Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis patients

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Abstract

Background. Osteoprotegerin (OPG) is a glycoprotein that inhibits osteoclast differentiation and activity. OPG-deficient mice develop severe osteoporosis and medial arterial calcification. The expression of OPG is detected in early atherosclerotic lesions in non-uraemic patients. We examined whether serum OPG is associated with aortic calcification in haemodialysis patients.

Methods. Serum OPG was measured in 102 patients who were undergoing haemodialysis. The aortic calcification index (ACI) was assessed by computed tomography scans.

Results. Serum OPG level, measured by enzyme-linked immunosorbent assay, was significantly greater in patients with higher ACI than in those with lower ACI. There was a direct relationship between ACI and serum OPG levels and a positive association between OPG and ACI (r = 0.483, P < 0.0001). Multiple regression analyses indicated that serum OPG levels were independently associated with the severity of aortic calcification (P < 0.0001).

Conclusions. These findings show that serum OPG levels are associated with the extent of vascular calcification, suggesting that OPG may be involved in the development of vascular calcification in haemodialysis patients.

Keywords: arteriosclerosis; calcinosis; glycoprotein; renal dialysis

Introduction

Osteoprotegerin (OPG) is a decoy receptor that blocks the interaction of nuclear factor-κB with its ligand, thereby inhibiting osteoclast differentiation and activity [1]. A previous study demonstrated that the expression and production of OPG were regulated by various cytokines and hormones [2]. It has been shown recently that OPG-deficient mice develop severe osteoporosis and medial arterial calcification of the aorta and renal arteries [3] and that the development of osteoporosis and arterial calcification is completely prevented by the restoration of the gene [4]. On the other hand, OPG immunoreactivity was detected not only in the normal vessel wall, but also in early atherosclerotic lesions in humans [5]. These findings suggest that OPG may play an important role in the development of vascular disease. A recent clinical study has reported that serum OPG levels are associated with the presence and severity of coronary artery disease in non-uraemic patients [6], suggesting that OPG may be related to the severity of vascular calcification. In this study, we assessed the severity of aortic calcification by computed tomography (CT) scans and examined if serum OPG levels were associated with aortic calcification in haemodialysis patients.

Subjects and methods

Patients

Of patients on regular haemodialysis in the Dialysis Unit of Minami Senju Hospital, 102 agreed to undergo tests to measure the extent of aortic calcification and serum OPG. The patients had been on haemodialysis for ≥3 months prior to the study, using the same membrane and the same dialysis procedure for ≥2 months. Tests were performed in the morning before the first weekly haemodialysis session. At that
time, blood pressures and biochemical profiles were determined using a conventional method, as described previously [7]. Diabetes was considered present if a patient was on insulin or oral antihyperglycaemic agents or had a fasting glucose level >126 mg/dl. Of the cohort, 12 were receiving antidiabetic treatments (seven received insulin injections and five were on sulphonylurea drugs). Hypertension was defined by a systolic blood pressure >140 mmHg, diastolic pressure >90 mmHg, or both, the current use of antihypertensive treatment or a combination of the three. Of the cohort, 86 subjects were receiving antihypertensive treatment with a calcium-channel blocker (60), an angiotensin-converting enzyme inhibitor (12), an α-blocker or a combination of those drugs [27]. Hyperlipidaemia was defined as a total cholesterol level >220 mg/dl, the current use of lipid-lowering treatment or both. In all, 22 subjects were receiving a lipid-lowering drug (3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitor). Each subject gave informed consent to participate in the study.

Assessment of aortic calcification

As described previously [7], the abdominal aorta was studied by non-contrast CT scanning in consecutive sequential 8 mm slices and the aortic calcification index (ACI) was estimated as the proportion of aortic circumference covered by calcification. By this method, arteriosclerosis was quantified morphometrically in the cross section with the most extensive aortosclerosis. The arithmetic mean values of three measurements were calculated and used for analysis. To optimize the reproducibility of the results, all scans were done by the same investigator using the same CT equipment. The ACI was independently checked in a blinded manner. Reproducibility was absolute for the patients studied. The patients were divided into four groups according to their ACI: group I (ACI 0–10%), group II (ACI 11–30%), group III (ACI 31–50%) and group IV (ACI 51–100%).

