Journal of Oral Science, Vol. 50, No. 4, 367-376, 2008

Review

Root resorption and the OPG/RANKL/RANK system: a mini review

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(Received 28 February and accepted 30 October 2008)

Abstract: Odontoclastic root resorption is a significant clinical issue in relation to orthodontic tooth movement, and resorption of the roots of primary teeth is an intriguing biological phenomenon. The functional coordination of the OPG/RANKL/RANK system seems to contribute not only to alveolar remodeling, but also to resorption during orthodontic tooth movement and physiological root resorption. Serum OPG and s-RANKL are related to regulation of bone homeostasis by the OPG/RANKL/RANK system, and determination of their concentrations might be useful for predicting the rate of bone remodeling during orthodontic tooth movement, the net effect between bone remodeling and root resorption, and the degree of root resorption. It is therefore rational to speculate that a study of the levels of OPG and s-RANKL in blood and GCF, in relation to the degree of root resorption during orthodontic tooth movement, using healthy experimental animals and a carefully planned and organized experimental design, may be able to answer this intriguing question. (J. Oral Sci. 50, 367-376, 2008)

Keywords: root resorption; osteoprotegerin; RANKL; RANK; serum.

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Introduction

Odontoclastic root resorption is a significant clinical issue in relation to orthodontic tooth movement, and resorption of the roots of primary teeth is an intriguing biological phenomenon. Several investigators have attempted to clarify the precise cellular mechanisms whereby root resorption takes place, and recent studies have shown that the RANKL/RANK/OPG proteins are involved in the molecular events that occur during both physiological and orthodontic root resorption.

Osteoprotegerin (OPG), receptor activator of nuclear factor – (KB) ligand (RANKL), and its cognate receptor RANK, are protein-ligands that share homologies with members of the tumor necrosis factor receptor superfamily and function as paracrine regulators of osteoclastogenesis and bone metabolism (1-5). OPG is a member of the TNF (tumor necrosis factor) receptor superfamily and represents a mature protein of 380 amino acids. In contrast to all other TNF receptor superfamily members, OPG lacks trans-membrane and cytoplasmic domains and is secreted as a soluble protein. OPG mRNA is known to be expressed in a number of tissues (6,7), but OPG protein is secreted mainly by cells of osteoblastic and other lineages (8). The major biological action of OPG is inhibition of osteoclast differentiation, inhibition of osteoclast resorptive function, and stimulation of osteoclast apoptosis (9).

RANK is a 616-amino-acid peptide on the cell surface of osteoclast precursors (2). RANKL is a 317-amino-acid peptide. It is produced by osteoblastic lineage cells and activated T cells. When RANKL is expressed by cells of osteoblastic lineage, it is cell-bound, and when expressed

by T-lymphocytes it is soluble (s-RANKL) (1). RANKL mRNA is expressed most highly in bone and bone marrow, as well as in lymphoid tissues. The role of RANKL, together with another very important protein ligand, M-CSF (which binds to its receptor c-fms), is to promote osteoclast formation, fusion, differentiation, activation and survival, thus enhancing bone resorption (10-19).

The biological effects of RANKL are produced when it binds to RANK. The biological effects of OPG are opposite to the RANKL-mediated effects, because OPG acts as a soluble receptor antagonist, which neutralizes RANKL and therefore prevents RANKL-RANK interaction (20) (Fig. 1). The aforementioned biological procedures can explain why the resorptive activity of osteoclasts, induced by soluble RANKL or cell-bound RANKL, is completely inhibited by the simultaneous addition of OPG (21). Conclusively, OPG, RANKL and RANK form a key network that regulates bone metabolism and osteoclast biology.

