Histologic Evaluation of Human Pulp Tissue after Orthodontic Intrusion

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Abstract

Introduction: The forces applied during orthodontic treatment bring about effects on the teeth and surrounding tissues. The aim of this study was to evaluate the possible changes in the human pulp tissue resulting from orthodontic intrusion in a 21-day period using histologic examination. Methods: The sample consisted of 17 young individuals of both sexes between the ages of 12 and 19 years. A total of 34 premolars were evaluated with orthodontic indication of extraction. Because it is a split-mouth study, in each patient, intrusion force of 60 g was applied randomly on 1 of the dental elements experimental group for 21 days. The counterpart control group received no force. After extractions, these dental elements were fixed in 10% formaldehyde, processed automatically, submitted to histotechnical preparation, and stained with hematoxylin-eosin for analysis under optical microscope. Results: The paired Fisher exact test (P ≤ .05) showed a significant increase of fibrous tissue in the experimental group. The nonparametric paired Wilcoxon test (P ≤ .05) showed a significant increase in the number of pulpal nodules in the elements of the experimental group and showed no difference in the number of blood vessels between the groups. Large-caliber vessels and congested elements were observed in 8 of the experimental group elements. Conclusions: The orthodontic intrusion force, in these conditions, caused vascular changes in the pulp tissue and also increased the presence of fibrosis and the number of pulp calcifications in the experimental elements. (J Endod 2014;40:1537–1540)

Key Words
Dental pulp, dental pulp calcification, endodontics, orthodontics, pulpitis, tooth movement


Methods

Ethical Considerations

This study was approved by the Ethics Committee on Research of the University of Maringá-Unininga under protocol no. 0004/11. Individuals selected and invited to participate received all instructions before the procedures and consented by signing an informed consent form.

Sample

The sample consisted of 34 upper first premolars of 17 individuals who sought care at the Clinic of Specialization in Endodontics of the Unity of Post Graduate Inga-Unininga Faculty-Posto Fundo, Rio Grande do Sul, Brazil. We included patients of both sexes (9 females and 8 males) with the following characteristics: age between 12 and 19 years, no previous orthodontic treatment, and good oral hygiene. Patients presenting with systemic disease; patients who used drugs for chronic conditions; and those whose premolars had incomplete apicogenesis, caries, restorations, endodontic treatment, and periodontal problems were left out.
The first upper premolars selected for the study had indication for tooth extraction by the orthodontic planning. Because it is an in vivo split-mouth study, in each patient only 1 element was tested, and the counterpart element served as the control. Thus, the sample was divided into 2 groups: the control group (n = 17) and the experimental group (n = 17). Patients with first premolars whose side of the mouth presented lesser crowding were selected for the experimental group during the clinical examination. In cases of similar bilateral crowding, the samples were randomly divided by lot.

Clinical Procedures

Before and after the installation of orthodontic appliances, pulp vitality was verified using a sensitivity test with Endo-Ice (−50°C) (Maquira, Maringá, Paraná, Brazil), and a record with periapical radiography was made. At the beginning of the experiments, the elements 16 and 26 were pulled apart with elastics for 2 days. In the next session, metallic bands were adapted, prepared, and welded. Then, we proceeded to transfer molding with alginate for making plaster models on which transpalatine bars welded with stainless steel wire 1.0 (Morelli, Sorocaba, São Paulo, Brazil) were made. Once ready, the bars were fixed with glass ionomer to the elements 16 and 26 and with light-curing resin to the second upper premolar on the side of the control element for the maximum possible anchorage. On the first experimental premolar, an Edgewise Standard bracket (Morelli) was glued.

Subsequently, a cantilever partial arc was made for the experimental side with 0.019 × 0.025-inch of stainless steel wire (Morelli) from the first upper molar to the first premolar, not involving the second premolar, for application of intrusion force.

Then, the partial arc was activated, and the force was measured with a mouth dynamometer (Morelli) until a magnitude of 60 g was reached. After 21 days, the extractions were made, and the first premolars were stored in 10% formalin for 45 days at most.