Serum OPG measurement

Fasting serum samples were collected before dialysis and stored at −80°C until use. Serum OPG levels were determined using a sandwich enzyme-linked immunosorbent assay (Immunodiagnostik, Bensheim, Germany). This immunoassay uses two highly specific antibodies against human OPG. The intra- and interassay coefficients of variation were 8% and 12%, respectively. All samples were measured in duplicate and the results were averaged. Normal values for a large number of normal subjects of different ages, obtained using the same assay method, have been reported recently [8]. Serum OPG levels in presumed healthy subjects were 66.6 ± 38.4 pg/ml.

Biochemical analysis

EDTA-plasma was used for biochemical assays, including lipids, albumin, calcium, phosphorus and C-reactive protein (CRP). Serum levels of these parameters were measured using automated methods. Serum intact parathyroid hormone (iPTH) was measured using a radioimmunoassay (Nichols Institute Diagnostics, San Juan, CA, USA).

Statistical analysis

All data are presented as means ± SD. Comparisons between groups for the variables studied 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 four groups according to the extent of ACI were probed by chi-square analysis. A multivariate regression analysis was applied to evaluate the independently determinant factors for aortic calcification, including serum OPG levels. Statistical analyses were performed using the StatView statistical software package (SAS Institute, Cary, NC, USA).

Results

The study cohort included 64 men and 38 women. Their ages ranged from 33 to 84 years (mean: 60.0 ± 10.9 years). The mean duration of dialysis was 8.3 ± 7.0 years. The prevalence of cardiovascular risk factors was 78% for hypertension, 38% for diabetes, 6% for hyperlipidaemia and 25% for current smoking. On the basis of ACI measurements, we had 27 patients in group I, 37 patients in group II, 21 patients in group III and 17 patients in group IV. No significant differences were detected in ages between patients grouped according to ACI values. Clinical characteristics and blood chemistries in the patients studied are shown in Table 1. With the exception of CRP, there was no

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>26</td>
<td>37</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.7 ± 12.2</td>
<td>61.8 ± 9.6</td>
<td>61.2 ± 10.2</td>
<td>64.8 ± 8.6</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>7.9 ± 6.3</td>
<td>7.9 ± 7.5</td>
<td>9.8 ± 7.8</td>
<td>8.0 ± 6.7</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.7 ± 1.0</td>
<td>9.8 ± 1.0</td>
<td>9.6 ± 0.9</td>
<td>9.8 ± 1.2</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.6 ± 1.2</td>
<td>5.4 ± 1.2</td>
<td>5.4 ± 1.0</td>
<td>5.3 ± 1.2</td>
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<tr>
<td>iPTH (pg/ml)</td>
<td>183.3 ± 135.1</td>
<td>133.9 ± 136.9</td>
<td>205.3 ± 181.4</td>
<td>268.7 ± 249.6</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>162.4 ± 33.0</td>
<td>163.8 ± 32.0</td>
<td>159.8 ± 32.4</td>
<td>166.6 ± 32.2</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>106.6 ± 58.7</td>
<td>112.6 ± 57.7</td>
<td>121.9 ± 78.8</td>
<td>141.9 ± 124.4</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.3 ± 0.4</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>OPG (pg/ml)</td>
<td>158.0 ± 58.3</td>
<td>247.0 ± 108.3</td>
<td>266.6 ± 105.6</td>
<td>319.7 ± 82.7</td>
</tr>
<tr>
<td>ACI (%)</td>
<td>3.9 ± 3.3</td>
<td>18.7 ± 6.5</td>
<td>38.7 ± 5.4</td>
<td>65.7 ± 13.0</td>
</tr>
</tbody>
</table>
significant difference in the serum levels of albumin, calcium, phosphorus, iPTH, total cholesterol and triglyceride between the four groups. To examine the relationship between circulating OPG and vascular calcification, we measured serum OPG levels in all 102 patients (mean: 238.4 ± 104.3 pg/ml; range: 49.6–487.6 pg/ml). The serum OPG levels were significantly correlated with age (r = 0.325, P < 0.001). There was no correlation between serum OPG levels and the duration of dialysis. In univariate analysis, the ACI values were positively correlated with serum OPG levels (r = 0.483, P < 0.001), CRP (r = 0.47, P < 0.0001) and iPTH (r = 0.264, P < 0.001). Serum OPG tended to be greater in patients with severe aortic calcification than in those with mild calcification. As shown in Figure 1, increasing serum OPG levels were related to the severity of aortic calcification.