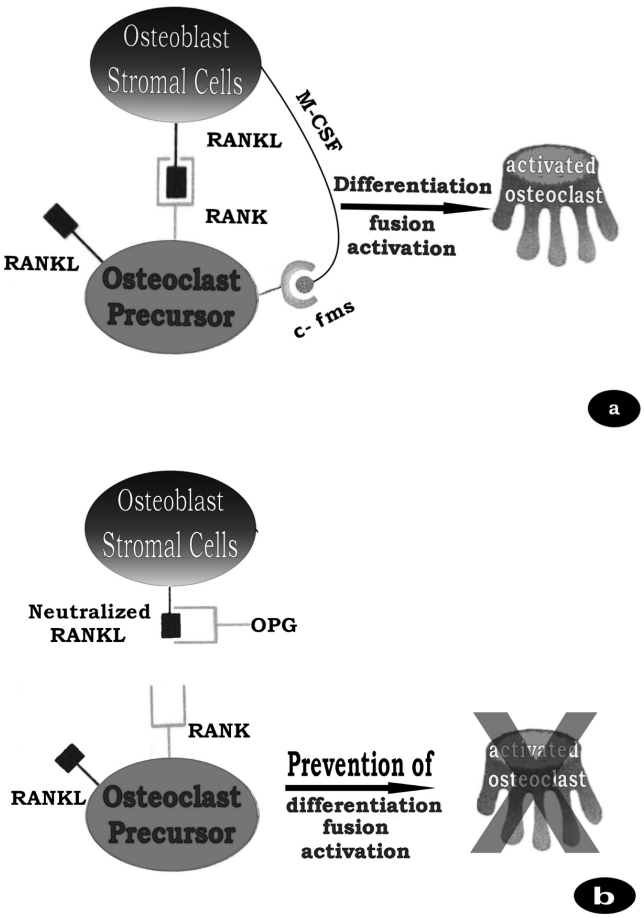

Fig. 1 The major biologic actions of the OPG/RANKL/RANK system: a) activation of osteoclast precursors by binding of RANKL and RANK, b) neutralization of RANKL by OPG and prevention of RANKL-RANK interaction.

These ligands also appear to be key regulators of bone remodeling during orthodontic tooth movement (9). OPG is considered to be a key negative regulator of osteoclastogenesis in the periodontal ligament (PDL) during tooth movement. PDL cells synthesize both RANKL and OPG, and inactivation of OPG may play an important role in the differentiation of osteoclasts. RANKL is expressed in PDL fibroblasts and osteoblasts on the compressed side of the PDL, and it seems that osteoclast differentiation is critically regulated by RANKL, produced as a local factor by osteoblasts/stromal cells, in response to mechanical stress. During alveolar bone resorption, RANKL has been detected in osteoblasts, odontoblasts, osteoclasts and other cells in the PDL (22-24). RANK has been detected in multinucleated osteoclasts and osteoclast precursors, and OPG in almost all osteoblasts, odontoblasts and mesenchymal cells in the periodontal ligament. However, no osteoprotegerin-positive osteoclasts have been reported (22-24). It has been shown that when the RANKL gene is transferred to periodontal tissue, osteoclastogenesis is activated and the rate of orthodontic tooth movement is significantly increased. Recent studies have also demonstrated that orthodontic forces change the levels of OPG and RANKL and that mechanical strain plays an important role in the regulation of OPG synthesis and RANKL expression. Cyclic tensile strain induces a magnitude-dependent increase in OPG synthesis and a concomitant decrease in RANKLmRNA expression and RANKL release from osteoblasts (8,25,26).

The functional coordination of the OPG/RANKL/RANK system seems to contribute to not only alveolar remodeling, but also physiological root resorption and root resorption during orthodontic tooth movement. The cells that are recruited on the tooth surface in order to remove the hyaline zone, induced by high orthodontic forces, have almost identical morphologies to osteoclasts and mediate root resorption upon differentiation to an osteoclast-odontoclast phenotype. They are generally smaller in size, have fewer nuclei, and form smaller resorption lacunae, but apart from a lack of expression of calcitonin receptors, which have not been detected in odontoclasts (27), no other difference has ever been recognized between odontoclasts and osteoclasts, either structural or histochemical (28-31). The cellular mechanisms of root resorption appear to be quite similar to those of osteoclastic bone resorption (27,32-39). PDL subjected to orthodontic forces and experiencing root resorption demonstrate changes in levels of OPG and RANKL (32,40), and it has been proposed that PDL cells, in cases of severe external apical root resorption, may produce a large amount of RANKL and up-regulate osteoclastogenesis (33,41).

