Effects of modified Portland cement and MTA on fibroblast viability and cytokine production

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ABSTRACT

Objective: The aim of this study was to investigate the effects of a new Portland Cement formulation (CPM) comparing it to Angelus MTA on cell viability and IL-1β and IL-6 release by mouse fibroblasts. **Methods:** Polyethylene tubes filled with these materials were placed into 24-well cell culture plates with mouse fibroblasts. Empty tubes were used as control. After 24 hours, MTT assay was used to evaluate the cell viability. For cytokine assay, mouse fibroblasts were incubated in 24-well flat-bottom plates with set material disks at the bottom or without material, as control. After 24 hours, culture media were collected for cytokine evaluation by using ELISA. **Results:** CPM and Angelus MTA did not inhibit the cell viability. Both materials induced IL-6 and IL-1β release and the amount was statistically significant compared with the control group. **Conclusions:** Both materials were not cytotoxic in fibroblast culture and induced IL-6 and IL-1β release.

Keywords: MTA. Cytotoxicity. Dental materials.

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Introduction

Mineral trioxide aggregate (MTA) was developed by Torabinejad in the early 1990's; the first study on this material was published by Lee et al.[1] The main MTA components are tricalcium oxide, tricalcium silicate, bismuth oxide, tricalcium aluminate, tricalcium oxide, tetracalcium aluminoferrite and silicate oxide. It was introduced to be used in pathological or iatrogenic root perforations and in root-end cavities.[1-9]

Studies have shown that MTA promotes favorable tissue reactions that are characterized by absence of severe inflammatory response, presence of a fibrous capsule, and induction of mineralized tissue repair.[8-13] However, MTA has working properties that are less than ideal. The resulting cement from the mixing of powder with water is difficult to manipulate and its setting time has been reported to be almost 3 hours, whereas the working time is less than 4 minutes.[14,15] Additional moisture is also required to activate the setting of the cement.[14]

In 2004, CPM ('Cimento Portland Modificado' or Modified Portland Cement) was developed in Argentina (Egeo S.R.L., Buenos Aires, Argentina), which is stated to be similar to MTA. The powder also consists of thin hydrophilic particles that form a colloidal gel in presence of moisture, that becomes solid to form a hard cement in one hour. The main components are tricalcium silicate, tricalcium oxide, tricalcium aluminate and other oxides.[3] However, according to the manufacturer, calcium carbonate was added to reduce the pH after set from 12.5 to 10.0 aiming to limit the surface necrosis but allowing the alkaline phosphatase action.

There are some experimental models used to evaluate the biocompatibility of endodontic materials such as cell culture,[16] which has the advantage of being relatively inexpensive, rapid and reliable.[17,18] However, there have been no studies in the literature evaluating cell viability and cytokine production induced by CPM. Thus, the aim of this study was to determine the effects of the CPM and Angelus MTA on cell viability in fibroblasts and to assess the effects of these materials on IL-6 and IL-1β releasing.

Materials and Methods

Cell culture

L929 mouse fibroblasts were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) streptomycin (50 g/mL), and 1% antibiotic/antimycotic cocktail (300 U/mL, 300 mg/mL streptomycin, 5 mg/mL amphotericin 100 g/mL) (GIBCO BRL, Gaithersburg, MD) under standard cell culture conditions (37 °C, 100% humidity, 95% air and 5% CO_2).

Test material

The materials used in this study were CPM (Egeo SRL, Buenos Aires, Argentina) and Angelus MTA (Angelus, Londrina, Brazil), that were prepared according to the manufacturers' recommendations.

