Original Article

Effects of Intrusive Force on Selected Determinants of Pulp Vitality

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ABSTRACT

Objective: To determine the activity of aspartate aminotransferase (AST) in the pulp of orthodontically intruded teeth and to test the sensitivity of these teeth by means of electrical pulp testing (EPT).

Materials and Methods: The study sample consisted of 21 healthy subjects who needed extraction of first premolars for orthodontic reasons. In every subject, one premolar included in a 0.016″–0.022″ stainless steel spring from the first molar and loaded by the force was regarded as a test tooth. The magnitude of the intrusive tipping force for every tooth was calculated with the use of ANSYS 10.0 software. The contralateral premolar was used as a control tooth. After 7 days, the spring was removed, and EPT was applied to test and control teeth. The teeth were extracted, and the dental pulp was removed. AST activity in the pulp was determined spectrophotometrically at 20°C.

Results: Estimated mean AST activity values ranged from 0.572 ± 0.097 U/mg in the test teeth to 0.348 ± 0.053 U/mg in the control teeth ($P < .01$). The EPT test showed significant differences between test and control teeth ($P < .001$). The mean estimated magnitude of the intrusive tipping force was 61 ± 4.5 g.

Conclusion: Seven days of orthodontic intrusion can cause metabolic changes in the pulp expressed by increased AST activity. The increased threshold in the pulp reaction to EPT indicates changes in the neural response of the pulp. (*Angle Orthod.* 2009;79:1114–1118.)

KEY WORDS: Aspartate aminotransferase; Dental pulp; Pulp vitality; Orthodontic intrusion

INTRODUCTION

The response of dental pulp tissue to orthodontic treatment has become a matter of particular interest in recent years.[1] However, no firm agreement is evident in the literature regarding the consequences of orthodontic movement for pulp viability. Several research groups have described orthodontically affected pulp reactions from circulatory vascular stasis to necrosis.[2–7] Other authors have reported a significant increase in functional pulpal blood vessels, as well as an increase in the angiogenic growth factors of affected pulp.[8–11]

The most commonly used parameters in the study of tissue response to orthodontic force have been measurements of pulpal vasculature and changes in blood flow. Human pulp blood flow has been shown with the use of laser Doppler flowmetry to decrease significantly or to remain stable after intrusive force activation.[12–15] Kvinsland[11] on the other hand, using fluorescent microspheres, showed a substantial increase in pulpal blood flow.

In medicine, clinical enzymology is used to aid in the diagnosis of localized inflammatory lesions before the development of overt clinical symptoms. Aspartate aminotransferase (AST) is an enzyme that normally is confined to the cell wall but is released to the extracellular environment upon cell death.[16] AST activity has been shown to be elevated significantly in the pulp

of orthodontically treated teeth, reflecting metabolic changes in dental pulp.[17]

Electrical pulp testing is a simple noninvasive method that is used by clinicians to obtain information about tooth vitality. Electrical stimulation induces a response from fast-acting, low-threshold, myelinated A fibers. In orthodontic patients, for whom force application might have the physiological status of the pulpal element, the response of pulp to electrical stimulation becomes inconsistent.[18–20]

Of possible force vectors that can be applied to the teeth during orthodontic treatment, intrusion is thought to have the greatest impact on the apical region[3,21–24] in that it may cause "strangulation" of the pulp by occluding the apical blood supply.[25]

To date, no studies have evaluated AST activity in the dental pulp during orthodontic tooth intrusion, and no data have been generated about the response of intruded teeth to electrical pulp testing (EPT).

Our study aimed (1) to measure AST activity, to determine changes seen in dental pulp during the early phases of orthodontic intrusion by fixed appliances, and (2) to evaluate pulp sensitivity in the same subjects, on the basis of pulp response to an electrical stimulus.

MATERIALS AND METHODS

The study sample consisted of 21 healthy subjects. Ages of subjects ranged from 11 to 21 years (mean age, 15.5 ± 0.5 years). Inclusion criteria consisted of the following: need for extraction of premolars, for orthodontic reasons; need for orthodontic fixed appliance therapy; no general diseases indicated; no use of anti-inflammatory drugs 1 month before the study; periodontal probing depth not exceeding 3 mm in the whole dentition; and no evidence of marginal periodontal bone loss after panoramic radiographic examination.

