Acta Odontologica Scandinavica, 2008; 66: 327–333

informa
healthcare

ORIGINAL ARTICLE

Nifedipine regulated periodontal ligament remodeling: An experimental study in rats

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Abstract

Objective. The aim of this study was to investigate the effects of nifedipine on periodontal ligament remodeling during orthodontic tooth movement. ***Material and methods.*** Sixty male Sprague-Dawley rats were randomly divided into an orthodontic group and groups that received either 10 mg/kg or 40 mg/kg nifedipine (NIF). Immunohistochemical staining and image analysis were used to investigate the expression of matrix metalloproteinase (MMP)-1, -10, -13 and collagen-I in PDL, and maxillary 1st molar displacement was measured. ***Results.*** Expression of MMP-1, -10, and -13 was significantly decreased in both NIF groups, while collagen-I expression was markedly increased. NIF significantly inhibited tooth movement. ***Conclusions.*** NIF affects the expression of MMP-1, -10, -13 and collagen-I and tooth movement induced by orthodontic force in rats, thus indicating that calcium channels might be important in mediating PDL remodeling.

Key Words: *Matrix metalloproteinase, nifedipine, orthodontic tooth movement, periodontal ligament remodeling*

Introduction

Orthodontic force induces structural and biochemical changes in periodontal ligament (PDL) space so that PDL remodeling and tooth movement can take place [1]. Many layers of networked signals occur in PDL remodeling that change mechanical force into molecular events. For example, mechanical force regulates a number of different cellular metabolisms, including collagen synthesis, collagenase activity, and cell division [2–4]. As a family of proteinases, matrix metalloproteinases (MMPs) play a central role in PDL remodeling [5]. It has been shown that the expression levels of MMP-2, MMP-9, and TIMP (MMPs specific inhibitors, tissue inhibitors of metalloproteinases) 1–3 increase transiently at both the tension and compression sides during orthodontic tooth movement [6]. MMP-13 and MMP-8 are differentially regulated by tension and compression *in vivo* and *in vitro* [7]. Although much progress has been made in the study of PDL remodeling, mechanistic details remain unclear.

Calcium channels are one mediator of signal pathways and are important in PDL fibroblast signal transduction [8]. Elevation in calcium ion concentration ($[Ca^{2+}]_i$) occurs in PDL fibroblasts subjected to mechanical strain *in vitro* [9]. However, there are no available data concerning calcium channel-mediated PDL remodeling induced by orthodontic force.

The purpose of this study was therefore to investigate the effects of nifedipine (NIF), a calcium channel blocker [10], on the expression of MMP-1, -10, -13, collagen-I, and tooth movement, and to explore the roles of calcium channel blocker in orthodontic PDL remodeling.

Material and methods

Animals

Sixty male 9-week-old Sprague-Dawley rats (body-weight 250–280 g) obtained from Harbin Medical University animal center were used in this study. The animals were acclimatized for 1 week in plastic cages with a standard 12 h light–dark cycle. They were fed a diet of soft laboratory food to minimize any discomfort after orthodontic appliance insertion

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(Received 29 December 2007; accepted 16 May 2008)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2008 Informa UK Ltd. (Informa Healthcare, Taylor & Francis As)
DOI: 10.1080/00016350802208398

and also the risk of appliance displacement. The study was approved by the institutional ethics committee of Harbin Medical University.

The rats were randomly divided into three study groups ($n = 20$ in each group): (1) an OD group: orthodontic group; (2) a NIF10 group: orthodontic with NIF 10 mg/kg by gastric feeding twice daily; and (3) a NIF40 group: orthodontic with NIF 40 mg/kg by gastric feeding twice daily. The rats in each group were divided into five subgroups (1 day, 3 days, 7 days, 14 days, and 21 days).

Placement of orthodontic appliances

Each rat was anesthetized with an intraperitoneal injection of 10% chloral hydrate (350 mg/kg). The orthodontic appliance was a replication of the appliance presented by Leiker et al. [11]. Orthodontic force was applied by a 5 mm length of 0.3 mm nitinol closed coil spring (Grikin Advanced Materials, Beijing, China) running between the right upper 1st molar and incisor. The spring was fixed in place using 0.2 mm steel ligature wires (Grikin Advanced Materials) surrounding the molar and incisor. Owing to the lack of undercuts in incisor area, a cervical groove was prepared on the tooth, in which the ligature wire was seated on both incisors. Each spring was activated, as previously described, to produce an initial measured force of about 50 g [11,12]. The left upper side was the control side.

