Effects of Mineral Trioxide Aggregate on Human Dental Pulp Cells after Pulp-capping Procedures

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Abstract

Introduction: Pulp-capping procedures are routinely performed. The control of infection and biocompatibility of the pulp-capping materials are important factors in determining the treatment outcome. Calcium hydroxide has been considered the gold standard for this procedure. However, previous reports have reported the causes of failures with the use of calcium hydroxide. Mineral trioxide aggregate (MTA) has proved to be effective in the process of pulp capping. **Methods:** Human dental pulp stromal cells (DPSCs) were cultured on gray MTA, and the levels of gene expression, secretion of vascular endothelial growth factor, and the surface morphology were analyzed. **Results:** MTA promoted cell survival and proliferation, which was significantly different from the controls in human DPSCs. MTA upregulated the expression of transcription factors like Runx2 and genes like osteocalcin, alkaline phosphatase, and dentin sialoprotein, which are important odontoblastic genes, thereby showing the ability to promote differentiation of the pulpal cells into odontoblast-like cells, which, in turn, are responsible for dentin bridge formation. MTA approximately induced a 1.7-fold increase in the secretion of angiogenic factors like vascular endothelial growth factor, which is important in the process of tissue healing and regeneration. The differences between the control and the MTA groups were statistically significant. Scanning electron microscopic studies revealed the differences in the surface morphologies between the control and the MTA groups. **Conclusion:** Overall, this study supports the biocompatible nature of MTA and the possible mechanism of dentin bridge formation along with tissue repair, all of which contribute to a successful treatment outcome. *(J Endod 2010;36:1042–1047)*

Key Words

Dental pulp stromal cells, differentiation, mineral trioxide aggregate, odontoblasts, vascular endothelial growth factor

Pulp capping can be best described as capping of the exposed pulp and is indicated for reversible pulp tissue injury after physical or mechanical trauma in developing or mature teeth. The control of infection and biocompatibility of the pulp-capping materials are important factors in determining the treatment outcome (1). Ultimately, the goal of treating the exposed pulp with an appropriate pulp capping material is to promote the dentinogenic potential of the pulpal cells (1). Dentin-bridge formation can occur under a number of pulp capping materials. Historically, calcium hydroxide has been studied and used extensively in clinical situations. Despite its wide use, calcium hydroxide is not ideally suited for pulp capping. Calcium hydroxide used in direct pulp-capping procedures has three major causes of failure: (1) the porosity of the dentinal bridge that is produced, (2) poor adherence of calcium hydroxide to the dentin, and (3) its inability to provide a long-term seal against microleakage (2).

In addition, it has been noted that calcium hydroxide destroys a thin layer of the underlying pulp tissue, leaving a necrotic layer, because of its high pH (3). Hence, there have been recent attempts to develop more effective pulp-capping materials. One of these materials is mineral trioxide aggregate (MTA), which appears to have more reliable effects and has proved more beneficial than materials previously used. MTA consists of 50% to 75% wt calcium oxide and 15% to 25% silicon dioxide. The two components together comprise 70% to 95% of the cement (4). MTA has proved to have a wide range of applications, including pulp capping (5–7), perforation repair (2, 8), apexification procedures (9), and root-end filling (10, 11). Direct pulp-capping experiments have reported that MTA induced the formation of dentin bridges with little or no inflammation (12) and has been shown to generate a greater frequency of dentin bridge formation than the other materials used in pulp capping (13). It has proved to be the material of choice for this procedure.

Previous research has shown the effects of MTA on cementoblasts and osteoblasts at the molecular level (14). MTA has also been shown to permit cementoblast attachment and growth and the production of mineralized matrix gene and protein expression (15). There have been a few reports suggesting the anti-inflammatory effects of MTA on the pulp (12, 16). However, the effects of MTA on the dental pulp stromal cells (DPSCs) have not been studied in detail. Furthermore, the precise mechanism of dentin bridge formation after exposure and the pulpal response to MTA have not yet been fully elucidated.

During dentin formation, odontoblasts synthesize and secrete several noncollagenous proteins into the dentin extracellular matrix (17). Of these, dentin sialoprotein (DSP) and alkaline phosphatase (ALP) are considered to play a regulatory role in the mineralization of reparative dentin and are therefore considered as specific markers for the odontoblast phenotype (17). It has been shown that the DPSCs secrete large amounts of angiogenic factors like vascular endothelial growth factor (VEGF) and Fibroblastic Growth Factor-2 (FGF-2) (18). These angiogenic factors are important because they play a critical role in tissue development, cell migration, and inflammation and wound repair (19). VEGF also provides important information related to the func-

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tionality of the cells (18). Because pulp capping is a form of tissue repair, it would be necessary to investigate the effects that MTA may have on the secretion of these angiogenic factors, particularly VEGF and whether it contributes to the process of dentin bridge formation. Hence, the aim of this study was two-fold: (1) to delineate the possible mechanism of action of MTA in the process of dentin bridge formation and (2) to determine the effects MTA has on the human DPSCs when placed in direct contact.

