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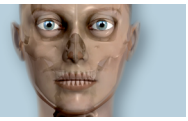

ORIGINAL ARTICLE

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Metabolic changes of human dental pulp after rapid palatal expansion

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Structured Abstract

Objectives – To investigate rapid palatal expansion (RPE)–induced metabolic changes in human dental pulp by measuring the expression and activity of aspartate aminotransferase (AST).

Methods – mRNA and protein levels of AST in human dental pulp were measured by quantitative real-time polymerase chain reaction and Western blot, respectively. Furthermore, the activity of AST was measured by a full automatic biochemical analyzer.

Results – AST mRNA and protein levels were found to be expressed in normal dental pulp. Moreover, the expression of AST was increased significantly after 14 days of RPE and then decreased at 1 month in retention. Three and 6 months after RPE, the AST expression level was gradually decreased to its baseline level. Similarly, AST activity was significantly elevated after 14 days of RPE, which was then down-regulated at 1 month in retention but was still kept at a higher level as compared with the control group. The enzymatic activity of AST was slowly decreased to its baseline level at 3 and 6 months in retention.

Conclusions – These results showed that significant reversible metabolic changes occurred in dental pulp during RPE, which revealed the high capacity of the pulp tissue for adaptation to this orthopedic method.

Key words: aspartate aminotransferase; dental pulp; rapid palatal expansion

Introduction

Rapid palatal expansion (RPE) is an orthopedic technique frequently used to correct maxillary constriction and posterior cross-bite (1). As Angle first reported on RPE in 1860, there has been widespread use of palatal suture opening as a procedure to orthopedically expand the constricted maxilla. A single activation of the expansion screw produces about 1–3 pounds (1.36–4.55 kg) of force (2). Each subsequent turn produces a cumulative effect. Accordingly, the jackscrew appliance can produce cumulative loads of 16.6–34.8 pounds (7.54–15.8 kg) or more after several daily turns (3). High forces are produced during active expansion of the mid-palatal suture by RPE. Hence, it is important that orthodontists sufficiently understand every aspect of the mechanism and consequences of RPE. Researchers have investigated skeletal, sutural, dental, and otolaryngologic effects of this orthopedic method (4–8).

In recent years, the response of dental pulp tissue to orthodontic treatment has become a matter of particular interest (9–11). Although some researchers have examined changes in pulp vitality (12), pulpal blood flow (13, 14), and other histological, histomorphometrical, and histopathological changes in dental pulp tissue induced by RPE (15, 16), only a few studies have focused on RPE-induced dental pulp changes in a metabolic aspect.

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 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 (17). Elevation of AST activity has not only been monitored in gingival crevicular fluid during orthodontic treatment (18), but also been found in the pulp of orthodontically treated teeth, which reflects the metabolic changes in dental pulp during orthodontic processes (19).

Hence, this study was initiated to evaluate, by enzymatic monitoring, whether significant metabolic changes occurred in dental pulp during the RPE procedure.

Materials and methods

Teeth and treatment

A total of 100 intact upper first premolars, which were extracted from 22 boys and 28 girls (aged 10–14 years; mean 12.8 ± 1.7 years) because of dental crowding or protrusion, were used in this study. All patients gave their informed consent before providing the samples. The protocol was reviewed and approved by the Ethical Committee of Shandong University (Supplementary).

The volunteers were selected based on the following criteria: 1) no major systemic diseases; 2) good general and periodontal health (probing depth values not exceeding 3 mm in the whole dentition and no evidence of periodontal bone loss after a radiographic periapical examination); 3) no caries; 4) no use of anti-inflammatory drugs in the month before and during the study.