Tissue preparation

Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (350 mg/kg) and fixed by cardiac perfusion with freshly made 4% paraformaldehyde, pH 7.2. Both sides of the maxillae and surrounding tissues were dissected and fixed in the same fixative for an additional 24 h at 4°C. The specimens were then decalcified in 15% ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, St. Louis, Mo., USA) pH 7.4, under microwave irradiation for 1 week, until the specimen had become soft enough for further processing. The demineralized tissues were dehydrated through ascending concentrations of ethanol. Subsequently, the tissue blocks were labeled, processed for embedding in paraffin wax, and then cut into 1 or 5 μm thick mesiodistal serial sections.

Immunohistochemical staining

For detection of MMP-1, -10, and -13, tooth and surrounding tissue sections were dewaxed by immersion in two changes of xylene and rehydrated through a graded ethanol series to double-distilled water. To inhibit endogenous peroxidase activity, they were incubated with 3% hydrogen peroxide in methanol for 15 min, followed by a single wash in distilled water, and heat-mediated antigen retrieval. The buffer (0.01 mmol/l sodium citrate buffer, pH 6.0) was heated to boiling point using a stainless steel pressure cooker. A metal rack with array slides was put into the pressure cooker and the slides were immersed in the buffer for 2 min [13]. The slides were rinsed three times in phosphate-buffered saline (PBS) and incubated with: mouse monoclonal antibody MMP-13 (clone VIII A2; Lab Vision Corporation, Fremont, Calif., USA) at a 1:100 working dilution; mouse monoclonal antibody MMP-10 (BA0609; Boster Biotechnology, Wuhan, China) at a 1:40 working dilution; or mouse monoclonal antibody MMP-1 (BA1270; Boster Biotechnology, Wuhan, China) at a 1:40 working dilution, all at 4°C overnight, prior to sequential incubation for 20 min with diluted and ready-to-use anti-mouse IgG (Boster Biotechnology). Immunoreactivity was visualized by development with 0.1% 3, 3'-diaminobenzidine (DAB; Boster Biotechnology) for 2–3 min. All dilutions were performed using PBS, pH 7.4. The sections were then rinsed in tap water for 5 min and counterstained with hematoxylin for 30–40 s. Stained sections were dehydrated with absolute alcohol, submerged in xylene and mounted on slides for light microscopy examination. Replacement of the first antibody with PBS served as the negative control. Breast carcinoma tissue sections served as the positive control for MMP-1, -10, and -13.

Masson staining method

A modified Masson staining based on traditional Masson staining [14] was used to detect collagen-I. The tooth and surrounding tissue sections were deparaffinized and rehydrated, then oxygenized with periodic acid for 20 min, rinsed in tap water for 5 min and washed three times with double-distilled water. The washed sections were submerged in achromatic color solferino solution for 10–20 min, washed three times in double-distilled water, and rinsed in tap water for 10 min. After the final rinse, each section was washed twice in 0.5% acetic acid, submerged in 0.5% aniline blue for 0.5–1 min, washed three times in 0.5% acetic acid, consecutively dehydrated with absolute alcohol, submerged in xylene, and mounted on slides.

Measurement of orthodontic tooth displacement

A plaster replica of the maxillae including the molars was made for each rat. An impression taken using polysiloxane (Dentsply Dental, Tianjin, China) immediately after removal of the appliance was used to make a cast using dental stone (Dentsply Dental). The mesiodistal space between the distal surface of the 1st molar and the mesial surface of the 2nd molar was measured on the cast with sliding calipers. All of

the rats initially had tight contacts between the molars.

Image observation and statistical analysis

A light microscope (Nikon, Japan) was used to observe the positive signal and measure the light density. The PDL area consisted of the mid-third of the 1st molar root on both the compression and tension sides. Grey-degree difference was a representation of positive degree of MMP-1, -10, -13, and collagen-I. Background grey degree was examined for each section to eliminate the difference between sections. The level of MMPs and collagen-I in the three groups was evaluated with the analysis of variance of split plot design. The statistical package SAS 9.1.3 (SAS Institute, N.C., USA) was used to process the data. The effect of NIF on the amount of tooth movement in the three groups was evaluated with factorial analysis of variance. The statistical Package for Social Sciences, version 10.0 (SPSS, Chicago, Ill., USA) was used to process the data. The level of significance was set at $p < 0.05$.

