HISTOLOGICAL STUDY OF WISTAR RAT TISSUE RESPONSE TO WHITE MINERAL TRIOXIDE AGREGATE

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Abstract

Since the invention of MTA, there have been many studies on its biocompatibility. Most of these studies were done on animals - dogs, rats, pigs, horses, etc. Our study was designed to verify and certify the datas on the biocompatibility of MTA made some studies, quantification and sequencing inflammatory manifestations after initial inoculation. We used 10 experimental Wistar rats, with ages between 3-6 months, weighing between 250-300gr. Our study used white MTA product of Angelus ™(Brazil), which consists 80% of Portland cement and 20% bismuth oxide. Following inoculation could detect microscopic tissue inflammation in various stages, whose amplitude decreased over time until healing. The study is part among similar studies done on rats, with whose results we have compared ours. MTA produces a response of moderate to severe disorders that reduce the longer time intervals. At 12 weeks after inoculation there was an almost complete mucosal healing, with persistent low number of lymphocytes and connective thick collagen type fibers.

Key words: MTA, subcutaneous, histology, experiment, Wistar rats

INTRODUCTION

The materials used in odonto-periodontology are often placed in close contact with pulp tissue and periodontium, therefore it is necessary that they are non-toxic and biocompatible to the host tissues. A number of biocompatibility and mutagenicity studies have shown that MTA is a bio compatible material[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23].

PURPOSE

Our study was designed to verify and certify the data on the biocompatibility of MTA made studies, quantification and sequencing the inflammatory manifestations after inoculation.
A total of 10 rats were included in the study, 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 according to the manufacturer, 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.

Maintenance procedures and handling of animals complied with "Principles of Laboratory Animal Science"[24] and "Guide for the Care and Use of Laboratory Animals"[25].

Sampling method

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

- rats were anesthetized with the same mixture anesthetic
- If there are still imposed itself, local shaving was carried out,
- Local desinfection was made with iodized solution,
- Samples were discovered using surgical scissors,
- MTA sample were removed with a 10 mm area submucous tissue from both ends of the tube with MTA.
- Samples were submersed in 10% chloroform
- Wound was sutured with no. 4 silk thread
- Exanguination folowed for blood tests. For this purpose we used needles of 23/0.4 mm, 5ml syringes. Collected blood was drawn into the marked vacutanks and transported to the lab.

Histological method

In this morpho-pathological study a total of 40 mucosal bioptic samples were taken from the back of 40 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)

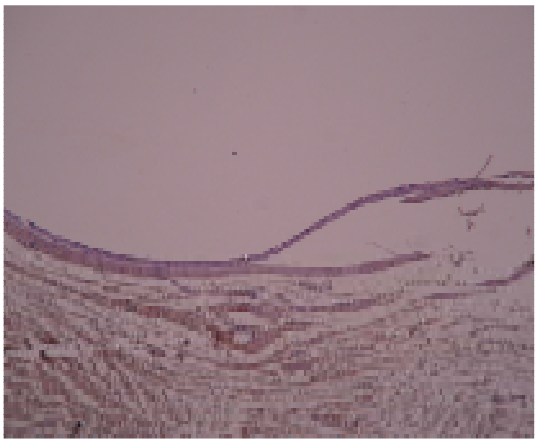


Fig.1 mucosa coming from the control group HE 40X

Analysis of the main sample group - MTA + sterile water (Fig.2-7)

Morphological examination of mucosal biopsies from the inoculation of MTA + sterile water, revealed the existence of a 2-week chronic granulomatous inflammatory reactions to foreign body. On microscopic images, uncazeouse epitelioide granulomas are present. They are made of central macrophage cells, grown in size and called epitelioide, and peripheral adult lymphocyte type cells were found. Among these cells multinucleated foreign body giant cells are noticed. (Fig.2)

