Histological evaluation of the phenobarbital (Gardenal™) influence on orthodontic movement: a study in rabbits

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Abstract

Introduction: The purpose of this study was to histologically evaluate the influence of phenobarbital on orthodontic tooth movement. **Methods:** Twenty-two New Zealand rabbits (Oryctolagus cuniculus) were divided into three groups: normal or non-tested (N), control (C), and experimental (E). In Group N (n = 2) no procedure was carried out, except to verify the condition of normality before treatment. In Groups C (n = 10) and E (n = 10) an orthodontic appliance was inserted between the first molars and lower incisors in order to promote a mesial molar movement. In Group E phenobarbital was administered during the course of the experiment, which differentiates it from the group C. The animals were sacrificed on days 7 and 14 so that anatomical sections could be prepared for further histological analysis. **Results:** Histologically no difference was observed between normal and experimental groups. **Conclusions:** Phenobarbital does not interfere with the orthodontic tooth movement.

Keywords: Tooth movement. Pharmaceuticals. Orthodontics.

INTRODUCTION

Orthodontic tooth movement occurs when forces are applied to teeth and are transmitted to the supporting periodontium, so as to change their position in relation to the surrounding structures, through alveolar bone remodeling.[19] That is to say, bone apposition in the area submitted to tension and bone resorption in the area under pressure.[17,19]

Although the histological mechanisms of this process have been studied for years, the mediators that initiate or facilitate them are not yet completely understood. As a result of this, several authors have found it necessary[1,5,19] to evaluate the interaction of medications which, acting at cellular level, could interfere with these mediators, and consequently interfere in the process of orthodontic movement.

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Studies have shown that many patients under dental treatment make use of medications.[12] Some of these patients may be under orthodontic treatment, and some of the medications used by them could influence orthodontic movement.[1,5]

Several medications have been studied with regard to the possibility of interfering in orthodontic tooth movement such as acetylsalicylic acid,[15] diazepam,[13] acetaminophen,[17] contraceptives,[18] corticosteroids,[21] indomethacin[24] and bisphosphonates.[5,8]

Phenobarbital is a recognized anxiolytic and anticonvulsant agent according to Davidovitch et al.[6]

Treatment with anxiolytic and anticonvusant drugs in ambulatory patients (not only during the epileptic state) may affect the action of several hormones, such as inhibition of antidiuretic hormone secretion, calcitonin, insulin, adrenocorticotropic hormone and PTH. One of the most remarkable side effects with this class of drugs is the alteration in calcium metabolism, which results in depletion of the serum level, in addition to inducing a condition of osteomalacia or rickets.[7]

Since anxiolytic and anticonvulsant drugs affect bone remodeling, they may also be capable of affecting orthodontic procedures due to the alteration in tissue reaction, and consequently, tooth movement.[6] Based on this premise the aim of this study was to perform a histological evaluation of the influence of phenobarbital (Gardenal™) on induced orthodontic tooth movement in rabbits.

MATERIAL AND METHODS

Twenty-two New Zealand (Oryctolagus cuniculus) rabbits were used, 11 males and 11 females, divided into the following groups: non-tested, control and experimental. The animals were healthy, ranging from 10 to 14 months of age, which corresponds to the young adult stage. They weighed on average 3 Kg, were provided and kept at the Pharmacy and Nutrition Institute phytopharmacology animal lab of the "José do Rosário Velano University – Unifenas" during the course of the experiment.

The animals were divided into five groups, named and characterized as follows:

- Non-tested Group: Represented by animals N1 and N2, which were not submitted to any orthodontic procedure or phenobarbital administration. They served as parameter for comparison with the other animals, and were sacrificed on the first day of the experiment.

- 7-day Control Group: Represented by animals C1, C2, C3, C4 and C5, which were submitted to the orthodontic movement, did not receive phenobarbital administration and were sacrificed after seven days of tooth movement.