Results

Effects of NIF on histological changes of PDL

Histological changes of PDL remodeling induced by orthodontic force are depicted in Figure 1. On the tension side of the OD group, the width of the PDL was increased, and collagen fibers were elongated parallel to the direction of tooth movement (Figure 1A). On the tension sides of the NIF10 and NIF40 groups, collagen was increased and collagen fibers were oriented to the orthodontic force (Figure 1B, 1C). On the compression side of the OD group, collagen fibers were disoriented and the amount of collagen was decreased (Figure 1D). Finally, on the compression sides of the NIF10 and NIF40 groups, the amount of collagen was increased (Figure 1E, 1F). However, on the control sides of all three groups, PDL showed radically oriented fiber collagen and fibroblasts.

Effects of NIF on collagenase (MMP-1 and MMP-13) expression

Figures 2 and 3 show MMP-1 and MMP-13 expression of the different groups on the third day. In the OD group, MMP-1 and MMP-13 were more strongly expressed on the tension and compression sides than on the control sides (Figure 2A, 2D, 3A, 3D). In the NIF groups, their expression decreased on the tension and compression sides (Figure 2B, 2C, 2E, 2F, 3B, 3C, 3E, 3F). As shown in Figure 2H and 3H, significant differences of MMP-1 and MMP-13 expression on the experimental sides were evident among the three groups ($p < 0.05$). Meanwhile, NIF did not significantly affect the control sides of the three groups ($p > 0.05$).

