The effect of mineral trioxide aggregate on dental pulp healing in the infected pulp by direct pulp capping

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This study aimed to clarify the effect of mineral trioxide aggregate (MTA) on pulp healing in the infected pulp by direct pulp capping (DPC). Thirty-six male ICR mice were divided into infected and uninfected groups. The pulp tissue was exposed to the oral flora for 24 h after pulp exposure in the infected group, or not exposed in the uninfected group, followed by sealing with MTA, calcium hydroxide cement (CH), or no DPC. Pulpal healing process was analyzed by hematoxylin-eosin staining and immunohistochemistry for nestin and Ki67. The active cell proliferation occurred on 1 week in the both MTA and CH groups, followed by the differentiation were not facilitated in the CH group. MTA is suggested to be a useful material for DPC with the infected and uninfected pulp tissue.

Keywords: Mineral trioxide aggregate, Dental pulp capping, Inflammation, Cell differentiation, Odontoblasts

INTRODUCTION

Direct pulp capping (DPC) is a treatment for accidental exposure of pinpoint-sized areas of pulp as a result of cavity preparation or traumatic injury with no pulp infection. Therefore, DPC is indicated in cases of normal pulp or reversible pulp inflammation, and it is not recommended for irreversible pulpitis that results from caries. However, irreversible pulpitis is difficult to diagnose histologically; the diagnosis is based on clinical symptoms and can therefore be inaccurate, and the result can be a poor outcome. The use of DPC for infected pulp is controversial, and new pulp capping materials must be developed, which requires additional experiments or clinical investigations. Mineral trioxide aggregate (MTA) has been used as a DPC material, and excellent clinical results have been reported, as opposed to results of the use of conventionally calcium hydroxide cement (CH). In most clinical reports, the use of DPC in normal or reversible pulpitis has been highly successful, which demonstrates the efficacy of MTA.

Due to unpredictable treatment results and limited research methods, few clinical and experimental studies have focused on DPC on pulp infected with bacteria. Recent clinical studies have demonstrated that MTA plays a role in blocking infection and induction of dentin production for infected pulp. In two investigations of the clinical effects of MTA application to the exposed pulp of permanent molars during caries progression, success rates were 93%–98% in 3-8 years. MTA expands slightly while setting; this may at least partly contribute to its good sealing ability. Moreover, apatite-like materials fill the MTA-dentin interfacial space. Apatite is deposited in collagen fibers, and the apatite interface layer has a taglike structure extending to dentinal tubules.

Furthermore, MTA has antibacterial action that may have positive effects on infected dental pulp. The antibacterial effects of MTA are probably related to the elevated pH that results from ionisation, which releases hydroxylated ions. This activity of MTA is, however, restricted to some facultative bacteria and does not affect strictly anaerobic bacteria, and it is also weaker than the action of zinc oxide eugenol cement. The antibacterial activity of the calcium hydroxide-based sealer Sealapex™ (Kerr, Orange, CA, USA) is superior to that of various MTA formulations and Portland cement. In summary, MTA’s antibacterial activity may not be as potent as traditional calcium hydroxide-based cements and sealers, but it helps reduce bacterial contamination in pulp wounds.

Dentin formation as dentin bridges is essential for the success of DPC, but according to one research
report, MTA has excellent biocompatibility and excellent dentin bridge system performance. MTA is highly biocompatible with pulp cells, minimizes pulp inflammation and promotes pulpal healing. In addition, it is reported to promote cell proliferation and odontoblast-like cell differentiation, which results in dentin bridge formation.

The purpose of this study was to elucidate the efficacy of MTA for infected dental pulp, which involved off-label use of DPC. To clarify the mechanism of direct efficacy of MTA for infected dental pulp, which involved (Fuji II, GC, Tokyo, Japan), based on a previous study (28-316-6).

Materials and Methods

All animal experiments complied with the guidelines by the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of the Environment and the Science Council of Japan and were carried out in accordance with the Act on Welfare and Management of Animals in Japan. All of the animal experiments were conducted in compliance with a protocol reviewed by the Institutional Animal Care and Use Committee of Niigata University, Niigata, Japan and approved by the university president (28-316-6).

