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Biocompatibility evaluation of alendronate paste in rat's subcutaneous tissue

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Abstract – Alendronate is a known inhibitor of root resorption and the development of alendronate paste would enhance its utilization as intracanal medication. Therefore, this study aimed to investigate the biocompatibility of experimental alendronate paste in subcutaneous tissue of rats, for utilization in teeth susceptible to root resorption. The study was conducted on 15 male rats, weighing ~180–200 grams. The rats' dorsal regions were submitted to one incision on the median region and, laterally to the incision, the subcutaneous tissue was raised and gently dissected for introduction of two tubes, in each rat. The tubes were sealed at one end with gutta-percha and taken as control. The tubes were filled with experimental alendronate paste. The animals were killed at 7, 15 and 45 days after surgery and the specimens were processed in laboratory. The histological sections were stained with hematoxylin-eosin and analyzed by light microscopy. Scores were assigned to the inflammatory process and statistically compared by the Tukey test ($P < 0.05$). Alendronate paste promoted severe inflammation process at 7 days, with statistically significant difference compared to the control ($P < 0.05\%$). However, at 15 days, there was a regression of inflammation and the presence of connective tissue with collagen fibers, fibroblasts and blood vessels was observed. After 45 days, it was observed the presence of well-organized connective tissue, with collagen fibers and fibroblasts, and few inflammatory cells. No statistical difference was observed between the control and experimental paste at 15 and 45 days. The experimental alendronate paste was considered biocompatible with subcutaneous tissue of rat.

Tooth avulsion, characterized by complete displacement of the tooth from the socket, accounts for about 0.5 to 16% of all cases of dental trauma (1).

After avulsion, the tooth should be repositioned in the socket in an attempt to re-establish normality (1). Immediate reimplantation (1–3) or maintenance of the avulsed tooth in storage media compatible for survival of these cells before reimplantation (1, 2) is fundamental for a successful tooth reimplantation procedure. However, usually, people let the tooth dry, keeping it wrapped in plastic or sometimes store the tooth immersed in solutions that do not allow for cell survival (4–6). This may lead to ankylosis and root resorption, both undesirable consequences of tooth reimplantation (1, 7).

Therefore, in an attempt to prevent or limit the root resorption and promote repair of the area, in case of late reimplanted tooth, it should be submitted to root surface treatment and endodontic therapy (1, 7). Currently, root surface treatment is performed with fluoride (7, 8). Calcium hydroxide is generally accepted as the intracanal medication of choice (8–11).

Even with such treatment possibilities, there are still many cases of root resorption, and the affected teeth are extracted over a period of 4–6 years (12, 13). Hence, the search for new substances that may inhibit or delay the effects of the root resorption is demanding.

We have previously reported that the use of a 10^{-5}M alendronate solution as intracanal medication enabled to limit root resorption in teeth submitted to late reimplantation, in a similar form as calcium hydroxide paste (7). However, due to the liquid presentation of the alendronate solution, its insertion and retention inside the root canal is rather problematic. Thus, the development of an alendronate paste would enhance its utilization as intracanal medication in teeth susceptible to root resorption.

Ideally, the experimental paste should be biocompatible with the surrounding tissues, since the concentration of alendronate is higher in the paste than in the solution tested by Mori et al. (7). The paste may then be tested in reimplanted teeth regarding its effectiveness to inhibit root resorption.

Considering the aforementioned aspects, this study aimed to investigate the biocompatibility of an experimental alendronate paste in the subcutaneous tissue of rats.

Materials and methods

The experimental alendronate paste used in the study contained alendronate as its main component. A saline solution was used as vehicle (Drogaderma - Presidente Prudente, SP, Brazil).

The study was conducted on 15 male rats (*Rattus, norvegicus, albinus, Wistar*) weighing ~180–200 g. All experimental procedures were approved by the Animal Research and Ethics Committee of Araçatuba Dental School, Unesp, Brazil. (Process 2007/2502). The animals were kept in cages identified according to the group and study periods. The cages were cleaned daily. The animals received solid food before and during the study, except for 12 h before surgery, and water *ad libitum*.

For surgical interventions, the animals were anesthetized with a mixture of ketamine (Dopalen – Sespo Industria e Comércio Ltda, São Paulo, Brazil) and xylazine (Anasedan – Agribrands do Brasil Ltda, São Paulo, Brazil), IM, in a dose of 0.05 ml/100 g of weight for each substance. Anesthesia was applied with disposable insulin syringes.

