BIOCOMPATIBILITY STUDY ON MTA MIXED WITH HUMAN BLOOD SERUM, HYSTOLOGICAL ASSESSMENT OF CELLULAR IMUNITY

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Abstract

Mineral trioxide aggregate (MTA) was developed and recommended initially as an endodontic material having been used for pulp capping, pulpotomy, apexogenesis, repair of root or pulp chamber perforations, or as a root canal filling material. According to it's patent, MTA contains calcium oxide (CaO) and silicon (SiO). When MTA powder is mixed with sterile water, calcium hydroxide and calcium silicate hydrate are formed. Blood serum was tested using distilled water in place to track the effects of cellular immunity, by optical microscopy histological analysis. At the same time study aims to analyze the existence of MTA-induced calcification process, sequencing the inflammatory manifestations initially occurred at the inoculation site.

Keywords: MTA, subcutaneous, histology, Wistar rats, blood serum

INTRODUCTION

Mineral trioxide aggregate (MTA) was developed and recommended initially as an endodontic material having been used for pulp capping, pulpotomy, apexogenesis, repair of root or pulp chamber perforations, or as a root canal filling material. MTA has been recognized as a biocompatible, bioactive material[1] hard tissue conductive[2], and inductive. It consists in a powder containing fine hydrophilic particles that set in the presence of moisture (sterile water). Several liquids have been used to hydrate MTA powder. According to it's patent[3], MTA contains calcium oxide (CaO) and silicon (SiO). MTA is currently marketed in 2 forms: gray (GMTA) and white (WMTA). The difference between the two is a lower amounts of iron, aluminum, and magnesium in white MTA than in gray MTA[4,5,7,8]. MTA was developed from the initial Portland Cement. The primary differences between MTA and PC are a lack of potassium and the presence of bismuth oxide in the first[6]. White MTA is primarily composed of tricalcium silicate and bismuth oxide[5]. When MTA powder is mixed with sterile water, calcium hydroxide and calcium silicate hydrate are formed[9].

PURPOSE

Our study was designed to verify and certify the data on the biocompatibility of MTA made some studies. Blood serum was tested using distilled water in place to track the effects of cellular immunity, by optical microscopy histological analysis. At the same time study aims to analyze the existence of MTA-induced calcification process, sequencing the inflammatory manifestations initially occurred at the inoculation site.

A total of 10 rats were included in the study which, according to the protocol, and fragments of mucosa were taken at 2, 4, 6 and 12 weeks. Morphological changes were compared with a control sample.

MATERIAL AND METHOD

This study was conducted at the Medical High Performance Research Center's Biobase, besides the College of Medicine and Pharmacy Oradea.

We used 10 Wistar experimental rats, with age between 3-6 months, weighing between 250-300gr (Fig.1). Our study used white MTA produced by Angelus™ company (Brazil), which comprises 80% of Portland cement and 20% bismuth oxide.

Samples of white MTA were prepared using the following procedure: sterile silicone tube for infusion of 2 mm external diameter was cut from inch to inch. MTA was prepared in aseptic conditions. Silicone Tubes were filled with MTA paste prepared with bhuman blood serum group B3 rh+, in aseptic conditions. Aditionaly, a laminar flow hood was used to sterilize samples. The samples were allowed to cure for 15 minutes, as described by themanufacturer.

Rats were anesthetized, then their backs were shaved. Incision site was disinfected with iodized solution and paravertebral incision of 2 cm was made with blade No. 11. In the chamber created submucous, settled one of the samples. Wound suture was made with a polyester or silk thread and needle number 4. Anesthetized rats were then placed in cages.

The animals were housed in cages with free access to food and water. Food was in the form of fodder grain to make known and analyzed. Health of the animals was monitored daily by trained personnel. Maintenance procedures and handling of animals

complied with "Principles of Laboratory Animal Science"[22] and "Guide for the Care and Use of Laboratory Animals"[23].

