



Odontoestomatología

ISSN: 0797-0374

ISSN: 1688-9339

Facultad de Odontología - Universidad de la República

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Odontoestomatología, vol. XXIV, no. 40, e316, 2022
Facultad de Odontología - Universidad de la República

DOI: <https://doi.org/10.22592/ode2022n40e316>

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Bone resorption in periodontal disease: the role of RANK, RANKL and OPG. A literature review

Reabsorción ósea en la enfermedad periodontal: el papel de rank, rankl y opg. Una revisión de la bibliografía

Reabsorção óssea na doença periodontal: o papel de rank, rankl e opg. Uma revisão da literatura

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DOI: 10.22592/ode2022n40e316



Abstract

Periodontitis is one of the most prevalent oral diseases. The study of its pathogenesis has significantly advanced in recent years. However, the details of the mechanisms involved in bone resorption are not clear yet. RANK, RANKL, and OPG are proteins that have a known role in other bone tissue diseases. This paper reviews the evidence of their potential participation in the pathogenesis of periodontal disease. More evidence is needed to understand the relationship between RANK, RANKL, OPG, and bone resorption in periodontitis.

Keywords: periodontal disease, RANK, RANKL, OPG.

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Resumen

La periodontitis es una de las enfermedades bucales de mayor prevalencia, el estudio de su patogénesis ha avanzado mucho en los últimos años, sin embargo, los detalles de los mecanismos involucrados en la reabsorción ósea no están aún esclarecidos. RANK, RANKL y OPG son proteínas que tienen un rol conocido en otras enfermedades del tejido óseo, en este trabajo se revisó la evidencia sobre su posible participación en la patogenia de la enfermedad periodontal. Es necesaria más evidencia para conocer la relación entre RANK, RANKL, OPG y la reabsorción ósea vinculada a la periodontitis.

Palabras clave: enfermedad periodontal, RANK, RANKL, OPG.

Introduction

Periodontal disease is a multifactorial infectious, inflammatory, immunologic, and chronic disease that responds to periodontopathic antigens. It results from the complex interaction between microorganisms and host defense mechanisms. Its development can be modified by environmental factors (tobacco), acquired conditions (systemic diseases), and genetic factors.⁽¹⁾

The primary clinical features of periodontitis include clinical attachment loss (CAL), bone resorption (BR), formation of periodontal pockets (PP), and gingival inflammation. In addition, there may be gingival enlargement or recession, bleeding on probing, increased mobility, tooth migration, or exfoliation.⁽²⁾

This article aims to review the currently available evidence on the role of RANK, RANKL, and OPG proteins in the pathogenesis of periodontal disease. Clarifying their role might lead to developing new treatments that contribute to the early diagnosis and better treatment and maintenance of patients with periodontal disease.

Resumo

A periodontite é uma das doenças bucais mais prevalentes, o estudo de sua patogênese avançou muito nos últimos anos, porém, os detalhes dos mecanismos envolvidos na reabsorção óssea ainda não estão esclarecidos. RANK, RANKL e OPG são proteínas que possuem papel conhecido em outras doenças do tecido ósseo, neste trabalho foram revisadas as evidências de sua possível participação na patogênese da doença periodontal. Mais evidências são necessárias para entender a relação entre RANK, RANKL, OPG e reabsorção óssea associada à periodontite.

Palavras-chave: doença periodontal, RANK, RANKL, OPG.

Methodology

A literature search was performed covering the period between 1977 in 2021 in PubMed, Scielo, and Medline databases using descriptors such as RANK, receptor activator of nuclear factor-kappa β , RANKL, receptor activator of nuclear factor kappa- β ligand, OPG, osteoprotegerin, periodontal disease, periodontitis, bone resorption.

Development

The initial response to bacterial infection is the inflammatory reaction that activates the innate immune system. The amplification of this response causes the release of a cascade of cytokines and other mediators and the spread of inflammation to underlying tissues.^(3,4) Failure to stop this inflammatory process at the gingiva can lead to the response expanding into the alveolar bone.⁽³⁾ The inflammatory process leads to connective tissue and alveolar bone destruction, the cardinal sign of periodontitis.⁽⁵⁾

The intrinsic mechanisms of tissue destruction and the genetic factors that make patients sus-

ceptible remain a mystery, despite advances in our knowledge of this disease.

