Osteoprotegerin Is Associated With Silent Coronary Artery Disease in High-Risk but Asymptomatic Type 2 Diabetic Patients

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OBJECTIVE — Osteoprotegerin (OPG) is an inhibitor of osteoclastogenesis, which has been recently involved in atherosclerosis. The relationship between coronary atherosclerosis and OPG has never been studied in asymptomatic type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — This is a nested case-control study; 162 asymptomatic type 2 diabetic patients were evaluated for silent myocardial ischemia using stress myocardial perfusion imaging; of 50 patients with positive results, 37 underwent coronary angiography, 20 of whom showed significant coronary artery disease (CAD group). Of 112 patients without silent myocardial ischemia, 20 subjects (NO-CAD group) were selected and matched by age and sex to patients with CAD. OPG, C-reactive protein, adiponectin, lipoprotein(a), albuminuria, and classical risk factors were measured.

RESULTS — The percentages of subjects with OPG levels above median and with nephropathy were higher in the CAD group than in the NO-CAD group (70 vs. 25%, $P = 0.004$ and 50 vs. 5%, $P = 0.001$, respectively). LDL cholesterol levels were higher and HDL cholesterol levels lower in the CAD compared with the NO-CAD group ($P = 0.033$ and $P = 0.005$, respectively). No other variables were associated with CAD. Logistic regression analysis showed that OPG values above median (odds ratio 8.31 [95% CI 1.18–58.68], $P = 0.034$) and nephropathy (21.98 [1.24–388.36], $P = 0.035$) were significant independent predictors of asymptomatic CAD in type 2 diabetic patients.

CONCLUSIONS — Our investigation reports the first evidence of an independent association of OPG with asymptomatic CAD in type 2 diabetic patients. The results of this nested case-control study with 20 cases need to be confirmed in a larger population.

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Diabetes is associated with a more than threefold increased risk of coronary artery disease (CAD) (1). Asymptomatic CAD is common in patients with diabetes (2–4) and is a strong predictor of future coronary events and early death, especially in this population (5,6). Early identification of diabetic patients with silent CAD can allow reduction of mortality and morbidity from cardiovascular events (7,8). However, screening all patients is not practical, and it is therefore important to find reliable predictors of silent CAD to identify those patients susceptible to benefit from further screening.

Very recently, osteoprotegerin (OPG), a key factor in bone remodeling (9), a member of the tumor necrosis factor receptor family, and a decoy receptor for the receptor activator of nuclear factor-κB ligand and tumor necrosis factor–related apoptosis-inducing ligand (10), was implicated in human atherogenesis. Epidemiological evidence shows an association of OPG with cardiovascular disease in the general population as well as with diabetes (11–13). Cross-sectional evaluations demonstrate a relation between OPG levels and the severity of coronary atherosclerosis (14–16). On the basis of these data, it appears that OPG could play an important role in atherosclerosis and could also be a marker of atherosclerosis lesions. So far, the relationship between CAD and OPG has never been studied in asymptomatic type 2 diabetic patients.

The aim of the present study was to investigate whether OPG levels are associated with the presence of angiographically documented asymptomatic CAD in patients with high-risk type 2 diabetes.

RESEARCH DESIGN AND METHODS
— From March 2001 to November 2003, a total of 162 high-risk type 2 diabetic patients (i.e., presenting at least one of the following conditions: age ≥60 years, active smoking, albuminuria, hypertension, dyslipidemia, family history of premature CAD, and peripheral arterial disease) with normal baseline electrocardiography (ECG) were evaluated to identify patients with asymptomatic CAD. Exclusion criteria were age <35 or >85 years, history of coronary events, symptoms of coronary events as defined

by the Rose questionnaire, abnormal result of resting ECG, chronic or acute disease, pregnancy, neoplasia, and contraindication to dipyridamole infusion such as asthma. Diabetes was diagnosed according to American Diabetes Association criteria (17). Participants were considered to have type 2 diabetes if they had no history of ketosis and if they did not start any insulin treatment either in the 2 years following the diagnosis or before the age of 40. Hypertension was diagnosed if one of the following conditions was present: systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg and/or presence of at least one antihypertensive medication. Patients with albumin excretion rate >30 mg/day and/or macroproteinuria were considered to have diabetic nephropathy. Peripheral arterial disease (PAD) was considered when one or more peripheral arterial pulse was abolished at clinical examination and/or when intermittent claudication and/or a past history of revascularization of the lower limbs were present.

All reported investigations have been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000 (available from http://www.wma.net/e/poling/17-c_e.html), and informed consent was obtained from patients before participating in the study.

