REVIEW ARTICLE

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RANK/RANKL/OPG during orthodontic tooth movement

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Structured Abstract

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Objectives – Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament (PDL) and alveolar bone. A precondition for these remodeling activities, and ultimately for tooth displacement, is the occurrence of an inflammatory process.

Materials and Methods – This review covers current knowledge regarding the role of the receptor activator of nuclear factor-kappa (RANK), receptor activator of nuclear factor-kappa ligand (RANKL), and osteoprotegerin (OPG) in periodontal tissue reactions, in response to orthodontic forces.

Results – It has been found that concentrations of RANKL in GCF increased during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF was significantly higher than in control sites. *In vivo* studies have shown the presence of RANKL and RANK in periodontal tissues during experimental tooth movement of rat molars, and that PDL cells under mechanical stress may induce osteoclastogenesis through upregulation of RANKL expression during orthodontic tooth movement.

Conclusions – Considering the importance of RANK, RANKL, and OPG in physiologic osteoclast formation, it is reasonable to propose that the RANKL/RANK/OPG system plays an important role in orthodontic tooth movement.

Key words: orthodontic tooth movement; RANK/RANKL/OPG; periodontal ligament; cytokines

Introduction

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament (PDL) and alveolar bone. A precondition for these remodeling activities, and ultimately for tooth displacement, is the occurrence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described, and a number of inflammatory mediators, growth factors, and neuropeptides have been demonstrated in periodontal supporting tissues. Their increased levels during orthodontic tooth movement have led to the assumption that interactions between cells producing these substances, such as nerve, immune, and endocrine system cells, regulate biologic responses following the application of orthodontic forces (1).

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Mechanical stress evokes biochemical responses and structural changes in a variety of cell types *in vivo* and *in vitro*. The overall objective of many investigations has been to further understanding of the mechanisms involved in converting molecular and/or mechanical stress to the cellular responses resulting in tooth movement. In sites at which inflammation and tissue destruction have occurred, cells may communicate with one another through the interaction of cytokines and other related molecules. Thus, it is important to more completely elucidate the complex cytokine cascade flow associated with inflammation-mediated tissue destruction at the molecular level (2).

The role of the receptor activator of nuclear factor-kappa (RANK), receptor activator of nuclear factor-kappa ligand (RANKL), and osteoprotegerin (OPG) system in inducing bone remodeling was recently demonstrated. The tumor necrosis factor (TNF)-related ligand, RANKL, and its two receptors RANK and OPG, have been shown to be involved in this remodeling process (3). RANKL is a downstream regulator of osteoclast formation and activation, through which many hormones and cytokines produce their osteoresorptive effect. In the bone system, RANKL is expressed on the osteoblast cell lineage and it exerts its effect by binding to the RANK receptor on osteoclast lineage cells. This binding leads to rapid differentiation of hematopoietic osteoclast precursors to mature osteoclasts. OPG is a decoy receptor produced by osteoblastic cells, and competes with RANK for RANKL binding. The biologic effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of activation of matrix osteoclasts, and induction of apoptosis. Thus, bone remodeling is controlled by a balance between RANK–RANKL binding and OPG production (4). This review covers current evidence regarding the role of the RANK/RANKL/OPG system in periodontal tissue reactions, in response to orthodontic force application.

Gingival crevicular fluid study during orthodontic tooth movement

Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate found in the gingival sulcus, where it tends to increase in volume with inflammation and greater capillary permeability. Serum is the primary source of the aqueous component of GCF; however, the gingival tissue through which the fluid passes, along with bacteria present in the tissue and gingival crevice, can modify its composition (5). With constituents of GCF being derived from a variety of sources, including microbial dental plaque, host inflammatory cells, host tissue, and serum, GCF varies according to the condition of the periodontal tissues. In addition to the cells, immunoglobulins, microorganisms, toxins, and lysosomal enzymes detected in GCF, the mechanism of bone resorption may also be related to the release of inflammatory mediators present in GCF. Recently, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in periodontal diseases (6), although only a few studies have focused on those involved in bone remodeling during orthodontic tooth movement. Mogi et al. (7) found that GCF concentrations of IL-1β and IL-6 were significantly higher in a group with severe periodontal disease compared with controls, and Yavuzyilmaz et al. (8) demonstrated the GCF IL-1β and TNF-α levels had a positive correlation to mean pocket depths, suggesting that the cytokines may be involved in the pathogenesis of periodontal diseases. Further, Mogi et al. (9) reported that an increased concentration of RANKL and decreased concentration of OPG were detected in GCF from patients with periodontitis, while the ratio of RANKL concentration to that of OPG in GCF samples was significantly higher for patients with periodontal disease than for healthy subjects. Taken together, these data suggest that RANKL and OPG contribute to osteoclastic bone destruction in periodontal disease.