Twenty untreated upper first premolars served as the control group. For patients who required opening of the palatal suture as the first stage of their orthodontic treatment, upper first premolars were extracted at 14 days, 1, 3 and 6 months after RPE completion and assigned to four groups, respectively. For the application of RPE, a Haas-type RPE appliance with meshpads was cemented on the first permanent molars

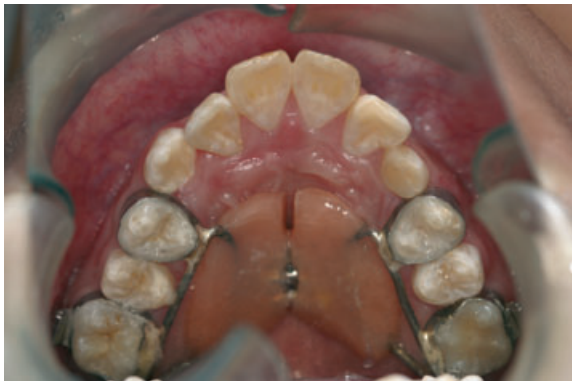

Fig. 1. The Haas-type rapid palatal expansion appliance. This is cemented onto the teeth and used to widen the maxilla by opening the mid-palatal suture.

and first premolars (Fig. 1). The jackscrew was activated with one-half turn (0.5 mm) by the operator after the placement, followed by one-quarter turn in the morning and one-quarter turn in the evening in the following days (20) until lingual cusps of the upper teeth were in line with the buccal cusps of the lower teeth. This step took about 14 days on average. After the active expansion phase, the appliance was kept as a retainer for 6 months. In each patient, after the first premolar being extracted, the other teeth were included in a straight-wire fixed orthodontic appliance.

Immediately after extraction, the teeth were longitudinally grooved with a diamond disk under copious water irrigation on the buccal and lingual surfaces 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. Based on the fact that AST is normally found in red blood cells (RBC), liver, heart, muscle tissue, pancreas, and kidneys, this procedure is necessary to remove the contamination of RBC-originated AST. The samples were divided into three parts, stored at −80°C, and then subjected to quantitative real-time polymerase chain reaction (qPCR), Western blot analysis, and AST activity assay, respectively. To distribute the whole pulp tissue evenly, we separated dental pulp samples in the sagittal plane.

Quantitative real-time polymerase chain reaction (qPCR)

To measure the mRNA level of AST, total RNA was isolated from dental pulp tissues using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA). After DNase treatment, 1 μg of the total RNA was reverse transcribed using SuperScript® VILO™ cDNA Synthesis kit (Invitrogen Corp.). Relative transcript levels were measured by qPCR in a 20 μl reaction volume, which included 10 μl SuperMix with Premixed ROX, 0.4 μl 10 μM forward primer (200 nM final), 0.4 μl 10 μM reverse primer (200 nM final), 100 ng template DNA, and DEPC-treated water up to 20 μl, using Roche LightCycler® 480 sequence detection system (Roche Diagnostics GmbH, Mannheim, Germany) by EXPRESS SYBR® GreenER™ qPCR SuperMix Universal (Invitrogen Corp.). A housekeeping gene β-actin was used as an internal control to quantitate and normalize the results. Primer pairs used for qPCR amplifications are listed below. Primers AST (accession NM_002079) forward (5′-TCCTCCACGCCTGTGCACAC-3′), reverse (5′-AGC-GAATGGCCCAGGCATCTC-3′); β-actin (accession NM_001101) forward (5′- GAGACCTTCAACAC CCCAGCC-3′), reverse (5′- GGCCATCTCTTGCTC-GAAGTC-3′) (21). The PCR program was as follows: 95°C for 2 min, then 40 cycles of 95°C for 15 s, 60°C for 1 min, and 72°C for 30 s. The specificity of the reaction is given by the detection of the Tms of the amplification products immediately after the last reaction cycle. The value $2^{-\Delta\Delta CT}$ was used to comparative quantitation. All PCRs were performed in triplicate.