Cytotoxicity testing

L929 fibroblasts were seeded into the 24-well plates ($3x10^4$ cells/1 mL medium per well). Cells were incubated for 24 hours in a humidified air atmosphere of 5% CO_2 at 37 °C. The test materials were placed in polyethylene tubes (BARD, C.R.; Bard Ireland Ltda., Galway, Ireland) with a 1.1-mm inner diameter and 10-mm length, and inserted into the fibroblast culture. Six wells were used for each material, and an empty tube was used as the control. Exposure of the cell cultures was stopped by discarding the exposed media after 24 hours. Viable cells were stained with formazan dye (3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) (Sigma Chemical Co, St Louis, MO). MTT was dissolved in phosphate-buffered saline at 5 mg/mL and filtered in order to sterilize and remove a small amount of insoluble residue. At the times indicated later, stock MTT solution (20 mL per 180 mL medium) was added to all wells of an assay, and plates were incubated at 37°C for 4 hours. The medium was then removed by the inversion of the plate and the dumping of 200 μL of isopropyl alcohol, which was added to the wells and mixed during 30 minutes in order to dissolve dark blue crystals. The blue solution was transferred to a 96-well plate, and the absorbance was read in the microplate reader by using a test wavelength of 570 nm.[19]

Cytokine assay

For cytokine assay, the tested materials were inserted into the wells of 24-well flat bottom plates (Corning) and condensed to disks that were approximately 1-mm thick and with the same diameter of the wells. The material was allowed to set for 2 weeks in cell culture medium at 37 °C. The medium was changed every day during this time.

L929 fibroblasts were seeded into the wells (10^6 cells/1 mL medium per well) with the material disks in the bottom. The plates were incubated for 24 hours. After incubation, the culture media were collected and analyzed for IL-1β and IL-6 content by ELISA (R&D Systems, Inc, Minneapolis, MN). Cells cultured without tested material served as negative controls.

Statistical analysis

The results were statistically analyzed by analysis of variance with Bonferroni correction ($p<0.05$).

Results

ELISA assay revealed that the average IL-6 (pg/mL) release was statistically higher when the cells were cultured in the presence of CPM and Angelus MTA than in the control for 24 hours, but they were not statistically different from each other ($p>0.05$) (Fig 1). IL-1β release for the Angelus MTA and CPM was statistically higher than for the control, but there were no statistically difference between them ($p>0.05$) (Fig 2). After 24 hours, CPM and Angelus MTA did not inhibit the cell viability, maintaining the same level as the control group ($p>0.05$) (Fig 3).

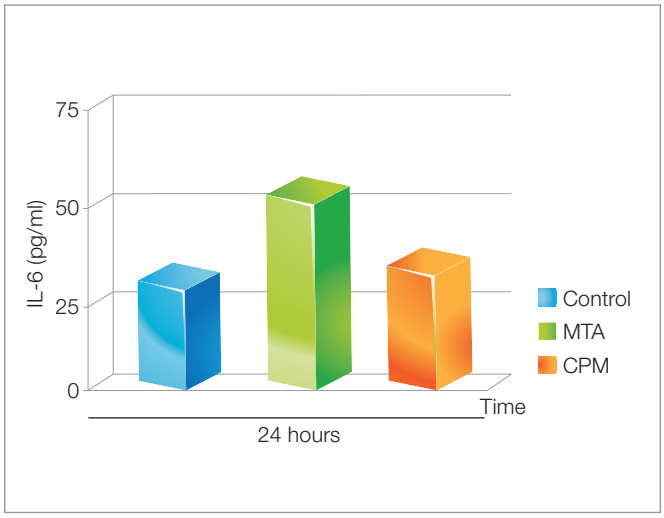

Figure 1. Mean levels of IL-6 raised when the cells were grown in the presence of the materials. There was statistically significant difference ($p<0.05$) between the experimental materials and the control group, but not between the materials.