Storey (10) proposed that the early phase of tooth movement involves an acute inflammatory response characterized by periodontal vasodilation and migration of leukocytes out of the capillaries. Recent research has led to the hypothesis that inflammatory mediators are released following mechanical stimulus, triggering the biologic processes associated with alveolar bone resorption and apposition (2). Among the local biochemical mediators are cytokines secreted by mononuclear cells and leukocytes. Cytokines can provoke the synthesis and secretion of numerous substances that form the molecular basis for cell-to-cell communication, including prostaglandins (PGs) and growth factors, thus interacting directly or indirectly with bone cells (2). Uematsu et al. (11) found that the levels of inflammatory mediators (IL-1β, IL-6, TNF-α, epidermal growth factor,

and b_2 microglobulin) in GCF were elevated during orthodontic treatment, and Grieve et al. (12) reported similar results for PGE and IL-1β. Further, Lowney et al. (13) described an increase in TNF-α in GCF from teeth undergoing orthodontic forces. As noted above, inflammatory mediators have been detected in GCF samples during orthodontic tooth movement in the early phase. Nishijima et al. (14) found an increased concentration of RANKL in GCF during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF was significantly higher than in control sites in another study. Further, Kawasaki et al. (15) reported that the age-related decrease in amount of tooth movement may be related to a decrease in RANKL/OPG ratio in GCF during the early stages of orthodontic tooth movement. Consequently, analysis of GCF samples may provide a better understanding of the biochemical processes associated with tooth movement, potentially helping clinicians make therapeutic choices based on qualitative and quantitative information.

In vivo study during orthodontic tooth movement

In *in vivo* studies, experimental tooth movement has been shown to lead to significantly increased recruitment of cells that belong to the mononuclear phagocytic system. Saito et al. (16) indicated that there was a local increase in PGs in the PDL and alveolar bone during orthodontic treatment, and other studies have shown an arrest in tooth movement in experimental animals when non-steroidal anti-inflammatory drugs were administered (17). Further, when PGE_1 was administered locally or systemically to rats, accelerated bone resorption, and tooth movement were observed after the application of orthodontic forces (18). Therefore, PGs have been shown to play an important role in orthodontic tooth movement.

Macrophages have the ability to produce cytokines, such as IL-1β and IL-6, levels of which are known to increase during orthodontic tooth movement (16). The number and distribution patterns of RANKL- and RANK-expressing osteoclasts change when excessive orthodontic force was applied to periodontal tissue, and IL-1β and TNF-α were expressed in osteoclasts in pathologic status rat periodontal tissues (19). Shiotani et al. (20) have also shown the presence of RANKL in periodontal tissues during experimental tooth movement of rat molars. Therefore, it is suggested that in response to mechanical stress, RANKL is regulated by inflammatory cytokines in the PDL.

The number and distribution patterns of RANKL and RANK-expressing osteoclasts change when excessive orthodontic force is applied to periodontal tissues. Aihara et al. (21) and Kim et al. (22) showed the presence of RANKL in periodontal tissues during experimental tooth movement of rat molars. Kanzaki et al. (23, 24) demonstrated that transfer of the RANKL gene to the periodontal tissue activated osteoclastogenesis and accelerated the amount of experimental tooth movement in rats. In contrast, OPG gene transfer inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement. Therefore, it is suggested that PGE_2, inflammatory cytokines, and the RANKL–RANK system may be involved in regulation of orthodontic tooth movement (Fig. 1).

In vitro study in response to mechanical forces

The PDL lies between hard tissues cementum and alveolar bone where it functions as a cushion to withstand mechanical forces applied to teeth, thus it receives and responds to external forces. It is likely that PDL cells stimulated by forces of mastication, occlusal contacts, and orthodontic treatment produce local factors that participate not only in the maintenance and remodeling of the ligament, but also in the metabolism of adjacent alveolar bone.

