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 pulpal 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 ($P \leq .05$) showed a significant increase of fibrous tissue in the experimental group. The nonparametric paired Wilcoxon test ($P \leq .05$) 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 pulpal 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

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There is no conclusive evidence of the relation between orthodontic force and tissue reaction of the dental pulp (1), and although contradictory and poorly publicized, the issue has been the subject of studies for many years in human (2) and animal models (3, 4). Profitt et al (5) stated that light continuous forces have an effect on the periodontal ligament and have little or no effect on the pulp. However, the literature describes pulp reactions ranging from mild hyperemia to an increase in cases of necrosis associated with trauma previous to orthodontic treatment (6, 7). Movement type, dimension of force, mechanical method used, patient age, periapical condition, and observation time hinder a consensus between the methodologies observed (8).

The early detectable signs in the pulp tissue are the hemodynamic changes (9, 10) and circulatory disturbances (11, 12) with an increase in the density of blood vessel volume within the first hours after application of orthodontic force (13). After a few days, there was an increase of neural activity and the response threshold to electric stimulation of the pulp (14). Apoptosis and necrosis of pulp cells also increased subsequently because of changes in the pulp metabolism expressed by increased enzymatic activity (15).

The presence of macrophages, alteration of the odontoblast layer, edema of the connective tissue, and an increase in progenitor cells and fibroblasts are reports that indicate inflammation and an adaptive process of the pulpal tissue to mechanical aggression caused by the orthodontic force (16–18). Long-term studies show that the reduction of the expression of some proteins causes an impediment to the regeneration and restoration of the pulp structure (19).

The pulp calcifications are common occurrences in the population, and although estimates vary widely, some authors have reported the presence in at least 50% of all teeth. Generally associated with age, pulpal calcifications appear to increase in number and size in patients undergoing long-term supervised orthodontic treatment. Large nodules and total calcification of the root canal inner space related to orthodontic forces are not uncommon findings of dystrophic calcification (20). Thus, the aim of this study was to assess possible changes in the pulp tissue of human premolars resulting from orthodontic intrusion in a period of 21 days.

Methods

Ethical Considerations

This study was approved by the Ethics Committee on Research of the University of Maringá-Uningá 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 Orthodontics of the Unity of Post Graduate Inga-Uningá- Faculty-Passo 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.

TABLE 1. Histologic Findings Listed According to Sübay and Colleagues

		Inflammatory response			Soft tissue response				Hard tissue response			
Group	No. of teeth	1	2	3	1	2	3	4	1	2	3	4
Control group	17	17	0	0	15	0	02	0	17	0	0	03
Experimental group	17	17	0	0	06	04	10	0	17	0	03	03

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 before changing the acid. After 8 days, the teeth were cleaved in half (mesiodistal) and remained for 5 days in the hydrochloric acid. Before the automatic processing, in the ASP 300S machine (Leica Biosystems, Melbourne, Australia), the teeth were placed in flowing water for 1 hour. We used 70% ethanol, 80% ethanol, 90% ethanol (2 changes), absolute alcohol (3 changes), xylene (3 changes), and paraffin (3 changes) in the processing.

Finally, the samples were embedded in paraffin and wax in order to carry out the histologic cuts of 3.5 μm and the histotechnical preparation. The material was also automatically stained with hematoxylin-eosin in the Autostainer XL machine (Leica Biosystems).

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 Sübay 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 mm^2, and we used 3 high-magnification fields (0.3 mm^2) 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 pulpal nodule count.

Results

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

Inflammatory Response

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