Original

The Stromal Cell-derived Factor-1 Expression Protected in Periodontal Tissues Damage during Occlusal Traumatism

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Abstract: Mechanical stress (MS) during hyperocclusion results in elimination of the alveolar hard line, enhancement of bone resorption, and shedding of a tooth, resulting in trauma of occlusion. Several studies have indicated that MS induces cytokine and chemokine expression during alveolar bone absorption in periodontitis and orthodontic treatment. However, it remains unknown regarding the effect of hyperocclusal MS on maintenance of alveolar bone metabolism. Using *in vivo* and *in vitro* hyperocclusion models, we investigated the effect of MS on relationships between the expression of stress-dependent and osteoblastogenesis-associated chemokines. In the *in vitro* model with 6-weeks old mice, MS upregulated the expression of stromal cell-derived factor-1 (SDF-1) in periodontal tissues, a self-renewal chemokine in bone marrow stem cell day 4 after MS. In contrast, in the *in vivo* model with 30-weeks old mice, SDF-1 expression was significantly upregulated at day 1 after MS in periodontal tissues. MS induced the expression of SDF-1 in the PDL root branch and of the SDF-1 receptor CXCR4 in the dental pulp and bone marrow on day 4 in an *in vivo* mice model. MS upregulated the expression of SDF-1 in PDL cells. SDF-1 binds to CXCR4, resulting in MS-resistant alveolar bone protection during occlusal traumatism.

Key words: Alveolar bone, Occlusal traumatism, Periodontal tissues, SDF-1

Introduction

Early contact and lateral rolling of abutment teeth in partial dentures exert intermittent mechanical stress (iMS) on the periodontal tissue. MS with regular occlusions suppresses tooth movement, and excessive MS is associated with disappearance of alveolar bone wiring and expansion of the periodontal ligament (PDL). Further, MS reportedly caused damage to periodontal tissue, including bone resorption and loss of teeth, thereby causing occlusal trauma¹⁻³⁾. The regulatory role of MS in the process of alveolar bone remodeling has been considered crucial^{4,5)}.

Chemokines are basic, heparinous, 92-99-amino-acid-long cytokines; they have a low-molecular weight of approximately 8-14 kDa. Approximately 50 types of N-terminal cysteine residual sequences responsible for intramolecular disulfide bonds have been identified. Chemokines are divided into the following four main subfamilies: C, CC, CXC, and CX3C⁶⁻⁸⁾. Chemokines play functional roles in the migration and infiltration of leukocytes into tissues in inflammatory and immune reactions as well as in the maintenance of ecological homeostasis and pathology. Stromal cell-derived factor-1 (SDF-1) is a chemokine for the imperative maintenance of hematopoietic stem cells in the bone marrow, and is reportedly involved in the movement of teeth by orthodontic forces and in the migration of blood stem cells. SDF-1 bind into its chemokine receptor CXCR4. This ligand-receptor complex plays im-

portant roles in the development, leukocyte migration, and metastasis processes. SDF-1 reportedly promoted new bone formation upon topical administration in a rat skull defect model. It has also been recently reported that SDF-1 and bone morphogenetic protein-2 (BMP-2) induce bone formation⁹⁾.

Here, we postulated that hyperocclusion MS induces the production of protective chemokines, including SDF-1, to maintain alveolar bone density and quality during occlusal trauma. Using *in vivo* and *in vitro* hyperocclusion models, we examined the effects of iMS on the relevance between chemokine expression in PDL and the maintenance of the bone marrow.

Materials and Methods

The protocol was reviewed and confirmed by the Research Ethics Committee (Approval No. 116). All animal experiments and follow the necessary procedures the guidelines established by the Animal Care Committee.

Cell Culture

Mouse PDL cells isolated from healthy mouse incisors and molars, as described previously¹⁰⁾. To avoid contamination by the gingivae and dental pulp, only the middle of each root was used. The cells were cultivated in a silicon-rubber chamber in α -minimum essential medium containing 10% fetal bovine serum at 37°C in a humidified atmosphere with 5% CO₂.

