ORIGINAL

Comparative study of mineral trioxide aggregate implanted in rat tibial bone defect

Estudo comparativo de agregado de trióxido mineral implantado em falha óssea na tíbia de ratos

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ABSTRACT

Objective

The objective of this study is to use morphometry to compare rat bone tissue response to two types of mineral trioxide aggregate: gray and white.

Methods

Mineral trioxide aggregate was implanted in tibial bone defects of 16 adult, male Wistar rats. Gray mineral trioxide aggregate was implanted in the left hind leg and white in the right hind leg. Another 16 animals were used as controls. Tissue samples were collected for morphologic and morphometric analyses 2, 4, 6, 8 and 16 weeks

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after the implant. The histological slides were stained with hematoxylin and eosin and analyzed with the software TPS Dig 1.38. The results were analyzed statistically using ANOVA followed by the Tukey test ($p<0.05$).

Results

The mineral trioxide aggregate was evidenced only in the periosteal region and adjacent soft connective tissue. Mineral trioxide aggregate was not found in the bone matrix. Osteoblast proliferation and formation of primary bone in the fracture region occurred in the same patterns and proportions as those of the control animals. No type of cell infiltrate was observed in the periosteal region that could indicate an inflammatory process. Abnormal deposition of collagen fibers or significant amounts of newly formed vessels was also not observed. Morphometrically, there were no significant differences between the two types of mineral trioxide aggregate.

Conclusion

The two types of mineral trioxide aggregate did not interfere significantly with the bone regeneration process, thus they were compatible with the bone tissues in the experimental conditions used.

Indexing terms: Biocompatible material. Bone implant. MTA. Rats.

RESUMO

Objetivo

Avaliar comparativamente, por meio de morfometria, a resposta tecidual óssea em ratos a dois tipos de Agregado de Trióxido Mineral: cinza e branco.

Métodos

Utilizaram-se 16 ratos, machos, adultos, da variedade Wistar, para implantes de Agregado de Trióxido Mineral em falhas ósseas nas tíbias. Na pata esquerda implantou-se o Agregado de Trióxido Mineral cinza e, na direita, o branco; outros 16 animais foram utilizados como controle. Ao final de duas, quatro, oito e 16 semanas pós-implante foram retiradas amostras de tecido para análises morfológica e morfométrica. As lâminas histológicas coradas em Hematoxilina e Eosina foram analisadas histomorfometricamente pelo software TPS Dig 1.38. Os resultados foram tratados estatisticamente por ANOVA seguido do teste de Tukey ($p<0,05$).

Resultados

O Agregado de Trióxido Mineral foi evidenciado somente na região do periósteo e tecido conjuntivo frouxo adjacente. Não se observou Agregado de Trióxido Mineral na matriz óssea. Na região da fratura, observou-se proliferação osteoblástica e formação de osso primário nos mesmos padrões e proporções daqueles observados nos animais do grupo controle. Na região do periósteo, não se evidenciou nenhum tipo de infiltrado celular que pudesse indicar o processo inflamatório. Não se observou deposição anormal de fibras colágenas ou vasos neoformados em quantidades significativas. Morfometricamente não houve diferenças significativas entre os dois tipos de Agregado de Trióxido Mineral.

Conclusão

Os dois tipos de Agregado de Trióxido Mineral não interferiram significativamente no processo de regeneração da falha óssea, mostrando-se, nas condições experimentais utilizadas, compatíveis com os tecidos ósseos.

Termos de indexação: *Materiais biocompatíveis. Implante ósseo. MTA. Ratos.*

INTRODUCTION

Many materials have been used as retrograde fillers. Among them, mineral trioxide aggregate (MTA) stands out. It is a new dental material that presents very promising results according to recent research[1-5].

The process of periradicular tissue repair after surgery in the apical region of the root has been very well described[6-9]. Regeneration of trabecular bone occurs after bone excision, with formation of functional periosteum and cortical lamina. Surgery success depends on the regeneration of the active periodontal apparatus, including cement, periodontal ligament and alveolar bone[1,10,11]. This may occur when the root canal is exposed after resection and filled with material that not only seals the canal to prevent infiltration of bacteria or their products but also allows the formation of a normal periodontium in the outer surface[12]. In this sense, it is essential to use a material that seals and adapts well and is biocompatible.