Role of the OPG/RANKL/RANK system during physiological root resorption

Immunohistochemical studies have shown that RANKL is expressed by odontoblasts, pulp and PDL fibroblasts, and cementoblasts (42,43). RANK is expressed by multinucleated odontoclasts, localized near the dentine surface in resorption lacunae, or by mononucleated precursors (44); OPG is expressed by odontoblasts, ameloblasts and dental pulp cells (45,46). As in osteoclasts, RANKL is also expressed in odontoclasts, suggesting an autocrine or paracrine effect of this regulator on these cells (44).

The resorbing activity of odontoclasts is related to expression of the OPG/RANKL/RANK system by PDL cells. It has been shown that PDL cells, isolated from either non-resorbing deciduous teeth or permanent teeth, express OPG, but not RANKL. In contrast, PDL cells derived from resorbing deciduous teeth predominantly express RANKL and less OPG. Similar to osteoclasts, odontoclasts express both RANKL and RANK. RANKL regulates odontoclast differentiation and dose-dependently increases odontoclast resorbing activity. OPG suppresses the RANKL-induced activation of resorbing activity in odontoclasts (27,42,47-49).

In the dental follicle environment, the ratio of OPG to RANKL supports, rather than inhibits, osteoclastinogenesis. Cytotrophic factors released from the dental follicle and/or the stellate reticulum, such as parathyroid hormone-related peptide (PTHrP), interleukin-1α and transforming growth factor-β1, stimulate the expression of RANKL during permanent tooth eruption. Among these factors, parathyroid hormone-related protein (PTHrP) controls regulation of the relative expression levels of RANKL/OPG on dental follicle cells, as well as in human PDL cells. PTHrP increases RANKL and downregulates OPG expression via a cAMP/PKA protein kinase-independent pathway, consequently leading to physiological root resorption of deciduous teeth and succesful eruption of permanent teeth (50,51). Another factor, macrophage colony-stimulating factor (M-CSF or CSF1), which is a hematopoietic growth factor, is involved in the differentiation and activation of localized preodontoclasts. It is expressed by odontoblasts, ameloblasts and dental pulp cells, and its mechanism of action appears to involve upregulation of RANK and downregulation of OPG gene expression (46,52).

The exact mechanism includes the mediation of T-cells, odontoblasts and fibroblasts (Fig. 2). Under the influence of these locally produced cytokines, T-cells can be activated, express RANKL, and induce differentiation and activation of preodontoclast cells (53). In addition, odontoblasts and fibroblasts, which express RANKL, interact with mononuclear progenitors and produce active odontoclasts. A similar cascade of events leads to physiological root resorption when there is no permanent successor. Cytokines, IL-β (interleukin-β), prostaglandin E2, TNF-α or hormones such as dexamethasone and 1,25 $(OH)_2D_3$, induced by the weakened PDL, stimulate expression of RANKL by PDL fibroblasts and, consequently, the recruitment of active odontoclasts and the beginning of the resorption process.

Role of the OPG/RANKL/RANK system during pathological (orthodontic) root resorption

During orthodontic tooth movement, on the compressed side of the tooth, RANKL expression is induced (9,22). RANKL activates osteoclastogenesis, and this is better demonstrated by the acceleration of tooth movement, which is achieved after transfer of the RANKL gene to the periodontal tissue (25). In contrast, it seems that on the tensile side of an orthodontically moving tooth there is an increase in OPG synthesis. It has been reported that application of tensile stretching to osteoblasts results in induction of OPGmRNA in periodontal ligament cells (54-56), and this up-regulation of OPG synthesis is reportedly magnitude-dependent (8). Such tensile strain also induces a decrease of RANKL release and RANKL mRNA expression in cultured osteoblasts. The expression of RANKL is not affected by OPG synthesis. There is no difference in RANKL expression between OPG-deficient and normal mice after application of orthodontic forces, although there is severe alveolar bone resorption in OPG-deficient animals (9,57). Conclusively, the relative expression of OPG and RANKL on the tensioned and the compressed sides of the tooth regulates bone remodeling during orthodontic tooth movement.