- 14-day Control Group: Represented by animals C6, C7, C8, C9 and C10, which were submitted to orthodontic movement, did not receive phenobarbital administration, and were sacrificed after seven days of tooth movement.

- 7-day Experimental Group: Represented by animals E1, E2, E3, E4 and E5, which in addition to being submitted to orthodontic movement, received phenobarbital (Gardenal Sanofi Aventis, São Paulo, Brazil Lot 042389), and were sacrificed 7 days after tooth movement.

- 14-day Experimental Group: Represented by animals E6, E7, E8, E9 and E10, which in addition to being submitted to orthodontic movement, received phenobarbital, and were sacrificed 14 days after tooth movement.

In the Experimental Groups the medication was administered orally, once a day. Each animal received 30 International Units of medication per kilo of weight; on average, each animal received 90 International Units.

The appliance used to promote tooth movement was similar to that described by Ruellas,[18] and consisted of a pre-fabricated closed stainless steel coil spring (Morelli, Sorocaba, Brazil), which extended from the mandibular first permanent molars to the incisors on both sides (Fig 1) under 80 cN of force application ± 5cN, measured with a tensiometer (Ormco, Glendora, USA).

After euthanasia, the mandibles were separated from the soft tissues, and prepared for histological sections.

Histological evaluation

The structures were histologically evaluated with an optical microscope (Nikon Eclipse E 600,Tokyo, Japan) and the regions of interest photographed with a digital camera (Nikon Coolpix 4500, Tokyo, Japan).

RESULTS

The results demonstrated that animals not submitted to orthodontic movement, they presented periodontal support with normal characteristics. Animals subjected to orthodontic movement, both control and experimental groups presented with areas of pressure and tension (Figs 2-5).

Tissue reactions in the pressure and tension sides of the periodontal ligament were similar in both control and experimental groups at 7 and 14 day period (Figs 2-5).