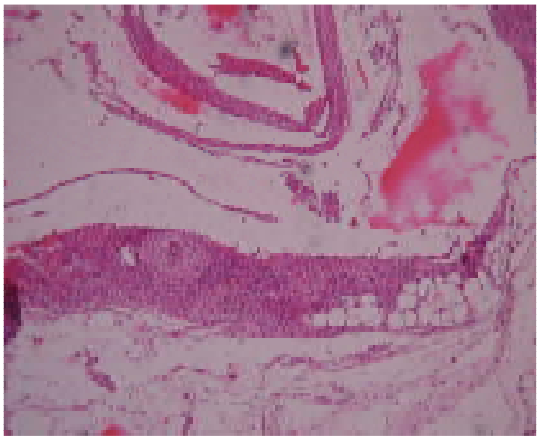


Fig 2. Granulomatous inflammation MTA +sterile water
HE -40X-2 weeks

It can be seen the inflammatory reaction (at the bottom) with morphological aspects of normal mucosa (on top). (Fig.2)
On a bigger resolution with 100X lens examination, local absence of normal mucosa is remarkable. This was initially ulcerated, in the acute faze, and 2 weeks later the existence of a healing process can be noticed. (Fig.3) immune response is mild.

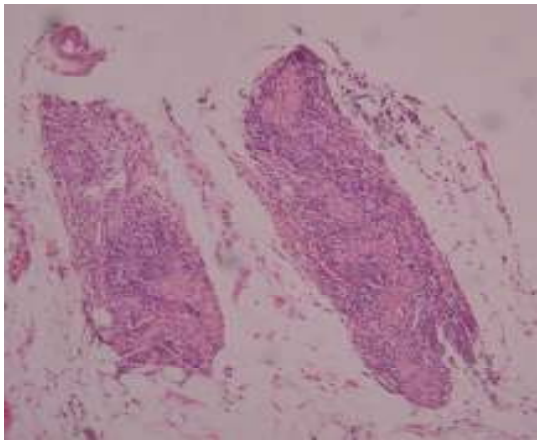
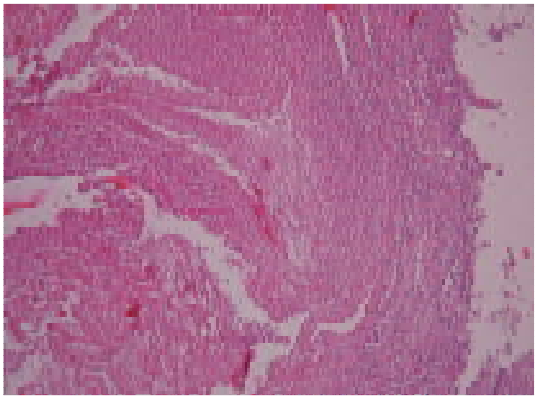


Fig 3. MTA+sterile water granulomatous inflammatory reaction
HE 100X-2 weeks

The analysis at 4 weeks (Fig.4) shows that the inflammatory reaction is lowering, the lymphocytes type inflammatory cells are reduced and adult fibroblasts are also present in the area. Inflammatory cells show both a direct role in the healing process through their lytic action and an indirect role through cytokine.

On the right side of the picture stands mucous existence of a new and deeper existence is remarkable repair process "per primam".

In all biopsies examined was noticed a single case of overinfection



near the inoculum. In this case the inflammatory response was massive, being mainly represented by acute inflammatory cells (neutrophils segmented) and the tissue necrosis. The author has considered the intensity of inflammation as severe type.

Fig. 4 Acute phase inflammatory response 40X-4 weeks

Dynamic analysis of this case reveals to 4 weeks (Fig.5) an inflammatory reaction transition type called granulation tissue. Granulation tissue is characterized by a mix of acute inflammatory cells and fewer chronic phase and the presence of a neoformation capillaries. Healing delay of about 2 weeks when compared with sterile tissue samples is supposed to be due to the local infectious type delaying factor.

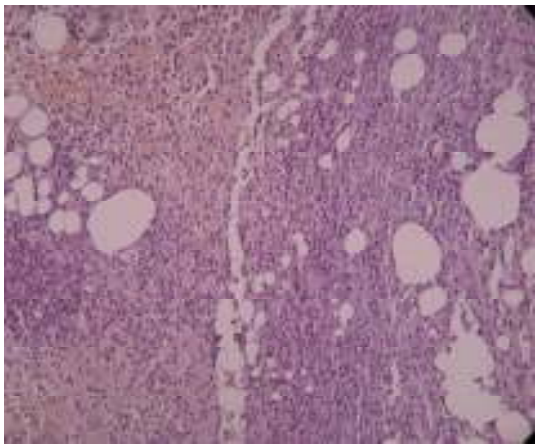


Fig. 5. MTA simple granulation tissue 40X-4 weeks

On mucosal biopsies from the rats sacrificed at 4 weeks is revealing the presence of fibroblasts (connective tissue cells of the young). Inflammatory cells play important roles in the healing process; the direct role is played by their lytic and phagocytic action, and indirectly through chemokines, they attract other cells necessary for the healing process.