Many studies have been done to assess MTA[1,13]. The results obtained were excellent: MTA is an excellent sealant[13,14], has alkaline pH[15] and adapts well[16]. Biological assessments using cell cultures[17,18], cortical brain cells of rodents[19], subcutaneous implants in rats[20], tooth implants in dogs[21], monkeys[22] and implants in guinea pig mandibles[2] showed excellent results.

MTA is composed of SiO_2, K_2O, Al_2O_3, Na_2O, Fe_2O_3, SO_3, CaO, Bi_2O_3 and MgO. In addition to the oxides, there are other compounds (insoluble crystalline silica residues, calcium oxide and sodium and potassium sulfates) that are responsible for the chemical and physical properties of this aggregate[23].

Its use is not restricted to the retrofilling process. It is also used to fill root canals in special situations, direct pulp cappings[24], repair of root or furcation perforations and resorption defects[25]. In other words, the main objective of this material is to seal areas that communicate the inside of the tooth with the outside.

Commercial MTA used in pulp capping is gray. For some researchers, this color could be cosmetically inconvenient. Today white MTA is also available. Camilleri et al carried out an experimental work comparing the two types of MTA and both presented similar results[26].

This study used morphometry to compare rat bone tissue response to two types of mineral trioxide aggregate (MTA), gray and white, after various implant durations. Both types of MTA was well tolerated by many tissues and presented good mechanical properties to be used in bone fractures. However, there are no scientific studies showing that MTA has no cytotoxic and/or genotoxic properties.

METHODS

Thirty-two adult, male, albino, non-isogenic Wistar rats (Rattus norvegicus) were divided into two groups: one group (16 animals) called Implanted Group and one group (16 animals) called Control Group. The study was approved by the Research Ethics Committee of the Center of Life Sciences of the Pontifical Catholic University of Campinas, protocol number 087/05.

The animals in the Implanted Group were further divided into 4 groups (n=4) according to the duration of the implants in their right and left hind legs. The materials remained implanted for 2, 4, 8 and 16 weeks. The tibias of the animals in the Control Group were fractured but they did not receive implants. This group was also divided into four groups (n=4) called 2, 4, 8 and 16 weeks.

After being weighed, the animals were submitted to general anesthesia by giving them 1.5mL/kg of body weight of a solution of xylazine hydrochloride (Virbaxyl® 2%) + Ketamine (Francotar®) administered intramuscularly. They were then placed supine in a restraining plate and had their hind legs shaved.

After local asepsis, a skin incision of roughly 1.0cm was done with sharp pointed tip scissors parallel to the tibial axis. The muscle tissue was sectioned with a scalpel until the periosteum was exposed. A low rotation per minute mini motor and a 3.0mm diameter drill bit were used to open a cavity in the upper third of the tibia in order to introduce the test body. This procedure was done on the two hind legs of each animal of the Implanted Group and only on the right hind leg of each animal of the Control Group.

The animals of the Implanted Group were submitted to the following procedures: the right hind leg received the white MTA test body and the left hind leg received the gray MTA test body.

Later, the muscle tissue and skin were sutured and an antiseptic (aqueous polyvinylpyrrolidone-iodine solution - PVP-I) was applied on the skin. In the first 48 hours following surgery, the animals received a single daily intraperitoneal dose (0.05mL) of the analgesic sodium dipyrone (500mg/mL). The animals had free access to food and water at all times.

Once each of the postoperative periods was over, the animals were killed by deepening the plane of anesthesia with a 40mg/kg of body weight dose of a 10% chloral hydrate solution. Immediately after euthanasia, bone tissue specimens were collected and fixed in a 10% buffered formaldehyde solution for 48 hours.

The bone tissue specimens were decalcified by placing them in a saturated solution of EDTA which remained under continuous agitation for 30 days. Next, they were histologically processed to obtain cuts with a thickness of 5 micron which were subsequently stained with hematoxylin-eosin. The histological slides were examined under a light microscope and photographed to obtain the results.