Stress protocol and myocardial perfusion imaging

Antihypertensive treatments with β-adrenergic blocking agents or calcium antagonists were stopped 72 h before the test, and patients were asked to abstain from caffeine-containing foods, beverages, and medications for a minimum of 12 h before the test. Dipyridamole (0.75 mg/kg) was infused intravenously over a period of 4 min to each patient and was followed by a graded exercise test on an ergometer bicycle according to classical standards (18). Patients tried to reach at least 85% of the theoretical maximal heart rate, taken as the maximal exercise test level. The workload from a 30-watt initial level was increased by 30 watts every 3 minutes. The cardiac rhythm was monitored continuously while a 12-lead ECG was recorded. The exercise tolerance test was considered to be positive in case of a horizontal or descending ST-segment depression >1 mm. None of the patients experienced clinical symptoms of CAD during the test.

^{99m}Tc-sestamibi (7 mCi - 259 MBq) was injected at peak exercise for patients performing maximal exercise or 7 min after initiation of the dipyridamole infusion for the others and, when rest images were acquired, at rest. A same-day stress-rest imaging protocol was used. Stress and rest images were acquired in the procubitus station 1 h after injection of ^{99m}Tc-sestamibi. Acquisition was performed using a double-head gamma camera (GE-SMV DST-XL) with low-energy high-resolution parallel-hole collimators. Acquisition parameters were as follows: 64×64 matrix, 6°/step for 180° (16 steps over 90° per head), 40 s acquisition time/step, and image magnification of 1.33×. Stress and rest acquisitions were gated (10% R-R interval acceptance window, eight gated intervals). Images were reconstructed (butterworth filter order 4, cutoff frequency 0.25/cm) and short axis, horizontal long axis, and vertical long axis sections were obtained. No automatic movement correction was applied: movement was detected during acquisition; for any significant movement, another data recording was acquired. If an antero-septo-apical or lateral hypoactivity was observed, a second acquisition in the decubitus station was performed to detect attenuation artifact. Myocardial perfusion imaging (MPI) reconstructions were divided into 20 segmental regions. Inside a segment, a hypoperfusion was considered to be significant if the mean activity in the segment was <70% of the maximal myocardium activity (19). Two experienced nuclear medicine physicians blinded to the clinical data reviewed the scintigraphic images.

When stress images showed a significant defect in at least three segments (i.e., ≥15% of the whole left ventricular myocardium), they were scored as abnormal, and a resting study was performed. For resting studies, patients were injected with 21 mCi (777 MBq) of ^{99m}Tc-sestamibi 3 h after stress. MPI was regarded as positive for patients having significant stress hypoperfusion in at least three segments either reversible or not. When images showed smaller defects (i.e., <15% of the whole ventricular myocardium), images were scored as normal. Defects were classified as reversible (normalization after injection) or fixed (persistent defect after injection). Left ventricular ejection fraction was calculated using commercially available software (QGSspect).

Coronary angiography

In patients with highly positive results of stress ECG and those with positive results of MPI, diagnostic coronary angiography was recommended. The same angiographer performed all the procedures within 1 month from the time of the scintigraphy. All coronary angiograms were reviewed and analyzed by two independent, well-trained angiographers blinded to all clinical data. The degree of stenosis was visually estimated in multiple views. Major analyzed coronary arteries were the left main, the left anterior descending, the left circumflex, and the right coronary arteries. Diagonal, acute, and obtuse marginal branch vessels were considered major and analyzed if they supplied enough myocardium to be potentially suitable for revascularization (≥2.0 mm lumen diameter). A ≥50% narrowing in luminal diameter for one of the three major epicardial coronary arteries or for the left main coronary artery was considered hemodynamically significant, and the patient was classified as having CAD.

Laboratory procedures

Venous blood samples were taken from subjects after fasting for 12 h. Total cholesterol, HDL cholesterol, and triglyceride levels were measured in serum by routine enzymatic methods (KonePro; Konelab, Epoo, Finland). Lipoprotein(a) [Lp(a)] concentrations were determined by immunonephelometric assay using a Behring Nephelemeter 100 (Behring Diagnostic, Marburg, Germany).

Highly sensitive C-reactive protein (hs-CRP; from 0.5 to 20 mg/l) was determined by latex-enhanced immunoturbidimetric method on an Olympus AU2700 biochemistry analyzer (Rungis, France). OPG and adiponectin were determined by ELISA (Biovendor Laboratory Medicine, Brno, Czech Republic). OPG values in type 2 diabetic patients were compared with those obtained in 30 healthy individuals (age 38.6 ± 12.1 years; 11 men/19 women) without diabetes or symptoms and history of CAD. HbA_{1c} (A1C) was measured by routine high-performance liquid chromatography–based ion-exchange procedure (HA-8140; Menarini, Rungis Cedex, France).

