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Expression of MMP-2 and TIMP-1 during rapid maxillary expansion in rats

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ABSTRACT

Objective: The aim of this study was to investigate the expression of MMP-2 and TIMP-1 during midpalatal suture expansion in rats.
Design: 72 male Wistar rats were randomly divided into 2 groups: the experimental group and the control group. In the experimental group, opening loops were applied across the midpalatal suture with an initial force of 50 g, whereas in the control group, rats were subjected to sham installation of opening loops without activation. On day 1, 4, 7 and 14, nine rats from each group were sacrificed, and the maxillae were dissected and prepared for Immunohistochemistry (IHC) and RT- PCR examination of MMP-2 and TIMP-1 expression.
Results: The results of IHC and Real Time PCR revealed that both protein and mRNA expression of MMP-2 and TIMP-1 were significantly increased after midpalatal expansion, and the ratio of MMP-2/TIMP-1 was also significantly enhanced.
Conclusions: The data suggested that MMP-2 and TIMP-1 might play an important role during the midpalatal suture remodeling process of maxillary expansion.

1. Introduction

Rapid maxillary expansion, or palatal expansion, has been a clinical practice in orthodontics to correct maxillary width deficiency (Hass, 1961). It is generally accepted that midpalatal suture remodeling plays a major role in the expansion process, but the underlying molecular mechanisms mediating the palatal suture remolding under tensile force are still poorly understood.

Matrix metalloproteinases (MMPs) are secreted by connective tissue cells and regulate embryonic development, morphogenesis and tissue remodeling through degradation of extracellular matrix collagens (Birkedal-Hansen et al., 1993). MMPs are secreted inactive by connective tissue cells to prevent their catabolic functions, and must be activated before function (Nagase, 1997). Their effect can be blocked by tissue inhibitors of metalloproteinase (TIMPs), a specific regulator secreted by the same cells, which affect the expression and activity of MMPs through molecular interaction. They represent a family consisting of at least four distinct members (TIMP-1,2,3,4) (Kubota, Matsuki, Nomura, & Hara, 1997). TIMPs specifically regulate the expression and activity of MMPs by binding to their active parts, and TIMP-1 has a particularly strong inhibitory effect on fibroblast and polymorphonuclear leukocyte–derived MMPs and proMMP-2 (Borkham-Kamphorst, Alexi, Tihaa, Haas, & Weiskirchen, 2015). MMPs and TIMPs may play a central role in regulating the production of ECM molecules (Kouki et al., 2004). As demonstrated by other

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researchers, expansion force applied to the cranial sutures for 2 days could enhance the accumulation of proline without alteration of the total protein content, which indicated the simultaneity of protein synthesis and degradation (Miyawaki & Forbes, 1987; 2005a, 2005b). The activation process of MMP is very complex, which initiates with MMP-2 and subsequently others (Aimes & Quigley, 1995; Hatori et al., 2004). MMP-2 (gelatinases A) has been shown to play a role in remodeling ECM molecules during embryonic and postnatal appositional bone formation (Collins et al., 2005a, 2005b). Previous studies demonstrated that exogenous cyclic forces induced the expression of MMP-2 gene in the physiologically fused cranial sutures, which was not found without mechanical stresses (Al-Mubarak, Da Silveira, & Mao, 2005). However, little is known regarding the expression of TIMP-1 in the suture under expansion force, and their relevance with MMPs might be important to tissue remodeling.

The purpose of the present study was to explore protein and gene expression of MMP-2 and TIMP-1 during midpalatal suture expansion in rats.

