Aspartate aminotransferase activity in pulp of orthodontically treated teeth

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This study examines the aspartate aminotransferase activity in the pulp of orthodontically treated teeth. Seventeen healthy male and female subjects (ages: 14.5-19.6; mean 16.8 ± 1.6 years) who needed extraction of the maxillary first premolars for orthodontic reasons were enrolled in the study. One randomly chosen maxillary first premolar, included in a straight-wire fixed orthodontic appliance and supporting orthodontic force, was considered as the test tooth. The contralateral first premolar, included in the orthodontic appliance but not subjected to mechanical stress, was used as the control tooth. After a week of treatment, the dental pulp tissues were extracted from both experimental teeth. Aspartate aminotransferase activity was significantly elevated in the test teeth as compared with the control teeth. These results demonstrate that in the early phases of treatment, orthodontic force application to the teeth can lead to significant metabolic changes in the pulp of these teeth. (Am J Orthod Dentofacial Orthop 2004;125:88-92)

The dental pulp tissue response to orthodontic force has been evaluated in recent years. The clinical impact of such studies was based on evidence that alteration in the pulpal tissue can have a deleterious effect on the long-term vitality of a tooth. Previous studies have reported that injuries produced by orthodontic force can be permanent and that the dental pulp could also lose its vitality; other authors have reported that orthodontic force probably has no significant long-lasting effect on the dental pulp. During orthodontic stress, changes in dental pulp tissue respiration have been reported. Recently, a detectable increase in the dental pulp blood flow in rats caused by an orthodontic force appliance was reported by Kvinnsland et al. Apoptosis in dental pulp tissues of rats undergoing orthodontic stress has been described by Rana et al. Other studies have reported an increase of angiogenesis in human dental pulp tissue during orthodontic treatment.

Aspartate aminotransferase (AST) is an intracellular, cytoplasmic enzyme that is released extracellularly upon cell death; hence, its activity in the extracellular environment can be considered as an indicator of cell necrosis. However, basal levels of AST in the extracellular environment can also be detected as a consequence of physiological tissue turnover. AST activity has been monitored in the gingival crevicular fluid during periodontal inflammatory or orthodontic treatment. It has also been detected in both healthy and inflamed dental pulp tissue.

To date, no studies have evaluated AST activity in dental pulp during orthodontic tooth movement. Many previous studies have focused on specific dental movement, such as intrusion or extrusion; however, the orthodontist is faced with clinical conditions that involve complex 3-dimensional movements of the teeth, rather than specific dental displacement. Derringer et al described a significant angiogenic increase in pulp tissue of teeth supporting 3-dimensional orthodontic forces by a flexible archwire. Hence, the present study was designed as a cross-sectional assessment to determine, by measuring AST activity, whether significant metabolic changes can occur in dental pulp during the early phases of orthodontic treatment by fixed appliances in young, systemically healthy subjects.

MATERIAL AND METHODS

Seventeen orthodontic patients, 11 females and 6 males (ages: 14.5-19.6; mean 16.8 ± 1.6 years), whose maxillary first premolars would be extracted because of dental crowding, were included in the study. The following inclusion criteria were used: (1) need for fixed appliance therapy, (2) good general and periodontal health, (3) no use of anti-inflammatory drugs in the month before the study, (4) probing depth values not exceeding 3 mm in the whole dentition, and (5) no
evidence of periodontal bone loss after a radiographic periapical examination. During the study, the subjects were not allowed to take anti-inflammatory drugs that could affect the results. Informed consent was obtained from the patients, or the parents of those under 18 years of age, and the protocol was reviewed and approved by the Ethical Committee of the G. D’Annunzio University Medical Faculty.

In each patient, the maxillary first premolars were included in a straight-wire fixed orthodontic appliance. One first premolar was randomly chosen as the test tooth, and force was applied. The contralateral first premolar was used as the control tooth. Both the test and the control teeth were unrestored, asymptomatic, and with no signs of premature contact. They had no radiographic evidence of caries, periapical pathology, or root resorption. No difference was found in the number of root canals between the test and the control teeth in the patients. Orthodontic brackets (MBT, 3M-Unitek, Monrovia, Calif) were placed on the buccal surfaces of the teeth in the maxillary arch, including the incisors, the canines, and all premolars; hence, the bands werebonded on the first molars. A monolateral orthodontic appliance, 0.018-in, circular cross-sectional dimension (MBT, 3M-Unitek), was then mounted from the central incisor to the first molar in the same quadrant as the test tooth to activate the orthodontic appliance. The brackets on the test teeth were placed so that, when placed into their slots, the archwires gave the required forces. These forces, measured as previously described, were from 30 to 90 g. The entire orthodontic appliance was put in place in a single clinical session. After 7 days of treatment, the experimental teeth were extracted under local anesthesia, and pulp samples were removed. Immediately after extraction, the teeth were longitudinally grooved under copious water irrigation on the buccal and lingual surfaces with a diamond disc so as not to penetrate the canal and then split in half with cutting pliers; care was taken in extirpating the pulp samples from the teeth. The samples were placed in plastic vials and were immediately washed 2 or 3 times in ice-cold, heparinized, sterile saline solution to remove all blood, which normally shows AST activity. The samples were stored at −80°C.