Pulp capping procedures

Thirty-six ICR mice (6 weeks old) were divided into two groups: the infected and uninfected models. Each model was further divided into three subgroups according to the pulp capping materials: calcium hydroxide cement (CH group), MTA (MTA group) and glass ionomer (GI [control] group). Anaesthesia was induced via the intraperitoneal injection of 350 mg/kg of 8% chloral hydrate (Wako, Osaka, Japan) and 1/4 round carbide burs in a high-speed handpiece at 200,000 rpm were used to create class I cavities on the occlusal surfaces of both maxillary first molars. Haemorrhage was controlled with sterile cotton pellets. For the infected model, the cavities were maintained without any further treatment (such as air drying, etching, or filling) for 24 h.

Our preliminary microbiological study showed that certain bacterial species found in the plaque on teeth moved into the dental pulp and proliferated 24 h after pulp exposure, suggesting the onset of a possible infection in the pulp tissue (data not shown). Subsequently, the exposed pulp was irrigated with sterile saline solution and capped first with calcium hydroxide cement (Life™, KerrHawe, Bioggio, Switzerland) in the CH group or MTA cement (ProRoot® MTA, Dentsply Sirona) in the MTA group and then with a self-curing GI cement filling (Fuji II, GC, Tokyo, Japan), based on a previous study demonstrating the clinical application of MTA followed by GI cement for successful vital pulp therapy. In the uninfected model, after dental pulp exposure, the teeth were immediately capped with calcium hydroxide cement in the CH group or MTA cement in the MTA group and then by a GI cement filling. In the GI (control) groups of both models, the cavities were sealed with only glass ionomer cement.

Tissue preparation

Materials were collected in groups of three animals 1 week (MTA group: n=6; CH group: n=6; GI group: n=6) and 2 weeks (MTA group: n=6; CH group: n=6; GI group: n=6) after pulp capping in each model. Under deep anaesthesia by an intraperitoneal injection of 8% chloral hydrate (350 mg/kg) and after transcardial perfusion with physiological saline at each stage, the animals were perfused with 4% paraformaldehyde in a 0.1-mol/L phosphate buffer (pH=7.4). The specimens were dehydrated through an ethanol series and embedded in paraffin after decalcification in a 10% ethylenediamine tetraacetic acid (EDTA)–disodium salt solution for 3 weeks at 4°C. Sagittal sections 4 μm thick were cut and mounted on glass slides coated with Matsunami adhesive silane (Matsunami Glass Industry, Osaka, Japan) and stained with hematoxylin-eosin.

Immunohistochemistry

For immunohistochemistry studies, we used a mouse anti-rat anti-nestin monoclonal antibody diluted 1:500 (Millipore, Temecula, CA, USA; catalogue number MAB353) and a rat anti-mouse Ki67 monoclonal antibody diluted 1:100 (Dako Japan, Tokyo, Japan; catalogue number M7249). We followed the procedure detailed by Quispe-Salcedo et al.

Numbers of Ki67-positive cells and statistical analysis

The numbers of Ki67-positive cells in the coronal and root pulp of each specimen (medial side only) were calculated separately. We did not distinguish endothelial cells with inherent pulpal cells. Subsequently, Ki67-positive cells per unit area were measured by means of image analysis and measurement software WinROOF (version 6.5.3, Mitani, Tokyo, Japan). All data were calculated as means and standard deviations in each group. The numbers of cells in the coronal and root pulp were compared between groups in one-way analysis of variance after the confirmation of data normality and homogeneity of variance, followed by the Bonferroni test for multiple comparisons with statistical software (Bell Curve, Japan). The samples showing non-normal distribution were compared using Kruskal–Wallis test.