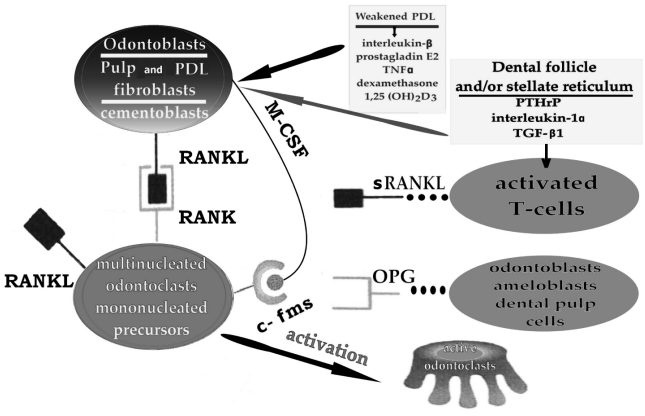

Fig. 2 Cascade of events related to physiological root resorption.

Nevertheless, it is suggested that this RANKL to OPG ratio in periodontal ligament (PDL) cells also contributes to root resorption during orthodontic tooth movement. The compressed PDL cells in cases of severe external apical root resorption may produce a large amount of RANKL and up-regulate osteoclastogenesis. This explains the greater increase of RANKL and decrease of OPG in cases of severe root resorption (26,39,41) (Fig. 3).

Generally speaking, when periodontal tissue is subjected to orthodontic forces and experiences root resorption, changes in the levels of OPG and RANKL, PGE, IL-1b, IL-6, and TNF-a (40,58) can be demonstrated. However, in an experimental study by Low et al. (40), where root resorption was induced by application of heavy orthodontic force to rat molars, levels of RANKL mRNA appeared to be lower than those of OPG. Nonetheless, when RANKL was detected, it was only in association with orthodontic forces. Low et al. (40) suggested that the apparent absence of RANKL mRNA could be related to the role of this protein in osteoclast differentiation, with the presence of multinucleated cells separate from the root surface, but in the PDL. In addition, they considered it likely that much of the OPG mRNA detected in their study was too far from the root surface to influence osteoclast differentiation.

The aforementioned conflicting results can also be explained by the findings of an investigation conducted by Nishijima et al. (26), who determined in ten adolescent patients the levels of RANKL and OPG in the gingival crevicular fluid (GCF) of experimental and control teeth 0 h, 1 h, 24 h, and 168 h after application of retracting force. They found that GCF levels of RANKL were significantly higher and the levels of OPG significantly lower in the experimental teeth than in the control teeth at 24 h, whereas no significant differences were evident at 0 h, 1 h, or 168 h. This return of the RANKL level to normal at 168 h was attributed to the experimental design (elastomeric chain), which did not provide a continuous and consistent force. Indeed, the method used by Low et al. (40) to induce root resorption included closed coil springs, which are also unable to provide continuous and consistent force; consequently, the levels of RANKL and OPG might have been different if measured at different time points after initiation of tooth movement. Using an *in vitro* study model, Nishijima et al. (26) also demonstrated that continuous and consistent compression force significantly increased the secretion of RANKL and decreased that of OPG in human PDL cells, but in a time – (for up to 48 h) and force – (up to 2 g/cm^2) dependent manner (39).

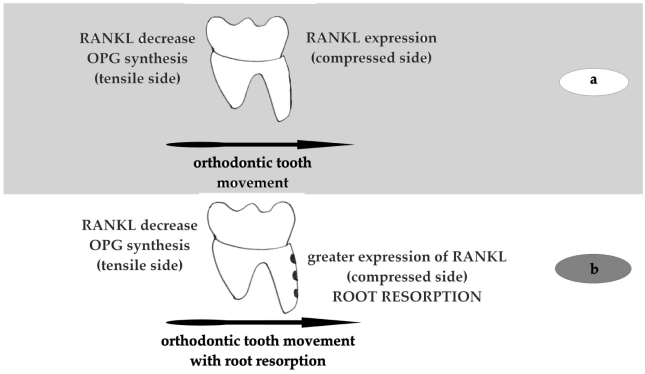

Fig. 3 Regulation of bone remodelling by RANKL/OPG. There is a greater increase of RANKL in cases of severe root resorption.