Immediately before the biochemical analysis, the specimens were weighed and homogenized in 1 mL of 10 mmol/L potassium phosphate buffer, pH 7.0, and 0.1% sodium cholate. This homogenate was centrifuged at 100,000 × g for 60 minutes at 4°C, and the supernatant was recovered, diluted to a volume of 2 mL with the phosphate buffer, and used for the enzymatic activity determinations. A spectrophotometer (8453 UV-Visible, Hewlett Packard, Woldgrohn, Germany) with a 1-cm path length was used in the assays, which were carried out at a constant temperature of 30°C. One milliliter of supernatant was taken for the assay of AST activity determination. The supernatant was incubated for 15 minutes in a substrate containing 100 mmol/L L-aspartate, 100 mM 2-oxoglutarate, 0.2 mmol/L reduced nicotinamide adenine dinucleotide (NADH), 400 mM/mL malate dehydrogenase and 100 mmol/L sodium phosphate buffer (pH 7.4), in a total volume of 3.0 mL. In the presence of AST, L-aspartate and 2-oxoglutarate exchange an amino group to yield oxalacetate and L-glutamate. The rate of this reaction was monitored by an indicator reaction in which the oxalacetate formed is reduced to L-malate by an excess of malate dehydrogenase, with the simultaneous oxidation of NADH. The NADH consumed was monitored as change in absorbance at 340 nm. A value of 6.22 was considered as the NADH millimolar absorptivity in computing enzyme activity units (U) (1 U = 1 μmol of NADH released per min at 30°C), with results expressed as AST activity/mg of pulp tissue (U/mg). Moreover, for each analysis, a background control was used, consisting of the reagent and the buffer without the sample, whose value of absorbance variation per minute was subtracted from the experimental result.

The Statistical Package for Social Sciences program (SPSS, Chicago, Ill) was used for the data analysis. Each data set was tested for normality with the Shapiro-Wilk test and Q-Q normality plots; equality of variance was also tested with the Levene test and Q-Q normality plots of the residuals. Each data set met the required criteria for using parametric analyses. A paired t test was used to assess the significance of the differences in the AST activity between the experimental teeth. The confidence interval (CI) at 95% of the difference between the mean values of the enzymatic activities of the 2 groups is also reported. The paired mean difference in the AST activity between the groups, calculated for each subject, is expressed as test-control. These differences were used to test the strengths of the straight-line relationship between age and enzymatic activities with the Pearson r correlation coefficient. A P value less than .05 was used to reject the null hypothesis.

RESULTS

The AST activity was 6.7 ± 1.9 U/mg of pulp tissue (range, 4.6 to 11.4 U/mg) in the test teeth; in the control teeth, the enzymatic activity was 3.6 ± 1.4 U/mg of pulp tissue (range, 1.3 to 6.2 U/mg) (Fig). The paired t test showed that the 2 groups differed significantly in AST activity (t16 = 7.4, P < .01). The 95% CI of the
differences of the mean values of the AST activities of the groups was 2.4 to 4.3 U/mg of pulp tissue. The Pearson r correlation coefficient gives a nonsignificant straight-line relationship between age and AST activity increments ($r = 0.44$; $P > 0.1$).

**DISCUSSION**

This cross-sectional study examined the AST activity in the pulp tissue of orthodontically treated teeth compared with untreated control teeth. The results show that significant increases in enzymatic activity (that did not correlate significantly with patient age) are detectable in the pulp tissue of teeth subjected to orthodontic forces (Fig).

Factors such as individual patient response, root surface area, and frictional losses in the fixed appliance have been described as the major sources of variable results from previous studies.\(^7\) The use of a control in the same patient, chosen in the present study and others,\(^7,8,15\) and the exclusive use of maxillary premolars are effective in controlling individual patient response and root surface area. The frictional losses in the appliance still remain difficult to standardize, because we attempted to maintain parity with the normal clinical situation when the teeth were bonded and a flexible archwire was engaged into the brackets, giving a continuous but reduced active force as tooth alignment occurred. On the other hand, this study design had the advantage of accurately reproducing the clinical effects of a week of initial archwire 3-dimensional forces that are of direct clinical relevance.