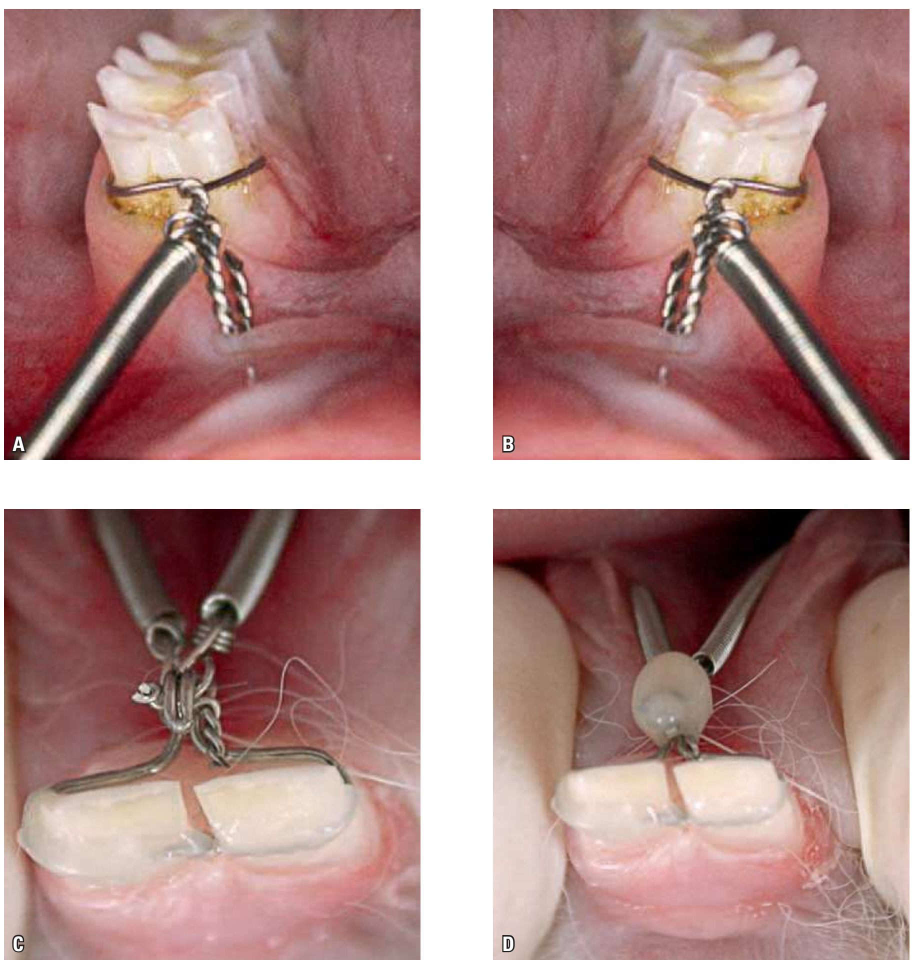

FIGURE 1 - Appliance used to induce tooth movement: **A** and **B**) coil spring tied to the first molars; **C**) coil spring tied to the mandibular incisors; **D**) complete assembly of the appliance.

DISCUSSION

Phenobarbital is a derivative of barbituric acid, and was synthesized for the first time in Germany, in 1912, and patented under the brand name of Luminal.[7] It acts on the GABA receptor, blocking the entry of calcium into the presynaptic terminals, inhibiting the transmission of the neurotransmitter glutamate.[16] As all barbiturates, chemically phenobarbital is a cyclic diamide of six carbons.

According to the literature, the treatment with drugs that have an anxiolytic and anticonvulsant action may affect the action of various hormones.[7,16]

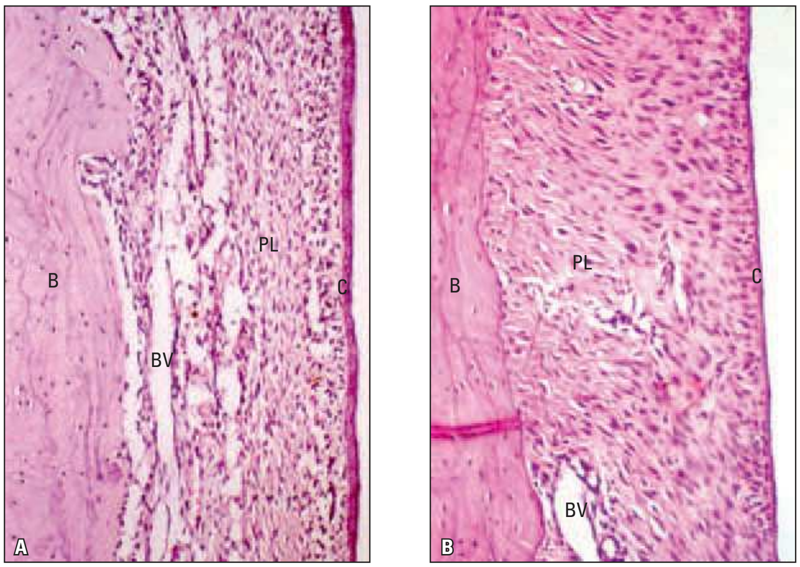

FIGURE 2 - **A**) Area of inter-radicular pressure, Group C, after 7 days of orthodontic movement. Note the narrowing of the periodontal ligament space, disorganized fibers and cells, discrete inflammatory infiltrate, dilated vessels, bone surface without resorption lacunae, partially covered with osteoblasts. **B**) Area of inter-radicular pressure, Group C, after 7 days of orthodontic movement. Note the discrete increase in the ligament thickness, stretched fibers, discrete inflammatory infiltrate, irregular bone surface with areas of active resorption. Normal cement surface.

