Serum Osteoprotegerin Levels Are Associated With the Presence and Severity of Coronary Artery Disease

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Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor family, has been identified as a regulator of bone resorption.1 Recently, it has been demonstrated that OPG is expressed by a variety of tissues, including the cardiovascular system (heart, arteries, veins), lung, kidney, and immune tissues, as well as bone,1,2 and that the expression and production of OPG are regulated by various cytokines and hormones.3 It has been shown that OPG-deficient mice develop severe osteoporosis and medial arterial calcification of the aorta and renal arteries,4 and that the development of osteoporosis and arterial calcification was completely prevented by restoration of the gene.5 OPG is also expressed in vascular cells such as coronary smooth muscle cells and endothelial cells in vitro.6 In endothelial cells, OPG has been demonstrated to act as an anti-apoptotic factor.7 Moreover, OPG immunoreactivity was demonstrated not only in the nondiseased vessel wall, but also in early atherosclerotic lesions in human tissues.8 These findings suggest that OPG may play an important role in the development of vascular disease. A recent clinical study reported that there is a significant correlation between elevated serum OPG levels and cardiovascular mortality,9 suggesting that OPG may contribute to the progression of coronary artery disease (CAD). In this study, we assessed the severity of CAD by coronary angiography and examined whether serum OPG levels are associated with the progression of CAD.

Methods

The present study involved 201 patients who underwent coronary angiography. All patients fulfilled the criteria of stable chest pain and/or signs of myocardial ischemia on exercise electrocardiography for clinical indication for cardiac catheterization. Patients with an acute coronary syndrome were excluded. At the time of a physical examination, blood pressure, body mass index (BMI), and a hematological and biochemical profile were determined. Age and history of cigarette use were assessed through an interview preceding the physical examination. Ninety-six subjects were receiving antianginal drug (isosorbide dinitrate). Diabetes was considered present if a patient was treated with insulin or oral agents or had a fasting glucose level ≥126 mg/dL (7.0 mmol/L). Twenty-seven subjects were taking antidiabetic drug treatment; 7 subjects were taking insulin injections and 20 were taking sulfonylurea (20 subjects). Hypertension was defined by systolic blood pressure ≥140 mm Hg.

Background—Osteoprotegerin (OPG) is a secretory glycoprotein that belongs to the tumor necrosis factor receptor family. OPG-deficient mice develop severe osteoporosis and medial arterial calcification of the aorta and renal arteries. OPG immunoreactivity was demonstrated in the normal blood vessels and in early atherosclerotic lesions. A recent clinical study suggests that there is a significant correlation between elevated serum OPG levels and cardiovascular mortality. We examined whether serum OPG levels are associated with the progression of coronary artery disease (CAD).

Methods and Results—Serum OPG levels were examined in 201 patients who underwent coronary angiography because of stable chest pain. The number of diseased vessels was used to represent the severity of CAD. Serum OPG levels were measured by ELISA and were significantly greater in patients with significant stenosis of the coronary arteries than in those without stenosis. As the severity of CAD increased, there was a significant increase in serum OPG levels. Serum OPG levels were 0.94±0.34, 1.04±0.38, 1.19±0.38, and 1.44±0.54 ng/mL (medians 0.91, 0.99, 1.09, and 1.37) for the subjects with normal coronary arteries or luminal irregularities, 1-vessel disease, 2-vessel disease, and 3-vessel disease, respectively. Multivariate logistic regression analysis revealed that serum OPG levels were significantly associated with the presence of CAD (odds ratio, 5.2; 95% confidence interval, 1.7 to 16.0).

Conclusions—Our data show that serum OPG levels are associated with the presence and severity of CAD, suggesting that OPG may be involved in the progression of CAD. (Circulation. 2002;106:1192-1194.)

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diastolic blood pressure $\geq 90$ mm Hg, the current use of antihypertensive treatment, or a combination of the 3. One hundred fifty subjects were receiving antihypertensive treatment with a calcium channel blocker (78 subjects), an angiotensin-converting enzyme inhibitor (11 subjects), $\beta$-blockers (7 subjects), diuretics (2 subjects), or a combination of these drugs (52 subjects). Hyperlipidemia was defined as total cholesterol level $>240$ mg/dL (6.2 mmol/L), the current use of lipid-lowering treatment, or both. Fifty-two subjects were receiving lipid-lowering drug (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor). Written informed consent was obtained from all patients.

**Coronary Angiography**

Quantitative coronary angiography (QCA) was performed with the use of an automated edge detection system Cardiovascular Measurement System (MEDIS) by experienced cardiologists who were blinded to the clinical and biological data. Significant coronary stenosis was defined as a $\geq 50\%$ diameter narrowing on the basis of the QCA measurement. The variability in repeat measurements of percent diameter stenosis was 2.7%. The severity of CAD was represented as the number of diseased vessels.

**Serum OPG Measurement**

Fasting serum samples were collected and stored at $-80^\circ$C until use. Serum OPG levels were determined using a sandwich ELISA (Cosmo Bio) as previously described. $^{10}$ Monoclonal antibody against human OPG, designated clone OI-19, was used to capture OPG from serum. Captured OPG was detected with peroxidase-labeled anti-human OPG monoclonal antibody, designated clone OI-4, and tetramethylbenzidine substrate. All samples were measured in duplicate and the results were averaged. The detection limit of this assay system was 0.03 ng/mL, and the intra- and interassay coefficient of variation values were <3.2% and <5.4%, respectively.

