Orthodontic Force Accelerates Dentine Mineralization during Tooth Development in Juvenile Rats

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Malocclusion, the improper positioning of the teeth and jaws, is among the most important global oral health burdens. People with malocclusions may require orthodontic treatment to correct the problem. Orthodontic treatment is a way of straightening or moving teeth, to improve the appearance of the teeth and how they work. It is generally best carried out in children aged 9 to 12 years, whose teeth are mainly young permanent teeth with incomplete root formation. However, the relationship between orthodontic force and tooth development has not been fully understood. In this study, we sought to investigate the effects of orthodontic force on dentine formation and mineralization during the development of young permanent teeth. Standardized orthodontic tooth movement was performed with the orthodontic appliance in five-week-old rats. To obtain longitudinal assessment of dentine formation, tetracycline was administered on the operation day and 1, 3, 7, 14 or 21 days afterward. We found that the distance between two tetracycline stripes, which indicates the amount of dentine formation during orthodontic treatment, increased with time. Importantly, no significant difference was detected in dentine formation between treated and control rats. In contrast, immunohistochemical analysis showed that the expression of dentin sialoprotein, a marker of odontoblast differentiation and mineral apposition, was significantly elevated in crown and root dentine after orthodontic treatment. In conclusion, orthodontic treatment does not affect the dentine formation of young permanent teeth, but it promotes the activation of odontoblasts and accelerates the dentine mineralization. These results suggest the safety of early orthodontic treatment.

Keywords: orthodontic tooth movement; dentine formation; dentine mineralization; dentin sialoprotein

Received February 25, 2010; revision accepted for publication June 22, 2010. doi:10.1620/tjem.221.265

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potential effects of orthodontic treatment on the tooth development.

Thus, in the present study, we sought to investigate the effects of orthodontic force on the root dentin formation and mineral apposition in an experimental model of orthodontic tooth movement in juvenile rats.

Materials and Methods

Experimental animals
All animal experiments were approved by the Animal Welfare Committee of School of Stomatology, the Fourth Military Medical University, China. Sprague-Dawley rats aged 5 weeks were maintained in a standard 12-h light/dark cycle and had access to food and water ad libitum. All animals were acclimatized for three days before the experiment started. In order to prevent breakage of the appliance, all the animals were fed on powder form fodder during the experimental period.

Experimental tooth movement
On the operation day, the rats were anesthetized by an intraperitoneal injection of 1% pentobarbital sodium (Westang Biotechnology, Inc., Shanghai, China) with a dosage of 30 mg/kg body weight during the setting and adjusting of the orthodontic appliance. The orthodontic appliance was composed of a Ni-Ti-closed coil spring (3M Unitek Co., Monrovia, California, USA), which was inserted between the upper incisors and the upper right first molar (Fig. 1). The contralateral side molar was kept intact to serve as internal control. As the upper incisors continuously grew during the experiment, we imbedded the stainless wire into a groove that was made around the maxillary first molar and the upper right first molar to prevent the detachment of the appliance. During the experiment, we checked the appliance every day, and adjusted it every week to make sure the force level was approximate 10 cN and to keep the force at the same direction.

Tissue preparation
At 1, 3, 7, 14 and 21 days after orthodontic force application, animals were sacrificed for histological evaluation. Prior to sacrifice, the rats received an overdose of anesthetic and tissues were perfused with 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.4) by direct perfusion into the left ventricle. After the fixation, the maxilla was dissected in halves at the intermaxillary suture and further fixed in the same fixative for 24 hours at 4°C followed by decalcification in 8% EDTA (pH 7.4) at 4°C for 2 weeks. Tissues were processed to paraffin embedding. Serial parasagittal sections of 4 µm were prepared for general histological examination after staining with hematoxylin and eosin (H-E) and immunohistochemistry (IHC).

Immunohistochemistry (IHC)
IHC was performed as previously described (Jiao et al. 2009). Briefly, paraffin sections were treated with 0.3% H₂O₂ in methanol solution followed by treatment of hyaluronidase for 1 h at 37°C. After incubation in 10% normal goat serum to avoid nonspecific immunoreactions, the sections were stained overnight at 4°C using primary antibodies against dentin sialoprotein (DSP; goat, Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA, 1:50). DSP is one of the dentine-specific matrix proteins. The expression of DSP is considered as a biochemical marker of odontoblasts and dentine mineralization.

