The RANKL: Osteoprotegerin (OPG) ratio as a new biomarker for coronary artery disease

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Abstract

Nasolacrimal There is a strong need for biomarkers to identify patients at risk for future cardiovascular events related with progressive atherosclerotic disease. Ideally, increasing knowledge of the mechanisms of atherosclerotic plaque destabilization should be translated in clinical practice. Systemic approaches are pursued to discover serum biomarkers that are applicable to define patients at risk for future cardiovascular events. Elevation in inflammatory markers, such as C-reactive protein, predicts outcomes of patients with acute coronary syndromes. Osteoprotegerin (OPG) protects the skeleton from excessive bone resorption by binding to receptor activator of nuclear factor-κB ligand (RANKL) and preventing it from binding to its receptor, receptor activator of nuclear factor-κB. Emerging evidence from in vitro studies, mouse genetics and clinical studies attributed to OPG an important role in vascular biology. But conflicting results have been obtained about association of serum level of OPG or RANKL with coronary artery disease (CAD). Based on their role in inflammation and matrix degradation and the fact that atherosclerotic plaque formation is an inflammatory process; we hypothesized that RANKL:OPG ratio could be a better biomarker for CAD.

Keywords
Osteoprotegerin, RANKL, Cardiovascular disease
Introduction

Every year, >19 million patients worldwide experience a sudden cardiac event (acute coronary syndromes and/or sudden cardiac death). A large portion of this population has no prior symptom. There is considerable demand for diagnosis and treatment of the pathologic conditions that underlie these sudden cardiac events. Traditional risk factors, combined with risk scores such as the Framingham score, can predict outcome for groups of patients but lack discriminative power to identify individual subjects who are at risk to experience a cardiovascular event in the near future. In other words, some people experience a cardiovascular event while by Framingham scores they were not considered to be at high risk group (1, 2). It has been established that acute clinical manifestations of atherosclerotic disease, such as myocardial infarction or stroke, are not the result of slowly progressing luminal narrowing. Instead, these are a consequence of acute disruption (rupture or erosion) of the atherosclerotic plaque, leading to exposure of thrombogenic plaque components to the bloodstream, with superimposed thrombus formation. The newly formed thrombus suddenly accelerates the degree of luminal stenosis or may totally occlude the lumen, giving rise to a myocardial infarction. On the other hand, superimposed thrombus can cause distal embolization, for example, in symptomatic atherosclerotic carotid disease (1, 3).

Plaque rupture is the most common type of plaque complication, accounting for ~70% of fatal acute myocardial infarctions and/or sudden coronary deaths. Several retrospective autopsy series and a few cross-sectional clinical studies have suggested that thrombotic coronary death and acute coronary syndromes are caused by the plaque features (2, 3). It is crucial to define risk factors and biomarkers with strong prognostic and diagnostic value. Serologic biomarkers are being investigated and, if proven, could be a future target for anti-tumor therapy (4, 6).

Osteoprotegerin (OPG) is expressed in many tissues apart from osteoblasts, including heart, kidney, liver, spleen, and bone marrow. Its expression is regulated by most of the factors that induce RANKL expression by osteoblasts. Although there are contradictory data, in general, upregulation of RANKL is associated with downregulation of OPG, or at least lower induction of OPG, such that the ratio of RANKL to OPG changes in favor of osteoclastogenesis. Many reports have supported the assertion that the RANKL/OPG ratio is a major determinant of bone mass. OPG also appears to protect large blood vessels from medial calcification, based on the observation of renal and aortic calcification occurring in OPG knockout mice. Furthermore, the absence of OPG in OPG/apolipoprotein E double knockout mice accelerates the calcific atherosclerosis that develops in apolipoprotein E knockout mice, suggesting that OPG protects against this complication of atherosclerosis (4-6).
Hypotheses

The numerous studies have attempted to use serum level of OPG or RANKL as a predictor of subclinical atherosclerosis or acute coronary syndrome but conflicting results have been obtained. But, determination of either OPG or RANKL serum level could not be a good predictor of cardiovascular disease. In our view, inhibitory effect of OPG on RANKL is the main cause of these disconformities. Therefore, we hypothesized that concomitant determination of both serum level of RANKL/OPG and calculation of RANKL:OPG ratio could be a robust biomarker for subclinical atherosclerosis or acute coronary syndrome. We evaluated the originality of our idea by searching the important resources of medical articles such as Pubmed and Scopus. We didn’t find identical idea in this resource and relevant articles were reviewed in the next section: "Evaluation of the hypothesis".