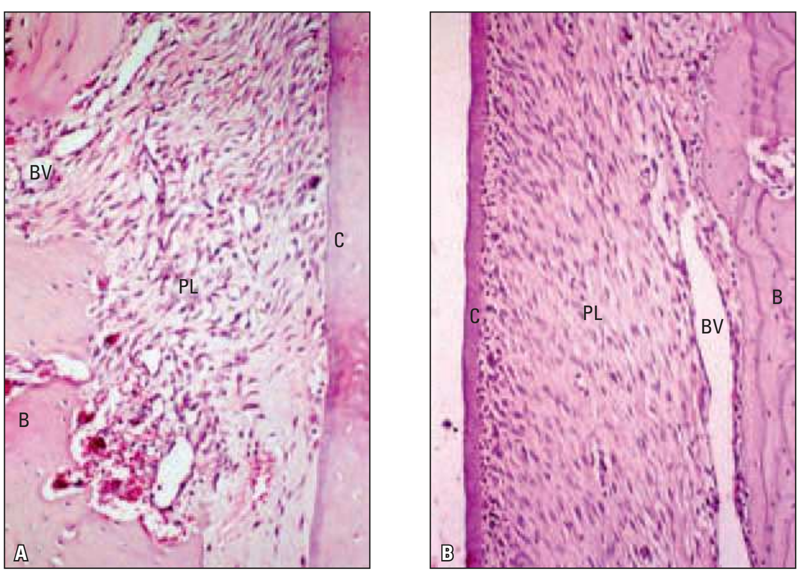

FIGURE 3 - **A**) Area of inter-radicular pressure, Group E, after 7 days of orthodontic movement. Note the narrowing of periodontal ligament space, disorganized fibers and cells, moderate inflammatory infiltrate, dilated and hyperemic vessels, area of intense maxillary bone resorption. **B**) Area of inter-radicular traction, Group E, after 7 days of orthodontic movement. Note the discrete increase in ligament thickness, stretched fibers, discrete inflammatory infiltrate, irregular bone surface without active resorption. Normal cement surface.

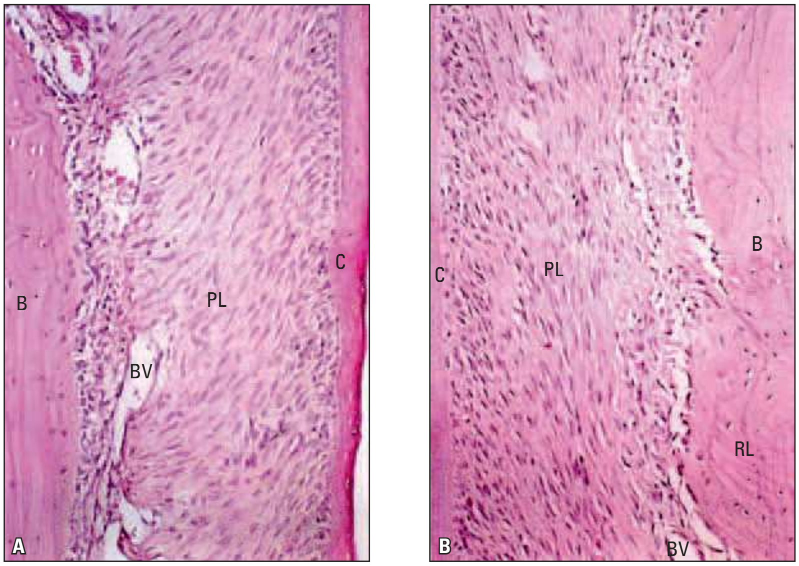

FIGURE 4 - **A**) Area of inter-radicular pressure, Group C, after 14 days of orthodontic movement. Note discrete narrowing of periodontal ligament space, with slightly compressed cells, there is rich cellularity and there are dilated blood vessels. Note discrete mononuclear leukocytes. **B**) Area of inter-radicular tension, Group C, after 14 days of orthodontic movement. Note discrete increase in ligament thickness, rich cellularity and few blood vessels. Predominant fusiform cells, cement surface preserved and covered with cementoblasts, notable reversal lines.