Before the study was begun, informed consent was obtained from patients and from parents of those younger than 18 years of age. The protocol was approved by the Ethical Committee of Kaunas University of Medicine.

For every subject, one randomly selected maxillary or mandibular premolar was included in a spring from the first molar and was loaded with intrusive force. This tooth was regarded as a test tooth (TT). A contralateral premolar was not subjected to mechanical stress and was used as a control tooth (CT). Both the TT and the CT were unrestored and asymptomatic with no evidence of caries, periapical radiolucency, or root resorption. Orthodontic brackets (Roth 0.022″ × 0.030″; Dentaurum Group, Ispringen, Germany) were bonded in the center of the buccal surfaces of the TT.
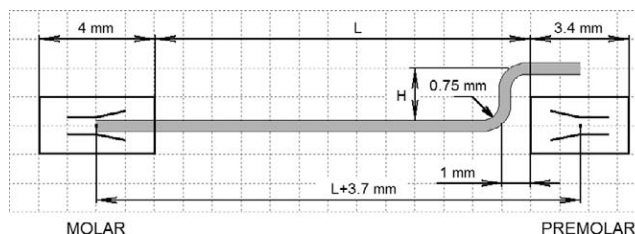
Figure 1. Calculation of the magnitude of the intrusive tipping force performed with the software program.

Crown orthodontic bands with buccal tubes were cemented onto the first molars. To avoid side effects (tipping, extrusion), first molars were connected with a palatal or lingual arch into a rigid unit. A spring (0.016″ × 0.022″ stainless steel; Dentaurum) was fabricated for every patient to generate intrusion force for the TT toward its longitudinal axis. Tipping and torque moments were reduced to the minimum.

The magnitude of the intrusive tipping force for every experimental tooth was calculated with the use of ANSYS 10.0 software (Finity Element Analysis System; ANSYS Inc., Canonsburg, Pa). Different lengths (L) from molar to premolar determined different force magnitude. The spring was ligated to the bracket with the use of wire ligature and was bent on both sides to avoid medial-distal displacement of teeth (Figure 1).

The spring was removed after 7 days of intrusion, and the test of electrical stimuli was applied to both test and control teeth. Electrical stimulation was provided by Pulptester PT1 (UAB, Lumen, Lithuania) with toothpaste used as the conduction medium. The amplitude of generated impulses was characterized by a gradual linear increase on the 1 to 200 unit scale (1 unit equal to 1 μA).[26] Because of a slow increase in electrical stimulation, Pulptester PT1 provides threshold sensations with least discomfort for the patient.

Every tooth was isolated with cotton rolls and was dried thoroughly before testing, to ensure stimulation of pulpal rather than periodontal nerve fibers. The tip of the probe was coated with toothpaste and applied to the buccal cusp of the TT or the CT on the pulp horn projection. This has been shown to be the most effective site of electrical testing because of its close proximity to the pulp horns.[18–20,26–28] During testing, current flow was increased slowly from the initial zero current state by adjusting the variable voltage control. Readings were recorded as the perception threshold stimulating current in microamperes. Testing was repeated after a 3-minute interval to reduce subjective fatigue and to minimize the possibility of nerve accommodation. When the threshold for healthy pulp was set at 20 μA (as recommended by the manufacturer), kappa values for repeat recordings of pulp response in test and control teeth were 0.9 and 1.0, respectively.

Examination procedures were performed by the same operator.

After EPT, the teeth were extracted with the patient under local anesthesia, and pulp samples were removed from both test and control teeth. For evaluation of AST activity, extracted teeth were grooved longitudinally on the buccal and lingual surfaces under extensive water irrigation with the use of a diamond bur, with caution taken to avoid penetrating the canal space, and then were split in half. Pulp samples were washed two times with ice-cold, heparinized, sterile saline, then were dried and frozen at $-25°C$.