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Figure 1. *In vivo* expression of stromal cell-derived factor-1 (SDF-1) and CXCR4 in PDL tissues, dental pulp, and bone marrow of hyperocclusion mouse models at 6 and 30 weeks. Hematoxylin-eosin (HE) staining and immunohistochemical staining of SDF-1 and CXCR4 in hyperocclusion mouse models at 6 weeks (A) and 30 weeks (B) are shown. Scale bars indicate 100 μ m.

***In vitro* Hyperocclusion Model**

Mouse PDL cells were cultivated in a 50-cm² silicon chamber at a density of 5×10^5 cells/cm². Attachments for silicon chamber was stretching apparatus (STB-140; Strex Co. Ltd., Osaka, Japan) and then subjected to intermittent uniaxial stretching for 1-7 days (iMS; conditions, 60 s/return; resting time, 29 s; stretch length, 1.6 mm; stretch ratio, 105%)

***In vivo* Hyperocclusion Model**

Experiments with animals were performed in accordance with the ethical guidelines for animal experiments established by Fukuoka Dental College (Approval No. 000233). Six- and 30- weeks of age ddY mice were used that purchased from Kyudo Animal Co, Ltd., Tosu, Japan. Anesthesia was induced by 2% isoflurane (Abbott Laboratories, Abbott

Park, IL, USA) and an air mixture gas flow of 1 l/ min using an anesthesia gas machine (Anesthesia machine SF-B01; MR Technology, Inc., Tsukuba, Ibaraki, Japan). To stimulate hyperocclusal MS, three maxillary molars in right side were attached a stainless steel wire using methyl-methacrylate resin (Super-Bond; Sun Medical Inc., Shiga, Japan). Stainless steel wire (0.3 mm in diameter) was glued to the occlusal surfaces of the right maxillary molars.

Immunohistochemistry

Tissue blocks were obtained from the mandible and soaked in 10% EDTA for 12 h at 4°C. After decalcification for 3 weeks at 4°C, the tissue blocks were washed overnight in phosphate-buffered saline containing 30% sucrose, buried in OCT compound, and frozen in dry ice/isopentane. Bone slices (thickness, 6 μ m) were performed using a Frigocut

Figure 2. *In vivo* expression of LNGFR (p75) and CXCR4 in the periodontal tissues of the hyperocclusion mouse models at 6 and 30 weeks. Immunohistochemical staining of LNGFR and CXCR4 in hyperocclusion mouse models at 6 weeks (A) and 30 weeks (B) is shown. Scale bars indicate 100 μm. Inlet in (A) is a low magnification image.

CM1850 (Leica, Wetzlar Germany). Slices were incubated with primary antibodies in a humidified chamber overnight at 4°C. Antibodies were visible using an Alexa Fluor-conjugated IgG secondary antibody (1 mg/ml, Molecular Probes, OR, USA) after incubation for 30 min at room temperature. Nuclear staining was performed using 4,6-diamidino-2-phenylindole (Dojindo, Kumamoto, Japan).

Western Blot Analysis

Cells were dissolved in a lysis buffer (pH, 7.5) containing 20 mM Tris-HCl, 200 mM NaCl, 1% Triton X-100, 1 mM DTT, and protease inhibitors (Roche, Basel, Switzerland). The protein content of the samples was assayed using a protein assay kit (Pierce, Hercules, CA, USA), in accordance with the manufacturers' protocol. Protein samples (20 μg) were subjected to 5%-20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electrophoretically transferred to a polyvinylidene fluoride membrane at 100 V for 1 h at 4°C. The membrane was incubated overnight at 4°C with antibodies against the targeted chemok-

ines at 1:500 dilutions in a 5% dry skim milk solution. The blots were then incubated with a secondary antibody for 30 min at room temperature, followed by incubation for 1 h with horseradish peroxidase-conjugated goat anti-rabbit or mouse IgG diluted at 1:2,500 in 5% dry skim milk-TTB and developed using an enhanced chemiluminescence system (GE, Arlington Heights, IL, USA).

Statistical Analysis

Data are expressed as the means and standard error of the number, *n*, of cells. Differences were assessed using paired one-way analysis of variance, as appropriate. *P* < 0.05 was considered statistically significant.