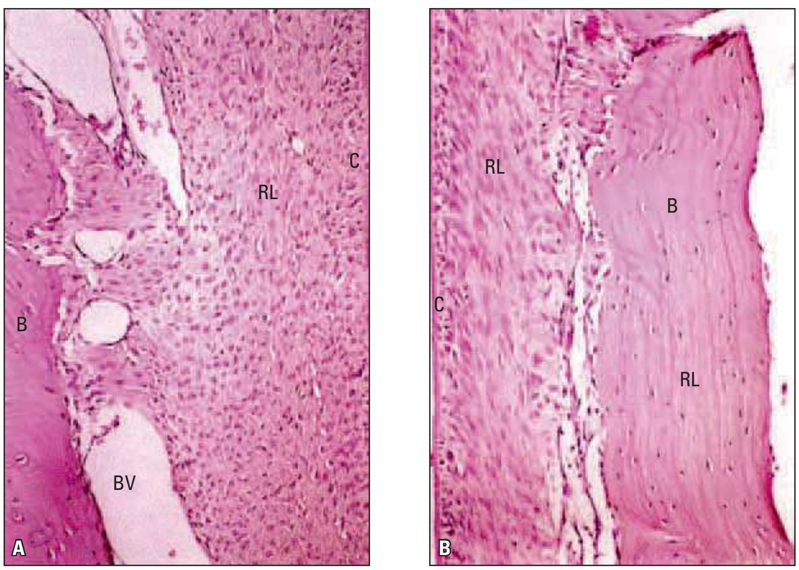

FIGURE 5 - **A**) Area of inter-radicular pressure, Group E, after 14 days of orthodontic movement. Note discrete narrowing of periodontal ligament space, with slightly compressed cells, there is rich cellularity with ovoid and irregular cells. Well dilated and non hyperemic blood vessels. **B**) Area of inter-radicular traction, Group E, after 14 days of orthodontic movement. Note discrete increase in ligament thickness, rich cellularity with fusiform cells, without organization and stretching of collagen fiber bundles, cement surfaces without resorption lacunae.

One of the most remarkable side effects in therapy with this class of drugs is the alteration in calcium metabolism, which results in depletion of the serum level, in addition to inducing a condition of osteomalacia or rickets.[7,16] Since it alters the metabolism of calcium, phenobarbital may have an influence on tooth movement. Therefore, the present article proposed to evaluate the influence of this medication on induced tooth movement.

For this purpose, a New Zealand (Oryctolagus cuniculus) breed of rabbits, frequently used in several studies on the effects of drugs on orthodontic tooth movement was used.[18,21,20] In order to induce the tooth movement similar to what would be seen clinically, a coil spring was extended from the maxillary incisors to the molars.

Tooth movement was evaluated after 7 and 14 days which reflects the intermediate and final periods of tooth movement in the rabbit periodontium.[18]

When the histological preparations from the animals not submitted to orthodontic tooth movement were analysed, the supporting periodontium presented characteristics of normality. There was a uniformity in the periodontal ligament width along the roots of the teeth, intense vascularization, high cellularity with various types of cells, particularly fibroblasts, in addition to lymphocytes, undifferentiated mesenchymal cells, osteoblasts and cementoblasts distributed in the amorphous fundamental substance, as referred to by Junqueira and Carneiro[9] and Ten Cate.[22]

The bony tissue was shown to be of the lamellar type, covered by a non lamellar bone layer. Superficially to the lamellar bone, in some areas, there was deposition of a thin layer of osteoid tissue covered by osteoblasts aligned along it. The presence of osteocytes distributed throughout the bone was also observed. This condition of normality demonstrated in the normal animals was mentioned by Junqueira and Carneiro.[9]

In the histological preparations of the animals submitted to orthodontic movement, both in the control and experimental groups, the presence of area of pressure in the periodontal ligament region corresponding to the mesial surface of the first permanent molar and in the area under tension on the distal surface was characterized, in agreement with findings of other experimental studies on orthodontic tooth movement.[11] Tissue reactions on the pressure and tension sides of the periodontal ligament were similar in the control and experimental groups both at 7 and 14 days, and were characterized as described below.

