Elevated Serum Osteoprotegerin Levels Are Associated With Vascular Endothelial Dysfunction in Type 2 Diabetes

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The majority of diabetes-related mortalities are due to macrovascular complications (1). The early detection of atherosclerotic complications is important in reducing mortality and morbidity from cardiovascular events (2). Endothelial dysfunction is an early process in atherosclerosis and a predictor of cardiovascular events (3,4). Furthermore, endothelial dysfunction is detectable by measuring the flow-mediated dilation (FMD) of the brachial artery (5).

Osteoprotegerin (OPG) is a key cytokine that belongs to the tumor necrosis factor receptor family and inhibits RANKL (receptor activator of nuclear factor κB ligand)–mediated osteoclastic bone resorption (6,7). OPG is expressed in various tissues (8). In endothelial cells, OPG may act as an antiapoptotic factor by binding to the tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) (9,10).

OPG knockout mice develop vascular calcification and osteoporosis (11). Epidemiologic studies have suggested that serum OPG levels are correlated with age, diabetes, hypertension, cardiovascular mortality (12), and the presence and severity of coronary artery disease (13,14). However, the relationship between OPG and endothelial dysfunction has not been proven. In this study, we evaluated the relationship between serum OPG levels and endothelial dysfunction in type 2 diabetes.

RESEARCH DESIGN AND METHODS

We enrolled 104 type 2 diabetic patients (mean age 53.1 ± 8.1 years, 67.6% male) using the following exclusion criteria: *1*) a history of myocardial infarction, angina, stroke, or peripheral artery disease; *2*) presence of carotid stenosis ≥30%; *3*) ankle-brachial index <0.9; and *4*) serum creatinine levels >1.5 mg/dl. Diabetic nephropathy was diagnosed if the urinary albumin excretion rate was ≥30 mg/day and categorized as either microalbuminuria (30–299 mg/day) or macroalbuminuria (≥300 mg/day). The FMD of brachial artery was performed to examine endothelial function, as previously described, using high-resolution ultrasound (15). Lipid profiles, fibrinogen, and high-sensitivity C-reactive protein (hsCRP) were determined. Total homocysteine was measured by polarization immunoassay method using an Abbott IMX analyzer (Abbott Diagnostics). Serum OPG levels were determined by enzyme-linked immunosorbent assay (Oscotec). The intra- and interassay coefficients of variation for OPG were 6.9–9.0% and 6.0–9.0%, respectively. Insulin resistance was defined by the homeostasis model assessment of insulin resistance (HOMA-IR) (16). The protocol was approved by the local ethical committee.

Statistical analysis

Data are presented as means ± SD. A P value <0.05 was considered significant. The differences between groups were compared using either paired Student's t test or ANOVA. The correlation of OPG as the dependent variable was determined by using linear regression analysis. HOMA-IR, OPG, and C-reactive protein (CRP) levels are presented as log-transformed values because the data were not normally distributed. The odds ratio (OR) for endothelial dysfunction according to OPG tertiles was determined using logistic regression analysis.

RESULTS

The median value of FMD (7.18%) was used as a cut-off point for endothelial dysfunction. The median and mean values of OPG were 369.4 and 388.8 ± 151.2 pg/ml, respectively. OPG values were significantly higher in subjects with than in those without endothelial dysfunction (442.5 ± 160.2 vs. 335.1 ± 121.0 pg/ml, $P < 0.0001$). Micro- and macroalbuminuric patients comprised 25.3 and 13.3% of the overall sample, respectively. OPG levels were directly correlated with urine albumin excretion rate (361.3 ± 137.2 vs. 402.7 ± 100.7 vs. 480.4 ± 236.6 pg/ml, respectively, $P = 0.046$). Log(OPG) strongly correlated with albuminuria ($r = 0.219$, $P = 0.013$), serum creatinine ($r = 0.191$, $P = 0.027$), fasting glucose ($r = 0.173$, $P = 0.041$), FMD ($r = -0.375$, $P < 0.001$), log(hsCRP) ($r = 0.285$, $P = 0.002$), fibrinogen ($r = 0.327$, $P < 0.001$), and log(HOMA-IR) ($r = 0.214$, $P = 0.015$). Similarly, age showed a positive relationship with OPG ($r = 0.158$, $P = 0.056$). In multiple regression analysis after an adjustment for age, sex, BMI, hypertension, smoking, and albuminuria, log(OPG) was significantly correlated with FMD ($\beta = -0.363$, $P < 0.001$), log(hsCRP) ($\beta = 0.264$, $P = 0.006$), fibrinogen ($\beta = 0.301$, $P = 0.005$), and log(HOMA-IR) ($\beta = 0.206$, $P = 0.045$). The ORs for endothelial dysfunction increased with increasing tertiles log(OPG)