Laboratory Procedures

Before the full execution of the study, the methodology was applied to 12 premolars (6 patients) serving as a pilot project for evaluating the method. The decalcification process used solution comprising sodium EDTA, tartrate of sodium and potassium, hydrochloric acid, and deionized water associated with use of a greenhouse. Each seventy-two hours, the descaling solution was replaced and, to speed up the process, we used 2 heating periods in a descaling machine.

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Analysis of Results

Once ready, the histologic slides were reviewed by a single calibrated examiner who was an experienced pathologist using the Zeiss Axioplan 2 microscope (Carl Zeiss, Oberkochen, Germany) with increases of 50×, 100×, and 400×. Because it was a double-blind study, the examiner and recorder had no information about which group each histologic slide belonged to (ie, control or experimental).

The cells and the structures of the coronal pulp of all elements were observed in the different regions of the pulp according to the inflammatory response, response of the soft tissues, and response of the hard tissue and qualitatively classified according to Súbay et al (23) as follows:

1 Inflammatory response
   a. No or few inflammatory cells in the pulp
   b. Mild to moderate inflammatory cell infiltration
   c. Severe infiltration of inflammatory cells.

2 Soft tissue response
   a. There is no detectable change in the structure of the pulpal tissue.
   b. Alteration (aspiration) of the odontoblast layer in some region
   c. Change of the pulpal tissue into fibrous tissue
   d. Some level of necrosis of the pulpal tissue

3 Hard tissue response
   a. Absence of reparative dentin
   b. Formation of additional reparative dentin
   c. Small nodules in the pulp tissue
   d. Large nodules in the pulp tissue

In addition, we performed a count of the number of blood vessels and pulp calcifications by high-magnification fields (400×). Each high-magnification field corresponds to 0.1 mm²; and we used 3 high-magnification fields (0.3 mm²) per slide for the counting and 1 high-magnification field per slide to make the record of the images.

The results were analyzed using the SPSS 15.0 software for Windows (SPSS Inc, Chicago, IL) using averages; we also used the Fisher exact and nonparametric Wilcoxon tests, both at the level of significance of 5%. The first 12 samples of the pilot project were reanalyzed by the examiner and included in the total sample, obtaining a weighted kappa coefficient (±1) of 0.92 for the vessel count and of 0.98 for the pulp nodule count.

Results

The histologic findings were classified according to Súbay et al (21) (Table 1).

Inflammatory Response

We did not detect the presence of inflammatory cells in any element in the sample.
Soft Tissue Response

The soft tissue was abnormal in 11 elements (64.7%) in the experimental group, showing fibrous tissue in 10 elements (58.8%) and odontoblast aspiration in 4 elements (23.5%). Both conditions were associated in 3 elements. The paired Fisher exact test ($P \leq .05$) showed a significant result for an increase in fibrous tissue in the experimental group ($P = .026$).

Hard Tissue Response

The presence of reparative dentin was not detected in any element of the sample. Pulpal nodules (Fig. 1) were observed in 6 elements of the experimental group (mean = 48.6 and median = 12.5) and in only 3 counterparts of the control group (mean = 14.6 and median = 2).

The nonparametric paired Wilcoxon test ($P \leq .05$) showed that the increase in the number of pulp nodules ($P = .028$) in the elements of the experimental group was statistically significant.

Vascular Alterations

Vasodilation was the vascular abnormality more evident in the experimental group because in 8 elements of the experimental group was identified the presence of high-caliber vessels and congested (Fig. 2). The number of blood vessels showed no significant difference between the 2 paired groups ($P = .43$) in the nonparametric Wilcoxon test ($P \leq .05$).

Discussion

The objective of this study was to simulate a step of the orthodontic treatment as closely as possible, trying to approximate the results of clinical practice, and, therefore, the procedures were performed in humans. Factors such as split-mouth study design, sample (young individuals of both genders) with good general health, allied to the criteria of inclusion/exclusion of dental elements, made the experimental group and the control group quite similar, favoring comparisons (22).