To evaluate the dental pulp response to tooth movement, investigators have used several models. Some of these have examined tissue respiration,\(^2\) blood flow,\(^5\) in vitro angiogenic properties,\(^7,8\) and histomorphometric aspects\(^19\) of the pulp after orthodontic stress on teeth. However, enzymatic monitoring during alterations of the pulp of teeth supporting an orthodontic force has not been used to date.

Previous studies have shown that the dental pulp tissue respiration rate is depressed by an average of 27% as a result of 3 days of orthodontic force application,\(^5\) or 33% after a 7-day rest period in teeth that had previously undergone 3 days of orthodontic force.\(^20\) These authors concluded that an orthodontic force of very short duration can cause biochemical and biological pulpal tissue alterations. Moreover, apoptosis in rat dental pulp tissue under orthodontic stress for 3 days to 2 weeks was evaluated by Rana et al,\(^6\) who observed that maximum apoptosis occurred after 3 days. In accordance with these data, Kvinnsland et al\(^5\) reported a substantial increase in the dental pulp blood flow after 5 days of orthodontic force appliance in a rat model; they used a semiquantitative method to assess their results. More evidence regarding pulp tissue changes produced by orthodontic force has been provided by Nixon et al,\(^19\) who reported that, in a rat model, within 2 weeks of force application, there was an increase in capillary numbers that was correlated with the time and the magnitude of the force applied. Although Kvinnsland et al\(^5\) and Nixon et al\(^19\) considered extrusive tooth movement exclusively, human pulpal blood flow has also been reported to be reduced if brief intrusive forces are applied to the teeth.\(^14\)

AST is widely distributed in tissues, with the highest levels in the heart and the liver, and its serum levels are considered to be biochemical markers of myocardial infarction or hepatitis.\(^17\) AST activity in dental pulp tissue has been found to be significantly increased in reversible pulpitis and decreased in irreversible pulpitis, as compared with healthy controls.\(^13\) These results demonstrate that AST in dental pulp tissue might have a role in the early events leading to inflammatory changes.

The AST activity in the control teeth was similar to that previously reported by Spoto et al\(^13\) for healthy pulp tissue, and the increase in the enzymatic activity in the test teeth after a week of orthodontic treatment is supported by the results of the other studies mentioned above. We can hypothesize that a reduction in the rate of tissue respiration\(^2,20\) and apoptosis\(^5\) during orthodontic treatment, described in several studies and detailed above, can produce modifications in the dental pulp tissue that lead to this increase in AST activity. Moreover, even if an increase in the dental pulp blood flow during such treatment has been described,\(^5,19\) we can exclude contamination from blood AST because each pulp sample was washed in an appropriate saline solution. It is of interest that vacuolization and severe odontoblastic degeneration have been observed in pulp
of teeth undergoing orthodontic extrusion of a 7-day duration.\textsuperscript{15} We can speculate that this degeneration is responsive to the AST activity increase in the test teeth, as compared with the control teeth; this hypothesis is supported by histological studies\textsuperscript{21-23} that have correlated dental pulp alterations with strangulation and stasis of blood flow after orthodontic force has been applied to the teeth. This reduction in blood flow has been implicated in a decrease in available oxygen\textsuperscript{2} and a reduction of the pulp tissue alkalinity\textsuperscript{24}; the latter phenomenon could then alter the membrane permeability.\textsuperscript{25} Considering that AST is a strictly intracellular, cytoplasmic enzyme,\textsuperscript{9} the increase in its extracellular activity in the test teeth, as compared with the control teeth, is consistent with the findings mentioned above.

Different pulpal reactions to orthodontic forces have been correlated with patient age and size of the apical foramen.\textsuperscript{26-28} These results are consistent with a relationship between the biological effects of orthodontic forces and the maturity of the tooth.\textsuperscript{26-28} In the present study, using a contralateral tooth as a control probably reduced the effect of the size of the apical foramen on the AST activity difference between the experimental groups. The exclusive use of maxillary teeth should also be considered relevant. Although positive correlations between age and the rate of tissue respiratory depression\textsuperscript{2} or other pulp tissue alterations\textsuperscript{26-28} have been reported during orthodontic treatment, we failed to find any significant correlation between AST activity increase and patient age. This result, however, is not unexpected, because the age range of the patients in this study was 14.5 to 19.6 years—more restricted than the age ranges in other studies.\textsuperscript{2} Thus, the lack of correlation between AST activity changes in the pulp of orthodontically treated teeth and patient age found herein should be interpreted with caution.

**CONCLUSIONS**

Although our results are supported by those of previous studies, more data are required to further assess the role of AST on dental pulp tissue in the early phase of orthodontic treatment.

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