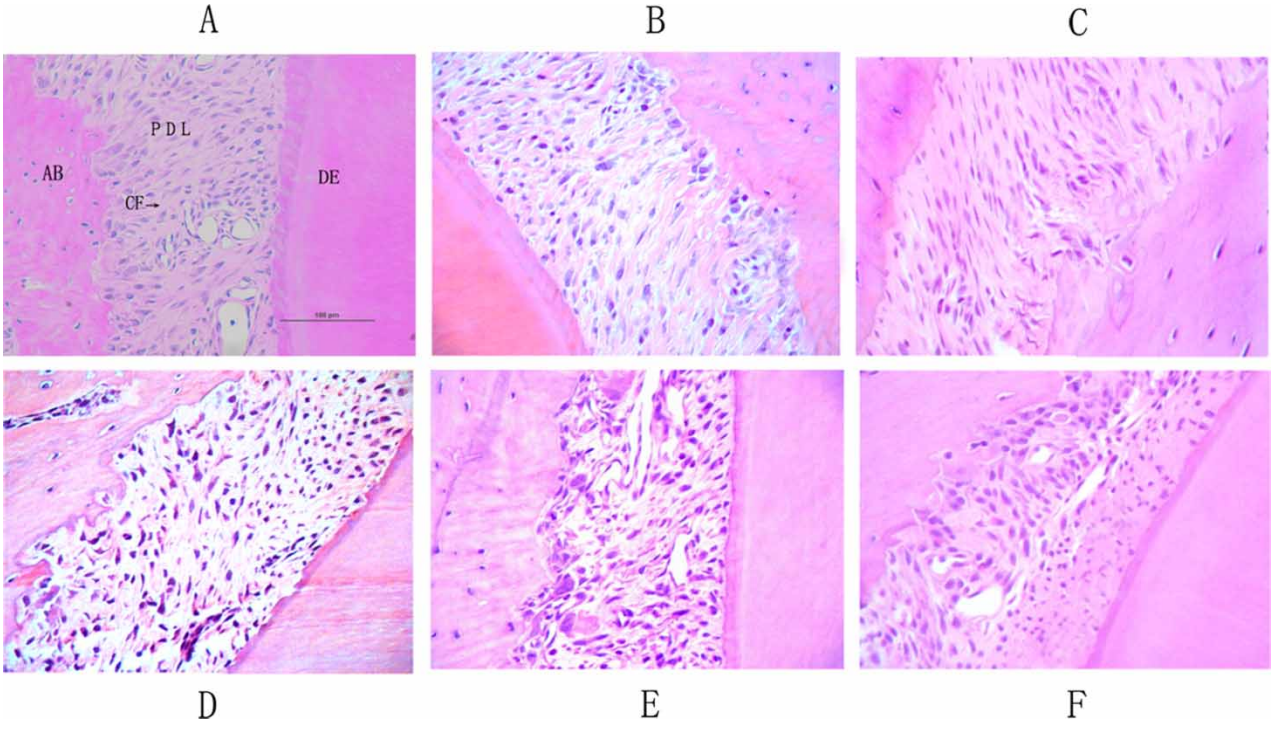

Figure 1. HE stain images of the mesiodistal sections of maxillary 1st molars (distal-buccal root) from experimental sides on day 3. (A) and (D) Tension and compression sides of the OD group. On the tension side of the NIF10 (B) and NIF40 (C) groups, the amount of collagen was increased and collagen fibers were oriented. On the compression sides of the NIF10 (E) and NIF40 (F) groups, the amount of collagen was increased, and collagen fibers were disoriented. PDL: periodontal ligament; DE: dentin; AB: alveolar bone; CF: collagen fibers.

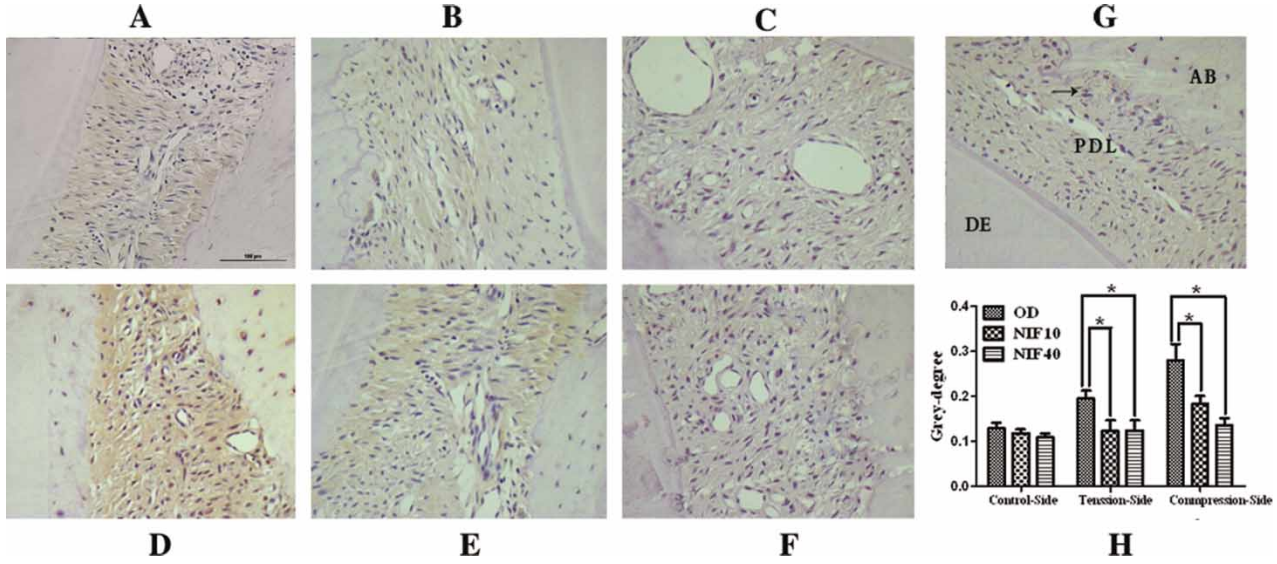

Figure 2. Immunohistochemical detection of MMP-1 expression in mesiodistal sections of maxillary 1st molars (distal-buccal root) on day 3. In the OD group, MMP-1 expression was stronger on the tension (A) and compression sides (D) than on the control side (G). In the NIF groups, MMP-1 level decreased on the tension and compression sides. (B) and (C) Tension side of the NIF10 and NIF40 groups. (E) and (F) Compression side of the NIF10 and NIF40 groups. (H) A significant difference of MMP-1 expression on the experimental sides among three groups was evident ($p < 0.05$). There was no significant difference of MMP-1 expression on the control sides among the three groups ($p > 0.05$). PDL: periodontal ligament; DE: dentin; AB: alveolar bone; arrowhead: positive signal.

