ELSEVIER

Bone 41 (2007) 446–455

BONE

www.elsevier.com/locate/bone

Local delivery of osteoprotegerin inhibits mechanically mediated bone modeling in orthodontic tooth movement

Matthew D. Dunn [a], Chan Ho Park [b,c], Paul J. Kostenuik [d], Sunil Kapila [a,*], William V. Giannobile [b,c,e,*]

[a] Department of Orthodontics and Pediatric Dentistry, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Periodontics and Oral Medicine and Center for Craniofacial Regeneration, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
[c] Department of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, MI, USA
[d] Metabolic Disorders Research, Amgen, Inc., Thousand Oaks, CA, USA
[e] Michigan Center for Oral Health Research, Ann Arbor, MI 48106, USA

Received 27 November 2006; revised 16 February 2007; accepted 16 April 2007
Available online 8 May 2007

Abstract

Introduction: The RANKL–OPG axis is a key regulator of osteoclastogenesis and bone turnover activity. Its contribution to bone resorption under altered mechanical states, however, has not been fully elucidated. Here we examined the role of OPG in regulating mechanically induced bone modeling in a rat model of orthodontic tooth movement.
Methods: The maxillary first molars of male Sprague-Dawley rats were moved mesially using a calibrated nickel–titanium spring attached to the maxillary incisor teeth. Two different doses (0.5 mg/kg, 5.0 mg/kg) of a recombinant fusion protein (OPG-Fc), were injected twice weekly mesial to the first molars. Tooth movement was measured using stone casts that were scanned and magnified. Changes in bone quantity were measured using micro-computed tomography and histomorphometric analysis was used to quantify osteoclasts and volumetric parameters. Finally, circulating levels of TRAP-5b (a bone resorption marker) was measured using enzyme-linked immunosorbent assay.
Results: The 5.0 mg/kg OPG-Fc dose showed a potent reduction in mesial molar movement and osteoclast numbers compared to controls ($p<0.01$). The molar movement was inhibited by 45.7%, 70.6%, and 78.7% compared to controls at days 7, 14, and 21 respectively, with the high dose of OPG. The 0.5 mg dose also significantly ($p<0.05$) inhibited molar movement at days 7 (43.8%) and 14 (31.8%). While incisor retraction was also decreased by OPG-Fc, the ratio of incisor to molar tooth movement was markedly better in the high-dose OPG group (5.2:1, $p<0.001$) compared to the control group (2.3:1) and the low-dose OPG group (2.0:1).
Conclusions: Local delivery of OPG-Fc inhibits osteoclastogenesis and tooth movement at targeted dental sites.

Keywords: RANKL inhibitor; OPG; Osteoclast; Micro-computed tomography; Tooth movement

Introduction

Bone deposition and resorption result from the interaction between bone-forming osteoblasts and bone-resorbing osteoclasts. Cells of the osteoblast lineage are not only involved in bone formation, but also regulate osteoclast formation, activation, and survival. This regulation is indirectly mediated by receptor activator of nuclear factor κB ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily. RANKL is produced by osteoblast lineage cells, periodontal ligament (PDL) cells, and by T lymphocytes. RANKL binds to a receptor called RANK, which is located on the surface of osteoclasts and osteoclast precursors. The binding of RANKL to RANK induces osteoclastogenesis, activates mature osteoclasts, mediates their attachment to bone, and promotes their survival. The activity of RANKL is controlled by a soluble decoy receptor called osteoprotegerin (OPG), which binds

* Corresponding authors. S. Kapila is to be contacted at Orthodontics and Pediatric Dentistry, 1004 Dentistry, 1011 N. University Ave., Ann Arbor, MI 48109-1078, USA. Fax: +1 734 763 8100. W.V. Giannobile, Michigan Center for Oral Health Research, University of Michigan Clinical Center, 24 Frank Lloyd Wright Dr., Lobby M, Box 422, Ann Arbor, MI 48106, USA. Fax: +1 734 998 7228.
E-mail addresses: skapila@umich.edu (S. Kapila), wgiannob@umich.edu (W.V. Giannobile).

8756-3282/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2007.04.194

to RANK and inhibits osteoclast formation, activation, and survival [1].