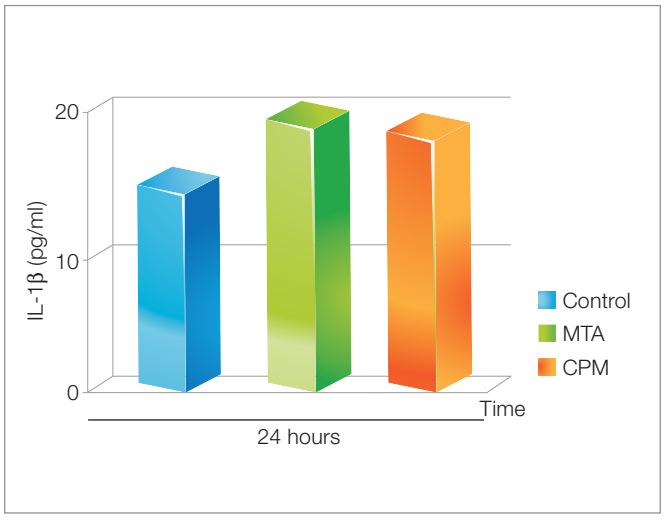

Figure 2. There was difference between the experimental materials and the control group ($p<0.05$), but not between the materials ($p>0.05$) for the levels of IL-1β.

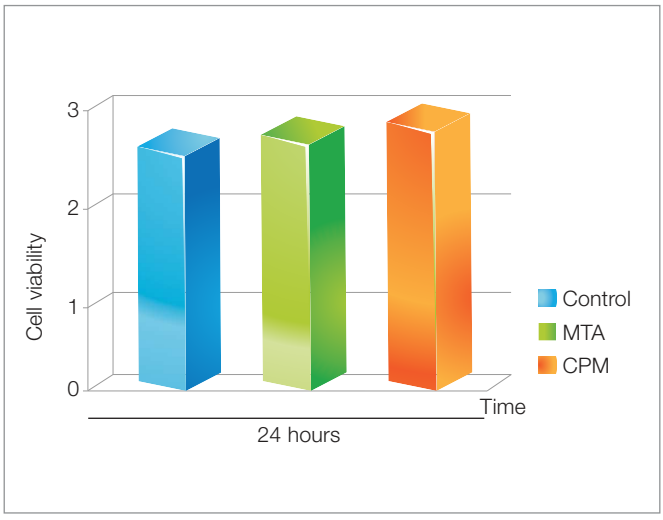

Figure 3. Viability of fibroblasts was not statistically different ($p<0.05$) between the experimental materials and the control group. These results were expressed as means of the absorbance (A_{570nm}) ± SD of each material and the control group.

Discussion

Endodontic materials should have adequate biological and physicochemical properties. The toxic effects of materials used for endodontic therapy are of particular concern once they can cause degeneration of the periapical tissue and delay wound healing.[20]

In this study, cell viability was determined by MTT assay based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium MTT salt into dark blue formazan crystals. Simplicity, rapidity, and precision are advantages of this method. In addition, it does not require radioisotopes.[19,21] Statistical analyses of the MTT assay data showed no significant difference to the CPM in 24 hours. MTA has been recommended to seal all pathways of communication between the root canal system and the external surface of the tooth. The results in the present study agree with previous work showing that MTA was not cytotoxic.[22-25]

Concerning CPM, according to the manufacturer, this material has similar or better physical, chemical and biological characteristics compared to MTA, with the same clinical indications.[26] In this study, the CPM cytotoxicity was not statistically different from the control group. The result can be explained by the similarity in the composition of CPM and MTA based on Portland cement. The pH reduction from 12.5 to 10.0 did not affect cell viability.

Synthesis of cytokines is complex, and their expression and effects are governed by many factors including other cells and chemical mediators.[27] Previous studies have shown that MTA stimulated IL-1β production by osteoblasts.[28,29,30] IL-1β is a cytokine that mediates bone resorption and it is synthesized by various cells including macrophages close to the bone resorption and osteoclasts.[31] In this study, all materials induced statistically more IL-1β release than the control group. IL-6, on the other hand, is a cytokine that mediates the host response to injury and infection, and it is secreted during the inflammatory process in order to regulate various aspects of the immune response, the acute phase of the reaction, and the control of blood infection.[32] Animals depleted of IL-6 showed larger periapical lesions than normal rats.[32] According to these results, all materials induced statistically more IL-6 release than the control group, which shows that they can play an important role on controlling the inflammation and promoting the healing process.[5]

It was possible to conclude that CPM and Angelus MTA did not inhibit L929 fibroblasts viability. Both materials induced statistically more IL-1β and IL-6 releasing than the control group.

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