Sections were then incubated in biotinylated rabbit anti-goat secondary antibodies for 2 h at room temperature, followed by rinsing in PBS and incubation in avidin-biotin-peroxidase complex for 2 h. After a final wash, sections were reacted for peroxidase enzyme activity using 3, 3-diaminobenzidine. The specificity of immunolabeling was verified by controls in which the primary antibody was omitted. Sections were counterstained with methyl green solution.

Tetracycline label
To mark the areas of dentin formation during experiment, we gave each animal an intraperitoneal inject of tetracycline 30 mg/kg at the onset of the experiment and one day before termination of the experiment.

After sacrifice, the group marked with tetracycline was preserved in absolute ethanol and sectioned undecalcified for histomorphometry. We sectioned the upper first molar at the mid-root level which was parallel to the occlusion plane and measured the dentine formation of mesial-buccal root, also the largest root. To measure the amount of dentine formation, we used Orthoplan Ploemopak microscope equipped with incident fluorescent light (detector wavelength, 460 nm; Leitz, Wetzlar, Germany), with which the tetracycline stripes surrounding the newly formed dentin could be readily seen.

Statistical analysis
To evaluate the integrate grey (IG) of immunoreactions, we selected 3 sections from different rats respectively and 5 visual fields from each section at each time point. Mean dentine apposition and mean IG were computed for each treatment group using Image-Pro Plus 6.0. Statistical difference between treatment means was assessed by Student’s t-test. The level of statistical significance was set at P < 0.05.

Results
Tetracycline fluorescence staining
Dentine and alveolar bone were green under fluorescence microscope. In the dentine, there were two inaurate tetracycline stripes (Fig. 2A). The stripe distinct from dental pulp cavity was obvious since the onset of the experiment.
Effect of Orthodontic Force on Dentinogenesis

ment (Fig. 2C, D), whereas the stripe adjacent to dental pulp cavity did not present itself until the day before termination of the experiment (Fig. 2E, F). Additionally, the distance between tetracycline stripes increased in a time-dependent manner (Fig. 2B). However, there was no significant difference between the treated and control groups.

Hematoxylin and eosin (H-E) staining
In the control group, the cell morphology of odontoblast layer was cubical. The predentin layer was thin (Fig. 3A). In contrast, in the treated group, odontoblast layer was regularly arranged and presented tall columnar cell morphology after orthodontic force application. The predentin layer developed much thicker (Fig. 3B).

Immunohistochemistry (IHC)
Clear immunoreactions for DSP were observed in dentinal tubules in the coronal matrix beneath the cusps as well as in odontoblasts and predentin. At 1st day and 3rd day, no significant difference was detected between treated and control groups. At 7th day, immunoreactive DSP was clearly observed, and the signals were stronger in the treated group (154.21 ± 8.02 gray levels) than the control group (169.70 ± 7.08 gray levels; \( P = 0.001 \)) (Fig. 4A, B). At the 14th day, immunoreactions for DSP in the treated group in coronal dentinal tubules reached the highest level (139.45 ± 8.65 gray levels).
gray levels) while its signals in root dentin were comparatively weak (165.11 ± 4.58 gray levels) (Fig. 4C-F). Moreover, positive reactions were found in the dental pulp of coronal part and the upper half of the root area. Intensive reactions in odontoblasts were widely observed in both coronal and root dentin areas (Fig. 4B, D, F). At the 21st day, intense signals for DSP were observed in odontoblasts in root dentin while the reactions in predentin of the coronal area were relatively weak. The expression of DSP in root dentine lagged behind those in crown dentine. Statistical analysis showed that the immunoreaction for DSP was significantly stronger in the treated group than that in control group (Fig. 4G, H).

Discussion

In this study, we asked whether orthodontic force might affect the dentine development of young permanent teeth. Using tetracycline to label bone growth, we first found the amount of dentine formation increased in a time dependent manner during the orthodontic tooth movement, but there was no significant difference with or without orthodontic treatment. H-E staining revealed that odontoblast layer was regularly arranged and presented tall columnar cell morphology, indicating that the odontoblasts were more active after orthodontic force application. The predentin layer developed much thicker. It was postulated that more extracellular matrix of dentin was secreted to form predentin.

Some explanations may account for the high resistance of incomplete root formation to orthodontic treatment: (1) the alveolar bone around the incompletely developed root is under-developed so that the root is easy to move; (2) cell layer covering the root surface, surface layer of the uncalcified matrix, precementum and predentin may have biological protection against root resorption; (3) with regard to immature teeth, force to move the teeth may speed up the rate of teeth growth (Xu and Baumrind 2002). During the treatment, root shortening and root elongation may happen at the same time.