Number of nestin-positive cells and statistical analysis

The percentage of nestin-positive perimeters in the total perimeter along the pulp–dentin border was calculated on weeks 1 and 2 with ImageJ software (ImageJ 1.45s, National Institutes of Health, Bethesda, MD, USA). The statistical analysis was the same as described for Ki67-positive cells.
RESULTS

Histological and nestin immunohistochemical evaluation of the dental pulp in the infected model

Inflammatory cell infiltration was limited in the exposed and coronal pulp in the MTA group (Fig. 1A, C). Intense inflammatory cell infiltration was recognised in the coronal pulp of the CH and GI groups (Fig. 1E, G, I, K). Intact coronal and root odontoblasts showed intense immunoreactivity to nestin within the cell bodies and the bottom of the cell processes (Fig. 1B, D, F, H, J, L). One week after the creation of the cavities, numerous inflammatory cells such as neutrophils appeared in the dental pulp in all groups. Nestin-positive reactions were observed from pulpal floor to the root apex in the MTA group, whereas the pulpal floor lacked positive reactions in the CH and GI groups. Two weeks after the procedure, nestin immunoreaction was the same as that after the first week in the MTA group, whereas nestin-positive areas were reduced in the CH and GI groups. The nestin-positive areas within the total perimeter in the mesial pulp are shown in Fig. 1J, L. After the second week, pulpal healing was significantly worse in the GI group than in the MTA group.

Histological and nestin immunohistochemical evaluation of the dental pulp in the uninfected model

One week after cavity creation, inflammatory cell infiltration in the uninfected MTA group was less extensive than in the infected MTA group (Fig. 2A, C). Nestin-positive reactions were observed along the pulp–dentin border throughout the dental pulp in the MTA group. In the CH and GI groups, inflammatory reactions were intense (Fig. 2E, G, I, K), and nestin-positive areas were reduced. Two weeks after cavity creation, nestin-positive reactions were unchanged in all groups. The percentages of nestin-positive areas within the total perimeter are shown in Fig. 3. The percentage of nestin-positive areas in the infected coronal pulp was significantly higher in the MTA group than in the GI group after the second week. The percentage of nestin-positive areas in the uninfected coronal pulp was significantly higher in the MTA group than in the CH and GI groups after the first week. The percentage of nestin-positive areas was significantly higher in the MTA group than in the CH and GI groups after the first and second weeks.

Cell proliferation in the dental pulp of infected and uninfected models evaluated with Ki67 immunohistochemistry

The number of Ki67-positive cells in the infected coronal and root pulp was significantly higher in the MTA group than in the GI group after the first week and higher in the MTA group than in the CH and GI groups after the second week in Fig. 4M, N. The number of Ki67-positive cells in the root pulp was significantly higher in the CH group than in the GI group after the first week.

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Fig. 1  (A, C, E, G, I, K) Hematoxylin-eosin staining and (B, D, F, H, J, L) nestin immunoreactivities in the direct pulp capping with infection, (A, B, E, F, I, J) 1, (C, D, G, H, K, L) 2 weeks after the operation in the (A–D) GI, (E–H) CH, and (I–L) MTA groups. Scale bars=(A, C, E, G, I, K) 100 μm; (B, D, F, H, J, L) 500 μm; (Insets) 50 μm. D, dentin; DP, dental pulp; OB, odontoblastlike cells.
DISCUSSION

The results of this study demonstrate that MTA works more effectively against the infected pulp during DPC and promotes earlier pulpal healing than does either GI alone or calcium hydroxide cement. The expression of nestin and Ki67 levels was correlated with the histological data in the chronological changes during the pulpal healing process. The nestin-positive reaction is a useful marker of odontoblast differentiation. Nestin-positive newly differentiated odontoblast-like cells were aligned from pulpal floor to root apex in the MTA group 2 weeks after cavity creation, whereas odontoblast-like cell differentiation was not activated in the CH group. Of interest was that the Ki67 immunohistochemistry evaluation showed greater cell proliferation in both infected and uninfected MTA groups after 1 and 2
Fig. 4 Cell proliferation in the GI, CH, and MTA groups evaluated by immunohistochemistry. Quantitative analysis of the number of Ki67-positive cells in the (M) coronal and (N) root dental pulp during weeks 1 and 2 and (A–L) immunohistochemistry for Ki67 on (J and K) weeks 1 and (L and M) 2 in the (A, D, G, J) GI, (B, E, H, K) CH, and (C, F, I, L) MTA. Scale bars=(A–L) 50 μm.