Sampling method

Rats were sacrificed at 2-4-6, respectively 12 weeks using the following method:

1. rats were anesthetized
2. If there are still imposed itself, local shaving was carried out,
3. Local desinfection was made with iodized solution,
4. Samples were discovered using surgical scissors,
5. MTA sample were removed with a 10 mm area submucous tissue from both ends of the tube with MTA.
6. Samples were submersed in 10% chloroform
7. Wound was sutured with no. 4 silk thread

Histological method

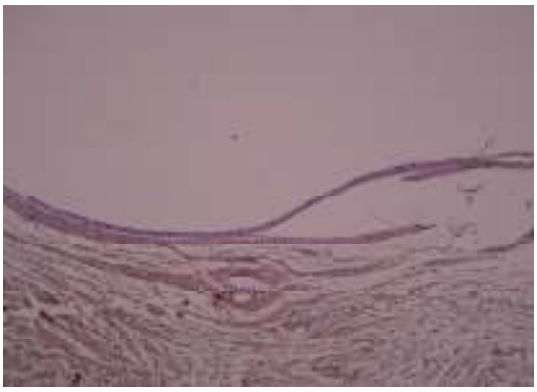
In this morpho-pathological study a total of 10 mucosal bioptic samples were taken from the back of 10 Wistar experimental rats. For histopathological analysis of the products, preservation and histological staining was necessary. We used haematoxiline-eozine staining technique. This results in: the nucleus is blue, the cytoplasm is colored pink, pink collagen fibers appear orange, red blood cells are yellow.

Histological results

Control group analysis results (Fig.1)

To lighter the MTA effects on cell imunology, a control group of 3 Wistar rats were inoculated with an empty piece of the same silicon tube we used to fill with MTA and inoculate on the main group, following the mucosa to be examined later. In the biopsies obtained 2 weeks later, scuamos multilayer epithelium can be noticed, which has a basement membrane and a variable number of cell layers (4-6 layers). Few inflammatory cells were noticed. (Fig.1)

Fig.1 mucosa coming from the control group HE 40X



Analysis of the main sample group - MTA + blood serum(Fig.2-5)

In this batch was used inoculum of human blood serum group B3 RH+ combined with MTA. Analysis of the biopsies from mucosa reveal a small number of inflammatory cell lympho-plasmocytes type. It can be seen even at a low microscope objective analysis of mucosal epithelial cells. In the stands of submucosa the existence of macrophage cells and rare lymphocytes and plasmocytes type cells can be noticed. At the examination of biopsy pieces 2 weeks after innoculation of MTA + serum (Fig.2), calciphycated deposits are remarkable on the mycroscopical images.

Fig.2. MTA+serum light inflammatory reaction and calcium deposits – HE 100x, at 2 weeks

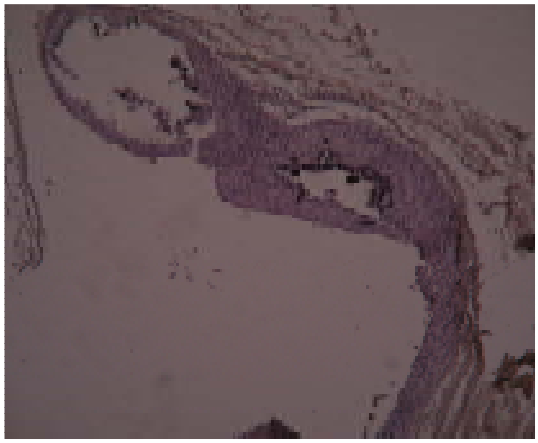


Fig.3. MTA+serum medium inflammatory reaction and calcium deposits – HE 100x- 4 weeks

In this images (fig.2-4) stands a particular phenomenon of immune response. Although the number of inflammatory cells at 2 weeks was reduced (Fig.2), 4 weeks increased lymphocytes and plasma cells is noted in the submucosa. (Fig.3)

An explanation of the phenomenon described, may be due to inflammatory reaction to this calciphycated deposits .

