The effects of mineral trioxide aggregates on cytokine production by mouse pulp tissue

Marcelo José Barbosa Silva MSc,[a] Leda Quercia Vieira PhD,[b] and Antônio Paulino Ribeiro Sobrinho DDS,[c] Belo Horizonte, MG Brazil
UNIVERSIDADE FEDERAL DE MINAS GERAIS

Objective. The objective of this study was to determine the effect of MTA on the expression of cytokines in mouse pulp tissue.
Study design. Pulp tissue was exposed to MTA, and the expression of CCL5/RANTES, CCL2/MCP1, IL-1α, IFN-γ, TNF-α, IL-4, and IL-6 was evaluated by RT-PCR at 10 and 20 days after exposure. Control groups were not exposed to MTA.
Results. We found no detectable expression of CCL2, IL-4, and IL-6 in the tissue from either group, while TNF-α was expressed at high levels 20 days after exposure ($P < .05$). CCL5 and IL-1α mRNA expression was lower in the MTA-treated group 10 days after treatment ($P < .05$). At 20 days after the surgical procedure, IFN-γ mRNA expression was also lower in the MTA-treated group ($P < .05$).
Conclusions. These findings suggest that MTA down-regulates the inflammatory cytokines CCL5, IL-1α and IFN-γ and may have an anti-inflammatory effect. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:e70-e76)**

MTA (mineral trioxide aggregate) has been widely used in endodontic treatment. It has shown good results when used as a repair material[1-4] for inducing bone, dentine, and cemental deposition.[5-7] Frequently, fibroblasts, osteoclasts, and periodontal cells are detected in contact with its surface.[8-12]

Pulpotomy and pulp capping are techniques that aim at preserving the vitality of pulp tissue, thus avoiding more invasive endodontic procedures. Recently, MTA has been introduced as a potentially alternative dressing material that promotes the healing of pulp wounds in the coronal or apical part of the tooth, maintaining pulp integrity after pulp capping and pulpotomy, without having any cytotoxic effects.[13] Inflamed periradicular and pulp tissues, upon which MTA is generally applied, contain several cells involved in local immune responses.[5] The early inflammatory response is characterized by the presence of polymorphonuclear cells and monocytes; during the chronic phase, T and B lymphocytes, natural killer, and plasma cells are also found.[14,15]

Several biocompatibility evaluations of MTA materials have been reported in the literature.[16] MTA causes adhesion and cytokine release by the human osteoblast-like cell line MG-63.[11] Moreover, this cell line, when exposed to MTA, was viable, attached normally, and proliferated at rates comparable to control cells cultivated in the absence of MTA. Additionally, the collagen production was also similar to that of controls.[17] Expression of interleukin (IL)-1α, IL-6, and IL-11 by the cell line MG63 in the presence of MTA was also evaluated. Under these conditions, there was no induction of IL-1α. Since this cytokine mediates bone resorption, it is expected that biocompatible materials should not induce its production. However, IL-6 and IL-8 were detected, suggesting that MTA promotes bone turnover. IL-6 stimulates differentiation and recruitment of osteoclasts, and is expressed by osteoblasts when stimulated by growth factors. IL-8, on the other hand, stimulates recruitment of osteoclast precursors and stimulates angiogenesis.[11]

Recently, we investigated the production of tumor necrosis factor (TNF)-α, IL-10, and IL-12 by macrophages exposed to MTA, in vitro, as these cytokines are involved at the onset of the inflammatory process (TNF-α), during the connection between innate and the adaptive immune response (IL-12), and in the regulation of the inflammatory process (IL-10).[18,19] We showed that MTA did not affect the production of these

This work was supported by CAPES and CNPq (grant number 350567/1995-6). A.P.R.S. and L.Q.V. are fellows of CNPq.
[a]Postgraduate Student, Department of Biochemistry and Immunology, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.
[b]Associate Professor, Departmento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
[c]Associate Professor, Departamento de Odontologia Restauradora, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
Received for publication April 29, 2007; returned for revision January 9, 2008; accepted for publication January 21, 2008.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2008.01.025

cytokines, either alone or in conjunction with gram-positive or gram-negative bacteria and interferon (IFN)-γ.[20] In another study, we demonstrated that phagocytosis and the ability of macrophages to eliminate microbes were not affected by MTA.[21]

