Evaluation of Bone Tissue Response to a Sealer Containing Mineral Trioxide Aggregate

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Abstract

Introduction: This study analyzed bone tissue reactions to MTA Fillapex (Ângelus Industria de Produtos Odontológicos Ltda, Londrina, Brazil) compared with an epoxy resin–based material in the femur of Wistar rats. **Methods:** Bone tissue reactions were evaluated in 15 animals after 7, 30, and 90 days ($n = 5$ per period). Three surgical cavities were prepared on the femur and filled with 0.2 mL MTA Fillapex, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), or no sealer (negative control). By the end of each experimental period, 5 animals were randomly euthanized. The samples were histologically processed and analyzed using a light microscope. The presence of inflammatory cells, fibers, and hard tissue barrier formation was evaluated. Differences among the groups and between the 3 experimental periods were evaluated by using 2-way analysis of variance followed by the Bonferroni post hoc test ($P \leq .05$). **Results:** MTA Fillapex scored significantly higher for neutrophils at 7 days than at 90. At 7 days, the same occurred when comparing MTA Fillapex with AH Plus. The presence of lymphocytes/plasmocytes significantly decreased over time in all groups. Macrophages, giant cells, eosinophils, and fiber condensation presented no differences among groups and periods. Within 90 days, all groups presented complete hard tissue barrier formation. **Conclusions:** The presence of mineral trioxide aggregate in MTA Fillapex composition did not improve the bone tissue repair. The presence of sealers provided the re-establishment of the original bone tissue structure and the inflammatory response decreased over time, so they can be considered biocompatible. *(J Endod 2015;41:62–66)*

Key Words

Biocompatibility, endodontic sealers, endodontics, MTA Fillapex, root canal filling

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Mineral trioxide aggregate (MTA) was introduced as a root-end filling material in the 1990s. Its biocompatibility and sealing ability have been shown in numerous studies (1–5). Because of its favorable properties, it was also indicated for use in direct pulp capping, pulpotomy, apexification, apexogenesis, and revascularization treatments (2, 6–9). However, the long setting time and poor handling characteristics still compromise the use of MTA as a root canal filling material (5, 10). Therefore, some manufacturers have recently added specific components to improve MTA handling properties.

A currently available endodontic sealer that contains MTA is MTA Fillapex (Ângelus Industria de Produtos Odontológicos Ltda, Londrina, Brazil). This sealer is composed of salicylate resin, resin diluent, natural resin, bismuth oxide, silica nanoparticles, and MTA. Its physical properties (11–23) and biocompatibility (24, 25) are already known. Nonetheless, to date, no studies have been conducted to analyze histologically the effect of MTA Fillapex in direct contact with bone tissue.

Therefore, the aim of the present study was to analyze bone tissue reactions to MTA Fillapex compared with an epoxy resin–based material (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany) in the femurs of Wistar rats. The hypothesis tested was that the use of MTA Fillapex would improve the bone tissue repair because of MTA's favorable biological properties.

Materials and Methods

The present study was approved by the Research and Ethics Committee of the School of Dentistry of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil (no. 21226). Bone tissue reactions to MTA Fillapex and AH Plus were evaluated in 15 animals after 3 experimental periods (ie, 7, 30, and 90 days; $n = 5$ per period).

Animals were anesthetized with 0.008 mL/100 g ketamine (Virbac do Brasil Industria e Comércio Ltda, São Paulo, Brazil) and 0.004 mL/100 g 2% xylazine hydrochloride (Virbac do Brasil Industria e Comércio Ltda). The right femur was used for intervention. Trichotomy was performed, and the area was disinfected with alcohol iodine solution. A 5-cm-long incision was made on the skin, tissues were separated by layers, and the periosteum was incised with a scalpel. Three 6-mm-diameter cavities were prepared on the cortical surface of the femur, approximately 6 mm apart from one another, with a slow-rotation trephine number 6 bur under constant irrigation with saline and aspiration. The bur was positioned perpendicularly to the femur and triggered until reaching the bone marrow. Surgical cavities were randomly designated to AH Plus, MTA Fillapex, and negative control (empty cavity) groups.

Sealers were prepared according to the manufacturers' instructions and introduced into insulin syringes (Injex Indústria Cirúrgica Ltda, Ourinhos, Brazil); 0.2 mL was immediately inserted into the respective cavity. The wound was sutured in layers (Vicryl Ethicon; Johnson & Johnson, São José dos Campos, SP, Brazil). After experimental procedures, 50 mg/kg opioid analgesic (Tramal 50; Pfizer Indústria Farcamcêutica, Guarulhos, Brazil) was injected intramuscularly.