Effects of NIF on MMP-10 expression

Figure 4 depicts MMP-10 expression of different groups on the third day. In the OD group, orthodontic force induced MMP-10 increasing on the tension and compression sides (Figure 4A, 4D). In the NIF groups, MMP-10 expression decreased with increasing NIF dose on the tension (Figure 4B, 4C) and compression sides (Figure 4E, 4F). NIF-mediated regulation of MMP-10 expression on both the tension and compression sides was statistically evident by the third day (Figure 4H; $p < 0.05$). There was no significant difference of MMP-10 expression on the control side among the three groups ($p > 0.05$).

Additionally, in all groups, MMP-1 and MMP-13 expression was stronger than MMP-10 expression.

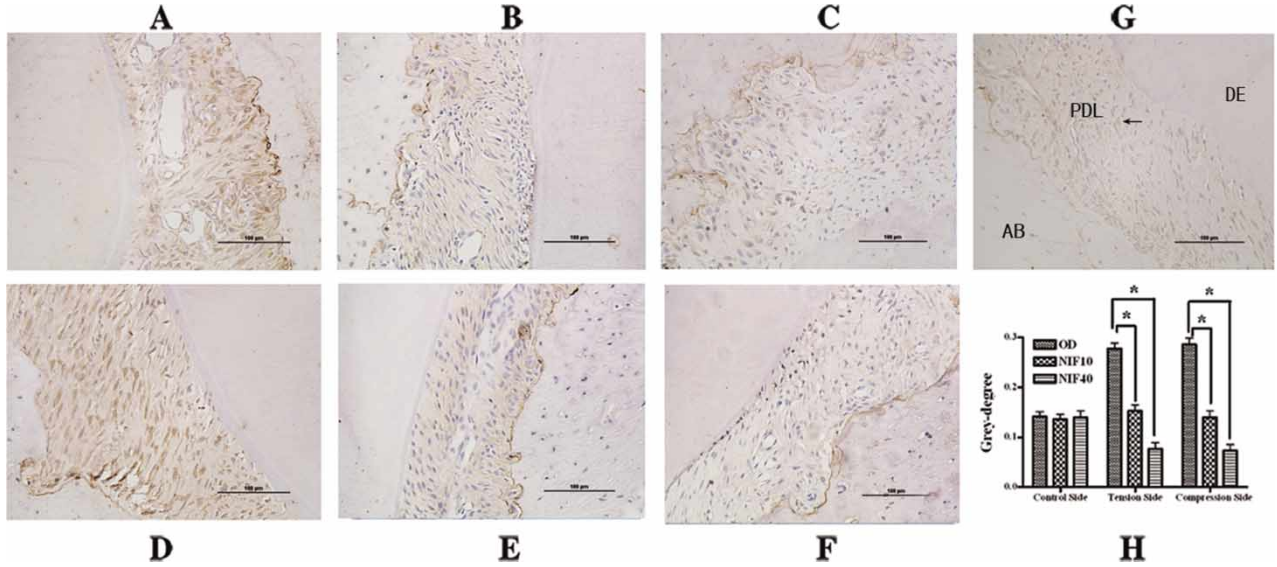

Figure 3. Immunohistochemical detection of MMP-13 expression in mesiodistal sections of maxillary 1st molars (distal-buccal root) on day 3. In the OD group, MMP-13 was stronger on the tension (A) and compression sides (D) than on the control side (G). In the NIF groups, MMP-13 decreased on the tension and compression sides. (B) and (C) Tension side of the NIF10 and NIF40 groups. (E) and (F) Compression sides of the NIF10 and NIF40 groups. (H) A significant difference of MMP-13 expression on the experimental sides among three groups was evident ($p < 0.05$). NIF did not produce a marked influence on the control sides of the three groups ($p > 0.05$). PDL: periodontal ligament; DE: dentin; AB: alveolar bone; arrowhead: positive signal.