Results

Intermittent MS in PDL cells transiently decreases and gradually increases SDF-1 gene expression

We have reported that iMS loading of human PDL cells for two days

(A)

(C)

(B)

30 weeks

Figure 3. *In vitro* hyperocclusion models of the expressions of SDF-1 mRNA, protein, and secretion using hyperocclusion mouse models at 6 and 30 weeks. (A) qRT-PCR analysis of SDF-1 mRNA expression induced by intermittent mechanical stress (iMS) using the stretcher of a silicon chamber in hyperocclusion mouse models at 6 and 30 weeks. (B) Expression of iMS-induced SDF-1 protein in hyperocclusion mouse models at 6 and 30 weeks. (C) iMS induced secretion of SDF-1 protein in the hyperocclusion mouse model at 6 and 30 weeks.

upregulated the expression of the bone resorption associated cytokines and chemokines genes compared with unloaded cells in an *in vitro* model using DNA microarray analysis⁽¹¹⁾. In contrast, iMS also downregulated the expression of SDF-1 gene, a self-renewal chemokine in stem cells of the bone marrow on day 2 after stimulation in human PDL cells.

We furthermore investigated the time course of iMS-induced SDF-1 expression using the *in vivo* hyperocclusion model of young (6 weeks-old) and aged (30 weeks-old) mice. No significant inflammation was observed by hematoxylin-eosin staining until day 7 after MS loading (Fig. 1) in two types of young and aged mice. Although little change in the expression of SDF-1 was identified by immunohistochemical staining until day 2 following hyperocclusion MS loading, the expression of SDF-1 gradually increased in PDL tissues after day 4 in the *in vivo* hyperocclusion model with 6 weeks-old mice. In contrast, MS rapidly increased the expression of SDF-1 in PDL tissues on day 2 after MS in the hyperocclusion model with 30 weeks-old mice.

The hyperocclusional MS also increased the expression of CXCR4, SDF-1 receptor in the dental pulp and alveolar bone marrow on day 4 or 2 in the hyperocclusion models at 6 or 30 weeks-old mice, respectively.

Co-localization of CXCR4 and LINGER (p75) in bone marrow

To investigate the properties of MS-induced CXCR expression, we examined the expressions of mesenchymal cell marker proteins in the periodontal tissues in the hyperocclusion model mice. CXCR4 expression was almost co-localized in a mesenchymal cell marker, LINGER (p75)-positive cells in the dental pulp and bone marrow of the hyperocclusion mice model.

The co-expressions of LINGER (p75) and CXCR4 were increased on day 4 in the hyperocclusion model with 6 weeks-old mice (Fig. 2). In contrast, MS rapidly increased both the expression of LINGER (p75) and CXCR4 on day 2 after MS stimulation in PDL tissues of the hyperocclusion model with 30 weeks-old mice.

Time-dependent expression of SDF-1 in PDL cells stimulated by iMS

To disclose the acute response to MS, we used quantitative PCR, ELISA, and Western blots to examine changes in the expression of SDF-1 on days 0, 1, 2, 3, and 5 following MS loading in PDL cells. In the *in vitro* hyperocclusion model, iMS loading significantly upregulat-

ed the expression of SDF-1 mRNA on day 5 after stimulation in PDL cells with 6 weeks-old mice (Fig. 3A). In contrast, MS quickly upregulated the expression of SDF-1 mRNA on day 2 after stimulation in PDL cells with 30 weeks-old mice. SDF-1 protein expression was slightly increased at day 3 after MS in PDL cells with 6 or 30 weeks-old mice (Fig. 3B). SDF-1 was also significantly produced at day 5 after iMS from PDL cells with 6 weeks-old mice (Fig. 3C). SDF-1 secretion was lower concentration in 30 weeks-old mice than that of 6 weeks-old mice day 0 before iMS. SDF-1 rapidly produced at day 2 after iMS from PDL cells with 30 weeks-old mice, but a few at 5 day after iMS in PDL cells with 6 weeks-old mice.