Immediately before AST activity measurements were taken, the residual liquid was removed from pulp specimens with filter paper; specimens were weighed with the A&D Precision Balance HA-202M (A&D Co. Ltd., Tokyo, Japan) and were homogenized in 1 mL of 10 mmol/L potassium phosphate buffer, pH 7.0, and 0.1% sodium cholate, at 0°C, with the use of a glass/glass homogenizer.

Homogenates were stored for 30 minutes on ice and then were centrifuged at maximum speed for 5 minutes (Eppendorf Centrifuge 5414; Lehman Scientific, Wrightsville, Pa). Further on, 400 μL of supernatant was mixed with 1 mL of reaction medium containing 100 mM aspartate ("Sigma"), 10 mM 2-oxoglutarate ("Fluka"), 400 mU/mL malate dehydrogenase ("Sigma"), and 0.2 mM NADH ("Sigma"), pH 7.4.

Determinations of AST activity were carried out at room temperature (20°C) with a spectrophotometer Heλios α (Thermo Electron Corporation, Waltham, Mass) and the addition of NADH. Oxidation of NADH was monitored as a decrease in absorbance at 340 nm.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS Inc., Chicago, Ill). Each data set was tested for normality with the Shapiro-Wilk Test. The paired *t*-test was used to assess the significance of differences in AST activity and EPT response between experimental teeth. Confidence intervals (CIs) at 95% of the difference between mean values of enzymatic activities of the two groups were reported. The paired mean difference in AST activity and EPT response between groups, calculated for each subject, was expressed as Test − Control. These differences were used to test the strengths of the straight-line relationship between patient ages, dental root number, pulp weight, AST activity, EPT response, and force magnitude with Spearman's correlation coefficient. The receiver operating characteristic (ROC) test was used to determine the critical value of the orthodontic force. Binary regression analysis was used to test the relationship between critical orthodontic force and EPT response.
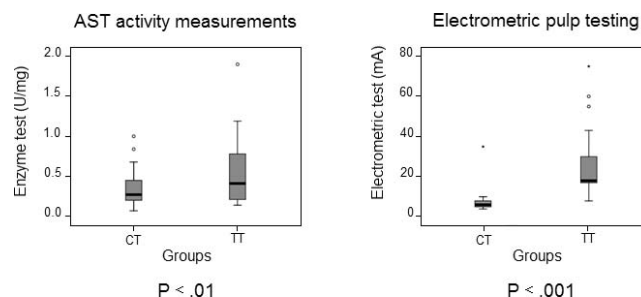
Figure 2. Distribution of AST activity and EPT values in orthodontically intruded and control teeth.

RESULTS

The mean magnitude of the intrusive tipping force for every treated tooth was 61 g (standard deviation [SD], 19.8; range, 34 to 106 g). The mean AST activity in pulp tissue was significantly higher in test teeth than in control teeth, at 0.57 (SD = 0.44) U/mg and 0.35 (SD = 0.24) U/mg, respectively ($P < .01$). The 95% CI of the differences in mean values of AST activities of the two groups was 0.069 to 0.379 U/mg.

The EPT test showed significant differences between test and control groups: 26.95 (SD = 17.92) μA and 7.76 (SD = 6.48) μA, respectively ($P < .001$). The 95% CI of differences in mean values of the EPT response of groups was 12.9 to 25.5 μA. The distribution of AST activity values and EPT values in TT and CT is presented in Figure 2.

A straight-line relationship was noted between measurements for TT and CT, in terms of AST activity and EPT responses (r = 0.6, $P < .01$, and r = 0.6, $P < .01$, respectively). However, no significant correlation was observed between AST activity and EPT responses in orthodontically intruded teeth (r = 0.135 and $P = .56$, respectively). No relationship was observed between the orthodontic force magnitude and patient age, dental root number, pulp weight, and AST activity and EPT response. However, when the EPT response threshold was set at 20 μA, the cutpoint for orthodontic force defined by the ROC test was equal to 65 g (sensitivity, 0.7; specificity, 0.8). Binary logistic regression analysis showed that for those teeth with EPT responses above 20 μA, the chances of receiving an orthodontic force greater than 65 g were 11 times greater than for teeth that presented EPT responses lower than 20 μA (OR, 11.0; 95% CI, 1.137 to 106.43).

