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Aloe vera as vehicle to mineral trioxide aggregate: study in bone repair

Aloe vera como veículo ao mineral trióxido agregado: estudo em reparo ósseo

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Resumo

Objetivo: Mineral trióxido agregado (MTA) foi associado a *Aloe vera* para se verificar a ação coadjuvante desta planta medicinal no processo de neoformação óssea em tíbia de ratos. **Material e método:** 36 ratos machos (*Rattus norvegicus*) foram utilizados, divididos em dois grupos com 18 animais em cada. Dois defeitos ósseos circunferenciais com aproximadamente 5 mm de diâmetro foram feitos na tíbia direita de cada animal: o defeito superior foi preenchido com coágulo sanguíneo em ambos os grupos para servir como controle e o defeito inferior foi preenchido com MTA e *Aloe vera* (grupo E1) e MTA e água destilada (grupo E2). Sete, 15 e 30 dias após a cirurgia, seis animais de cada grupo foram submetidos à eutanásia e a tíbia direita de cada animal foi retirada para análise histológica. **Resultado:** Histologicamente, o grupo experimental E1 apresentou melhores resultados para as duas variáveis, inflamação [em sete dias (p = 0,045)] e formação óssea [em sete dias (p = 0,018) e 30 dias (p = 0,034)], em comparação com os resultados do grupo E2. **Conclusão:** A associação entre o MTA e *Aloe vera* demonstrou potencial para reduzir os efeitos da cascata inflamatória e promover a neoformação óssea, tornando-a uma proposta promissora para uso futuro no tratamento endodôntico.

Descritores: Regeneração óssea; medicamentos fitoterápicos; inflamação; histologia; endodontia.

Abstract

Aim: Mineral trioxide aggregate (MTA) was associated to *Aloe vera* to verify the coadjutant action of that medicinal plant in the bone neoformation process in tibia of rats. **Material and method**: 36 male rats (*Rattus norvegicus*) were used, divided into two groups of 18 rats each. Two circumferential bone defects with approximately 5 mm in diameter were made on the right tibia of each animal: the upper defect was filled with blood coagulates in both groups to serve as experimental control and the lower defect was filled with MTA and *Aloe vera* in experimental (group E1) and MTA and distilled water in experimental (group E2). Seven, 15 and 30 days after surgery, six animals from each group were euthanized and the right tibia of each animal was removed for histological analysis. **Result**: Histologically, experimental group E1 presented better results for the two variables, inflammation [at seven days (p=0.045)] and bone formation [at seven days (p=0.018) and 30 days (p=0.034)], compared to the E2 group. **Conclusion**: The association of MTA and *Aloe vera* showed potential to reduce the effects of the inflammatory cascade and promote bone neoformation making it to a promising proposal for future use in endodontic therapy.

Descriptors: Bone regeneration; phytotherapeutic drugs; inflammation; histology; endodontics.

INTRODUCTION

There has been much scientific research to find substances with chemical, physical or biological properties capable of promoting tissue repair. In the 1990s, studies by Professor Mahmoud Torabinejad, at the University of Loma-Linda, California, USA, made a bioactive cement called Mineral Trioxide Aggregate (MTA). Initially conceived to seal communications between the tooth and the external periodontal surface, this material was a great advance in the development of tissue repair techniques in dentistry[1].

The marginal adaptation properties, ability to induce cementogenesis and osteogenesis, antimicrobial capacity, biocompatibility, radiopacity, absence of cytotoxicity and sealing properties conferred by this material has been shown[2]. Its clinical use could be widened because of these characteristics,

as in pulpotomy, direct pulp capping, apexifications, sealing perforation, retro-restorations and endodontic complications such as root and furcation perforation[3]. Regarding the MTA, manufacturers recommend the use of distilled water as vehicle. However, other vehicles have been tested for use in combination with MTA[4,5].