Discussion

We investigated the hyperocclusion MS-induced expression of chemokines associated to alveolar bone metabolism in PDL and their receptors in the bone marrow cells in *in vitro* with MS-treated PDL cells and *in vivo* models with traumatic hyperocclusion. The *in vitro* and *in vivo* models similarly revealed the following results during hyperocclusion, MS significantly upregulated the expression of SDF-1 in PDL tissue on 4-7 days in 6weeks-old mice or 2-7 days in 30 weeks-old mice after MS loading, indicating in quick protection to MS damages in PDL tissues in aged mice compared to that of young mice. The MS-induced SDF-1 upregulation may effective against stress and the initiation of osteoblasts differentiation from mesenchymal bone marrow cells, resulting in the maintenance of alveolar bone density and quality during occlusal traumatism. MS loading for 48 h has previously reported to lead to a transient decrease in the expression of MS-protective and osteogenic chemokines in human PDL cells using DNA microarrays. MS in present experiments particularly induced SDF-1 expression in PDL tissue of the *in vivo* mouse models with young and aged mice. We found that the time course of MS-induced SDF-1 expression is likely to dramatically increase following transiently decrease in mouse PDL tissues. Furthermore, both the expression and localization of SDF-1 *in vivo* were similar to those recognized in the *in vitro* models. The data indicated that CXCR4, a SDF-1 receptor also increased by MS in mesenchymal cells of alveolar bone marrow, resulting in protection of hyperocclusal damages in periodontal tissues. The SDF-1 has been known to be upregulated the MSCs in the portion of bone repair after femoral bone fracture¹²⁾. The circulating osteoblast progenitor cells in BMCs have reported to be recruited in bone formation via SDF-1/CXCR4 axis¹³⁾.

These findings suggested that the alveolar bone maintenance against MS induced the osteoblasts differentiation from mesenchymal bone marrow cells via upregulation of SDF-1 in damaged PDL tissues, resulting in the quick initiation in old mice compared to young mice occlusal traumatism.

MS loading is well known to induce receptor activator of nuclear factor kappa-B ligand (RANKL) expression in PDL both *in vivo* and *in vitro*^{3, 14)}. Jung *et al.* reported a possible mechanism for localizing stem cells in the appearing bone marrow, whereby accrued expression of SDF-1 in the local bone marrow environment along with diminished SDF-1 in serum may create a homing gradient¹⁵⁾. The role provided that SDF-1 in BMP2-induced osteogenic differentiation of MSCs as a perturbing signal affects the differentiation of MSCs toward osteoblastic cells in response to BMP2 stimulation¹⁶⁾. Together with our present data, these effects propose that SDF-1 is involved in alveolar bone metabolism via SDF-1/CXCR4 signaling during traumatic hyperocclusion.

It is known that SDF-1 expression is temporally and spatially associated with tooth eruption, arthritic bone destruction, osseous inflammation, and the developmental regulation of bone remodeling¹⁷⁻¹⁹⁾. Mesen-

chymal stem cells reportedly express CXCR4 and CCR2 and reacted to chemokine ligands, such as SDF-1 and MCP-1²⁰⁾. The *in vivo* and *in vitro* experimental models used in the present study indicated that the excessive MS associated with hyperocclusion as well as the orthodontic model mice with retraction and compressed stress resulted in the migration of SDF-1 in PDL tissues and its receptor CXCR4 in the bone marrow, including in the mesenchymal stem cells. These results demonstrate that SDF-1 plays not only an MS-dependent role in bone resorption but an MS-protective role in bone formation during alveolar bone remodeling.

Both SDF-1 mRNA and protein reportedly increase during bone graft repair, and an anti-SDF-1 antibody reportedly inhibits new bone formation²¹⁾. SDF-1 expression was increased around defects at already 1 week after injury, and BMCs were mobilized to the defects²²⁾. These results suggest that SDF-1 may regulate and thus promote alveolar bone formation during occlusal traumatism.

Furthermore, the level of PGE₂ and IL-1 β secreted from elder PDL tissues has been reported to be higher than that of young PDL tissues²³⁾. Our reported also indicated the excess MS during hyperocclusion protectively increased the expression of collagen type XII in aged mice compared to that of younger mice²⁴⁾.

These finding, together with our data suggested that excess MS to elder periodontal tissues quickly may defend and maintain their present characteristics and shape compare to that of younger.

In conclusion, we demonstrated that MS induces SDF-1 secretion from PDL tissues. SDF-1 binds to its receptor CXCR4 in mesenchymal stem cells in the bone marrow and might perform an important role in the maintenance of alveolar bone metabolism with inducing osteoblast differentiation.

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Conflict of Interest

The authors have no conflict of interest to declare.

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