2. Materials and methods

2.1. Animals and appliance installation

All experimental procedures involving animals were approved by the local Animal Experiment Ethics Committee of the State Key Laboratory of Oral Diseases of the Sichuan University (Protocol No.: SKLODLL2013A185). 72 5-week-old male Wistar rats weighing 70 ± 10 g were obtained from the experimental animal center of Sichuan University. Animals were randomly divided into two groups with 36 animals each: the experimental group and the control group. Animals in the experimental group were subjected to rapid maxillary expansion, and animals in the control group received sham device installation. The expansion appliances were made with 0.014 inch stainless steel orthodontic wire with two opening loops, and the initial width between the two retention arms were set according to the width of dental arch. The position of the retention arms were adjusted laterally with an orthodontic gauge that when they were compressed to the initial position they would generate an expansive force of 50 g. After force adjustment, the expansion appliance was bonded to the first and second molar on both sides by a light cured adhesive (3M Unitek, Monrovia, CA) as described by Bo Hou et,al(Bo Hou, Naomi Fukai & Bjorn R Olsen.,2007). (Fig. 1A and B) On day 1, 4, 7, 14 after installation, 9 rats from each group were sacrificed under deep anaesthesia, and the maxilla of each animal was carefully dissected and prepared for histalogical observation and PCR examination

2.2. Histology

Tissue samples were fixed in freshly prepared 4% paraformaldehyde for 24 h, and then decalcified in 0.5 M EDTA for 14 days, rinsed in phosphate–buffered saline for 1 day and finally embedded in paraffin. Semi-serial sections (6um) were mounted onto salinized glass slides. For morphological observation of midpalatal suture, serial coronal sections of midpalatal suture were stained with hematoxylin-eosin and then microphotographs were taken.

2.3. Immunohistochemistry

Sections were dewaxed in xylene and dehydrated through graded alcohol baths. After a rinse in tap water, sections were immersed in 3% hydrogen peroxide solution for 15 min to block endogenous peroxidase. Slides were then “pressure cooked” for 5 min in 0.1 M citrate buffer (PH6.0) for antigen retrieval and blocked in 10% normal goat serum for 20 min. Sections were incubated overnight at 4 °C with affinity–purified antibody against MMP-2 and TIMP-1 respectively (at a dilution of 1: 200, BOSTER). Negative–control sections were incubated with non-immune antibody. The immune reaction was detected according to the manufacturer’s instruments, by subsequent incubation with horseradish peroxidase (HRP)-labeled goat antirabbit antibody (Zhongshan Biotech China), biotinyl tyramide and HRP-labeled streptavidine (1:100, dilution Zhongshan Biotech China). Finally DAB (diaminobenzidine) was used for visualization of antibody binding. Cell nuclei were counterstained with hematoxylin.

2.4. Real-Time PCR

For Real-time PCR, the specimens removed from oral and nasal mucosa were frozen in liquid nitrogen and kept at −130 °C until further use. The levels of MMP-2 and TIMP-1 mRNA expression were detected using SYBR Green Real-time PCR method. Total RNA from each sample was isolated using Trizol reagent (Invitrogen, USA), followed by DNase I (Invitrogen, USA) treatment to remove DNA contaminant. One microgram of the total RNA was subjected

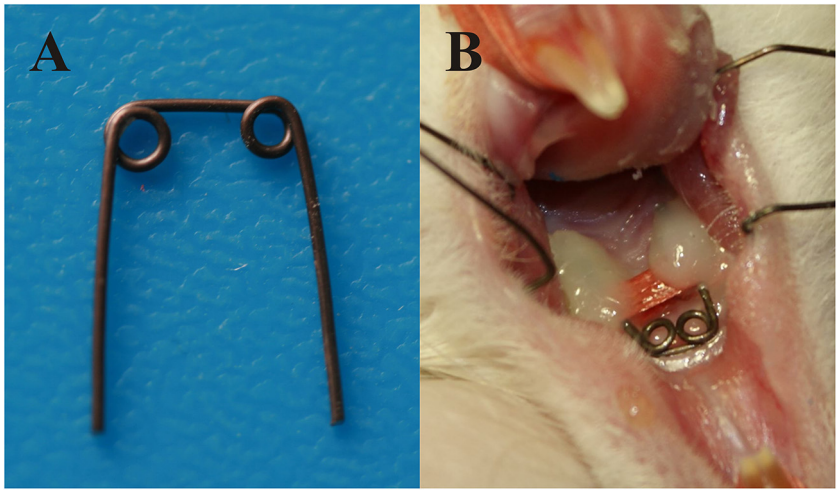

Fig. 1. A: midpalatal suture expainsion appliance, B: the appliance bonded on the tooth.