At 4 weeks (Fig.3) we can not notice any characteristic structures for granulomatous chronic foreign body inflammatory reaction. This phenomenon could be explained by self-recognition type of these calcium deposits and inflammatory response were due to the newly emerging structure of local irritation.

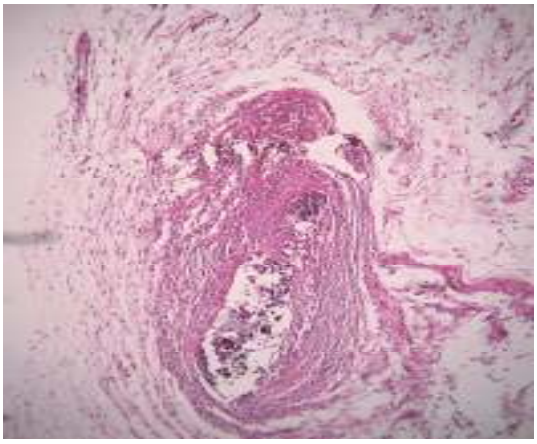


Fig.4- MTA+serum medium inflammatory reaction and calcium deposits – HE 100x- 4 weeks

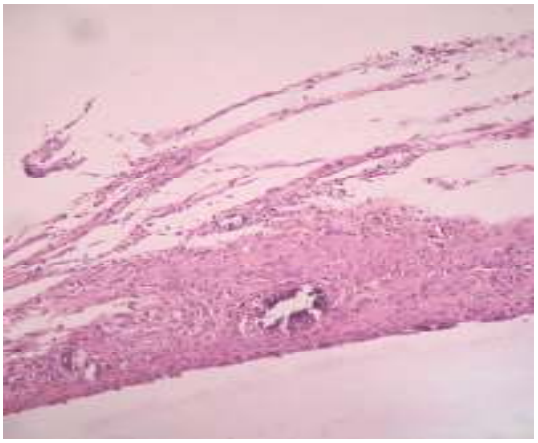


Fig.5 - MTA+serum medium inflammatory reaction and calcium deposits – HE 100x- 6 weeks

At 6 weeks (Fig.5) mucosal and submucosal thickening can be noticed, due to a process of healing by fibrosis. In the stands numerous capillaries of neoformation can be mentioned. In the center stands the image represented by an area of calcium deposits. In hematoxylin eosin staining the purple-magenta color represents the calcium deposits.

DISCUSSIONS

Many studies were made on subcutaneously inserted MTA's biocompatibility[10,11,12,13,14,15,16,17,18,19,20]. Some of these studies reported a tissular calcification response towards the MTA[12,13,14,15,16,17]. The most of these studies used the Von Kossa technique to detect de calcificated structures[12,13,14,15,16]. Most of them showed Von Kossa-positive structures sorounding the MTA samples, one week after subcutaneous implantation. Some studies found that these calcified structures grow bigger as time pass[12,15]. Both white MTA and gray MTA presents these calcifications around the MTA samples[12,13,14,15,16,17]. Also, there are studies wich hadn't described this tissue calcification process around the MTA samples[11,19,20,21].

CONCLUSIONS

2 weeks after inoculation are seen striking signs of inflammation in this tissue cellular elements characteristic of chronic granulomatous inflammatory reactions to foreign body. Inflammation in all groups at 2 weeks less than the control group, is average. A special notice for the calcium deposits wich is particular to the MTA combined with blood serum.

4 weeks after inoculation, while granulation tissue appears as healing tissue on MTA+ sterile water samples, on MTA+serum samples an increased number of cells, especialy limphocytes and plasmocytes can be noticed, probably due to the calcium deposits. Subsequently, the granulomatous healing is missing at this moment, beeing delayed for a latter period of time.

6 weeks after inoculation, a decrease in the number of lymphocytes and plasmocytes and repair processes become visible at mycroscopical observation. Healing process is a fibrous one. Calcium deposits are still visible.

Morphologically, 12 weeks after inoculation there was an almost complete mucosal healing, with persistent low number of lymphocytes in connective collagen type thick fibers. Calcium deposits are still visible as a mark of this type of samples.

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