Pulp capping and pulpotomy materials are constantly being evaluated and questioned, and experimental studies are warranted to add information about the behavior of these materials in living tissues, making them more reliable. Although the in vitro studies cited above seem to indicate that MTA presents good biocompatibility, there are no in vivo studies dealing with the MTA influence on the profile of cytokines produced by pulp tissue. In the present study, we assayed, in vivo, the expression of the CC chemokines CCL2/MCP-1 and CCL5/RANTES and the cytokines IL-1α, TNF-α, IL-4, IL-6, and IFN-γ in mouse pulp tissues exposed to MTA at 10 and 20 days after experimental pulpotomy.

Fig. 1. Isolation of the surgical field and removed crown.

MATERIALS AND METHODS

Animals

Wild-type C57BL/6 mice were purchased from CEBIO (Centro de Bioterismo, ICB, UFMG, Belo Horizonte, MG, Brazil). Mice were kept in a conventional animal house with barriers and a controlled light cycle. The experimental protocol was approved by our institution's animal ethical committee (protocol number 097/04, CETEA – UFMG).

All experimental procedures were carried out under general anesthesia. Mice were injected with 100 mg/kg of ketamine hydrochloride (Dopalen, Division Vetbrands Animal Health, Jacareí, SP, Brazil) and 10 mg/kg of xylazine (Anasedan, Agribrands do Brasil Ltda, Paulínia, SP, Brazil).

Surgical procedure

The permanent mandibular right central incisor was isolated according to the method previously described[22] and adapted for this experiment. Briefly, the tooth had its clinical crown removed at its gingival margin using a diamond bur coupled to a controlled rotation hand piece (Driller, São Paulo, SP, Brazil) (Fig. 1). The tip of an endodontic nickel titanium file was beveled so that it could be used to access the root canal. Pulpotomy was performed as follows: the root canal was prepared with a NiTi file with a tip diameter of 0.20 mm and 0.04 taper (#20/04 file; Profile System, Dentsply Maillefer, Ballaigues, Switzerland) at a rotation of 300 rpm. Half of the pulp tissue was concomitantly removed, leaving the apical half still viable. At this point, the gray MTA (Odonto-lógika, Londrina, PR, Brazil) was prepared according to the manufacturer's specifications and manipulated under aseptic conditions. MTA was compacted 3 mm into the root canals using a Schilder's plugger in the MTA-treated group. In the control group, MTA was not inserted in the root canal. Immediately, the cavity preparation of both groups was dried with a sterile cotton pellet and the root canal was sealed with photopolymerizated dental resin according to the manufacturer's recommendation (Bioplic, Biodinâmica Química e Farmacêutica, Ibiporã, PR, Brazil).

Twenty mice were used for each experimental group (MTA-treated and control) and time period (10 and 20 days) after the surgical procedure. In the MTA-treated group, the prepared region of the root canal next to the apical viable pulp (i.e., about half of the root canal) was filled with MTA. The control group was subjected to the same procedure, but the prepared region of the root was not filled with MTA. All teeth were sealed with dental resin. For each time point, 5 mice were used as unoperated controls (relative to normal pulp tissue).

Gene expression analysis

The mice were killed by cervical dislocation. The mandibles were removed and the incisor teeth were extracted. The pulp tissues from 5 mice were withdrawn from the root canals and pooled, representing 1 sample. The cytokine gene expression was determined using reverse transcription-polymerase chain reaction (RT-PCR). The total RNA from pulp tissues was extracted using the TRIzol reagent (GIBCO/BRL Laboratories, Grand Island, NY) and resuspended in 50 μL of diethylpyrocarbonate-treated water (DEPC) (Sigma Chemical Co., Louis, MO) containing 1 mM EDTA.

After total extraction, the concentration of RNA was quantified spectrophotometrically and normalized for a RNA concentration of 2 μg/μL. The total RNA was reverse transcribed at 37°C for 60 minutes in the presence of 5 μL RNA; 250 mM dNTPs; 50 mM TRIS-