Evaluation of the Tissue Reaction to Fast Endodontic Cement (CER) and Angelus MTA

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Abstract
Introduction: A new cement (CER; Cimento Endodôntico Rápido or fast endodontic cement) has been developed to improve handling properties. It is a formulation that has Portland cement in gel. However, there had not yet been any study evaluating its biologic properties. The purpose of this study was to evaluate the rat subcutaneous tissue response to CER and Angelus MTA. Methods: The materials were placed in polyethylene tubes and implanted into dorsal connective tissue of Wistar rats for 7, 30, and 60 days. The specimens were prepared to be stained with hematoxylin-eosin or von Kossa or not stained for polarized light. The presence of inflammation, predominant cell type, calcification, and thickness of fibrous connective tissue were recorded. Scores were defined as follows: 0, none or few inflammatory cells, no reaction; 1, <25 cells, mild reaction; 2, 25–125 cells, moderate reaction; 3, >125 cells, severe reaction. Fibrous capsule was categorized as thin when thickness was <150 μm and thick at >150 μm. Necrosis and formation of calcification were both recorded. Results: Both materials Angelus MTA and CER caused moderate reactions at 7 days, which decreased with time. The response was similar to the control at 30 and 60 days with Angelus MTA and CER, characterized by organized connective tissue and presence of some chronic inflammatory cells. Mineralization and granulations birefringent to polarized light were observed with time. The response was similar to the control at 30

Conclusions: It was possible to conclude that CER was biocompatible and stimulated mineralization. (J Endod 2009;35:1377–1380)

Key Words
Biocompatibility, connective tissue, MTA

Mineral trioxide aggregate (MTA) was introduced to be used in pathologic or iatrogenic root perforations, as well as in root-end cavities (1, 2). It has positive characteristics such as favorable tissue reactions, characterized by the absence of several inflammatory responses, the presence of a fibrous capsule, and the induction of formation of mineralized repair tissue (3, 4). It is also characterized by the hydrophilic nature of the particles from MTA powder that allows its usage even in the presence of moisture (1) and the similar behavior of calcium hydroxide when used in pulp capping, or pulpotomy, in animals and in humans (5–10).

The resulting cement from the mixing of powder to water is difficult to manipulate (9) because it takes 2 hours 45 minutes to set, although the working time is less than 4 minutes (11). Additional moisture is also required to activate the setting of the cement (9). Recently, a new type of cement was developed, CER (12), which is a formulation composed of Portland cement gel, water, barium sulfate, and an emulsifier (proprietary) whose function is to improve handling properties. The name CER comes from cimento endodôntico rápido or fast endodontic cement in English. A previous study has shown that CER has the same ability as Angelus MTA (Angelus Soluções Odontológicas, Londrina, Brasil) to release hydroxyl and calcium ions (12), the setting time of 7 ± 1 minutes compared with 15 ± 1 minutes for the Angelus MTA cement, and the coefficient of expansion similar to dentin (13).

Although CER cement apparently presents positive physical characteristics for being clinically used as MTA, there has been no study evaluating its biologic properties. Thus, the aim of this study was to evaluate the tissue response and the ability of CER cement, compared with Angelus MTA, to stimulate mineralization in subcutaneous connective tissue of rats.

Materials and Methods
Eighteen male 4- to 6-month-old Wistar Albino rats, weighing 250–280 g, were used. The animals were housed in temperature-controlled rooms and received water and food ad libitum. The care of the animals was carried out according to Araçatuba School of Dentistry-UNESP Ethical Committee, which approved the project before the beginning of the experiments.

Thirty-six polyethylene tubes (Abbott Lab of Brazil, São Paulo, SP, Brazil) with 1.0-mm internal diameter, 1.6-mm external diameter, and 10.0-mm length were filled with the tested materials. The Angelus MTA and CER (Ilha Solteira, Brazil) were prepared according to the manufacturer’s recommendations and inserted into the tubes with a lentulo spiral (Mailfeber Dentsply, Tulsa, OK). Eighteen polyethylene tubes remained empty and were used as control.

The animals received antisepsis with 5% iodine solution and were shaved under xylazine (10 mg/kg) and ketamine (25 mg/kg) anesthesia. The shaved backs received a 2-cm incision in a head to tail orientation with a number 15 Bard-Parker (Becton Dickinson, Franklin Lakes, NJ) blade. The skin was reflected, creating 2 pockets in 1 side of the incision, one in the cranial portion and other in the caudal portion distant 6 cm from each other, and another pocket in the opposite side of the incision. The tubes were implanted into the spaces created with blunt dissection, and the skin was closed with 4/0 silk suture.
After 7, 30, and 60 days from the implantation time, the animals were killed by overdose of anesthetic solution, and the tubes and surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0. The tubes were then bisected transversely, and both halves were cut again longitudinally with the use of a sharp blade to allow the surfaces to be readily kept in contact with the processing solutions. The specimens were processed for glycol methacrylate (GMA) embedding, serially sectioned into 3-μm cuts, and stained with hematoxylin-eosin (14). The 10-μm cuts were stained according to von Kossa technique or remained without staining to be observed under polarized light. Von Kossa technique was used to observe mineralized structures in the tissue, which was stained dark. Polarized light technique allows observing birefringent structures related to calcium carbonate crystals originated from the combination of calcium ions from the material with carbonic gas from the tissue (15).