Western blot analysis

To investigate the protein level of AST, Western blot analysis was used. Samples containing equal amounts of protein were electrophoresed in 5% stacking and 12% resolving SDS-PAGE gels and were electrotransferred onto polyvinylidene fluoride membranes (PVDF) (Amersham Biosciences, Uppsala, Sweden). The membranes were blocked with 5% (v/v) skimmed milk in Tris-buffered saline Tween-20 (TBS-T) at 37°C for 2 h and then incubated at 4°C overnight with a 1:2500 dilution of AST antibody (Abcam Ltd., Hong Kong, China) and anti-actin serum (Santa Cruz Inc., Santa Cruz, CA, USA), followed by incubation at 37°C for 1 h with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Inc.). The immunoreactive bands were observed using DAB Horseradish Peroxidase Color Development kit (Zhong Shan – Golden Bridge Biological Technology CO Ltd., Beijing, China).

Aspartate aminotransferase assay

Immediately before the biochemical analysis, the specimens were weighed and homogenized in 1 ml of 10 mM potassium phosphate buffer, pH

7.0, and 0.1% sodium cholate. This homogenate was centrifuged at 100 000 × *g* for 60 min 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. One milliliter of supernatant was taken for the assay of AST activity determination. A full automatic biochemical analyzer (Bayer express-plus 560) was used in the assays. The final results were converted into enzyme activity units and 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.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and analyzed using SPSS 12.0 software (SPSS, Chicago, IL, USA). Each data set was tested for normality with the Shapiro–Wilk test, and equality of variance was tested with the Levene's test. Each data set met the required criteria for using parametric analyses. After performing one-way analysis of variance (ANOVA) to compare the means among multiple groups, the independent samples *t*-test was used to analyze the difference between the control group and one of the experiment groups (control vs. 14 days or 1 or 3 or 6 months). To avoid the multiple testing problems, according to Bonferroni method (22), the significant level for each pairwise comparison was set at 0.0125.

Results

At various time points, total RNA were prepared, and equal amounts of RNA were analyzed by qPCR. As shown in Fig. 2A, AST mRNA was