RANKL action can be blocked by its antagonist, Osteoprotegerin (OPG), a TNF superfamily member homologous in structure to RANK.⁽⁶⁾ OPG binds to RANKL and prevents its interaction with RANK. There follows the cascade of molecular events leading to osteoclast differentiation

and bone resorption. Several factors regulate the concentrations of these proteins; in the absence of inflammation, the balance favors OPG. When there is inflammation, chemical mediators shift the balance in favor of resorption. In periodontitis, PGE2, IL-1 β , TNF- α , and IL-6 have been the mediators most closely related to bone resorption.⁽⁷⁾

Figure 1: Hormonal control of bone resorption. Schematic representation of the mechanism of action a) pro-resorptive factors and b) anti-resorptive factors. RANKL expression is induced in osteoblasts, activated T cells, synovial fibroblasts, bone marrow cells. RANKL subsequently binds to its receptor RANK, triggering a cascade of events that promotes osteoclast differentiation, activation, and survival. Conversely, OPG expression is induced by factors that block bone catabolism and promote anabolic effects. OPG binds to and neutralizes RANKL, leading to a block in osteoclastogenesis and decreased survival of pre-existing osteoclasts.

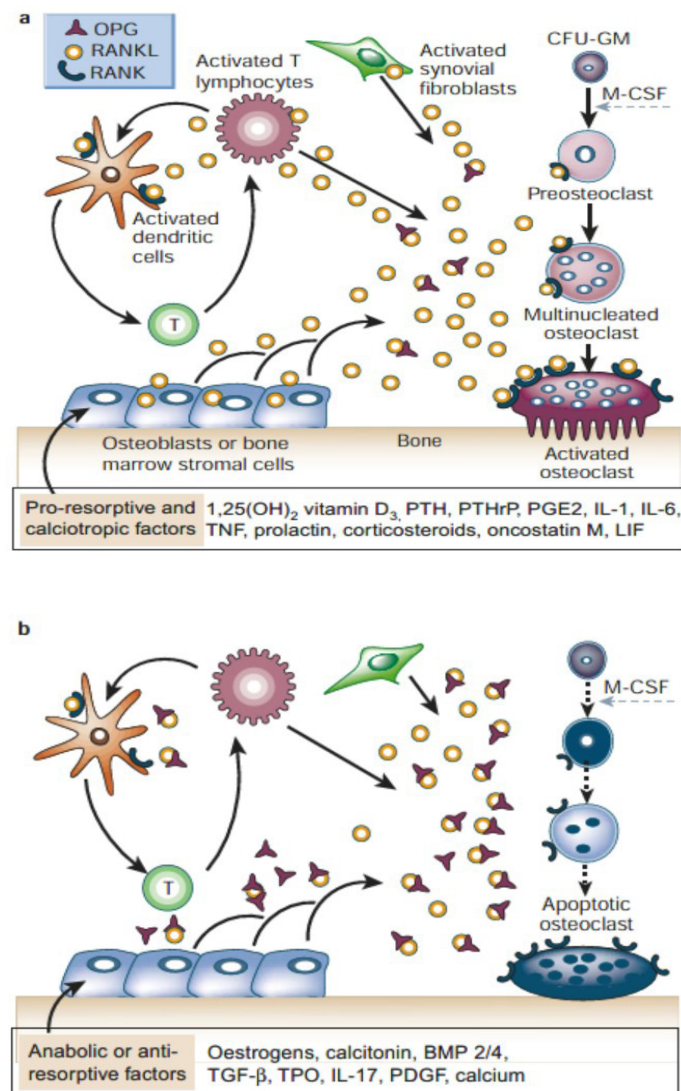


Image taken from Boyle et al. 2003.⁽⁸⁾

Sources of RANK, RANKL, OPG and their role in bone metabolism.

