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Cellular and Structural Changes of the Pulp in Rats after Continuous Orthodontic Force Application: A Pilot Study

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Abstract

Background: The application of continuous orthodontic force may have a harmful effect on pulpal tissues. Blood vessels may be damaged, causing hemorrhage, leading subsequently to tooth discoloration and even worse, and necrosis of the pulp. The aim of the present pilot study was to analyze the early histologic changes that occur in pulpal tissues of rats when molars were submitted to continuous orthodontic forces.

Methodology: The left and right mandibular healthy second molars of 10 male Wistar rats (n=10) were extracted. After healing the left mandibular first molars were submitted to continuous distal orthodontic forces of 17g for the duration of two observation periods, 7 and 14 days. The right mandibular first molars served as controls and were not submitted to orthodontic forces. In the animals of group 1, 5 (n=5) had orthodontic forces applied for 7 days, while in Group 2 (n=5) the forces of application lasted 14 days. After euthanasia the mandibles were dissected, fixed in formalin and decalcified in EDTA. They were then further processed for histologic analysis. Sagittal serial sections of 6 microns were cut through the pulps and stained with hematoxilyn and eosin.

Results: All baseline control teeth exhibited a normal healthy pulp morphology. The walls of the pulp were lined with a regular odontoblastic cell layer. In Group 1, some dilated and congested blood vessels were present in the coronal pulp tissues, while the walls of the pulpal chamber were lined with a regular odontoblast cell layer. In Group 2, a moderate to severe concentration of inflammatory cells was present at the coronal aspect of the pulp. The odontoblast palisades were interrupted in various locations of the pulp chamber and many dilated and congested blood vessels along with mild hemorrhage was observed. No hard tissue formation or necrosis was present. Statistical analysis demonstrated a significant difference between the two groups (Fisher´s exact test: P<0,05).

Conclusions: The application of continuous orthodontic force without using rest periods may have an early harmful effect on pulpal tissues in rats.

Keywords: Blood vessels; Hyperemia; Orthodontic force; Pulp tissues; Rat; Odontoblastic layer

Introduction

Pulpal microvasculature changes may happen after application of orthodontic forces beyond the physiological limits of pulp tolerance [1,2]. Blood vessels that supply pulpal tissues may be damaged causing hemorrhage, which may lead to tooth discoloration and possibly necrosis [2]. The forces generated on teeth during orthodontic therapy elicit release of inflammatory

mediators in pulp tissues causing odontoblastic vacuolization and other cell response, which can result in either resorption or tertiary dentin deposition, while it can also result in an increase in blood flow, reduction in pulpal volume, inflammation and calcitonin gene-related peptide (CGRP) expression [3-5]. Many variables including direction, intensity and duration of the force have an influence on the way the pulp reacts. In one study, the objectives focused on the long-term effects of orthodontic forces on pulpal tissues [6], but the information on early effects on the pulp is scarce. The aim of this pilot study was to qualitatively evaluate the early histologic changes in the pulp of rats when exposed to distal orthodontic movement. The null hypothesis postulated that there is no difference in the histologic pulpal features between two short periods, 7 and 14 days, of orthodontic force application.

Material and Methods

The protocol for this study received approval from the Institutional Research Ethics Committee of the Faculty of Odontology, University of Buenos Aires (Res.– N° 398/15) and CICUAL (ODON/FOUBA Nº 001/2018).

Animals

This study was conducted on 10 male Wistar rats (n=10) that had intact healthy teeth and a weight of approximately 250g. The operative procedures were in accordance with the National Academy of Science Animal Welfare Regulations, 1985; NRC, 1996; NIH, 2011). Every effort was made to minimize animal discomfort and limit the total number of animals for the experiment. For the duration of the experiment the animals were housed in metallic cages under controlled room temperature (25±2°C) with 12h light/dark cycles and access to food and water ad libitum. They were assigned to 2 groups of 5 animals each (n=5). The animals were anesthetized by administration of intraperitoneal injection of ketamine chloride (14 mg/Kg body weight) and acepromazine (10 mg/Kg body weight. In Group 1 an uninterrupted distal orthodontic force was applied for 7 days, while in Group 2 the force lasted for 14 days. Before force application, the left and right mandibular second molar of each animal were extracted using a slight modification of the procedures described [7]. For orthodontic force application, a unilateral expanding apparatus was used. On the left mandibular first molar of both experimental groups an orthodontic band was cemented with a glass ionomer cement (Ketac Cem, 3M). A stainless-steel open coil spring (Morelli, Sorocaba, SP, Brazil) was then attached between the cemented band and the left mandibular incisor (Fig.1A). Before placement of the coil spring, it was calibrated with an orthodontic dynamometer (Morelli) to exert a constant expanding force of 17g, thus producing a distal movement of the first molar. The first molars in the right mandible underwent no treatment and served as controls.