**Statistical Analysis**

All data are presented as mean $\pm$ SD. Comparisons between groups for study variables were done using the unpaired Student’s $t$ test for normally distributed parameters and the Mann-Whitney $U$-test for non-normally distributed data. The relationships between continuous variables were evaluated by linear regression. Differences between the 4 groups according to the extent of coronary angiographic disease were analyzed by Kruskal-Wallis test and Dunn’s test for multiple comparisons. We performed multivariate logistic regression analysis to adjust risk factors. The dependent variable was the presence of CAD. The independent variables were age, sex, BMI, hypertension, diabetes, hyperlipidemia, current smoking, and serum OPG levels. Odds ratios (ORs) are presented with 95% confidence intervals (CIs).

**Results**

The study group included 166 men and 35 women. Their ages ranged from 28 to 80 years (mean age 63 $\pm$10 years). The mean value of BMI was 23.6 $\pm$3.2 kg/m$^2$. The prevalence of cardiac risk factors was 74% for hypertension, 32% for diabetes, 26% for hyperlipidemia, and 36% for current smoking. On the basis of angiographic analysis, 140 (70%) of the patients were characterized to have significant stenosis. We found 59 patients with 1-vessel disease, 48 patients with 2-vessel disease, 33 patients with 3-vessel disease, and 61 patients with no diseased vessel or luminal irregularities. All patients were normocalcemic, and there was no significant difference in serum calcium levels among the 4 groups. With the exception of BMI, traditional CAD risk factors (age, male sex, hypertension, diabetes, hyperlipidemia, and current smoking) were significantly associated with the presence of CAD by multivariate analysis.

**Discussion**

In this study, we found that serum OPG levels were significantly increased as the severity of CAD increased, and that

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.0</td>
<td>1.0–1.1</td>
<td>0.080</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.7</td>
<td>1.1–6.7</td>
<td>0.037</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>0.9</td>
<td>0.8–1.0</td>
<td>0.173</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.6</td>
<td>1.6–8.1</td>
<td>0.003</td>
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<tr>
<td>Diabetes</td>
<td>2.7</td>
<td>1.1–6.8</td>
<td>0.031</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>3.7</td>
<td>1.4–10.0</td>
<td>0.009</td>
</tr>
<tr>
<td>Current smoking</td>
<td>2.4</td>
<td>1.0–5.6</td>
<td>0.047</td>
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<tr>
<td>OPG, ng/mL</td>
<td>5.2</td>
<td>1.7–16.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Serum OPG levels in subjects with no-, 1-, 2-, and 3-vessel disease. The central line represents distribution median, the boxes span from 25th to 75th percentiles, and the error bars extend from 10th to 90th percentiles. No-VD indicates normal coronary arteries or luminal irregularities; 1-VD, 1-vessel disease; 2-VD, 2-vessel disease; and 3-VD, 3-vessel disease including 2 cases of left main disease.

To examine the relationship between circulating OPG and CAD, we measured serum OPG levels in all 201 subjects. The mean serum level of OPG was $1.11 \pm 0.43$ ng/mL, with a range of 0.22 to 3.08. Serum OPG levels were correlated with age ($r=0.20; P<0.01$). There was no correlation between serum OPG levels and BMIs. We found no difference in serum OPG levels when stratifying the results by other CAD risk factors including sex, hypertension, diabetes, hyperlipidemia, and current smoking. Serum OPG levels were significantly greater in patients with clinically significant stenosis of the coronary arteries than in those without stenosis. Moreover, increasing serum OPG levels were related to the severity of CAD (Figure).

Multivariate logistic regression analysis revealed that serum OPG levels were independently associated with the presence of CAD (Table). A 1 ng/mL increase in serum OPG concentration was associated with an OR of 5.2 (95% CI, 1.7 to 16.0; $P<0.01$) for the presence of a coronary artery disease.
there is a significant association between serum OPG levels and the presence of CAD by multivariate logistic regression analysis. The mechanism by which serum OPG levels were increased in advanced coronary artery disease, however, is unknown. OPG functions as a soluble decoy receptor for receptor activator of nuclear factor-κB (RANK) ligand (RANKL or OPG ligand). RANKL is produced by osteoblastic lineage cells and activated T lymphocytes and stimulates its receptor, RANK, which is located on osteoclasts and dendritic cells. Thus, it modulates various biological functions such as osteoclast formation and survival. OPG, RANKL, and RANK act as key regulators of bone metabolism and the immune system. Because vascular diseases are promoted by immune-mediated mechanisms, OPG may be involved in the progression of atherosclerosis. OPG is also a receptor for the cytotoxic ligand TNF-related apoptosis-inducing ligand (TRAIL), a potent activator of apoptosis. One possibility is that OPG influences vascular disease by inhibiting TRAIL-induced apoptosis of vascular cells. Although the mechanism for the vascular effects of OPG is unknown, emerging evidence indicates OPG may act as a protective factor for vascular diseases. One hypothesis is that increased serum OPG levels may be a compensatory self-defensive response to the progression of atherosclerosis.

Consistent with previous studies, we found that serum OPG levels are positively correlated with age. This finding suggests that the factors associated with aging may regulate serum OPG levels. At present, there is no information about the main sources and the regulatory mechanism of circulating OPG. In this report, we studied the subjects with stable chest pain for which they underwent coronary angiography. Future studies should examine whether these differences in OPG levels have an implication for asymptomatic subjects or acute coronary syndrome. Our results, however, suggest that OPG may be involved in the progression of CAD, and that serum OPG levels may reflect certain stages of cardiovascular disease.

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References
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