RANK is expressed in osteoclast precursor cells. It has been found in cells of the bone marrow, spleen, intestine, thymus, kidney, brain, and lung,⁽⁹⁾ megakaryocytes,⁽¹⁰⁾ monocytes,⁽¹¹⁾ dendritic cells, CD4+ T cells, foreskin fibroblasts, and some Hodgkin's lymphomas.⁽¹²⁾

This protein induces osteoclast differentiation and also has the following functions: endothelial cell survival, dendritic cell maturation and function, chemotaxis, and enhancement of T-cell growth.⁽¹³⁾

In 2012, Giannopolou et al. used immunohistochemistry to study RANK, RANKL, and OPG positivity in periodontal tissues from patients with periodontitis. RANK expression was significantly higher than OPG expression in the periodontal pocket epithelium. In 100% of the cases, over 60% of the stained cells were positive for RANK and only 42% for OPG. In the inflammatory cell infiltrate, the expression of RANK and RANKL showed significant differences compared to the OPG ($p=0.001$ and 0.006 , respectively). OPG staining presented from 30–59% of labeled cells in 2 out of 14 (14.3%) cases and less than 29% of stained cells in 7 out of 14 (50%) cases, whereas 9 out of 14 (64.3%) cases for RANK expression and 8 out of 14 (57.1%) cases for RANKL expression had over 60% of labeled cells. They found no significant effect of tobacco consumption on RANK, RANKL, and OPG expression.⁽¹⁴⁾

Even at present, the sources of RANKL during physiological bone remodeling have not been elucidated. However, evidence suggests that the source changes throughout development so that hypertrophic chondrocytes provide RANKL for trabecular removal during endochondral bone formation, and trabecular osteocytes provide much of the RANKL needed for bone remodeling in response to mechanical stress.^(15,16) More than 30 years ago, receptors for osteoclastogenic hormones, such as parathyroid hormone (PTH), were found in cells with osteoblastic characteristics but not in osteoclast progenitors. Therefore, a theory was developed: osteoblasts control os-

teoclast function and differentiation.⁽¹⁷⁾ Further work showed that cell lines with osteoblastic characteristics or cell preparations rich in osteoblast progenitors support osteoclast formation in co-culture with osteoclast progenitors.^(18,19) Macrophage Colony Stimulating Factor or M-CSF and RANKL are factors produced by supporting cells that are essential for osteoclast differentiation in vivo. This explains the need for osteoblastic cells for osteoclast formation.^(20,21)

Osteocytes would also be an essential source of the RANKL that controls osteoclast formation during cancellous bone remodeling.⁽²²⁾ These form at various skeletal sites for different purposes and require different supporting cells in each case. Osteocyte RANKL is not required for tooth eruption or calcified cartilage resorption during endochondral bone formation. This leads to the conclusion that other cell types must supply the RANKL required for osteoclast formation in these processes.⁽²³⁾ Therefore, the role of osteocyte RANKL may be limited to physiological bone remodeling.

Several studies indicate that both T and B lymphocytes express RANKL.^(24–26) T lymphocytes would be the cells that mediate excessive bone resorption in inflammatory bone resorption by producing RANKL. This is a membrane-bound protein in osteoblasts, but its membrane-bound expression in T lymphocytes would be limited, and most of the protein in these cells could be activated in soluble form.⁽²⁷⁾

Teng⁽²⁸⁾ found RANKL expression in T lymphocytes from patients with aggressive periodontitis infected with *A. actinomycetemcomitans*. In fact, T lymphocytes isolated from the periodontitis lesion expressed RANKL.^(27,29) Authors such as Han, Kawai, and Vernal^(30–32) specified that Th1, Th17, and B lymphocytes function as the primary source of RANKL. In addition, all mesenchymal resident cells can also express this protein when attacked by bacteria.^(33,34) Strikingly, T-regulatory cells can attenuate their expression by other activated T cells.⁽³⁵⁾

A 2011 study by Belibasakis et al. demonstrated that exposure of T cells to *P. gingivalis* in vitro induced the production of RANKL, but not OPG. In addition, it also stimulated the production of the key inflammatory mediator PGE2. This indicates that *P. gingivalis* regulates T-cell function in a manner that promotes osteoclastogenesis and bone resorption.⁽³⁶⁾