In vitro studies have shown that the expression and production of some inflammatory mediators (PGE_2, IL-1β) are promoted by mechanical stimulation of the PDL (25). COX-2 is induced in PDL cells by cyclic mechanical stimulation and is responsible for the augmentation of PGE_2 production *in vitro* (26). In addition, Kanzaki et al. demonstrated that compressive force upregulated RANKL expression and induced COX-2 expression in human PDL cells *in vitro*. Nakao et al. (27) reported that intermittent compressive forces induced RANKL in PDL cells via IL-1β. Compression force significantly increased RANKL and decreased OPG secretion in human PDL cells in a time- and force–magnitude-dependent manner (14, 28). Further, Nakajima et al. (29) reported that in response to

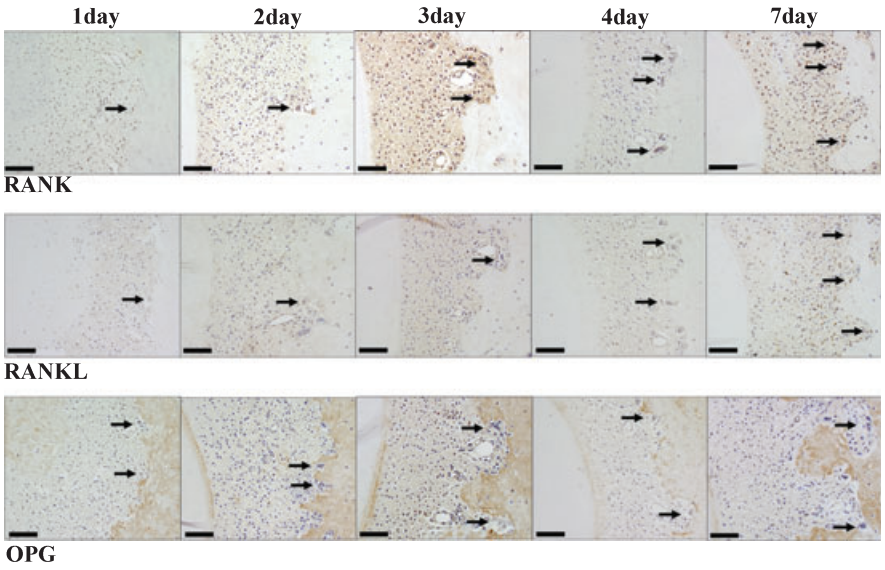

Fig. 1. Immunohistochemical staining for RANK, RANKL, and OPG in the PDL for days 1–7 of orthodontic tooth movement. Osteoclastic activity indicated by arrows. Scale bar = 50 μm.

compression force the production of RANKL increased via FGF-2 expression. These results suggest that PDL cells under mechanical stress may induce osteoclastogenesis through upregulation of RANKL expression during orthodontic tooth movement.

Low-power laser irradiation stimulates the velocity of tooth movement via the RANK/RANKL/OPG system

From the patient's point of view, accelerating tooth movement would be desirable during orthodontic treatment to reduce treatment duration. Literature shows various methods to stimulate bone remodeling such as drug injections (30), electric stimulation (31), and ultrasound application (32). Recently, various biostimulatory effects of low-energy laser irradiation have been reported in wound healing (33–35), fibroblast (36, 37), and chondral (38) proliferation, collagen synthesis (39–41), and nerve regeneration (42). Acceleration of bone regeneration by laser treatment has been the focus of recent studies (43, 44). In the field of orthodontics, low-energy laser irradiation has been utilized for several types of clinical orthodontic treatment, such as reduction of post-adjustment pain (45), or treatment of traumatic ulcers in the oral mucosa induced by an orthodontic appliance (46). However, scant information is available concerning the effects of low-energy laser irradiation on bone remodeling during orthodontic tooth movement.

Several investigations have been conducted on the effects of low-energy laser irradiation on bone tissues and these may relate to potential applications in orthodontic tooth movement. Saito and Shimizu (47) reported the stimulatory effects of low-energy laser irradiation on bone regeneration in the median palatine suture area during rapid maxillary expansion in rats. Ozawa et al. (48) demonstrated that laser irradiation stimulates cellular proliferation and differentiation of osteoblast lineage nodule-forming cells, especially in committed precursors, resulting in an increase in the number of differentiated osteoblastic cells as well as in bone formation. Kawasaki and Shimizu (49) reported that low-energy laser irradiation stimulated the amount of tooth movement and formation of osteoclasts on the pressure side during experimental tooth movement *in vivo*. Further, Fujita et al. (50) and Yamaguchi et al. (51) demonstrated that low-energy laser irradiation enhanced the velocity of tooth movement via (RANK)/RANKL and the macrophage-colony stimulating factor and its receptor (c-Fms) expression. Together, these findings suggest that low-energy laser irradiation accelerates bone remodeling and thus could potentially shorten the orthodontic treatment period.