The use of alternative therapies, such as plant medications, has held an important position in technological development, bringing greater scientific knowledge based on the chemical and pharmacological properties and main ingredients existing in plants. The worldwide growth of phytotherapy in preventive and curative programs has stimulated the evaluation of the activity of different plant extracts. However, the correct use of plants for therapeutic purposes requires plants to be selected for their effectiveness and safety, and scientifically validated as being for medicinal use. Brazil is outstanding as regards having the largest vegetal biodiversity on the planet, which represents a promising source of new compounds, with therapeutic activity[6].

Aloe vera is a plant of the Liliaceas family, popularly known as babosa and has potentially active components including amino acids, sugars, enzymes, vitamins and minerals that have important properties such as tissue penetration, anti-inflammatory effect, and immunoregulatory function, in addition to antimicrobial, healing and regenerative properties[7,8].

The objective of the present study was to propose a better alternative vehicle for the association with MTA, using the *Aloe vera* medicinal plant, coadjutant in its anti-inflammatory action and observing its bone inducing property, for future use in endodontic therapy and parendodontics surgeries.

MATERIAL AND METHOD

Animals Used

The research was carried out in compliance with Brazilian Federal Laws 9.605/1998 and 11.794/2008, in the Physiology Laboratory at the Department of Biophysics and Physiology, Health Science Center at UFPI in 2010. It was approved by the UFPI Ethics Committee for Animal Experimentation, no. 086/2010.

Thirty-six male rats of the Rattus norvegicus species were used, 230g average weight, supplied by the animal house of the Medicinal Plant Research Nucleus at the Federal University of Piaui. The animals were divided into two groups of 18 animals each: experimental group 1 (E1), which was tested MTA associated with *Aloe vera* and experimental group 2 (E2), wich was used MTA with distilled water. The animals were kept in propylene cages, routinely cleaned, and they were fed with standard diet: meal (Labina TM®, Purina, São Paulo, SP, Brasil) and water *ad libitum*.

Surgical Technique

The animals were pre-medicated with 0.2% acepromazin (Acepran®, Vetnil/Univet, Louveira, SP, Brasil) at 5 mg/kg intramuscular injection (IM). Fifteen minutes after inducing anesthesia, the following anesthetic combination was injected: 2% xilazin hydrochlorate (Anasedan®, Vetbrands, Porto Alegre, RS, Brasil) at 5 mg/kg IM, associated to quetamin chloridrate (Dopalen®, Vetbrands, Porto Alegre, RS, Brasil) at 100 mg/kg IM. The anesthesia was maintained with half the dose of the same anesthetic combination whenever necessary.

The hind right leg was then shaved, cleaned with topical polvidine (Riodeine®, Rioquímica, São José do Rio Preto, SP, Brasil) and the tibia was isolated with sterilized surgical sheets.

Surgical access was obtained to the right tibia of each rat by a 20 mm lenght linear incision, in the head-tail direction, using a number 15 scalpel blade. The skin, muscle and periosteum were cut to expose the bone surface.

Two bone defects were made (upper and lower) under abundant irrigation with 0.9% physiological solution (5 mL/Kg/h IV) and using a number 6 steel spherical drill (Dentsply, Petrópolis, RJ, Brasil) attached to a surgical microengine approximately 5 mm in diameter and as deep as the bone marrow canal. The upper bone defect in both groups was filled only with blood coagulate, and it was considered the experimental control. In the E1 group the lower bone defect was filled with MTA (MTA®, Angelus, Londrina, PR, Brasil) associated to *Aloe vera* (Alphaloe®, Jungconsult do Brasil, Bom Retiro, SC, Brasil) and in the E2 group the same orifice was filled with MTA associated to distilled water. Then the muscle was sutured first with absorbable suture, followed by the skin suture. All the animals received 0.2 mg/kg meloxicam (Maxicam®, Ourofino, Cravinhos, SP, Brasil) and 25 mg/kg sodium dipyrone (Finador®, Ourofino, Cravinhos, SP, Brasil) for immediate analgesia and a 0.1 mg/kg meloxicam dose was maintained for five days after surgery.