Multiple regression analyses were performed to adjust for the roles of different pathogenic factors on vascular calcification. As shown in Table 2, serum OPG and CRP levels were independently associated with the extent of vascular calcification. Moreover, serum iPTH levels and systolic blood pressure were also associated with the vascular calcification. No significant correlation was found between ACI values and other variables, such as serum levels of albumin, total cholesterol, triglyceride, diastolic blood pressure or the calcium × phosphate (Ca × P) product.

**Discussion**

In this study, we found that serum OPG levels were higher in haemodialysis patients and increased with the grade of aortic calcification. Multiple regression analyses indicated that serum OPG levels were independently associated with the severity of aortic calcification. However, the mechanism whereby serum OPG was related to the progress of vascular calcification in dialysis patients is unknown. As consistent with previous studies [8,9], serum OPG is positively correlated with age, suggesting that the factors related to aging may regulate serum OPG in dialysis patients. Cao et al. [10] have reported recently that OPG expression correlates with age-related bone loss in male C57BL/6 mice, suggesting increased osteoclast vs osteoblast activity, which may explain the imbalance in bone formation and resorption associated with aging. No significant difference was detected in age among patient groups divided according to ACI values. However, further studies are required to elucidate whether increased OPG in the patients with severe aortic calcification is partly due to advanced age.

OPG may be involved in the progression of atherosclerosis. In a recent experimental study [3], OPG-deficient mice exhibited medial calcification of the aorta and renal arteries as well as osteoporosis, suggesting that the regulation of OPG—its signalling pathway or its ligand—may play a role in the association between osteoporosis and vascular calcification. Moreover, Jono et al. [6] reported that serum OPG levels were significantly greater in patients with significant stenosis of coronary arteries than in those without stenosis. As the severity of coronary artery disease increased, there was a significant increase in serum OPG. Dhore et al. [11] have reported recently that OPG was expressed in non-diseased vessel walls and in early atherosclerotic lesions. In the presence of advanced calcified lesions, OPG was also present in bone structures. Taken together, OPG may act as a protective factor for vascular diseases. One possibility is that the increase of serum OPG may be a compensatory, protective response to the progression of vascular calcification.

A previous study [12] demonstrated that the acute-phase reaction, characterized by increased CRP, is a prominent risk factor for cardiovascular events in dialysis patients. During conventional dialysis, a concomitant inflammatory state may act as a cofactor in the development of cardiovascular calcification. Because vascular diseases are promoted by immune-mediated mechanisms, OPG may play a role in the pathogenesis of chronic inflammation. In this study, we found that CRP as well as serum OPG levels were independently associated with the severity of aortic calcification in our patients. In addition, serum OPG

![Fig. 1. Serum OPG levels in haemodialysis patients. The patients were divided into four groups according to their aortic calcification index (ACI): group I (ACI 0–10%), group II (ACI 11–30%), group III (ACI 31–50%) and group IV (ACI 51–100%).](image)
levels were positively correlated with CRP values \( (r = 0.342, P < 0.001) \). At present, there is no information concerning the main sources and regulatory mechanism of circulating OPG. However, our studies suggest that OPG may be involved in the progression of vascular calcification and that serum OPG levels may reflect certain stages of vascular calcification in patients undergoing maintenance haemodialysis.

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Conflict of interest statement. None declared.

References


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