Root resorption and the RANK/RANKL/OPG system

Many orthodontists consider external apical root resorption (EARR) to be an unavoidable pathologic

consequence of orthodontic tooth movement. EARR is often considered an iatrogenic disorder that occurs unpredictably with orthodontic treatment. This undesirable side-effect has been described as being the outcome of a sterile, complex inflammatory process that involves various disparate components including mechanical forces, tooth root and bone tissues, cells of the surrounding matrix, and certain known biologic messengers (52). Killiany (53) reported that EARR of >3 mm occurs at a frequency of 30% in a patient population, while 5% of treated individuals have >5 mm of root resorption. Harris et al. (54, 55) reported that the sum of the effects of the patients' sex, age, severity of the malocclusion, and the kind of mechanics applied accounted for little of the overall variation in EARR.

Orthodontic force applications induce a local process that includes all of the characteristics of inflammation (redness, heat, swelling, pain, and altered function). The inflammatory process, an essential feature of tooth movement, is actually the fundamental component behind the root resorption process (56). The process of resorption requires specific interactions between various inflammatory cells and hard tissues, whether bone, cementum or dentine, and is a multistep process. The underlying cellular processes involved in root resorption are thought to be similar if not identical to those occurring during bone resorption (57). Multinucleated clast cells are formed as a result of cellular injuries to bone, cementum, or dentine (58). The progenitor cells arrive at the resorption site via the bloodstream as mononuclear cells (derived from hemopoietic precursors in the spleen or bone marrow) and fuse prior to getting involved in the resorptive process. The pathogenesis of this process has been assumed to be the removal from the PDL of necrotic tissue compressed by an orthodontic load. It is believed that PGs are intimately involved in root resorption (59).

A search for risk factors affiliated with the development of EARR during orthodontic treatment has led to the suggestion that individual susceptibility, genetics, and systemic factors may be significant modulators of this process. Current research on orthodontic root resorption is directed toward identifying genes involved in the process, their chromosome loci, and their possible clinical significance. Al-Qawasmi et al. (60) reported evidence of a linkage disequilibrium of IL-1β polymorphism in allele 1 and EARR. Low et al. (61) reported that RANK and OPG regulated the root resorption process, while Yamaguchi et al. (28) reported that the compressed PDL cells obtained from patients with severe EARR produce a large amount of RANKL and upregulate osteoclastogenesis. Therefore, evidence suggests the occurrence of orthodontic root resorption involves a combination of genetic factors and RANKL (Fig. 2).

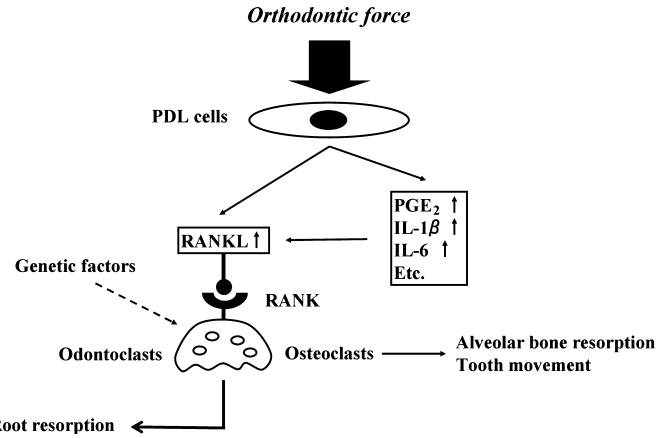

Fig. 2. Schematic representation of events related to inflammation in periodontal ligament when stimulated by orthodontic forces.

Conclusion

The multifunctional roles of RANK, OPG, and RANKL may provide an important link between bone remodeling, orthodontic tooth movement, and root resorption to other local and systemic conditions.

Clinical relevance

Recent studies show that orthodontic forces change the levels of OPG, RANK, and RANKL in GCF during orthodontic tooth movement. The rate of orthodontic tooth movement is significantly increased by the transfer of RANKL gene. It has also been reported that the compressed PDL cells in cases of severe EARR may produce a large amount of RANKL, and upregulate osteoclastogenesis. Therefore, the RANK/RANKL/OPG system may provide an important link between bone remodeling, orthodontic tooth movement, and root resorption during orthodontic tooth movement.

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