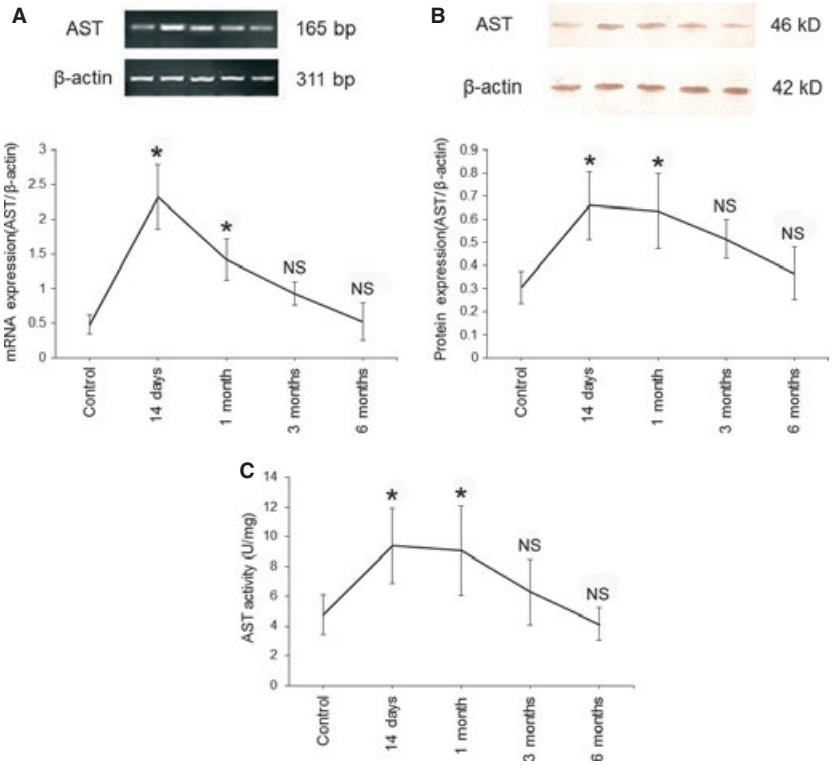

Fig. 2. Expression of AST mRNA and protein levels and its activity after RPE activation for different time periods. (A) Shown here are dental pulp cDNA samples amplified using conventional PCR (top) and quantitated results by qPCR (bottom) for AST from three separate experiments. (B) Tissue extracts from dental pulp tissue (equivalent amounts of protein) were subjected to Western blot analysis, using an anti-AST antibody. One representative experiment is depicted here (top). Densitometric analysis of three experiments performed (bottom). (C) AST activity in U/mg was measured by a full automatic biochemical analyzer. Data are presented as mean ± SD, $*p < 0.0125$ vs. control, NS: no significant vs. control.

found to be expressed in the normal dental pulp. After 14 days of RPE, the level of mRNA increased significantly ($p < 0.0125$), followed by a decrease at 1 month in retention ($p < 0.0125$). At 3 and 6 months in retention, the mRNA level progressively decreased to the control level ($p > 0.0125$).

Tissue extracts were prepared, and equal amounts of protein were analyzed by Western blot. Based on the Western blot analysis, as shown in Fig. 2B, AST protein, of about 46 kDa, was detected in normal dental pulp, and RPE induced up-regulation in the AST protein level. In consistence with the changes in mRNA level of AST, AST protein level increased after 14 days of RPE ($p < 0.0125$). Subsequently, the expression of AST protein was decreased at 1 month in retention ($p < 0.0125$) and showed no significant difference at 3 and 6 months after RPE when compared with the control group ($p > 0.0125$).

AST activity was measured by a full automatic biochemical analyzer (Fig. 2C). AST activity was 4.76 ± 1.32 U/mg in the pulp tissues of control group. The enzymatic activity was increased to 9.41 ± 2.54 U/mg in the pulp tissues after 14 days of activation ($p < 0.0125$). Similarly, AST activity was down-regulated but remained at a higher level when compared with the control group at 1 month in retention (9.06 ± 3.04 U/mg) ($p < 0.0125$). At 3 and 6 months in retention, AST activity (6.28 ± 2.21 U/mg and 4.13 ± 1.10 U/mg, respectively) was gradually decreased to the control level ($p > 0.0125$).

Discussion

This study measured the metabolic changes of dental pulp after RPE up to 6 months in retention by measuring AST mRNA and protein levels and its activity. The results showed that AST increase was found not only at the mRNA and protein levels, but also in the enzymatic activity after 14 days of activation and at 1 month in retention. AST was decreased to its baseline level at 3 and 6 months in retention.

Dental pulp is a specialized tissue located in the rigid dentinal layer of tooth. According to the study of Nixon, orthodontic forces do not have significant long-lasting effects on dental pulp (23). However, the relationship between orthopedic forces and dental pulp changes remains unclear. In our study, basal levels of AST mRNA and protein in dental pulp as a consequence of physiological tissue turnover could be detected and its activity was similar to that previously reported by Perinetti and Spoto (19, 24). After 14 days of activation, significant increase was found at both mRNA and protein levels, as well as in the enzymatic activity. These results were supported by previous studies (15, 16, 25, 26). Histological studies of Kayhan et al. and Taspinar et al. showed similar reversible vascular changes and relatively aseptic inflammatory changes in the pulp tissue after orthopedic force application (15, 16). Pulp inflammation might elicit a localized circulatory response that is restricted to a particular region and does not necessarily produce general circulatory changes in dental pulp. Tzannetou et al. also clearly demonstrated that, during orthodontic or orthopedic tooth movement, a relatively aseptic inflammatory process occurred, and the inflammatory mediators interleukin-1β (IL-1β) and β–glucuronidase (βG) were increased in the gingival crevicular fluid (24, 25). Inflammation is a local response of the host to tissue injury including chemical and/or physical stimuli, which alters the permeability of the cell membrane (26). Considering that AST is a strictly intracellular, cytoplasmic enzyme (17), the elevation in AST expression levels and its activity could be considered as a consequence of cell destruction that resulted from inflammation.