Piece Preparation and Histopathological Analysis

Seven, 15 and 30 days after surgery, six animals from each group were sacrificed in anesthetic deepened by sodium thiopental overdose (25 mg/kg) (Thiopentax, Cristália, Itapira, SP, Brasil). The right tibia of each animal was then removed and the material fixed in 10% formaldehyde for later decalcification by the Morse method, as previously reported by Morse[9].

The de-calcified pieces were dehydrated in solutions with increasing ethanol concentrations, diaphanized in xylol and blocked in histological resin. Cuts of 6mm were obtained and stained with haematoxylin-eosin and analyzed under an optical microscope.

The histological slides were analyzed by observing in 10 different fields of each histological cut under a NIKON microscope, model Eclipse E600 attached to a NIKON COOLPIX MDC video-camera with a 3.3 megapixel lens, using 10 and 20x magnification lenses to assess the presence of inflammatory infiltrate and bone neoformation (Table 1)[10,11]. The analysis was performed in a blinded manner by a single evaluator.

Statistical Analysis

The data were processed in the Statistical Package for the Social Sciences (SPSS) program version 16.0. Initially the Shapiro-Wilk normality test and the Levene test for homogeneity

of variances were applied. Due to be non-normally distributed data, the nonparametric Mann-Witney and Wilcoxon tests were applied with 5% level of significance ($p<0.05$).

RESULT

The results of bone neoformation and the inflammatory process for the control and experimental groups are shown in Table 2 and histological analyses are shown in Figure 1.

DISCUSSION

The study of bone repair has led many researchers to work in search of new knowledge, techniques and materials to assist this process. For this, biomaterials have been studied, such as the experimental material used in this research and, more recently, the mesenchymal stem cells[12].

The inflammatory process due to the surgical trauma seven days after the bone defect, was high and similar among the groups. This inflammatory reaction contributes positively to forming the bone callus, neovascularization and is due mainly to growth factors such as FGF, VEGF, PDGF, TGF, released especially by the coagulate formed in the first inflammatory phase[13].

The data shows that the inflammatory reaction decreased progressively in the control, E1 and E2 groups (Table 2). Along with this, histologically there were lower inflammatory process means for the experimental defects in both groups E1 and E2 compared to the defect in the control group at 7, 15 and 30 days after surgery (Figure 1). Although there were no statistical differences between E1 and E2 groups, the lower degree of inflammation in the defects where MTA was applied confirmed that this material is well tolerated and corroborated with other studies on lesions in bone tissue[14,15].

This decrease of inflammation observed may also be explained by the anti-inflammatory property of *Aloe vera*, through the immunomodulatory effect of the polysaccharides acetylated mannose (mannose-6-phosphate), acemannan and veraciglucans A, B and C, according to the findings of other studies[16-20].

Accorinte et al.[21] observed necrotic areas surrounding the bone tissue after using MTA, suggesting that MTA can cause some level of superficial necrosis when in contact with the pulp tissue. Indeed, the histological analysis showed areas of necrosis in E1 on the seventh day after surgery (Figure 1), an event also observed by Holland et al.[22], when they reported that after pulpotomies with MTA a thin layer of superficial necrosis is formed between the point of mineralized tissue and the material. Although these authors have found these results in pulp tissue, they corroborate with the findings of this research in bone tissue, for both are connective tissues. Necrosis was observed in a few specimens and was probably due to the high pH of the material or due to trauma of bone heating during drilling of bone to create the defects.