Once the images were captured, the periosteum thickness in the callus region was determined using the software TPS Dig version 1.38. ANOVA followed by Tukey test were used to compare the results statistically. *P* values below 0.05 were considered significant.

RESULTS AND DISCUSSION

According to Shahi et al, today there are four classical methods to assess the biocompatibility of a material: (a) cytotoxicity assessment, (b) subcutaneous implants, (c) endosseous implants and (d) *in vivo* assessment of periradicular tissue reaction in animals[27]. Endosseous implants were used in our experiment; however, since the material was a powder, it was dispersed by the adjacent connective tissue, allowing double assessment.

Histological assessments were done for all implant durations. Presence and intensity of inflammation and of cell infiltrate components and variation of periosteum thickness in the callus region were considered when tissue response was analyzed.

MTA was evidenced only in the periosteum region and adjacent soft tissue. It was not observed in the bone matrix (Figures 1B and 2B). Osteoblastic proliferation and primary bone formation in the same patterns and proportions as those observed in the Control Group 2, 4, 6 or 8 weeks after the implant (Figures 1, 2 and 3, respectively) were observed in the fracture region. Osteoblasts are easily identified by histological analysis in cuts stained with hematoxylin and eosin since they have a cuboidal shape, central nucleus and cytoplasmic basophilia and are typically located in the periosteum-bone matrix interface region. In an experiment to repair dental furcation perforation, Noetzel et al. report bone reorganization and deposition of connective fibers without significant changes[28]. Such results are in agreement with those of our study. Shahi et al.[27] also verified that white MTA was more biocompatible than gray MTA in the first 3 days after the implant but 3 weeks after the implant there was no significant difference between the two.

In the periosteum region, there was no type of cell infiltrate that could indicate an inflammatory process in response to the implanted MTA. Likewise, abnormal deposition of collagen fibers or significant amounts of newly formed vessels was not observed. There are reports in the literature of inflammatory changes associated with MTA, especially during the first week after the implant[27-29]. It is possible that an inflammatory process was present during this period in response to the surgical procedure but our results showed no inflammatory changes 2 weeks after the implant.

Regarding the thickness of the periosteum in the fracture region, no variation was observed in any of the Implanted Group animals when compared with those of the Control Group (Figures 1, 2 and 3). Morphometric and statistical analyses showed significant differences in periosteum thickness only between the different implant durations; however, there were no significant differences between the Implanted and Control groups (Figure 4). Changes in the thickness and cell constitution of the periosteum could indicate a more or less efficient repair process since the periosteum is involved with callus formation to consolidate the fracture and its subsequent resorption (bone remodeling). The results obtained for the Implanted Group 16 weeks after the implant did not show significant differences in relation to those obtained 8 weeks after the implant, even when they were compared with those of the Control Group (Figure 5).