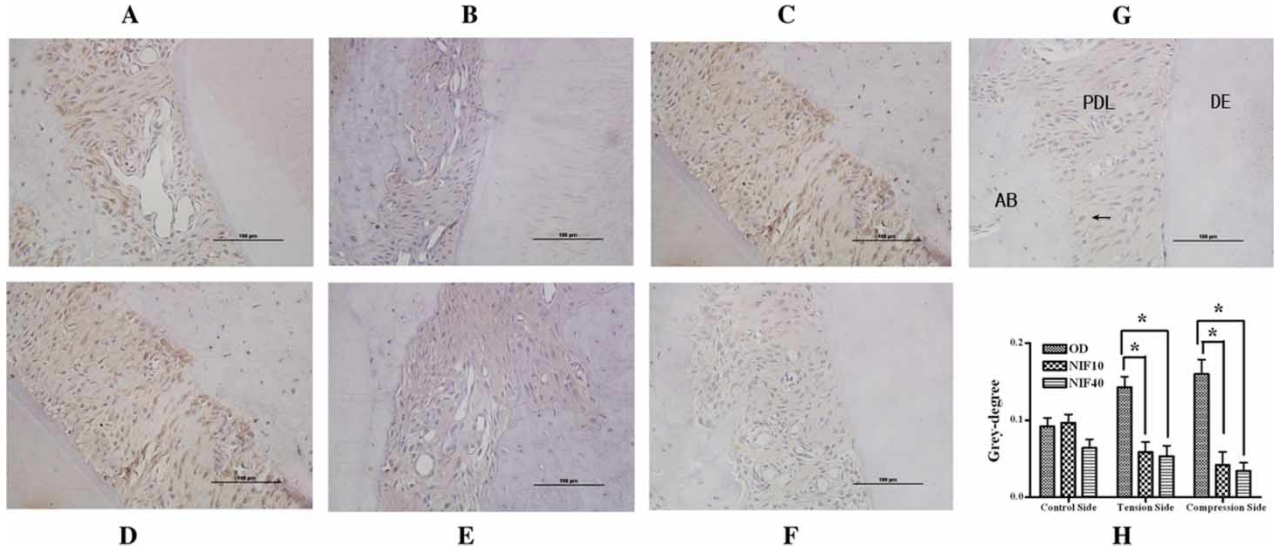

Figure 4. Immunohistochemical detection of MMP-10 expression in mesiodistal sections of maxillary 1st molars (distal-buccal root) on day 3. In the OD group, MMP-10 expression was stronger on the tension (A) and compression sides (D) than on the control side (G). In the NIF groups, MMP-10 level decreased with increasing NIF dose on the tension and compression sides. (B) and (C) Tension side of the NIF10 and NIF40 groups. (E) and (F) Compression side of the NIF10 and NIF40 groups. (H) The regulated role of NIF on MMP-10 expression on both the tension and compression sides was statistically evident by day 3 ($p < 0.05$). There was no significant difference of MMP-10 expression on the control sides among the three groups ($p > 0.05$). PDL: periodontal ligament; DE: dentin; AB: alveolar bone; arrowhead: positive signal.

Effects of NIF on the expression of collagen-I

Figure 5A displays the effect of NIF on collagen-I expression among the three groups on day 7. On both the compression and tension sides, the collagen-I level was upregulated in the NIF groups ($p < 0.05$). On the control sides, there was no significant difference in any of the groups ($p > 0.05$).

Effects of NIF on tooth displacement

Figure 5B shows the tooth displacement in OD and in two NIF groups. After 21 days, the 1st molar of animals in the OD, NIF10, and NIF40 groups moved 0.32, 0.25, and 0.21 mm, respectively. The slopes of the regression lines through the data points of each rat represented the rate of orthodontic tooth movement. Factorial analysis of variance showed significant inhibitory effects of NIF on tooth movement ($p < 0.05$).

Discussion

The PDL is the first tissue to react to force application; without remodeling of the PDL, orthodontic tooth movement is not possible [15]. Recent studies have shown that MMPs are key enzymes that play a crucial role in PDL remodeling during orthodontic tooth movement [16–18]. Collagenase activity was augmented in the course of orthodontic tooth movement in humans [16]. Apajalahti reported that MMP-8 levels in orthodontically treated teeth became significantly higher 4–8 h after force