Liu (2003) examined mRNA expression for RANKL at the cellular level using in situ hybridization and found that it expressed in inflammatory cells, mainly lymphocytes and macrophages.⁽²⁶⁾ In addition, proliferating epithelium in the vicinity of inflammatory cells expressed high RANKL mRNA levels.⁽²⁶⁾ Confocal microscopy analysis showed that both B and T cells, but not monocytes or fibroblasts, are the primary cellular source of RANKL in the bone lesion of periodontal disease.⁽³¹⁾ However, other cells may also be an important source in this process because they regulate its expression indirectly through the production of proinflammatory cytokines.⁽³⁷⁾ Activated B cells produce IgG as host protection but may also contribute to bone destruction by producing RANKL because this type of lymphocyte appears profusely in inflamed periodontal tissues.^(38,39) This occurs because B cells respond excessively to abundant bacterial antigens through the IgG antibody.^(40–42) This may contribute to the development of immune-mediated periodontal bone resorption,^(43,44) in addition to expressing high amounts of RANKL in gingival tissue with periodontitis.⁽²⁵⁾ B lymphocytes had a strong RANKL expression in animals injected with *A. actinomycetemcomitans*. Administering OPG-Fc to this model decreased bone loss,⁽³⁰⁾ suggesting that resorption related to B-lymphocyte stimulation is RANKL-dependent.^(30,45)

Crotti et al. (2003) worked with gingival tissue samples from patients with periodontitis and found that approximately 30% of CD3 T lymphocytes located in the inflammatory infiltrate expressed RANKL. They also found that CD68-positive macrophages expressed RANKL

in about 50% of cases and that reduced numbers of B CD22 lymphocytes appeared in the inflammatory lesion, and in the latter case, there was no RANKL expression.⁽⁴⁶⁾

In gingival tissue from patients with periodontitis subjected to immunohistochemistry, inflammation (moderate or strong) was associated with increased RANK and RANKL expression in inflammatory cells ($p=0.07$ and 0.08 , respectively). In fact, 9 out of 14 cases (64.3%) and 8 out of 14 cases (57.1%) showed over 60% of stained cells in the periodontal pocket area for RANK and RANKL, respectively. OPG expression was not related to inflammation.⁽¹⁴⁾ In 2009, Dereka et al.⁽⁴⁷⁾ also used immunohistochemistry to compare RANKL expression in tissues from diseased and healthy patients after non-surgical periodontal treatment. In the healthy samples, they found approximately 50% positivity in the infiltrate and less in the epithelium. In the samples with the disease, they found 75% positivity in the epithelium and 87% in the inflammatory infiltrate.

These results suggest that increased RANKL production may be associated with bone resorption and that lymphocytes are a major source of RANKL in periodontitis tissue. The expression of RANKL and osteoprotegerin on fibroblastic cells, including osteoblasts, periodontal ligament fibroblasts, and gingival fibroblasts, are mainly examined using cultured cells in vitro.⁽⁴⁸⁾

At a general level, OPG expression has been detected in cells of the skin, bones, arteries, and gastrointestinal tract,⁽³⁴⁾ endothelial cells of the macro- and micro-vasculature, and smooth muscle cells.^(49–51) There is evidence of the role of OPG in the vascular system, with expression observed in the heart, arteries, and veins.⁽⁵²⁾ Several growth factors and cytokines increase OPG expression in vascular smooth muscle cells, including TNF- α , IL-1 β , fibroblast growth factor, platelet-derived growth factor, and angiotensin II.^(51,53) Several of the above cytokines play an essential role in the pathogenesis of periodontal disease.⁽³⁾

OPG has been localized in specific secretory gra-

nules known as Weibel-Palade bodies (CWP) of endothelial cells, which also contain the glycoprotein von Willebrand's factor (vWF), and the adhesion molecule P-selectin.⁽⁵⁰⁾ Within CWP, OPG is associated with vWF, but not with P-selectin. When cytokines TNF- α and IL-1 β in vitro stimulate the endothelial cells, this complex is secreted into the surrounding medium. Additionally, there is evidence that OPG promotes leukocyte adhesion to endothelial cells, and this may be mediated by OPG interactions with the endothelial cell surface.⁽⁵⁴⁾

Connective tissue itself could be a source of OPG in gingival tissue. Nagasawa et al.⁽²⁹⁾ demonstrated that gingival fibroblasts produce OPG in response to LPS stimulation. The induction of gingival fibroblasts may be a defense mechanism that inhibits alveolar bone destruction during periodontal inflammation.