Reactions in the tissue in contact with the material on the opening of the tube were scored according to previous studies (14, 16–18) as 0, none or few inflammatory cells and no reaction; 1, less than 25 cells and mild reaction; 2, between 25–125 cells and moderate reaction; and 3, 125 and more cells and severe reaction. Fibrous capsules were considered to be thin when thickness was <150 μm and thick at >150 μm. Necrosis and calcification were recorded as present or absent. An average of value for each material was obtained from 10 separate areas. Results were statistically analyzed by Kruskal-Wallis tests.

Results

Control

At 7 days, a moderate chronic inflammatory cell infiltration consisting of lymphocytes and macrophages was present in a fibrous capsule (Fig. 1A). The fibrous capsule surrounding the tube was thin, with few chronic inflammatory cells at 30 and 60 days (Fig. 1B and C). The empty tubes were not positive to von Kossa stain, and no birefringent structure was observed under the polarized light (Fig. 1D–F).

Angelus MTA

At 7 days, a moderate inflammatory cell infiltration consisting of lymphocytes and macrophages was present in a fibrous capsule

Figure 1. Control. (A) Moderate chronic inflammatory cell infiltration and thick fibrous capsule formation (7 days; hematoxylin-eosin; original magnification, 10×) (B, C) Reduction in thickness of fibrous capsule and inflammatory cell near tube infiltration (30 and 60 days, respectively; hematoxylin-eosin; original magnification, 10×). Angelus MTA (D) Thick fibrous capsule and moderate inflammatory cell infiltration consisting of lymphocytes and macrophages (7 days; hematoxylin-eosin; original magnification, 10×) (E, F) The fibrous capsule surrounding the tube was thin, with few chronic inflammatory cells (30 and 60 days, respectively; hematoxylin-eosin; original magnification, 10×). CER (G) Thick fibrous capsule formation and moderate inflammatory cell infiltration (7 days; hematoxylin-eosin; original magnification, 10×) (H, I) Mild chronic inflammatory cell infiltration in a thin fibrous capsule (30 and 60 days, respectively; hematoxylin-eosin; original magnification, 10×).
The intensity of the inflammation was reduced at 30 and 60 days, with a thin fibrous capsule near the tube and almost no inflammatory cells (Fig. 1E, F). Granulations birefringent to polarized light and von Kossa positive were observed near the tube opening (Fig. 2E–H).

**Comparison among the Groups**

The data were compared in each period of time and are presented in Table 1.

**Day 7**

There was no statistically significant difference ($P > .05$) among the scores of the different groups (median score, 2).

**Day 30 and Day 60**

There was no statistically significant difference ($P > .05$) among the scores of the different groups (median score, 1).

**Discussion**

The empty tubes in the present study caused few reactions in subcutaneous connective tissues, similar to the results previously reported (10, 14, 15, 17–19).

The results observed with CER were similar to the Angelus MTA and control groups. A moderate chronic inflammatory response at day 7, which reduced with time, was observed in a thin fibrous connective tissue capsule surrounding the tube. Positive von Kossa areas and birefringent structures under polarized light were also observed, showing that the material stimulated the formation of mineralized tissue in subcutaneous tissues of rats via the calcium carbonate formation from the calcium of the material and carbon dioxide from the surrounding tissue (15).

After clinical application, the diffusion of hydroxyl ions from the calcium oxide present in the MTA formulation raises the pH at the surface of root adjacent to the periodontal tissues, possibly interfering with osteoclastic activity, and promotes alkalinization in the adjacent tissues, which favors the healing process (20). Calcium ions are important as a result of their participation in the activation of calcium-dependent adenosine triphosphatase (21). Calcium carbonate from the reaction of calcium ion with carbon dioxide crystals serves as a nucleus for calcification and favors mineralization (21). A rich extracellular network of fibronectin in close contact with these crystals strongly supports the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue (21). Calcium is also needed for cell migration and differentiation (22).
CER is composed of Portland cement in gel with water, barium sulfate, and an emulsifier whose function is to improve handling properties, which has the same ability as Angelus MTA to release hydroxyl and calcium ions (12), presents less setting time, and a coefficient of expansion similar to dentin (13). The behavior of the CER in the subcutaneous tissue of the present study was similar to that observed with Angelus MTA, including the mineralization stimulation, probably as a result of the presence of Portland cement in its composition. The similarity between the action mechanism of MTA and Portland cement in the subcutaneous tissue of rats has already been demonstrated (23). The favorable behavior of CER in the present study can also be explained by its ability to release calcium and oxide ions similar to MTA (12), which are important for the induction of the mineralization (15, 20–22).

In conclusion, the histologic response to CER was very similar to that reported for Angelus MTA. Other studies are necessary to better analyze the behavior of this material and confirm the observed data.

References


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CER, fast endodontic cement (cimento endodôntico rápido); MTA, mineral trioxide aggregate.