weeks than in the GI groups. Thus, in the MTA groups, the active cell proliferation began during the first week, followed by odontoblast-like cell differentiation during the second week, whereas the differentiation of odontoblast lineage cells did not occur, regardless of active cell proliferation in the root pulp after the first week, in the CH groups. GI cements are biomimetic materials with a wide range of use from a liner/base to atraumatic definitive restorations\textsuperscript{27,28}, although the \textit{in vitro} cytotoxicity of GI cements\textsuperscript{29} and the \textit{in vivo} studies showing pulpal inflammation caused by GICs\textsuperscript{30,31} have been reported. In contrast, a recent study demonstrated that GI cements caused mild pulp damage but did not affect dental pulp stem cell viability\textsuperscript{32}. Nevertheless, we cannot exclude the negative effects of a self-cured glass ionomer cement on the infected and uninfected dental pulp. Furthermore, we should consider the interactions of materials with different properties, such as alkaline-base and acid-base materials. In this study, potential interactions of materials with different pH are not evaluated. The results must be interpreted with caution and some limitations should be borne in mind. We would evaluate the effects of the experimental materials in the conditions where the interactions of materials with different properties is eliminated by interposing some material between them in the future.

Only a few studies of the effects of MTA on the infected pulp during DPC have been conducted. In an \textit{in vivo} study in 2015, a model of infected pulp was developed in rats\textsuperscript{33}. Results of \textit{in vivo} and \textit{in vitro} studies in which MTA was applied to this model suggested that MTA is capable of inducing hard tissue formation and a weaker inflammatory response than is CH, even in infected pulp\textsuperscript{14}. Whether the formed hard tissue is dentin bridge remains to be elucidated, as does the healing mechanism of the infected pulp. The infected model in our experiment is the first animal model in mice of DPC on the infected pulp. MTA may also be useful in DPC as a biofunctional material compatible with the infected pulp, and the healing process with MTA was better than that with CH.

Our results with the uninfected mouse model were consistent with those of previous reports\textsuperscript{35–37} in which different animals, such as rats, pigs, monkeys and dogs, were used to evaluate the effects of direct MTA capping on the uninfected pulp. All animal studies showed a high rate of dentin bridge formation after DPC with MTA. Similarly, in several studies of the clinical performance of MTA as a DPC medicament, MTA accelerated the healing of the damaged pulp and the formation of
the hard dentin bridge\textsuperscript{6,8,10}. Pulpal wounds treated with MTA were mostly free from inflammation after 1 week, and a compact, hard tissue barrier that steadily increased in length and thickness covered the wounds within 3 months after DPC. In the control teeth treated with calcium hydroxide cement, a hard tissue barrier formed less consistently, and the barrier that did form contained numerous tunnel defects. The presence of pulp inflammation up to 3 months after DPC was recognisable in specimens treated with calcium hydroxide cement\textsuperscript{38}. MTA resulted in a significantly better pulpal response, with less inflammation and a thicker dentin bridge 8 weeks after DPC\textsuperscript{10}.

Overall, the clinical outcome of DPC and pulpotomy with MTA seems better than the outcome with calcium hydroxide cement, although there are still a limited number of controlled prospective studies. It is noteworthy that the odontoblast-like cell differentiation in the uninfected coronal pulp was significantly more extensive in the MTA group than in the CH and GI groups and that this differentiation at the uninfected root pulp was more extensive in the MTA group than in the GI group 1 week after DPC. The findings indicate that calcium hydroxide cement and GI disturb the differentiation of odontoblast-like cells, at least in the uninfected coronal pulp. Thus, clinicians should consider the negative effects of DPC with calcium hydroxide cement or GI in cases of uninfected pulp. The negative effect of calcium hydroxide cement is supported by our findings that 2 weeks after DPC, cell proliferation in the root pulp was significantly less extensive in the CH group than in the MTA group.

CONCLUSION

DPC with MTA worked more effectively in infected pulp and promoted earlier pulpal healing than did DPC with GI alone and calcium hydroxide cement. Active cell proliferation occurred during the first week after DPC in both the MTA and CH groups; in the MTA group, this was followed by odontoblast-like cell differentiation during the second week, which did not occur in the CH group. MTA is therefore suggested to be a useful biofunctional material in DPC with infected and uninfected pulp tissue.

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CONFLICTS OF INTEREST

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

REFERENCES