It is also known that any alteration in pulpal blood flow or vascular tissue pressure can endanger the health of dental pulp. Anstendig and Kronman (27) observed fewer blood vessels when forces were applied. Stanley et al. (28) also suggested that too powerful a depressive force could shut off the arterial supply and thus caused devitalization of the pulp tissue. This reduction in blood flow has been implicated in a reduction in the pulp tissue alkalinity, which also could alter the membrane permeability (29).

Thus, increase in AST extracellular activity might reflect dental pulp alterations with strangulation and stasis of blood flow after RPE. We can also hypothesize that a reduction in the rate of tissue respiration (30, 31) and apoptosis (32) during orthodontic treatment may produce modifications in the dental pulp, which leads to an increase in AST. In addition, the heavier the force, the greater increase in AST could be observed.

As previously stated, Kayhan et al. (15) and Taspinar et al. (16) reported similar reversible vascular changes in the pulpal tissues of anchor upper premolar teeth with histological techniques. Cho et al. (12) reported reversible changes after RPE using an electric pulp tester and a cold test. Babacan et al. (13) found that the pulpal blood flow (PBF) was increased by nearly twofold during the first week of expansion when heavy forces applied with the expander accumulated on the anchor teeth. However, PBF decreased and tended to reach its initial values during the retention period. In our study, elevated levels of AST were also observed in the dental pulp at 1 month in retention, although AST activity was found to decrease gradually after the termination of force (14 days) and showed no difference at 3 and 6 months in retention when compared with the control group. It may indicate a normal healing process of the dental pulp tissue. From the clinical view, the elevated levels of AST might either be attributed to the 'relapse' tendency of the 2 maxillary segments and the bone remodeling around the anchor teeth, or as a result of the beginning of tooth movement, with decreasing orthopedic force levels. At 3 and 6 months in retention, expression levels and activity of AST were decreased to its baseline level, which is consistent to previous reports (12, 13, 15, 16). Our results not only demonstrated the biological changes in the dental pulp during and after the application of RPE, but also revealed the high capacity of the pulp tissue for adaptation to a set of aggressive external stimuli. Considering that AST might reflect the extent of inflammation and stasis of blood flow mentioned above, its decrease to a normal level at 3 and 6 months in retention might be an indicator of the restoring of the physiological conditions in the dental pulp. However, because AST level/activity is only one of the biochemical indicators of metabolic changes, more data are required to further assess the full re-establishment of the initial conditions of the pulp tissue.

Rate of tissue respiratory depression (30) or other pulpal reactions (33–35) to orthodontic forces has been correlated with patient age and size of the apical foramen. However, Perinetti failed to find any significant correlation between AST activity increase and patient age (19). In our study, the age range of the patients was 10–14 years – broader than the age ranges in other study (19). Thus, it is critical to evaluate the correlation of AST changes in the dental pulp after RPE with patient age and size of the apical foramen in the near future.

Conclusions

In conclusion, our results demonstrated that significant reversible metabolic changes occurred in dental pulp during the RPE procedure, which revealed the high capacity of the pulp tissue for adaptation to this orthopedic method.

Clinical relevance

Rapid palatal expansion (RPE) is an orthopedic technique frequently used to correct maxillary constriction and posterior cross-bite. It is important that orthodontists sufficiently understand every aspect of mechanism and consequence of RPE. Our results showed that significant reversible metabolic changes occurred in dental pulp during the RPE procedure, which revealed the high capacity of the pulp tissue for adaptation to this orthopedic method. However, because the AST levels/activity is only one of the biochemical indicators of the metabolic tissue changes, more data are required to further assess the full re-establishment of the initial conditions of the pulp tissue.

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