However, comparison of the experimental defects of E1 and E2 showed that the defects where MTA was associated to *Aloe vera* presented better results for the two variables inflammation and bone formation, compared to those where MTA was used associated to distilled water. The statistical difference observed at seven days for the variables, inflammation ($p=0.045$) and at seven and 30 days for bone formation ($p=0.018$ and $p=0.034$,

Table 1. Scores attributed to the inflammatory reaction and new bone formation after biological graft association of MTA and *Aloe vera*

*Score	Inflammatory grade	Bone neoformation
0	Blood Clot	
1	Absence or moderate infiltrate of inflammatory cells	Bone neoformation, filling tissue, blood vessels, fibroblasts and macrophages
2	Moderate infiltrate of inflammatory cells	Bone neoformation, differentiated connective tissue
3	High presence of inflammatory cells	Primary bone tissue

*Adapted to Dahlin et al.[10] (1988) and Hedner, Linde[11] (1995).

Table 2. Mean of the scores of bone formation and the inflammatory process in the tibia of *Rattus norvegicus* for the control and experimental groups (E1 and E2)

	Bone neoformation			Inflammatory grade		
	Day 7	Day 15	Day 30	Day 7	Day 15	Day 30
Control	1.1	1.6	2	2	1.5	1.4
E1	1.2	1.8	2.5	1.8	1.2	1.2
E2	0.3	1.3	1.6	2.5	1.5	1.2
$p_{\text{cont-E1}}$	0.564	0.564	0.102	0.083	0.157	0.317
$p_{\text{cont-E2}}$	0.317	0.414	0.317	0.633	0.317	0.157
$p_{\text{E1-E2}}$	0.018*	0.300	0.034*	0.045*	0.460	1

p – Level of significance ($p<0.05$)*. Laboratory of Physiology, Federal University of Piauí, Teresina, Brazil.

respectively) (Table 2 and Figure 1) showed that in spite of the initial tissue necrosis, the anti-inflammatory repairing capacity of *Aloe vera* was superior, corroborating findings by Vázquez et al.[17], Duansak et al.[18] and Prabjone et al.[20], when they emphasized the analgesic and steroidal capacity of *Aloe vera* in their studies.

The results obtained for the group where MTA was associated to *Aloe vera* showed bone neoformation (Table 2 and Figure 1) corroborating observations by Jittapiromsak et al.[23] who observed that acemannan (a *Aloe vera* polysaccharide) significantly increased pulp cell proliferation, bone morphogenetic protein-2,