Ethical considerations aiming to minimize patients’ discomfort and damage to their teeth guided the choice of orthodontic mechanics that are simple and had already been used in other experiments (14, 15, 22). Previous studies confirmed the greater convenience of histologic studies using human premolars because these elements are generally more suitable for extraction when there is orthodontic need (2, 14, 15, 18, 21–23). However, some authors use animal models citing the difficulty or inability to perform certain methodologies in humans (13, 16).

Figure 1. A photomicrograph showing a pulpal nodule (arrow) (200×).

Figure 2. A photomicrograph of elements in the experimental group showing large congested vessels (arrow) (200×).

The 21-day period used in this experiment, corresponding to the minimum interval between consultations during orthodontic treatment, resembles clinical practice and is considered sufficient for a tooth to move and complete its cycle of bone formation resorption provided that the orthodontic forces are not excessive (21). Pulp vitality tests before and after the application of orthodontic force showed no alteration in tooth sensitivity, and the patients reported no discomfort during the trial period. Likewise, there was no presence of visible alteration in the periapical radiographs performed in the beginning and in the end.

The results showed some kind of change in the pulp tissue in most elements of the experimental group after application of orthodontic intrusive force. We did not detect the presence of inflammatory cells in the elements evaluated in the same way as in other studies with similar forces (21, 23). On the other hand, the results of Raiden et al (22), with intrusive forces of 150 g, showed that inflammatory cells were present in twice as many samples of the experimental group compared with controls.

The odontoblast aspiration shown by some authors as one of the first visible pulp reactions to external stimuli was detected in 23.5% the elements of the experimental group ($n = 4$). A similar result (22.5%) was found by Subay et al (23). Ramazanzadeh et al (21) reported odontoblast aspiration in only 1 case (10% of the sample) in the group in which intrusion force was applied and concluded explaining that it happened because of the trauma caused by forceps at the time of tooth extraction.

In pulp coronary, calcification usually takes the form of discrete and concentric pulp stones, whereas in the radicular pulp calcification tends to be diffused. The cause of calcification of the pulp is completely unknown, and, in many cases, it occurs around degenerated cells, blood thrombi, or collagen fibers, which characterizes dystrophic calcification. Pulpal nodules (stones) are difficult to be classified as dystrophic because very often they occur in healthy pulps and do not necessarily relate to functional stress (20). The pulp calcifications, when in large number and size, can obliterate all or part of the pulp cavity and hinder the endodontic treatment. The significant increase in the number of nodules in the experimental group found in this study is supported by the literature, which informs us that the pulp calcifications increase in the presence of orthodontic force (21–23).

In this study, it was not possible to count fibroblasts; however, areas of dense connective tissue (fibrosis), when present, could be visualized. The presence of at least 1 area of fibrosis in 10 elements of the experimental group was significantly higher than in control group elements (22). Clinical cases involving pulp necrosis are cited as caused by trauma previous to orthodontic treatment, extremely high forces, the
extrusion of impacted teeth or orthognathic surgery (ie, situations in which the neurovascular bundle is broken) (5–7). Thus, the force of 60 g observed in the period of 21 days was not able to produce any case of necrosis of the pulp.

There was no statistical difference in the number of blood vessels between the groups, but the vasodilatation was evident in 8 elements, all belonging to the experimental group. Besides being of large caliber, most vessels were congested at histologic examination, which is to say, filled with blood cells. According to Kim (24), vascular congestion arises when part of the interstitial fluid is forced out of the pulp because of increased blood flow and characterizes a situation of acute inflammation.

At the end of this histologic study, it was concluded that, after 21 days, the application of orthodontic intrusion force caused vascular changes in the pulpal tissue; it also increased the presence of fibrosis and of the number of pulp calcifications.

Acknowledgments

The authors deny any conflicts of interest related to this study.

References