrows) surrounded osteodentin (H&E, x100).
- Fig. 6. Pulp capping with MTA after 2 week: The presence of mineralized particle surrounded by odontoblast-like cells beneath the exposure site under MTA (H&E, x40).
- Fig. 7. Pulpotomy with MTA after 4 week: Osteocyte are seen in the matrix of osteodentin (H&E, x400).
- Fig 8. Pulp capping with MTA after 4 week: A complete dentin barrier sealing the exposed pulp. The hard tissue contains predentin-like(arrows) unmineralized tissue layers (H&E, x100).

FIGURES



Fig. 1. Standard view of before treatment



Fig. 2. Standard view of after treatment