DISCUSSION

Results of this study show that 1 week after initiation of orthodontic intrusive load, a significant increase in AST activity (by 64%) was observed in the test teeth. Furthermore, an increased response threshold to EPT (by 3.5 times) was seen in the pulp tissue of affected

teeth. The positive correlation of AST activity with EPT results between test and control teeth has confirmed that the pattern of pulp response is the same with respect to the anatomic features of teeth such as pulp weight, root surface area, and so forth.

The increase in AST activity reported in our study correlates with results previously reported by Perinetti et al,[17] who observed enzymatic activity that was twice as high in the pulp tissue of orthodontically treated teeth as compared with untreated teeth. The levels of AST activity reported by Perinetti et al[17] are comparable with AST levels in teeth with reversible pulpitis.[29] Although no threshold levels have been defined for enzymatic activity associated with pulp inflammation, an obviously significant increase in AST levels in response to orthodontic intrusion as observed in our study indicates that certain reactive mechanisms were occurring in the pulp tissue.

Alterations in tissue respiration and the increase in pulp tissue apoptosis are presumed to be the factors that affect AST activity.[2,30,31] Furthermore, orthodontic load application can induce circulatory disturbance[12–15,32] and reduced oxygen levels in the pulp.[3,17] However, numerous studies on pulp tissue respiration disagree regarding the effects of application of orthodontic load on the pulp, showing either a decreased[2,30] or an increased rate of respiration.[33] The results of blood flow evaluation in orthodontically affected pulp are conflicting as well.[11–15,32] These processes, depending on the degree of their disturbance, may cause changes in metabolic cell activity, cell damage, or defense reactions. As suggested by many workers, changes in blood flow are temporary and return to normal within a few days. However, the increased enzymatic activity observed in our study might be due to orthodontic force–induced proliferation of pulp cells and activation of mitogenesis.[34] Oxygen is essential for the aerobic production of sources of cellular energy such as adenosine triphosphate (ATP) in mitochondria. One in vitro study of pulp cell responses to hypoxia[35] has suggested that hypoxia might enhance the mitochondrial function and proliferative activity of pulp cells in situ. Activation of mitogenesis was observed under various conditions to modestly disturb the mitochondrial oxidative phosphorylation system.[34] Severe injury to the pulp cells and their membrane structures seems unlikely in our study because if that were to occur, AST would be released from mitochondria and cells and, accordingly, its activity would be decreased.

We found that within 7 days of initiation of orthodontic tooth intrusion, the response threshold to electrical pulp testing was increased. Although this method of evaluation of pulp response is based on a subjective judgment provided by the patient, significant differences between measurements of control and test teeth suggest the possibility of certain alterations in the pulp. This could be explained as resulting from pressure or tension on apical nerve fibers.[19,20] As was suggested by Bender et al[18] in their study on posttraumatic teeth, no response to EPT occurs immediately after trauma caused by the concussion syndrome; however, this response normally returns over time.

Direct clinical evaluation of any inflammatory alteration in the pulp is impossible because of it is complicated anatomy. Therefore, most of the research that seeks to attain a thorough insight into changes elicited by orthodontic forces is based on laboratory experiments. The use of human material is restricted, and most of the findings of vascular, cellular, and neural changes in dental tissue incident to orthodontic tooth movement are derived from animal experimental models. Real life conditions may differ in many respects from experimental models because of the complexity of factors associated with the physiology of dental pulp.

It can only be hypothesized that changes in tissue respiration and possibly hypoxia that occur during orthodontic treatment may affect dental pulp tissue, leading to an increase in AST activity and the modified pulpal neural response. Additional studies should be undertaken to investigate this matter further.

CONCLUSIONS

- The permanent intrusive force applied for 7 days significantly increased the pulpal neural response and AST activity in treated teeth.
- The short duration of the experiment does not allow firm conclusions to be drawn about existing pulp damage, but it seems to be a clear indication of certain response reactions that occur in this tissue.

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