Evaluation of the hypotheses

Several groups investigated the effect of OPG and RANKL on promotion or inhibition of vascular calcification in vitro or in vivo and linkage between serum levels of both with incidence of atherosclerosis or ACS. Following is a review of these reports.

OPG-deficient (OPG-/-) animals develop osteoporosis. Surprisingly, the majority of the OPG-deficient mice developed severe medial calcifications of the renal arteries and the aorta that led to aneurysm formation and lethal vessel rupture and hemorrhage. The vascular abnormalities were completely abolished using an OPG transgene approach, but not following postnatal administration of OPG protein, suggesting local production is important in the inhibition of vascular calcification. The protective role of OPG in vascular calcification is also underscored by a rat model of vascular calcification, in which treatment with warfarin, an inhibitor of vitamin K-dependent γ-carboxylation, or supraphysiologic doses of vitamin D are used to induce diffuse vascular calcification. In these two models, simultaneous administration of OPG fusion protein with mineralization-inducing agents prevented arterial calcification (4, 7-11). OPG expression in human vascular smooth muscle cells (VSMCs) was significantly reduced in response to the ligand-activated PPARγ. This study provides a new insight into the understanding of the role of PPARγ in atherosclerosis and hypertension (12).

OPG has a protective role in the progression and calcification of advanced atherosclerotic lesions in the innominate arteries of apoE/-/- mice (13). A recent study demonstrated lowered serum OPG levels in persons with subclinical echogenic carotid plaques and identified an inverse relationship between serum OPG and plaque echogenicity (14).

In contrast, other studies represent association of high OPG level to cardiovascular diseases. Studies have shown an association between OPG levels and CAD in humans. In patients with cerebrovascular disease, OPG levels are independently associated with cardiovascular mortality but not with bone mineral density. In patients with stable angina, OPG levels are associated with significant coronary artery narrowing (9) and high expression of OPG was observed in few inflammatory cells present in the fibrocalcific plaques. (15) Plasma OPG measurements were correlated with baseline/follow-up coronary artery calcification (CAC) severity and predicted CAC progression (16) and also was increased with the severity of CAD in age-matched men and are higher in men with diabetes mellitus.(17). OPG was detected around apoptotic calcified regions of atherosclerotic arteries (18). Concentration of OPG increased within unstable atherosclerosis (19). Other reports identify high serum OPG as a risk factor of progressive atherosclerosis and incident cardiovascular disease (20), asymptomatic CAD in type 2 diabetics (21), developed HF after AMI (22), CAC and aortic plaque (23), subclinical atherosclerosis (24) and atherothrombotic ischemic stroke (25). Serum OPG level was related to the severity of stenotic coronary arteries and serum CRP levels (26). Kidney transplantation decreased OPG as an risk factor for vascular calcifications.(27). OPG levels are increased in ST elevation AMI (acute myocardial infarction) within 1 h of infarction. (28). Increased serum concentrations of OPG are present in patients with rheumatoid arthritis and are independently associated with severity of coronary-artery calcification in those with long-standing disease (29).

Increased expression of the RANKL in clinical and experimental atherosclerosis was reported, with enhanced T-cell expression of RANKL as an important feature of unstable disease (30). Large-scale epidemiological support for role of RANKL in cardiovascular disease was also reported. In the absence of a significant association between RANKL and atherosclerosis, the idea that RANKL promotes plaque destabilization and rupture is a highly appealing concept (12).

The functional importance of these data is difficult to interpret since the authors did not indicate OPG levels in relation to RANKL availability, which is required to assess its net biological effect on bone and vasculature, because OPG functions as a soluble “decoy” receptor that binds and inhibits RANKL (9). OPG may act as an active, although perhaps incomplete, self-defense
mechanism to counteract excessive atherosclerosis and calcification. So the concomitant measurement of RANKL/OPG could be a valuable biomarker for cardiovascular disease. Furthermore, usefulness of this ratio in prediction of some other pathologic conditions has been established. RANKL/OPG ratio is increased in severe osteolysis, mainly in primitive bone tumors and in bone metastases (31). It is demonstrated that at the time of diagnosis of juvenile dermatomyositis, untreated patients have an elevated RANKL/OPG ratio compared to that of healthy children (32). It is concluded that the baseline RANKL/OPG ratio has distinctive effects on 5-year radiographic progression in patients with early active rheumatoid arthritis (33).