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Abbreviations: CRP, C-reactive protein; FMD, flow-mediated dilation; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity CRP; OPG, osteoprotegerin; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Risk for endothelial dysfunction*

	OR (95% CI)		
Tertiles	1	2	3
Log(OPG)			
Model 1	1	2.5 (0.9–6.7)	5.2 (1.9–14.1)*
Model 2	1	2.3 (0.8–6.7)	6.3 (1.9–20.7)*
Model 3	1	2.7 (0.8–8.9)	5.4 (1.4–20.4)†
Log(hsCRP)			
Model 1	1	1.3 (0.5–3.6)	1.2 (0.5–2.8)
Model 2	1	1.5 (0.5–4.4)	1.6 (0.6–4.3)
Model 3‡	1	1.7 (0.5–5.3)	2.0 (0.7–5.9)

Model 1: unadjusted. Model 2: adjusted for age, sex, BMI, hypertension, smoking, and albuminuria. Model 3: adjusted for the variables in model 2 plus hsCRP, HOMA-IR, and antidiabetic and cardiovascular medication (including insulin, peroxisome proliferator–activated receptor γ agonist, ACE inhibitor, angiotensin receptor blocker, and statin). ‡Model 3: adjusted for the variables in model 2 plus HOMA-IR and antidiabetic and cardiovascular medication. *$P < 0.01$; †$P < 0.05$.

(OR 1, 2.5 [95% CI 0.9–6.7] and 5.2 [1.9–14.1], respectively), and the OR in highest tertile remained significant after a multivariable adjustment (1, 2.7 [0.8–8.9] and 5.4 [1.4–20.4], respectively). To compare the relative predictive value of OPG for endothelial dysfunction with inflammatory marker, ORs for endothelial dysfunction according to tertiles of log(hsCRP) levels were examined. However, ORs for endothelial dysfunction according to hsCRP levels were lower than those according to OPG levels. Also, unadjusted and fully adjusted hsCRP did not significantly predict endothelial dysfunction (Table 1).

CONCLUSIONS — Our study demonstrates that elevated OPG levels are significantly associated with endothelial dysfunction in type 2 diabetes. Patients in the highest tertile of OPG were five times more likely to have endothelial dysfunction than those in the lowest tertile. However, the correlation coefficient between OPG and FMD was low and the CI was broad; therefore, its prognostic value is limited.

Recent studies reported that high OPG is an independent risk factor of progressive atherosclerosis (17) and is a surrogate marker for peripheral artery disease (18). Also, soluble TRAIL is reduced in patients with coronary artery disease and is negatively correlated with CRP levels (19). In our study, OPG levels were significantly higher in patients with endothelial dysfunction compared with those without and showed significantly negative correlations with FMD. We hypothesized that elevated OPG may represent a decline of soluble TRAIL levels. Elevated OPG may represent a compensatory mechanism to protect against the apoptotic effects of TRAIL on the vascular wall. Therefore, OPG may be a surrogate marker for endothelial dysfunction.

OPG levels increase with age (14,20), diabetes (12,21), and renal failure (22) and are positively correlated with CRP, interleukin-6, fibrinogen, glycemic status, and insulin resistance (17,23,24). In our study, fasting glucose, creatinine, hsCRP, fibrinogen, and HOMA-IR showed significantly positive correlations with OPG. These results suggest that multiple factors, including age, glycemic status, renal function, insulin resistance, and inflammation, may contribute to elevated OPG levels.

Our study has several limitations. First, because our study was cross-sectional, we cannot prove a causal link between OPG and endothelial dysfunction. Second, because the total amount of OPG was measured, we cannot discriminate between free and complex forms of OPG. Finally, antidiabetic medications were discontinued 24 h before the study. Therefore, FMD and OPG levels might be influenced by a short-term change in plasma glucose levels.

In conclusion, our study demonstrates that elevated OPG levels are significantly associated with endothelial dysfunction in type 2 diabetes. Further studies are needed to prove whether the OPG/RANKL/TRAIL system has a causal role in atherosclerosis or is a compensatory response to protect against atherosclerosis.

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