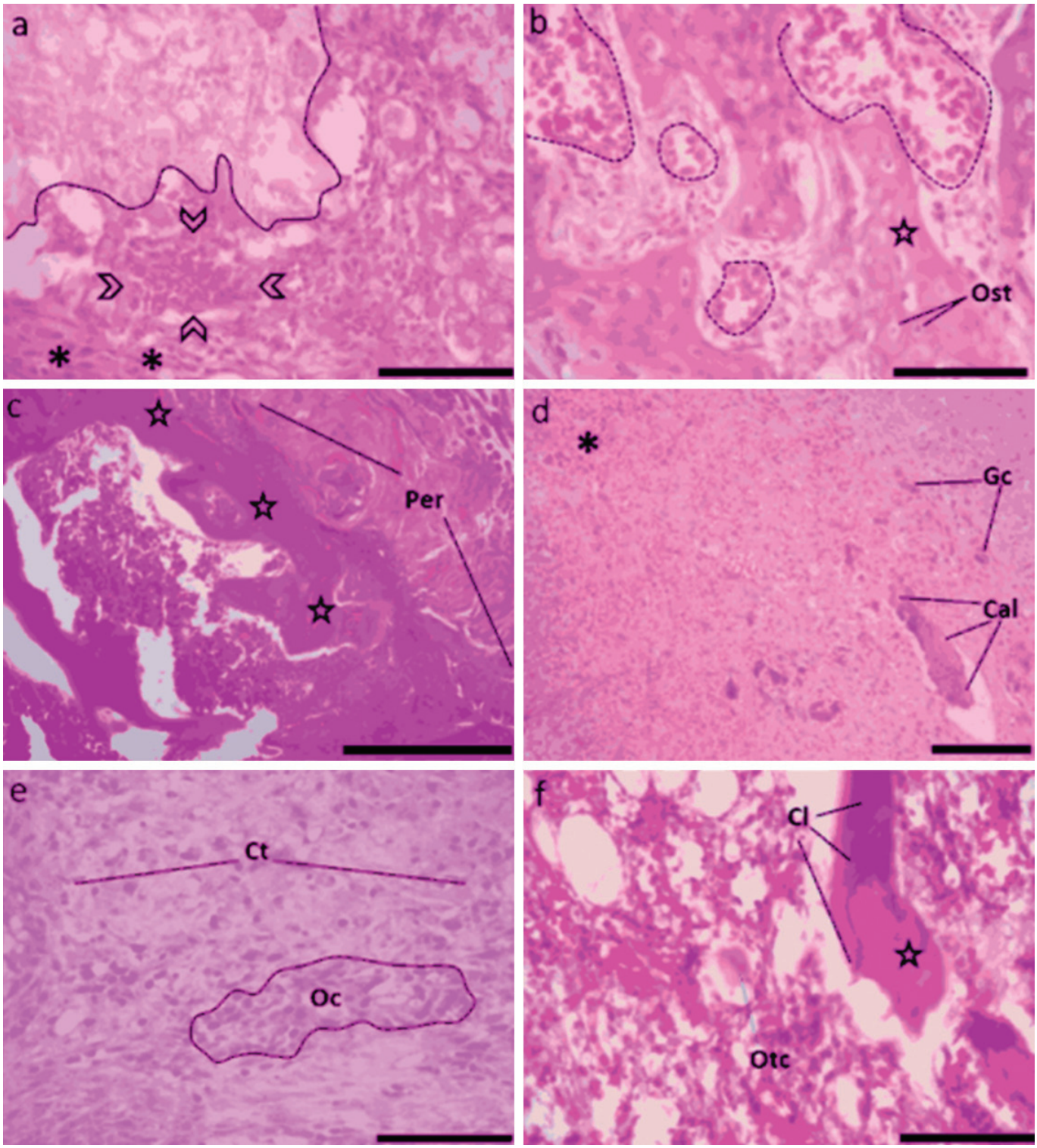

Figure 1. Photomicrographs of bone defects produced in the right tibia of *Rattus norvegicus*. (a) E1 group after seven days of surgery showing areas of necrosis (continuous line), presence of granulation tissue (*) and neovascularization (arrows). (b) E1 group after 15 days of surgery. Note the filling of bone tissue, bone trabeculae (✯) and dilated medullary spaces (dotted line). (c) E1 group with 30 days with the presence of mature neoformed bone trabeculae (✯) and periosteum (Per). (d) E2 group after 7 days of surgery filled with granulation tissue (*), multinucleated giant cells (Gc) and areas of calcification (Cal). (e) E2 group 15 days after surgery showing connective tissue (Ct), neovascularization and osteogenic cells in proliferation (Oc). (f) E2 group after 30 days of surgery. Note the presence of neoformed immature bone trabeculae (✯), cement line (Cl) and osteoclasts (Otc). Bars: a, b, e and f: 50μm; c: 25μm; d: 100μm.

alkaline phosphatase activity, dentin sialoprotein expression, and mineralization with a complete homogeneous calcified dentin bridge. Similarly, Jettanacheawchankit et al.[24] found that acemannan plays a significant role in the oral wound healing process via the induction of fibroblast proliferation and stimulation of keratinocyte growth factor-1 (KGF-1), vascular endothelial growth factor (VEGF), and type I collagen expressions. In a clinical trial with 40 patients with oral minor aphthous lesions, Babaee et al.[25] observed that *Aloe vera* 2% oral gel is not only effective in decreasing the recurrent aphthous stomatitis patients' pain score and wound size but also decreases the aphthous wound healing period.

The results obtained suggested that MTA associated to *Aloe vera* is a promising clinical protocol because of the potential demonstrated for this association in reducing the effects of the inflammatory cascade and promoting greater bone neoformation in the region of the bone defect in the animals researched, opening new perspectives for its future use in endodontic therapy.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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