Periodontal pathogenic bacteria affect the RANKL/OPG ratio in periodontal ligament fibroblasts differently from gingival fibroblasts. RANKL and OPG can be produced by periodontal ligament fibroblasts, with stimulation of periodontal pathogens increasing the RANKL/OPG ratio. Also, gingival fibroblasts produce OPG, but not RANKL, with the stimulation of periodontal pathogens decreasing the RANKL/OPG ratio.

⁽³⁴⁾ Sakata et al. performed RT-PCR and detected OPG mRNA in human gingival fibroblasts, periodontal ligament fibroblasts, and dental pulp cells but not in human gingival keratinocytes.

⁽⁵⁵⁾ IL-1 β and TNF- α increased the OPG mRNA in periodontal ligament fibroblasts, but IL-6 and TGF- β had little effect on OPG mRNA levels.⁽⁵⁵⁾

Lu et al. found low OPG expression in the inflammatory infiltrate of the gingival tissue of patients with periodontitis.⁽⁵⁶⁾ These proteins are also associated with various systemic diseases, such as osteoporosis⁽⁵⁷⁾ and rheumatoid arthritis⁽⁵⁸⁾ (among others), which in turn are associated with periodontal disease.^(59,60)

Discussion

RANK, RANKL and OPG levels in health and disease

Lu et al. compared RANKL and OPG expression in healthy and periodontitis patients. They found that immunohistochemical analysis showed no difference in the distribution of OPG-positive cells in the connective tissue of the healthy and diseased groups. OPG-positive cells were irregularly distributed in the diffuse inflammatory zone of the gingival connective tissue of the diseased samples. However, RANKL-positive cells were widely distributed in the connective tissue of patients with chronic periodontitis [mean periodontitis [mean $\pm$ standard deviation (SD), 53.70% $\pm$ 15.48%]. The Mann-Whitney U Test showed a significant difference $p < 0.01$ in the percentage of RANKL-positive cells between tissues from healthy and diseased individuals, but not for OPG levels.⁽⁵⁶⁾ In this study, the samples were taken from patients who had received no periodontal treatment within the three months preceding the study. The authors did not provide information on whether the patients had received dental scaling and smoothing treatment before surgical therapy.⁽⁵⁶⁾

Immunohistochemistry was performed, and RANKL was found to be associated with lymphocytes and macrophages. It was expressed at significantly higher levels in tissue from patients with periodontitis.⁽⁶¹⁾ Conversely, very few RANKL-expressing cells were present in healthy gingival tissue.^(31,62) Over 50% of T cells and 90% of B cells expressed RANKL in periodontitis tissue, whereas less than 20% of B or T cells showed RANKL expression in healthy gingival tissue. RANKL, but not OPG, concentrations were significantly higher in periodontitis tissue than healthy tissue.⁽³¹⁾ The samples were taken during surgical procedures; the authors did not specify if the participants had received any periodontal therapy before this procedure.

The immunohistochemical analysis of connective tissue from rats subjected to an experimen-

tal periodontitis process by ligation revealed a higher number of RANK and RANKL positive cells in the group of animals that had a 60-day periodontitis evolution compared to the same group at the beginning of the study and at 15 days (0 and 15 days of evolution respectively) ($p < 0.05$). The three groups had no significant differences in terms of the number of OPG-positive cells. The development of periodontitis was associated with increased levels of cells that are positive markers for bone resorption (RANK and RANKL). OPG possibly decreased due to the increase in lymphocytes observed in the infiltrate during the experimental period.⁽⁶³⁾

RANKL was highly expressed in leukocytes forming large cellular infiltrates in the granulation tissue of periodontitis lesions. Mild staining was also seen in extracellular tissue, indicating a connection between RANKL and connective tissue. RANKL expression was weaker in tissues without periodontitis.⁽⁴⁶⁾

Staining for OPG demonstrated that OPG was associated with cells lining blood vessels in both types of tissues; however, staining was much stronger in non-periodontitis tissues. Dual labeling was carried out for RANKL and T cells (CD3), B cells (CD22), and macrophages (CD68). Many T (CD3 expressing) cells were seen in the mononuclear cell aggregates in the periodontitis tissues. Approximately 30% of these cells expressed RANKL, and many non-CD3 cells also expressed RANKL. Fewer cells expressing CD3 were seen in the tissues obtained from non-periodontitis patients.⁽⁴⁶⁾