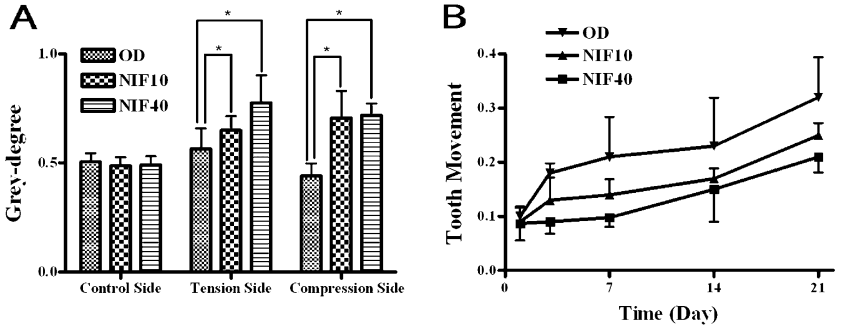

Figure 5. (A) Densitometric results from semi-quantitative immunohistochemical analysis (mean ± standard deviation) on day 7. The magnitude-dependent effects of tension and compression on expression of collagen-I are indicated. Expression decreased in the OD group, and level increased in the NIF groups ($p < 0.05$). On the control sides, there was no significant difference in any group ($p > 0.05$). (B) Effects of NIF on maxillary 1st molar mesial displacement of three experiment groups. After 21 days, the 1st molar of animals in the OD, NIF10, and NIF40 groups moved 0.32, 0.25, and 0.21 mm, respectively. The slopes of the regression lines through the data points of each rat represent the rate of orthodontic tooth movement. Factorial analysis of variance showed significant inhibitory effect of NIF on tooth movement ($p < 0.05$).

application than before activation [17]. Upregulation of MMP-1 and MMP-2 protein levels in a time-dependent fashion in gingival crevicular fluid during initial orthodontic tooth movement has been described previously [18]. Furthermore, MMP-13 increasingly occurs early, following the application of an orthodontic force in both PDL and alveolar bone in rats [19]. The present results are consistent with these previous observations; the expression of MMP-1, -10, and -13 on the experimental sides of the OD group gradually increased in the first three days (Figure 2A, 2D, 3A, 3D, 4A, 4D), usually returning to normal level by day 7.

Although it has been established that MMPs and collagen are altered in PDL remodeling from orthodontic force, the detailed mechanism remains uncertain. It has previously been reported that mechanical stretching stimulus in PDL fibroblasts results in the increase of intracellular calcium [9,20]. Mechanical force applied to PDL fibroblasts activates calcium-permeable channels and increases actin polymerization [21]. As calcium channel blockers, NIF and verapamil completely reverse such calcium enhancement, which suggests that an opening of the L-type calcium channel is involved in this process. However, it is not completely clear whether calcium channels are correlated to PDL remodeling during orthodontic tooth movement. Based on the collective foregoing information, we hypothesize that NIF can influence the expression of MMPs and tooth movement. We have demonstrated that NIF, a calcium channel blocker, can decrease the expression of MMP-1, -10, and -13, increase collagen-I level during PDL remodeling, and inhibit tooth movement induced by orthodontic force. In our study, MMP-1, -10, and -13 in the two NIF groups were expressed less than in the OD group on the third day. However, this influence of NIF was insignificant on other days. Meanwhile, MMP-1, -10, and -13 expression on the control sides did not differ significantly on the third day among the three groups (Figure 2H, 3H, 4H), which indicates that NIF can decrease MMP-1, -10, and -13 expression in PDL remodeling. In addition, we found that the expression of collagen-I was markedly increased on the seventh day and tooth displacement was significantly inhibited on the experimental sides in the presence of both tested concentrations of NIF (Figure 5A, 5B). These findings conform to the histological changes shown in Figure 1. Some clinical and experimental investigations have shown that NIF often causes gingival overgrowth [22,23]. The underlying mechanism is related to the inhibitory effects of NIF on gingival fibroblast intracellular calcium. Whether NIF also produces PDL overgrowth is unknown. Our findings demonstrate that NIF does not cause PDL overgrowth without orthodontic force. This is supported in a previous study [24] in which NIF seemed to have no influence on L-type calcium channels under resting conditions or on the blocking effects of NIF when calcium channels opened under depolarizing conditions. In our study, the calcium channels on PDL fibroblasts were mostly activated when orthodontic force was applied to teeth, and the blocking effect of NIF became stronger. The present result implies that orthodontic tooth movement is delayed in patients who are administered NIF.

It can be concluded that the calcium channel blocker NIF can affect the expression of MMP-1, -10, -13 and collagen-I during PDL remodeling, and inhibit tooth movement induced by orthodontic force. To some extent, our results indicate that calcium channels play an important role in PDL remodeling resulting from orthodontic force.

Acknowledgments

This work was supported by Heilongjiang Province Doctor Foundation of China (No. YJSCX2007-0328HCJ) and Heilongjiang Province Natural Science Foundation of China (D2004-40).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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