Thereafter, thirty sterile polyethylene tubes measuring 1.3 mm of internal diameter and 10 mm in length were sealed at one end with gutta-percha (Odahcam Dentsply Indústria e Comércio LTDA, Rio de Janeiro, Brazil). These tubes were filled with experimental paste introduced in the tubes with aid of a sterile Lentulo spiral (Dentsply-Maillefer, Ballaigues, Switzerland). The ends sealed with gutta-percha were used as controls.

Prior to the surgical insertion of the tubes into the animals' subcutaneous tissues, their dorsal regions were submitted to trichotomy and antisepsis with Periogard (Pfizer Ltda, São Paulo, Brazil). A surgical incision in the median region was then performed with a #15 blade (Embramac Exportação e Importação, São Paulo, Brazil). Laterally to the incisions, the subcutaneous tissue was raised and a gentle dissection was performed with blunt-ended scissors. The tubes were inserted in the subcutaneous tissue of animals and the incisions sealed with cyanoacrylate (Super Bonder® - Loctite, São Paulo, Brazil), as suggested by Moreira et al. (14).

After 7, 15, and 45 days, five animals were killed with an overdose of anesthetics. The tissues containing the tubes were detached and fixed in 10% formalin for 48 h. After fixation, the tubes were removed from the tissue. Both gutta-percha and alendronate paste were still present in the tubes. The specimens were processed and embedded in paraffin for histological processing. Longitudinal sections of 5 μm were taken at each 50 μm with microtome, totaling 15 sections for each specimen. The sections were stained with hematoxylin-eosin and analyzed by light microscopy.

The sections were microscopically evaluated regarding the presence or absence of inflammation. When present, the type of inflammatory cells, intensity of inflammation and its relationship with the materials were evaluated. The occurrence of destructive processes such as abscesses and tissue necrosis were also considered. Tissue proliferation was described, when present. The biocompatibility of the experimental paste with the surrounding tissues was classified according to already established scores, varying with the intensity of inflammatory process (Table 1). The criteria for classification of the different degrees of inflammation and tissue reactions were established in accordance with recommended standard practices for biological evaluation of dental materials (15).

Table 1. Classification scores used to distinguish the intensity of the inflammation in the subcutaneous tissue of animals

Scores	Level of inflammatory
1	none
2	slight
3	moderate
4	severe

The scores were assigned by a blinded and experienced examiner. The identification of the histological sections was occulted and the scores recorded on specific tables. Statistical analyses of the results were carried out using the Tukey test, with a significance level set at 5% ($P \leq 0.05$).

Results

Alendronate paste

At 7 day, the histological sections were characterized for the presence of collagen fibers and few fibroblasts. The inflammatory process was present in most sections; in some of them, inflammation ranged from moderate to severe and the cells most frequently observed were neutrophils.

At 15 day, a regression of the inflammation was observed, in most sections, however, it was slight or absent ($P \leq 0.05$). In some sections, there were few lymphocytes and macrophages. Connective tissue with collagen fibers, fibroblasts and blood vessels were present at 15 days.

At 45 days, a well-organized connective tissue was observed, with tissue proliferation into the tube in some of the cases. Inflammation was completely absent at 45 days, except for three cases presenting slight inflammation with few lymphocytes (Fig. 1).

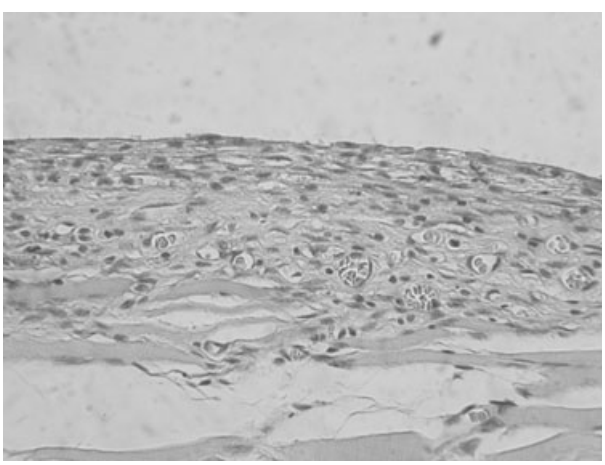

Fig. 1. Alendronate paste at 45 days. Note the presence of organized connective tissue with proliferation of blood vessels and the absence of inflammatory process. Original magnification ×57.5.

Table 2. Mean scores observed for each group, for each study period

Groups	Study periods		
	7 days	15 days	45 days
Alendronate paste	3.6[a]	1.5[b]	1.3[b]
Gutta-percha (Control)	1.3	1.1	1.0

Values with different superscript letters are statistically significant. [a]With statistically significant difference from[b].