Euthanasia and sample preparation

After each observation period the animals were euthanized with an anesthetic overdose. After vascular perfusion with saline, heparin and 10% neutral buffered formalin the mandibles were dissected and trimmed into blocks, thus isolating the experimental and control mandibular first molars. They were then postfixed in 10% neutral buffered formalin for 72h, decalcified in 10% ethylenediaminetetraacetic acid (EDTA) and rinsed in running tap water for 12h. After dehydration in ascending concentrations of alcohol, the specimens were cleared in xylene and embedded in paraffin. Sagital serial sections of approximately 6µm thick were cut through the pulps and stained with hematoxylin and eosin.

Evaluation

The histologic sections were examined with a Nikon Eclipse Ni photomicroscope (Nikon Instruments Inc, Melvielle, NY, USA) and microphotographs were obtained at different magnifications. The images were transferred to a computer and analyzed with Image J 1.38x image-analyzer software (National Institutes of Health, Bethesda, MD). The presence of dilated and congested blood vessels, inflammatory cells, fibrous tissue or necrosis, reparative hard tissue formation and morphological changes in the odontoblastic cell layer were determined by using predetermined criteria and a modified grading system [8]. A 1 to 4 scoring system, 1 being the best result and 4 the worst, was used (Table 1). The results were statistically analyzed by the Fisher´s exact test using the SPSS Version 17.0 (SSPS Inc, Chicago, IL). The significance level was set at $P<0.05$.

Results

The results of the histological evaluation are presented (Table 2). In both groups, the baseline control molars exhibited a healthy pulp morphology with normal distribution of blood vessels. In all specimens the pulpal walls were lined with a regular well-preserved odontoblast cell layer (Figure 1B and C).

In Group 1 (7 days) no inflammation, hard tissue formation, fibrous tissue or necrosis was observed. In deeper pulpal tissues some dilated blood vessels in the coronal pulp were observed, while the walls of the pulp chamber and the root canals were lined with a regular odontoblast cell layer (Figure 2A,B,C). In Group 2 (14 days), all specimens showed a moderate to severe concentration of inflammatory cells in the coronal aspect of the pulp. The odontoblast palisade was interrupted in various areas, especially in the coronal portion of the pulp chamber. In addition, many wide engorged blood vessels with isolated hemorrhagic sites (score 4) were seen (Figure 2D and E). Wide dilated vessels

and odontoblastic palisades were also observed at the level of the root pulpal tissues. (Figure 2F). The statistical analysis showed that Group 1 scored significantly better (P<0,05) compared to Group 2 with respect to hyperemia, mild hemorrhages, inflammation and odontoblast cell layer organization. Therefore, the null hypothesis was rejected.

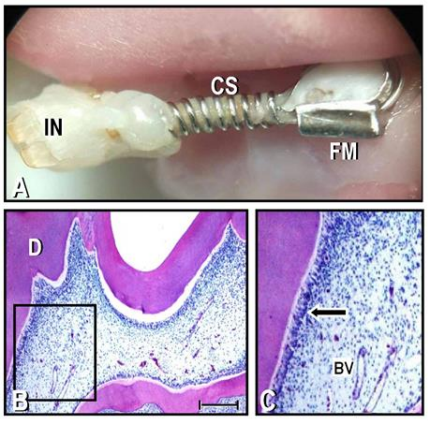

Figure 1*: A: Image of the orthodontic appliance that applied a distal force. The coil spring (CS) was attached between the mandibular incisors (IN) and the cemented band on the mandibular first molar (FM). B: Microphotograph of representative 14-day control mandibular first molar showing healthy pulp tissues with uninterrupted ondontoblastic palisades. D: Dentin. Bar: 100 µm. (Hematoxylin & Eosin; Original magnification x 200). C: High magnification from the square area in B. Note the presence of normal pulp cells and blood vessels (BV) and well-preserved odontoblastic palisades (arrow). Hematoxylin & Eosin; Original magnification x 400).*