By the end of each experimental period, 5 animals were randomly euthanized by CO_2 inhalation. The operated leg was disarticulated and dissected to isolate the femur. Then, with a slow-rotation diamond disc, the bone was transversally sectioned to separate the region of surgical cavities. Each fragment was individually stored in 10%

TABLE 1. Absolute and Relative Frequencies for Observed Histologic Features according to Periods and Groups

	MTA Fillapex, *n* (%)			AH Plus, *n* (%)			Control, *n* (%)		
Scores	**7 days**	**30 days**	**90 days**	**7 days**	**30 days**	**90 days**	**7 days**	**30 days**	**90 days**
Neutrophils									
1	0 (0)	1 (20.0)	5 (100.0)	2 (40.0)	2 (40.0)	5 (100)	1 (20)	2 (40)	5 (100)
2	1 (20)	3 (50.0)	0 (0)	3 (60.0)	3 (60)	0 (0)	2 (40)	3 (60)	0 (0)
3	3 (60)	1 (30.0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)
4	1 (20)	0 ()	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymphocytes/plasmocytes									
1	0 (0)	0 (0)	4 (80)	0 (0)	0 (0)	3 (60)	1 (20)	0 (0)	5 (100)
2	1 (20)	1 (20)	1 (20)	2 (40)	2 (40)	2 (40)	1 (20)	0 (0)	0 (0)
3	3 (60)	1 (30)	0 (0)	3 (60)	0 (0)	0 (0)	3 (60)	3 (60)	0 (0)
4	1 (20)	3 (50)	0 (0)	0 (0)	3 (60)	0 (0)	0 (0)	2 (40)	0 (0)
Macrophages									
1	3 (60)	1 (30)	5 (100)	1 (20)	2 (40)	5 (100)	2 (40)	4 (80)	5 (100)
2	1 (20)	1 (30)	0 (0)	3 (60)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	3 (40)	0 (0)	1 (20)	3 (60)	0 (0)	3 (60)	1 (20)	0 (0)
4	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Giant cells									
1	5 (100)	4 (90)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
2	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Eosinophils									
1	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fibers									
1	3 (60)	1 (10)	2 (40)	1 (20)	1 (20)	5 (100)	5 (100)	5 (100)	5 (100)
2	1 (20)	4 (90)	3 (60)	4 (80)	4 (80)	0 (0)	0 (0)	0 (0)	0 (0)
3	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Barrier									
1	5 (100)	0 (0)	0 (0)	4 (80)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)
2	0 (0)	1 (30)	0 (0)	0 (0)	3 (60)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	4 (70)	5 (100)	1 (20)	2 (40)	5 (100)	0 (0)	5 (100)	5 (100)

neutral-buffered formalin for 48 hours. After decalcification with 10% nitric acid, the samples were set in paraffin blocks and processed for histologic analysis. Sections with 5-μm thickness were cut transversely to the long axis of the femur, mounted on slides, and stained with hematoxylin-eosin. Slices were analyzed with a light microscope (Model Lambda LQT 2; ATTO Instruments Co, Hong Kong, China), using 40, 100, 200, and 400× magnification. The repair process was analyzed according to histologic features. The presence of inflammatory cells, fibers, and hard tissue barrier formation below the cavity access was evaluated by 2 blinded and calibrated examiners (κ = 0.79, $P < .001$).

Cellular events and fiber condensation were analyzed qualitatively according to the criteria described by Tavares et al (24). The cellular inflammatory component was determined by the presence of neutrophils, lymphocytes, eosinophils, macrophages, and giant cells. Hard tissue barrier formation was classified as follows: (1) absence: no hard tissue deposition on the cavity region, (2) partial: partial close of cavity by hard tissue deposition, and (3) complete: total close of cavity by hard tissue deposition.

Differences among the groups and between the 3 experimental periods were evaluated by using 2-way analysis of variance followed by the Bonferroni post hoc test. The significance level was set at .05.

Results

The study results are summarized in Table 1 and Figure 1. The histologic parameters used for assessing the results over the experimental periods are shown in Figure 2*A–F*.

The presence of neutrophils significantly decreased between 7 and 90 days in the MTA Fillapex group ($P < .001$). At 7 days, an acute response, characterized by the presence of neutrophils, scored significantly higher for the MTA Fillapex group compared with the AH Plus group ($P = .008$). The presence of lymphocytes/plasmocytes significantly decreased over time in the MTA Fillapex group ($P < .05$). For the AH Plus ($P = .009$) and control ($P < .001$) groups, the intensity of lymphocyte/plasmocyte infiltrate significantly reduced only from 30 to 90 days. Macrophages, giant cells, eosinophils, and fiber condensation presented no differences among the groups and periods ($P > .05$).

Comparison among periods showed that at 7 days all groups presented less deposition of the hard tissue barrier than at 30 and 90 days ($P < .0001$). At 90 days, all specimens presented complete formation of the hard tissue barrier. In the control group, the barrier was completed at 30 days after the intervention.

Discussion

A primary goal of endodontics is the maintenance or regeneration of the bony tissue supporting teeth in a healthy state. Therefore, the effect of endodontic sealers on bone has clinical relevance. The present investigation showed that the tested materials enabled osseous repair after 90 days.

Despite the attempt to standardize experimental models and evaluation parameters, there is still a great diversity of methodologies, which makes a comparison of the existent studies difficult, allowing contradictory conclusions. While evaluating new materials, it is

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