DSP is one of the most important dentine non-collagenous proteins. Its unique expression in odontoblasts and predentin just before the onset of mineralization suggests that it may participate in dentin formation. DSP is also thought to be a factor in the conversion of unmineralized dentin matrix into mineralized tissue (Butler et al. 1992). So the expression of DSP is related to state of odontoblasts and mineral apposition, which makes DSP as a biochemical marker of odontoblasts and dentine mineralization.

Expressions of DSP were detected in dentinal tubules, predentin and odontoblasts, especially at the cusped area. Our results showed intense immunoreactions for DSP in dentinal tubules in coronal regions at later stages after molar eruption, where the mechanical stress from early occlusal forces would occur (Baba et al. 2004). We also observed that the immunoreactions for DSP in the mesial part of cusps were stronger than those in the distal part, which may be related to the shape of the rat molar. The cusps of the rat molar tip distally and the mesial part may endure greater occlusal force. The expression of dentin matrix protein-1 (DMP1) in osteocytes, playing an important role in mineralized tissue formation (Gericke et al. 2010), were upregulated by mechanical stress, which was in accordance with observations in the expression of DSP (Gluhak-Heinrich et al. 2003). As it is possible that there might be considerable similarities between the formation of bone and dentine, one function of DMP1 and/or DSP may involve a response to the mechanical stress by osteocytes, odontoblasts and their processes.

The immunoreactions for DSP in crown dentine were strongest at the 14th day but relatively weaker at the 21st day. The increase in DSP expression seems to represent a period of accelerated growth rather than a permanent change. That is in accordance with the development stages of rat molar (Furuta et al. 1999). The Immunoreactions for DSP in mature dentine is weaker than those in the newly formed dentine. The expressions of DSP in root dentine...
Fig. 4. IHC for DSP after orthodontic tooth movement.
Dentin sialoprotein (DSP) immunostaining revealed the state of dentine mineralization after orthodontic force application. (A-D) Immunoreactions for DSP in crown dentine increased in a time-dependent manner and the expression of DSP in experimental group was stronger than that in control group. Thick arrows indicated the direction of tooth movement. DSP-positive predentin was shown by thin arrows. (A) Control group at 7 days; (B) Experimental group at 7 days; (C) Control group at 14 days; (D) Experiment group at 14 days. (E, F) Clear immunoreactions for DSP were observed in dentinal tubules in the coronal matrix beneath the cusps as well as in odontoblasts and predentin. (E) Control group at 14 days; (F) Experimental group at 14 days. Quantitative analysis of IG, which was inversely proportional to immunoreaction signal, showed that DSP expression in crown (G) and root (H) elevated prominently after orthodontic force application. *P < 0.05 vs. respective control group.
lagged behind those in crown dentine. That is because the root starts to develop when the crown completes its development.

In providing orthodontic care for pediatric patients, clinicians often question whether to begin treatment early—during either the primary or transitional dentition—or wait until all or most of the permanent teeth are present. Many clinicians, especially the advocates of any "early treatment" regimens, considered that the outcome of the early intervention and/or first phase of treatment will apply the minimal amount of treatment to achieve the maximum final outcome. It will ultimately lead to a better, more stable result than that would be achieved by starting treatment later. Our study chose juvenile rat as experimental animal so as to mimic early treatment. Rats aged 5 to 8 weeks are on the average at the same stage of maturation as adolescents aged 10 to 12 years, which is the peak of the adolescent growth spurt (Tjäderhane et al. 1995; Furuta et al. 1999). The results indicate that orthodontic force promotes the expression of DSP in young permanent teeth, suggesting the safety of early orthodontic treatment.

In summary, at later stages of tooth development, moderate orthodontic force could not obviously affect the dentine formation. However it promotes the activation of odontoblasts and increases the expression of DSP in dentine, thereby accelerating the dentine mineralization.

Acknowledgment

We thank Prof. Xiaodong Liu, Dr. Kai Jiao (Department of Oral Anatomy and Physiology and TMD, School of Stomatology, Fourth Military Medical University, China) and Dr. Mo Li (Institute of Orthopedics, Xijing Hospital, Fourth Military Medical University, China) for their assistance.

Competing Interests

The authors declare that they have no conflicts of interest.

References