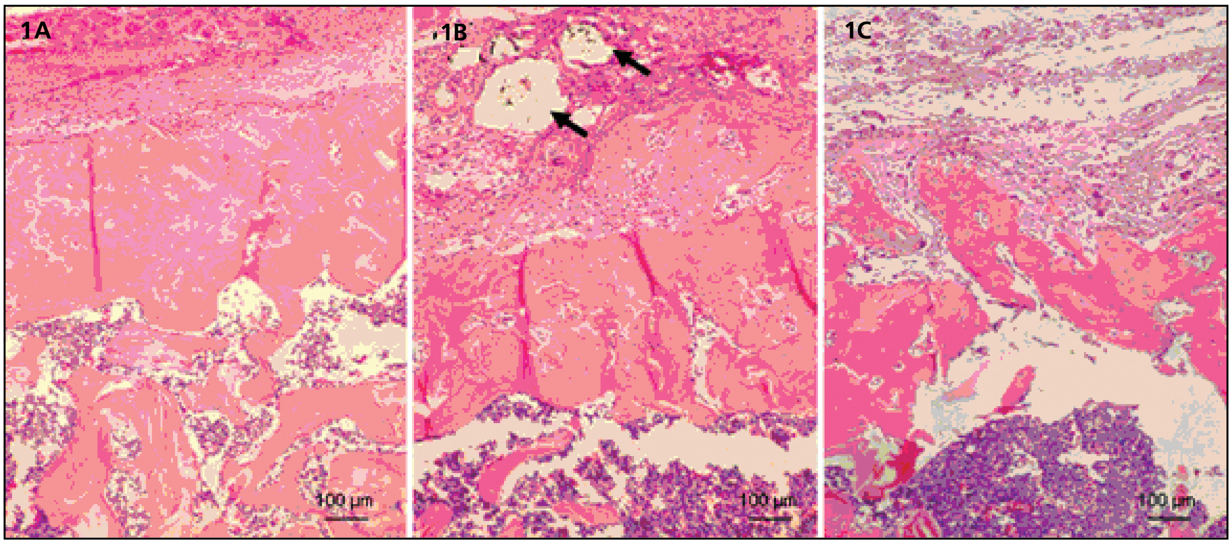

Figure 1. Microphotographs of the bone callus region 2 weeks after the fracture.
Note: A: Control Group; B: Group with Gray MTA implant (arrow); C: Group with White MTA implant; Hematoxylin-eosin; bar=100µm.

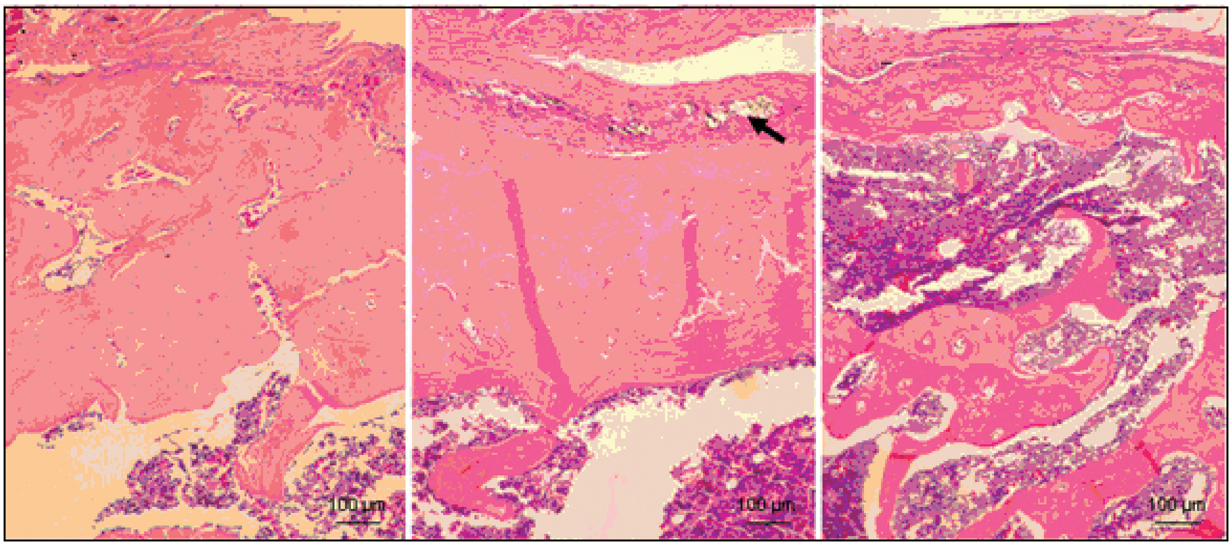

Figure 2. Microphotographs of the bone callus region 4 weeks after the fracture.
Note: A: Control Group; B: Group with Gray MTA implant (arrow); C: Group with White MTA implant; Hematoxylin-eosin, bar=100µm.