While the contribution of RANKL–OPG in bone turnover mediated via systemic and local biologic agents has been well studied [2,3], less is known of the contribution of these proteins to mechanically induced bone turnover. Several in vitro studies provide important insights into the potential role of mechanically regulated OPG and RANKL in modulating bone turnover in altered strain environments. Compressive mechanical loading of isolated PDL cells leads to a substantial upregulation of RANKL with little change or slight increase in OPG expression which in turn increases osteoclastogenesis when loaded cells are co-cultured with peripheral bone mononuclear cells [4,5]. Also, conditioned media from osteoblasts subjected to microgravity show an increase in the RANKL/OPG ratio, which is accompanied by increased osteoclastogenesis and bone resorption in mouse bone marrow cultures when compared to cells grown at 1 *g*. In contrast, dynamic tensile loading upregulates OPG mRNA and concentration of OPG in conditioned medium, while having little effect on the levels of RANKL in human PDL cells [6], and conditioned media of PDL cells subjected to cyclical tensile force inhibit osteoclastogenesis [7]. Similarly, human osteoblasts demonstrate increased OPG expression but also a decrease in soluble RANKL when subjected to cyclic tensile strain [8]. Moreover, osteoblasts cultured on artificial substrates and subjected to bending also demonstrate increased levels of OPG relative to RANKL [9]. Finally, ST-2 murine bone marrow stromal cells exposed to oscillating fluid flow show a maximal reduction in RANKL/OPG immediately after the end of flow with a significant increase in OPG and decrease in RANKL [10]. RAW 264.7 monocytes co-cultured with ST-2 cells and subjected to fluid flow showed a decrease in osteoclast formation when compared with control cells. Together, these studies demonstrate that different types of mechanical strains by differentially regulating OPG and RANKL result in important differences in the net osteolytic responses. Specifically, the findings suggest that while microgravity and cyclic compressive forces likely contribute to a net increase in osteolytic activity by enhancing the RANKL/OPG ratio, the reverse is true for cyclic tensile strain and oscillating fluid flow. While these in vitro studies may not precisely represent the complex nature of mechanical strains experienced by loaded bone in vivo, including that in the PDL during orthodontic tooth movement, they indicate that cells are capable of perceiving and responding differently to diverse strain histories.

Limited in vivo data also point to the role of RANKL and OPG in modulating mechanically mediated bone turnover. This includes the effects of the administration of recombinant human OPG in reversing the decrease in bone mineral content (BMC), bone mineral density (BMD), and bone strength back to normal levels in limbs of immobilized rats [11]. It has also been demonstrated that excess OPG actually impairs bone remodeling in a situation such as callus repair following fracture indicating that the ideal ratio of OPG to RANKL is dependent on the mechanical model being employed [12]. Finally, local OPG gene transfer to sites significantly diminishes while RANKL gene transfer significantly enhances orthodontic tooth movement possibly by respectively inhibiting or enhancing RANKL-mediated osteoclastogenesis [13,14]. Orthodontic tooth movement is a well-utilized in vivo model for determining the contributions of various exogenous and endogenous agents to mechanically mediated bone modeling [15–19]. It results from the application of forces to teeth that cause bone resorption under pressure and bone deposition under tension. The entire process is based on bone turnover in which the bone surrounding the roots undergoes degradation and active reparative mechanisms as a response to orthodontic forces.

Recently, a fusion protein OPG-Fc and other RANKL inhibitors have been shown to reduce bone resorption systemically and preserve bone in a variety of clinical and preclinical disease settings. Examples include primary osteoporosis, Paget's disease, rheumatoid arthritis, hypercalcemia of malignancy, osteolytic metastases, postmenopausal bone loss, and periodontal disease [1,20–27]. While the potential benefits of these RANKL inhibitors in systemic conditions resulting in significant bone loss are evident, there are also possible uses for local RANKL inhibition such as during mechano-modulation of bone modeling during orthodontic tooth movement, where it is often necessary to minimize unnecessary movement of teeth. The stability of anchor teeth, which tend to inadvertently move during treatment, is a critical shortcoming in orthodontics. In order to combat this undesirable movement, orthodontists have developed several mechanical methods of improving anchorage. However, these have substantial limitations including the need for compliance, discomfort, cost or lack of efficacy [28,29]. Given these disadvantages, a pharmacological approach aimed at utilizing the known biological mechanisms underlying tooth movement may provide an effective, non-compliant, non-visible means of anchorage. If the resorptive process of modeling during tooth movement can be inhibited, tooth movement may be inhibited as well. Accordingly, if OPG is involved in mechano-modulation of bone modeling, its local inhibition of RANKL may provide a novel pharmacological approach for preventing unneeded tooth movement that is highly desirable for preserving orthodontic anchorage.

In this study we tested the hypothesis that local delivery of OPG-Fc will inhibit mechanically induced bone modeling in a rat model of orthodontic tooth movement at the site of OPG-Fc delivery. The specific objectives of this study were to: (1) assess the magnitude of movement of molar and incisor teeth at sites closest and distant, respectively, to the administration of OPG-Fc; (2) quantify the bone resportive activity by histomorphometric assessment of osteoclasts; (3) determine the effects of OPG-Fc on bone density through micro-CT analysis; and (4) assay for serum levels of a bone resorption maker, TRAP-5b.

Materials and methods

Animals

A total of 39 male Sprague-Dawley rats (approximate weight 250–300 g) were utilized in this study. Thirty animals divided into groups of ten were subject to orthodontic forces in addition to volumetrically equivalent injections of 5.0 mg/kg human OPG-Fc (AMGEN, Inc., Thousand Oaks, CA), 0.5 mg/kg OPG-Fc, or phosphate-buffered saline (PBS) vehicle. Three animals received no appliances or injections and were sacrificed at baseline; three animals were vehicle-injected with no appliances, and three animals received high-dose OPG and no appliances. All injections were administered into the palatal mucosa