Materials and Methods

Cells and Reagents

Dulbecco modified Eagle medium Dulbecco's Modified Eagle Medium (DMEM) (Cellgro, Manassas, VA) (Cellgro, VA) was supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% sodium pyruvate, 1% streptomycin, and 1% L-glutamine (Invitrogen, Carlsbad, CA) and used for cultures of DPSCs. N-acetylcysteine (NAC) was purchased from Sigma (St Louis, MO). A fluorescein isothiocyanate conjugated annexin V/propidium iodide (FITC-PI) kit was purchased from Coulter Immunotech (Miami, FL) and composite restorative material (TPH^3) from Dentsply International (York, PA).

Cell Culture

Human dental pulp cells were obtained from the laboratory and maintained in DMEM supplemented with 10% (v/v) FBS and penicillin, streptomycin, and L-glutamine (100 units/mL, 100 μg/mL, and 2 mmol/L, respectively). Cells were incubated at 37 °C in an atmosphere of 5% CO_2.

Preparation of MTA

Gray MTA (ProRoot MTA; Dentsply Tulsa Dental, Tulsa, OK) was used in all the experiments. The material was mixed according to the manufacturer's instructions and then placed in either 12- or 24-well plates. The material was left to set completely for 48 hours at 37 °C in a humidified 5% CO_2, 95% air atmosphere; after this, the dental pulp cells were plated directly on the surface of the MTA.

Determination of Apoptosis

The cells were cultured under the various conditions as stated in the Results section and stained as previously described (18).

RNA Extraction

Human DPSCs were plated in 12-well plates at a concentration of 1×10^5 cells/well and maintained in DMEM with 10% FBS. Cells were cultured for different time points in the various conditions for 1, 4, and 7 days. Total RNA was isolated by Trizol reagent (Invitrogen/GIBCO BRL, Carlsbad, CA) at the various time points.

Real-time Reverse-Transcriptase Polymerase Chain Reaction and Enzyme-linked Immunosorbent Assay

These procedures were described previously by Rutherford et al (20) and Paranjpe et al (21), respectively.

Scanning Electron Microscopy

Gray MTA was prepared according to the manufacturer's instructions. Pellets of the material were made under sterile conditions and placed on slides. One pellet was placed per well in a 12-well plate. The other wells served as the positive (bone morphogenic protein-4 [BMP-4]) and negative controls (no treatment and composite). A total of 12 slides were prepared. DPSCs were plated in these mentioned conditions for the various time points and then were washed twice with distilled water and fixed with 2.5% gluteraldehyde overnight at 4 °C. They were then washed six times with distilled water and submitted for further treatment and sputter coating for a scanning electron microscopy (SEM) examination. Surface morphology of the specimens was then examined at a magnification of 250× and 500×.

Statistical Analysis

All experiments were performed in triplicate. Each value represents the mean ± standard deviation. Statistical significance was determined by using the Student t test when compared with the control group. Differences with p values (*) <0.05 were considered significant.

Results

MTA Did Not Affect the Viability of the Human DPSCs

The human DPSCs were cultured on the set MTA (as described in the Materials and Methods section). Cells cultured directly on the culture plates served as controls. N-acetyl cysteine (NAC) at a concentration of 20 mmol/L and composite restorative material (prepared according to manufacturer's instructions) were used as controls because NAC has been shown previously to promote cell survival and proliferation (18, 21) and composite to increase the levels of cytotoxicity (21, 22). Cells were cultured in the previously mentioned conditions for 24 hours, trypsinized, and then stained with Fluorescein isothiocyanate–Propidium Iodide (FITC-PI) and analyzed on the flow cytometer. These results (Fig. 1*A*) indicated that in the presence of MTA the percentage of viable cells was approximately 82%, which was similar to the control group at 80% and the NAC group at about 81%. In comparison, the composite group showed only about 21% of viable cells, which was similar to what has been reported previously. Therefore, these data suggest that MTA promotes cell survival similar to NAC and is, therefore, a biocompatible material. These results were verified using the Trypan blue stain (results not shown).

MTA Increases VEGF Secretion in Human DPSCs

DPSCs secrete VEGF (18, 19). VEGF is an important angiogenic factor, and disruptions in the regulation of this angiogenic response have been correlated with delayed healing of wounds (23). Hence, secretion of VEGF would be a crucial factor in the process of pulpal repair. Furthermore, the secreted levels of VEGF are closely correlated with the viability and functional competency of DPSCs (18), which is why we chose to analyze this angiogenic factor. As seen in Figure 1*B*, the levels of VEGF secretion were almost doubled when cells were grown in the presence of MTA as compared with the control cells. This shows that increased proliferation and survival induced by MTA in human DPSCs have important functional consequences for the cells.

MTA Induces Osteo-/Dentinogenic Gene Expression in Human Pulp Cells

The DPSCs contain a population of putative postnatal stem cells that have the capability to regenerate a dentin-pulp-like complex (24). The differentiation of the DPSCs into odontoblasts is essential because these cells are capable of laying secondary/reparative dentin, which, in turn, is able to protect the pulp from further damage that could be induced by a number of mechanisms. Odontoblasts are highly specialized cells aligned in a single layer at the edge of the dental pulp and are responsible for secretion and mineralization of the fibrillar extracellular matrix of the dentin (25). Odontoblasts are known to express specific proteins such as DSP, osteopontin (OPN), osteocalcin (OCN), type 1 collagen, and ALP (18, 26). The analysis of these genes along with an important transcription factor Runt-related transcription