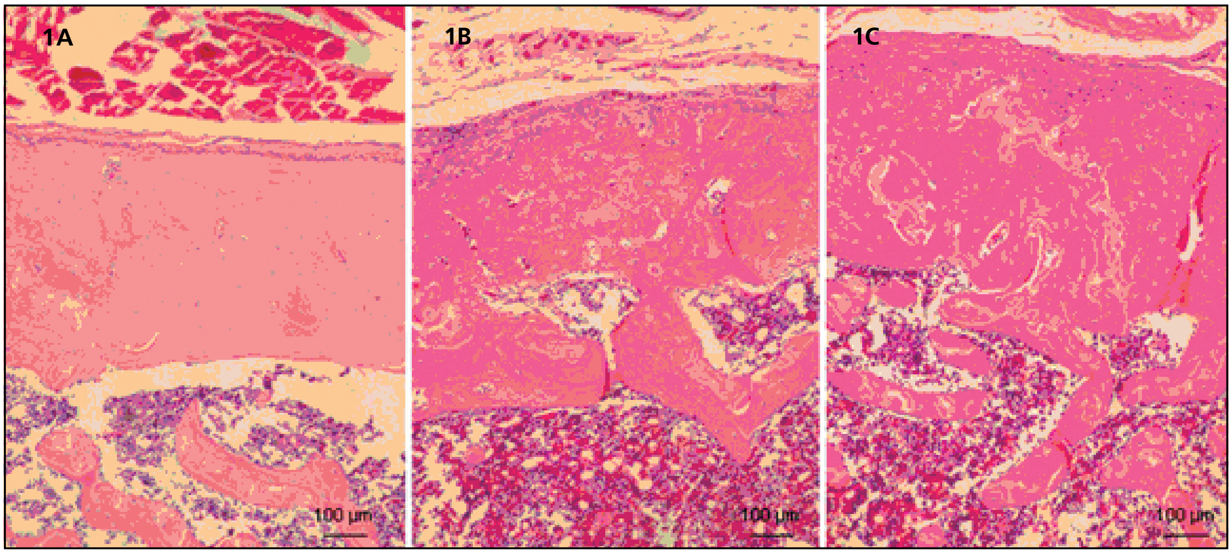

Figure 3. Microphotographs of the bone callus region 8 weeks after the fracture.

Note: A: Control Group; B; Group with Gray MTA implant; C: Group with White MTA implant; Hematoxylin-eosin, bar=100µm.

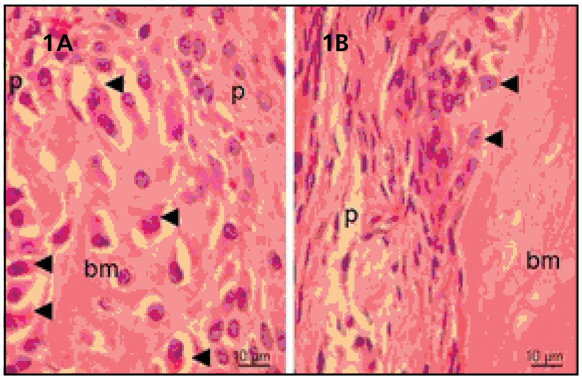

Figure 4. A: Osteoblastic proliferation in the fractured region of the tibia of animals (2-week group) that received gray MTA implant. Note that trabecular bone has a number of osteoblasts. B: Healing of the bone fracture in animals from the control group (2 weeks).

Note: bm: bone matrix; p: periosteum; arrow head: osteoblasts; Hematoxylin and Eosin; bar=10µm.

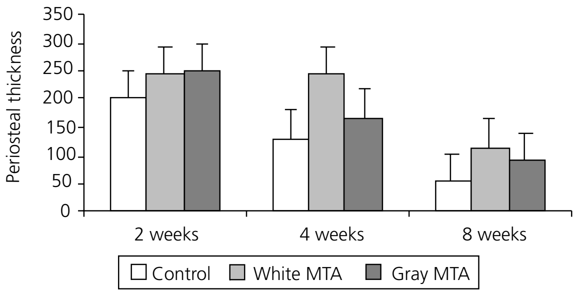

Figure 5. Mean periosteal thickness in the bone callus region 2, 4 and 8 weeks after the implant.

Note: The columns show the mean ± standard error (n=4). **p*<0.05 comparing control groups at different time periods (ANOVA followed by Tukey's test).

CONCLUSION

During endodontic treatment, filling material may leak to the periodontal bone tissue for different reasons.

Bone tissue responses to the two types of MTA were assessed quantitatively by comparing the two materials and the two groups of animals.

In the experimental conditions used in this work, the two types of MTA presented similar bone tissue responses and they did not differ from those of the Control Group. They also did not interfere significantly in the repair process of the bone fracture.

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