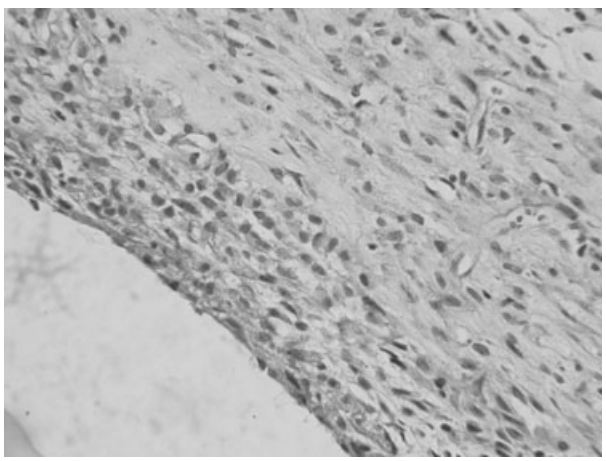

Fig. 2. Gutta-percha (control) at 7 days. Note the presence of organized connective tissue with proliferation of blood vessels and the absence of inflammatory process. Original magnification ×157.5.

Statistical analysis revealed that the inflammatory events occurring at 15 to 45 days were different from the events occurring at 7 days ($P \leq 0.05$) (Table 2).

Control

Microscopic analysis of the histological sections confirmed the biocompatibility of gutta-percha with the connective tissue (Fig. 2). There were no detectable signs of inflammation in all study periods. Only three sections, namely two at 7 days and one at 15 days, showed a mild inflammatory infiltrate. At 7 days, poorly organized collagen fibers and fibroblasts were common observations.

At 15 and 45 days, the connective tissue was well organized, with collagen fibers, fibroblasts and blood vessels in their normal distribution patterns. There were no statistically significant differences between any of the study periods for this group (Table 2).

The results for both groups showed statistical differences between them. The inflammatory events observed in the experimental paste group, at 7 days, were statistically different when compared to the events occurring in all study periods for the control group ($P \leq 0.05$). Nevertheless, no statistical differences were observed between the control group and experimental paste at 15 and 45 days.

Discussion

The guidelines suggested by the American Dental Association (16) and Fédération Dentaire Internationale (17) consider implantation methods as valid tests to investigate the biocompatibility of materials. Thus, implantation of materials in the connective tissue of small animals, such as rats, is considered an adequate test to evaluate material biocompatibility.

A biocompatible material should present low toxicity, without promotion of inflammatory response (15, 18). In the presence of inflammation, it should be insignificant (15, 18). According to Lawrence et al. (19), in implantation tests, the new material should be compared with a control material, which in turn, should be an acknowledged nontoxic material. Gutta-percha is considered to have acceptable biocompatibility, with none or low degree of toxicity (18); thus, it was used as control, for comparison of toxicity with the experimental paste employed.

We have previously demonstrated the efficiency of an alendronate solution to limit root resorption (7). The development of an alendronate paste would enhance its utilization as intracanal medication in teeth susceptible to root resorption and, therefore, in this study, we investigated the biocompatibility of an experimental alendronate paste.

According to the results of the present study, the experimental paste showed to be biocompatible with the surrounding tissues. Even though it may have promoted an inflammatory response at 7 days, the inflammation was nearly absent in the additional study periods. Moreover, there was no statistical difference between the results with the experimental paste and with the gutta-percha used as control.

In 2005, Moreira et al. (14) investigated the toxicity of an alendronate paste consisting of a mixture of 2 g of alendronate per ml of polyethylene glycol 400. The authors concluded that the alendronate paste was highly cytotoxic, *in vitro* as well as *in vivo*, which are contrary to our results. This discrepancy may be related to differences in concentration and vehicle used in the formulation of the experimental pastes of both studies. Various authors have suggested that the higher the concentration of alendronate, the higher the effect on clasts (20, 21). However, very high concentrations can also be toxic for other constitutive cells in the tissues (22). It is recommended that the alendronate be mixed with saline solution which does not alter the properties of the active substance (20–26). In our study, the alendronate concentration was lower than the concentration used by Moreira et al. (14) and the vehicle was saline.

In summary, the results show that the experimental alendronate paste appears to be biocompatible, showing similar results as the control group, and may be considered satisfactory for utilization as an intracanal medication. Additional studies on teeth susceptible to root resorption should also be carried.

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