Information is also available regarding the differences between juvenile and adult patients in relation to the production of these proteins during orthodontic tooth movement. It is suggested that the decrease in the amount of tooth movement with age may be associated with a decrease in the RANKL/OPG ratio during the early stages of orthodontic tooth movement in adult patients (59). The transition from adulthood to old age induces a shift in expression of RANKL and OPG that favors osteoclast formation (60,61) and, in humans, OPG has been reported to decrease significantly with ageing (62). Since the regulatory mechanism of cellular resorption of mineralized tissues, such as bone and teeth, is common (33), the shift in the expression of OPG and RANKL with age affects both bone remodeling and root resorption, and it remains to be clarified how root resorption is affected by the difference in the bone remodeling rate and the RANKL to OPG ratio.

Role of soluble OPG and soluble RANKL in blood and in GCF

OPG functions mainly as a soluble decoy receptor for RANKL. It is produced by a variety of tissues including bone, intestine, the cardiovascular system (heart, arteries, veins), kidney, lung, hematopoietic and immune cells (6,63,64), liver, stomach, brain and spinal cord, and thyroid gland (6,7). Its expression is modulated by various cytokines, peptides, hormones and drugs (65). Such cytokines up-regulating OPG expression include TNF-α, interleukin-1α, interleukin-18, transforming growth factor-β, bone morphogenetic protein, and steroid hormones such as 17β-estradiol (66-80). Glucocorticoids and immunosuppressant cyclosporine A, parathyroid hormone, prostaglandin E2 and basic fibroblast growth factor suppress the expression of OPG (11,72,81-84). The presence of OPG in serum is an absolute requirement for maintenance of bone mass by making unavailable sufficient quantities of RANKL, and several studies have investigated the clinical

use of OPG as an antiresorptive agent for treating a variety of bone disorders characterized by increased osteoclast activity (11,85-87).

RANKL exists functionally as both a membrane-bound protein and as a soluble protein (10). Also, mRNA for RANKL is expressed at high levels in bone, bone marrow and lymphoid tissue including fetal liver, lymph nodes, spleen and thymus (3). Lower levels can also be detected in heart, lung, thyroid and placenta. The soluble form of RANKL with M-CSF is able to induce osteoclast formation in the absence of cellular presentation. A possible explanatory mechanism is the differentiation of peripheral blood mononuclear cells and macrophage-like cells (88). As a soluble protein, RANKL is produced by activated T cells, and therefore bone resorption is regulated by the immune system, where T-cell expression of RANKL may contribute to pathological conditions such as periodontitis and autoimmune arthritis (2). It is suggested that agents such as OPG which inhibit RANKL's activity may be therapeutic for several diseases.

It seems that the OPG/RANKL/RANK system is instrumental for interactions among bone, vascular and immune cells. OPG and the soluble form of RANKL (s-RANKL) are present in the bloodstream, and measurement of their concentrations offers insights into the regulatory mechanisms of this system (89). For example, the level of s-RANKL is elevated in serum of OPG-deficient mice (90). Serum OPG levels are higher in postmenopausal women with osteoporosis and increased bone turnover (91), and it is suggested that this might be a homeostatic mechanism to limit rapid bone loss. In women and in men, ageing seems to increase the serum level of OPG (91), although OPG production by marrow stromal cells appears to decline with age (62).

RANKL and OPG in periodontal tissues are important determinants for regulation of bone remodeling during orthodontic tooth movement as well as root resorption. Determination of serum OPG and s-RANKL can give insight into the regulation of bone homeostasis by the OPG/RANKL/RANK system, and their concentrations might be useful for predicting the rate of bone remodeling during orthodontic tooth movement, the net effect between bone remodeling and root resorption, and the degree of root resorption. Although circulating OPG and s-RANKL originate from several sources and their concentrations may be altered by different coexisting pathological processes (89,92), it would be of great interest to investigate whether serum and GCF concentrations of RANKL and OPG can offer valuable information related to the degree of root resorption induced by orthodontic therapy. It is therefore rational that a study of the levels of OPG and s-RANKL in blood and GCF, in relation to the degree of root resorption during orthodontic tooth movement, using healthy experimental animals and a carefully planned and organized experimental design may be able to answer this intriguing question.

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