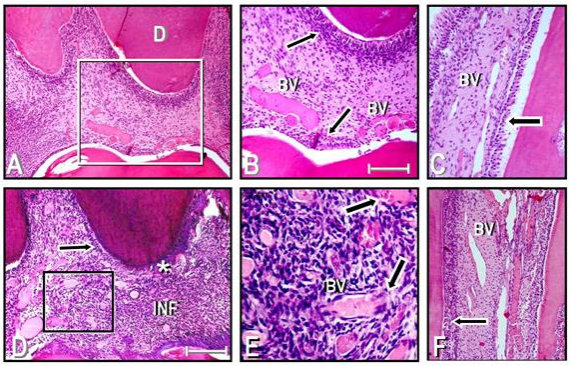

Figure 2*: A: Microphotograph of a representative specimen of Group 1 showing intense pulp cellularity and well-preserved odontoblastic palisades. (Hematoxylin & Eosin) Original magnification x100). B: Higher magnification from the square area in A. Several enlarged and congested blood vessels (BV) and a continuous odontoblastic layer (Arrow) can be seen. Bar: 100 µm. (Hematoxylin & Eosin; Original magnification x200). C: In the root canal the pulp appeared to be normal showing enlarged blood vessels (BV) and a continuous odontoblastic cell layer (arrow); (Hematoxylin & Eosin; Original magnification x 400). D: Microphotograph of a representative specimen of Group 2 showing a severe inflammatory cell concentration (INF) in the coronal pulp and many dilated blood vessels. The odontoblastic palisades (arrow) are interrupted at various locations and replaced by inflammatory cells (asterisk). Bar: 100 µm. (Hematoxylin & Eosin; Original magnification x200). E: Higher magnification from the square area in D, showing a mixed inflammatory cell population and many dilated and congested blood vessels (BV). The arrow points at a localized hemorrhagic area. (Hematoxylin & Eosin; Original magnification x400). F: In the root canals no inflammatory cells were observed. A continuous odontoblastic cell layer was present (arrow) but there are many large and dilated blood vessels (BV). (Hematoxylin & Eosin; Original magnification x 400).*

Table 1*: Scoring system used for histologic evaluation.*

Pulp inflammation:	1: Absent. 2: Mild, (<30 inflammatory cells) 3: Moderate, (30 – 50 inflammatory cells). 4:Severe, (>50 inflammatory cells).
Blood vessels	1: Absent. 2: Normal (normal diameter without congesti 3: dilated and congested vessels. 4: dilated and congested vessels plus hemorrhagic
Hard tissue formation	1: Absent. 2: Present in the pulp chamber. 3: Present in the root canal. 4: Present in the pulp chamber and the root canal.
Fibrous tissue	1: Absent. 2: Present in the pulp chamber. 3: Present in the root canal. 4: Present in the pulp chamber and the root canal
Odontoblast cell layer	1: Absent. 2: Regular (Presence of complete odontoblast cell layer). 3: Irregular (Interrupted along the odontoblast cell layer). 4: Atrophy of odontoblast cell layer.
Necrosis	1: Absent. 2: Present in half of the coronal pulp. 3: Present in the entire coronal pulp.

	4: Present in the coronal and root pulp tissue.

***Table 2**: Histologic scores of the two experimental groups.*

	Group 1 9n=5				Group 2 (n=5)			
	Score				Score			
	1	2	3	4	1	2	3	4
Pulp inflammation	0	0	0	0	0	0	1	4
Blood vessels	0	0	5	0	0	0	2	3
Hard tissue formation	0	0	0	0	0	0	0	0
Fibrous tissue	0	0	0	0	0	0	0	0
Odontoblast cell layer	0	5	0	0	0	1	4	0
Necrosis	0	0	0	0	0	0	0	0

Discussion

It is important to ask the question if continuous orthodontic forces can negatively affect pulpal tissues and if so, what is the initial response of the pulp? The knowledge of the changes reported here may help clinicians decide to incorporate an appropriate rest period between each force application during treatment. In the present study, we chose 7 and 14 days as observation periods in order to study the early effects of continuous orthodontic force application on the pulp of the rat mandibular first molar. The rat model is frequently used for different experiments in dental sciences since the pulp and periodontal tissue healing has similar features as in humans [9]. Although this study was conducted on a small sample size, the results generated valuable preliminary insights into the initial pulpal reactions to continuous orthodontic force. Our observations demonstrated that a distal force application of 17g during 7 and 14 days does not cause pulpal necrosis, however it caused blood vessel dilatation, congestion and an inflammatory response. These findings are in agreement [10] and are similar to what occurs in humans [11,12]. According [4], vascular pulp changes due to orthodontic force application can be triggered by neuropeptides, which are recognized as neurotransmitters or neuromodulators, thus inducing vasodilatation, congestion, plasma leakage and recruitment of inflammatory cells. In this respect, a neuropeptide such as CGRP can be triggered by orthodontic forces thus increasing bone morphogenetic protein expression in human pulp cells, which in turn stimulates dentin deposition by odontoblasts. This phenomenon along with prolonged cellular hypoxia may lead to degenerative pulp calcification [13-15]. However, these events were not observed in the current study. CGRP release can lead to initial vascular dilatation and congestion, two pulpal changes that were consistently observed in the specimens of both groups. The presence of congested vessels with mild hemorrhage were the result from the incremental increase in internal pulp pressure, which led to the rupture of the vessel walls. Although these features are prone to develop into pulp necrosis, this effect was not observed in the present study, probably because the force application was only applied for a short period of time. It is of interest to note that [16] did not find pulp alterations after 7 days of orthodontic force. On the contrary, our results demonstrated that pulp angiogenesis and dilated blood vessels may occur as early as 7 days of continuous force application. However, one must realize that other changes such as the deposit of reactionary dentin or pulp calcifications may also occur depending on the intensity and traumatic effects of the applied forces [13-15].

Conclusions

Within the limits of this pilot study, it can be concluded that the application of continuous orthodontic force without using rest periods may cause early detrimental changes to the pulp of rats.

Conflict of Interest

The authors declare no conflict of interest.

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None.

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