The pressure side in animals of the 7-day Control and Experimental Groups

Seven days after force application, a reduction in the width of the periodontal ligament space was observed, in addition to a discrete inflammatory infiltrate next to the bone surface, discrete hyaline zones, fibers and disorganized cells. Absence of alterations at the tooth margin (cement) and a discrete bone resorption with an irregular surface were also observed. Another condition found was lacunae without clasts in the majority of the laminae while in others, a discrete distant resorption with clasts in lacunae could be observed (Figs 2 and 3).

The tension side in animals of the 7-day Control and Experimental Groups

On the tension side, the presence of inflammatory infiltrate next to the bone was observed, as well as hyaline zones, increased periodontal ligament space, well distended periodontal ligament fibers, dilated vessels and discrete hyperemia. Some empty lacunae and distant clasts were also observed, as well as absence of bone and cement resorption, demonstrating a normal tooth surface and presence of bone formation with reversal lines (Figs 2 and 3).

These characteristics observed at 7 days are in agreement with those that presented in the literature by Ruellas,[20] demonstrating that there are no differences between the normal and experimental groups.

The pressure side in animals of the 14-day Control and Experimental Groups

Discrete reduction in the periodontal ligament space was observed with slightly compressed and irregular cells without an organized distribution pattern. No hyaline zones were observed, however, but rich cellularity and dilated blood vessels, some of them hyperemic, were observed. Discrete mononuclear leukocytes, bone and cement surface resorption were noted, with the bone surface being uniform at times and cut at others. The events are compatible with the healing process (Figs 4 and 5).

Areas of cement resorption occurs even with the use of light orthodontic forces, according to Kurol and Owman-Moll,[11] which explain all the histological characteristics observed on the pressure side at the 14-day period.

The tension side in animals of the 14-day Control and Experimental Groups

The space of the periodontal ligament presented with rich cellularity and a few blood vessels. Cells were predominantly fusiform and the fiber bundles were distended. The cement surface was preserved and covered with cementoblasts. The bony surface was slightly cut, without resorption lacunae, and reversal lines indicative of bone deposition were noted. No inflammatory infiltrate or hyaline zones were noted (Figs 4 and 5).

It is important to point out that Group E presented characteristics similar to those of Group C at 14-day period. However, a lower quality in the healing process in Group E could be noted as compared to Group C. These characteristics observed on the pressure and tension sides are corroborated by previous findings,[19,20] therefore demonstrating no difference between the control and experimental groups after 14 days.

Histological differences at 7 and 14-day period, were found by Chao et al[3] and Tenshin,[23] when prostaglandins were used. The authors found an increase in vascularization and significant changes in its morphology, representing greater bone resorption, thus obtaining double the movement, without promoting damage to the periodontal tissues.

Other medications have also been tested such as parathyroid hormone,[10] vitamin D metabolites,[4] indomethacin,[24] aspirin,[2] ibuprofen, acetaminophen[14] and bisphosphonates[14] and have demonstrated histological differences between control and experimental groups.

Although no differences were found in the periodontium of animals treated with phenobarbital, it is important for the clinician to be aware of patients being treated with this kind of drug particularly because there is no reference in the literature on the long-term effect on tooth movement.

The results of this study were based on subjective and overall analysis by an experienced and trained examiner involving various parameters described for optical microscopy. The use of optical microscopy and hematoxylin-eosin staining was shown to be practical, low cost and allowed overall analysis of all tissues, with a high degree of fidelity, without the need of any special and specific analytic or staining technique.

CONCLUSION

The results of this study demonstrate that phenobarbital did not interfere with tooth movement, as tissue reactions on the pressure and tension sides on both groups, were similar and in agreement with those reported in the literature. Nevertheless, a lack of organization was observed in the bone formation process of animals treated with phenobarbital in the 14-day period.

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