Dual staining for RANKL and CD68 showed that macrophages were present in periodontitis tissues, but these were less abundant than the CD3 cells. Approximately half of the cells expressing CD68 also expressed RANKL. In contrast, fewer CD68-expressing cells were identified in non-periodontitis tissues. Small numbers of CD22-positive cells were seen in non-periodontitis tissues, but RANKL was not expressed by any of these cells. Antibodies directed against Factor VIII were used to verify that cells expressing OPG in

the blood vessels were endothelial cells. Only weak expression of OPG protein was associated with Factor VIII expressing cells in the periodontitis tissues. In contrast, OPG was strongly expressed by Factor VIII expressing cells in the non-periodontitis tissues.⁽⁴⁶⁾ In this study, the samples were taken during a surgical procedure; the authors did not specify if the participants had received any treatment before the procedure.

Other techniques have been used to study the behavior of these proteins in periodontal disease. Bostanci et al. wanted to determine differential gene expression of RANKL and OPG in different clinical forms of periodontal disease.⁽⁶⁴⁾ They performed real-time quantitative PCR to study gingival tissues from 9 healthy patients and 41 patients with periodontitis. RANKL was not detected in any samples of healthy tissue, but was detectable in 25% of tissue samples from patients with gingivitis, in 54% of tissue samples from patients with chronic periodontitis, in 75% of tissue samples from patients with generalized aggressive periodontitis, and in 60% of tissue samples from patients with chronic periodontitis that received immunosuppressant therapy. In contrast, all of the tissue samples expressed detectable OPG levels. Compared with healthy tissues, RANKL expression was significantly ($p < 0.05$) induced in all periodontitis groups but not in gingivitis. In addition, RANKL levels in generalized aggressive periodontitis were much higher than in chronic periodontitis. Compared with healthy tissues, OPG expression was decreased in all disease groups, but this was statistically significant only in chronic periodontitis. Moreover, OPG levels in chronic periodontitis were 16-fold lower than in generalized aggressive periodontitis. Healthy tissue samples were taken during extraction and crown-lengthening procedures in healthy subjects before non-surgical periodontal therapy.⁽⁶⁴⁾

The level of RANKL mRNA in advanced periodontitis was higher than in moderate stages of disease or in healthy sites (Kruskal-Wallis

test, $p < 0.042$). Interestingly, it appears that the RANKL mRNA level in moderate periodontitis was relatively lower than in healthy sites. This might reflect moderate inflammation in normal gingival tissues obtained from healthy sites. The results showed that healthy sites expressed higher levels of OPG mRNA than in advanced periodontitis. The level of OPG mRNA in moderate periodontitis was significantly lower compared to other groups. Significant differences were found ($p < 0.05$) between all groups. RANKL is highly expressed in inflammatory areas and is associated with increased RANKL/OPG ratio compared to healthy subjects. Tissue samples obtained from diseased individuals were taken during perio-

dontal surgery procedures. The authors did not specify if the participants had received any periodontal therapy before this procedure.⁽²⁶⁾

Conclusions

RANK, RANKL, and OPG may play a major role in the pathogenesis of periodontal disease, as has been demonstrated for other diseases affecting bone metabolism. Several factors related to the investigation methodology, such as the timing of the sampling, must be considered. Therefore, more evidence and comparable methodology are needed to establish a clear relationship between these proteins and bone resorption associated with periodontitis.

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Conflict of interest declaration:

The authors have no conflict of interest regarding the publication of this paper.

Authorship contribution

1. Conception and design of study
2. Acquisition of data
3. Data analysis
4. Discussion of results
5. Drafting of the manuscript
6. Approval of the final version of the manuscript.

NA has contributed in 1, 2, 3, 4, 5, 6.

GV has contributed in 4, 5, 6.

VPP has contributed in 4, 5, 6.

LRB has contributed in 4, 5, 6.

RBM has contributed in 4, 5, 6.

Acceptance note:

This article was approved by the Editorial Committee of Odontoestomatología.