Discussion and conclusion

Despite advances in risk factor management, each year, 12 million patients die worldwide because of a cardiovascular event such as a myocardial infarction or a cerebrovascular accident. Histologic examination of atherosclerotic plaques obtained postmortem or during endarterectomy has identified plaque characteristics associated with adverse clinical events. When these characteristics are found in asymptomatic plaques, they are thought to confer vulnerability to becoming symptomatic of the plaque (1). Yet, even with aggressive thrombolytic, anticoagulant, and/or antiplatelet agents or interventional therapy, patients with acute coronary syndrome (ACS) still have a 12% to 16% incidence of major cardiac events at 4 to 6 months after hospital discharge (34).

Newly available biochemical markers, such as troponins, have improved our ability to detect cardiac injury and, thanks to the high sensitivity and specificity of this type of marker, now considered a “gold standard” in identifying myocardial infarction (MI), the percentage of patients with a diagnosis of MI has increased by a magnitude ranging from 23 to 195%. However, patients with normal troponin values are not necessarily free of the risk of major cardiac events (35). In fact, recent investigations have indicated that overall patient risk may be assessed earlier than before, and subjects at a higher risk of adverse cardiac events identified, thanks to the increased use of biochemical markers upstream from markers of necrosis, cellular adhesion molecules, acute phase-reactants (Creactive protein), plaque destabilization (Myeloperoxidase), and rupture (sCD40L), and markers of ischemia (Ischemia-Modified Albumin) and myocardial stretch (natriuretic peptides (1, 35, 36). The presence and extent of vascular calcification is highly correlated with cardiovascular disease.

RANKL and OPG are produced by various skeletal and vascular cell types in vitro, and are expressed in the skeleton and vascular wall in vivo. While the producing (osteoblastic cells) and target cells (osteoclastic cells) of RANKL and OPG within the skeleton plausibly explain coupling of bone formation to bone resorption, the characterization of the specific target cells for RANKL and OPG within the vascular system is just emerging. An important and unifying hypothesis is that the immune system with RANKL-producing T cells and RANK-expressing dendritic cells links bone metabolism and vascular biology, and that RANKL and OPG in both systems are modulated by ubiquitous cytokines (37). Furthermore, osteoblast-like cells have been indentified in vascular walls originated from vascular smooth muscle cells and share properties with bone osteoblast cells. It seems that vascular smooth muscle cells migrate from intima to media during progression of atherosclerotic lesions and during this migration their phenotype is changed to those of osteoblast cells (9).

Soon after development of sensitive detection systems for OPG protein, several studies have evaluated the role of circulating OPG serum levels as biochemical marker of bone turnover. But unexpected results are obtained showing high serum concentration of OPG in CAD (21, 23) while, earlier evidence established the inhibitory effects of OPG on vascular calcification (8). Recent comprehensive study suggested RANKL as a biomarker for ACS (12).

In conclusion, according to theses paradoxical reports, it seems that in ACS and some other pathological conditions, concentrations of RANKL and OPG are increased concomitantly, and OPG acts as a self-defense mechanism. In other words, the biological effects of OPG are opposite of the RANKL mediated effects, because OPG acts as a soluble inhibitor that prevents RANKL interaction and subsequent stimulation with its receptor, RANK and the physiological role of OPG may be dependent on its levels relative to RANKL. Therefore we hypothesized that measurement of RANKL:OPG ratio could be a better biomarker of ACS than serum level of either RANKL or OPG (Figure 1). Now, commercial ELISA kits to measure the plasma concentration of both OPG and RANKL are available. This is make it so easy to determine the RANKL:OPG ratio in plasma of normal group and CAD patients in human subjects.
Fig 1. Schematic diagram of RANKL and OPG role in the plaque stability. Soluble RANKL (as indicated in black color) and OPG (as indicated in gray color) are secreted in the atherosclerotic plaque and in the blood stream mainly by smooth muscle cells and endothelial cells. Soluble RANKL promotes OCL precursor (mainly monocytes/macrophages, dendritic cells, and SMCs) differentiation into OCL cells (as indicated in white color). OPG neutralizes the action of RANKL. The balance between these two soluble molecules regulates the bone resorption in calcified plaques, which is correlated to plaque rupture. A: Concentration of both RANKL and OPG are low in normal condition. OPG act as a soluble receptor of RANKL and inhibits effects of RANKL on its receptors. B: Once RANKL level is elevated OPG level is also elevated as a compensatory mechanism. C: In pathological condition OPG level is elevated but it is not enough to neutralize the action of RANKL because RANKL level is more elevated. Therefore in this condition the RANKL:OPG ratio is significantly increased.
References