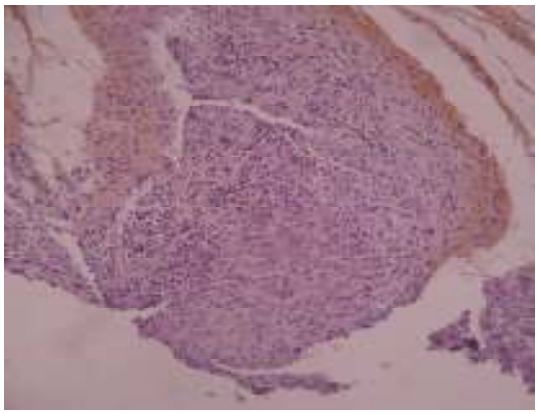


Fig.6 MTA fibroblasts present single-40X-6 weeks HE

At 12 weeks post inoculation(Fig.7), is remarkable the restoration of the tisue. Still can be noticed the thicker conjunctive tissue fibers. These are the type of collagen fibers. Among the connective tissue fibers that form a network of support, stands lymphocytes decreased in number. From the point of view of the author, the intensity of reaction is lowering, following in the near future to make a reorientation of thick collagen fibers.

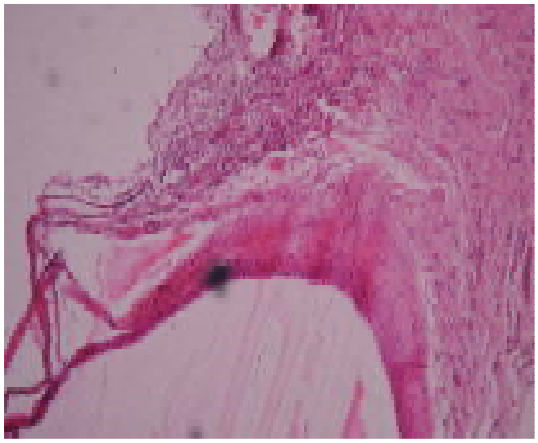


Fig.7 MTA simple, low-grade inflammation HE 40X-12 weeks

DISCUSSIONS

Sarkar & co[11] suggested that the biocompatibility, sealing ability, and the MTA's dentinogenetyc activity is due to the physico-chemical reactions between the MTA and tissue fluids during the formation of hydroxyapatite. The presence of various ions in a living tissue affects it[12]. Although the production of hydroxyapatite is a very desirable phenomenon and a sign of biocompatibility, hydroxyapatite can induce cell death and inhibit their proliferation when the concentration of Ca-P particles is too high. Gomes-Filho & co[26] experimenting on rats, compared the subcutaneous reaction of white MTA Angelus and light curing MTA. White MTA samples showed an minimum inflammatory response after 30 days and almost no inflammation at 60 days. Some studies have reported the calcification of the tissue as a response to MTA[14,15,16,17,18,19]. Others have reported the formation of calcified structures around the implanted MTA[26,20]. A recent study showed inflammatory tissue reaction similar mild to moderate when Portland Cement and white MTA were implanted in the

subcutaneous tissue of rats[26]. These studies have shown that the responses varies from subcutaneous necrosis to dystrophyc calcification. In addition, the initial response MTA produces moderate to severe disorders that reduce the longer time intervals.

CONCLUSIONS

At 2 weeks after inoculation are seen striking signs of inflammation in this tissue cellular elements characteristic of chronic granulomatous inflammatory reactions to foreign body, regardless of source group. Inflammation in all groups at 2 weeks less than the control group, is average.

At 4 weeks after inoculation granulation tissue appears as tissue healing.

At 6 weeks after inoculation to observe a decrease in the number of lympho-plasma cells and repair processes become visible. Healing is fibrous.

Of morphologically 12 weeks after inoculation there was an almost complete mucosal healing, with persistent low number of lymphocytes in connective fibers and collagen type thick.

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