Table 1—*Characteristics of the CAD and NO-CAD groups*

	CAD	NO-CAD	*P*
n	20	20	
Sex (men/women)	15/5	15/5	1.000
Age (years)	59.8 ± 9.5	60.9 ± 9.6	0.723
Duration of diabetes (years)	12.0 ± 6.6	10.5 ± 5.9	0.444
BMI (kg/m^2)	29.8 ± 4.5	29.0 ± 3.6	0.543
A1C (%)	8.9 ± 2.7	8.3 ± 2.0	0.434
Left ventricular ejection fraction	56 ± 8	61 ± 9	0.059
Total cholesterol (mg/dl)	211 ± 39	198 ± 38	0.281
LDL cholesterol (mg/dl)	131 ± 37	104 ± 41	0.033
HDL cholesterol (mg/dl)	45 ± 8	58 ± 17	0.005
Triglycerides (mg/dl)	145 (59–395)	142 (72–390)	
Subjects with triglycerides >150 mg/dl (%)	45.0	50.0	0.752
Nephropathy (%)	50.0	5.0	0.001
Retinopathy (%)	40.0	25.0	0.311
PAD (%)	20.0	0.0	0.035
Smokers (%)	35.0	20.0	0.288
Family history of CAD (%)	5.0	0.0	0.311
Hypertension (%)	95.0	85.0	0.282
Lp(a) (mg/dl)	13 (10–165)	21 (10–95)	
Subjects with Lp(a) levels >30 mg/dl (%)	30.0	40	0.507
hs-CRP (mg/l)	3.30 (0.37–28.24)	1.92 (0.06–13.40)	
Subjects with hs-CRP levels >median value (%)	60.0	35.0	0.113
Adiponectin (μg/ml)	7.17 (1.13–33.34)	11.55 (3.72–46.55)	
Subjects with adiponectin levels <median value (%)	60.0	40.0	0.206
OPG (pmol/l)	13.97 (1.31–20.04)	9.44 (0.10–25.88)	
Subjects with OPG levels >median value (%)	70.0	25.0	0.004

Data are means ± SD or median (range) unless otherwise indicated.

Statistical analysis

This study is a nested case-control study. The 20 patients with significant but asymptomatic CAD (CAD group) were age and sex matched to 20 patients with normal stress ECG and normal MPI (NO-CAD group). The two groups were compared in order to determine associates of CAD.

For continuous variables, differences between groups were compared using either the two-sample Student's *t* test or the Mann-Whitney *U* test as adequate. Variables with highly "skewed" distribution were dichotomized using either values generally admitted according to the current data of the literature when available (Lp[a] levels >30 mg/dl and triglyceride levels ≥150 mg/dl, respectively) or according to the median (hs-CRP, adiponectin, and OPG). The Pearson χ^2 test was used for comparison of frequency. A multiple logistic regression analysis with the presence of asymptomatic CAD as the dependent variable was performed. Because of the limited number of patients in each group, baseline variables were entered as possible predictor variables into the model to adjust for confounding effects if they were imbalanced between patients with and without CAD, indicated by $P \leq 0.05$. However, if a nominal variable was associated with CAD in univariate analysis at $P \leq 0.05$ but with little cases and was strongly imbalanced between the CAD and NO-CAD groups, it was not entered into the model (20). Odds ratios (ORs) were estimated, and the results were given as ORs and 95% CIs. Data were presented as means ± SD, unless otherwise stated. Values of $P < 0.05$ were considered significant.

RESULTS — Of the 162 diabetic patients, 50 (30.9%) were screened positive for silent myocardial ischemia: 8 (4.9%) had positive results of stress ECG, 34 (21.0%) had positive results of MPI, and 8 (4.9%) had positive results of the two tests. In 112 patients (69.1%), the results were negative. A total of 37 patients (4 subjects with positive results of stress ECG, 27 with positive results of MPI, and 6 with positive results of the two tests) gave informed consent for coronary angiography. Coronary angiography showed ≥1 significant lesion in 20 subjects (3 of 4 subjects with positive results of stress ECG, 11 of 27 with positive results of MPI and 6 of 6 with positive results of the two tests). Angiography showed no significant lesion in 17 patients (1 with positive results of stress ECG, 16 with positive results of MPI and 0 with positive results of the two tests). Of 20 patients with angiographically documented asymptomatic CAD (CAD group), monovessel disease was shown in 10 subjects (50.0%), bivessel disease was shown in 7 (35.0%), and multivessel disease was shown in 3 (15.0%). The 20 patients with CAD were age and sex matched to 20 patients with normal stress ECG and MPI.

The proportions of patients treated with diet alone, oral agents, insulin, or a combination of insulin and oral agents were 3 (15.0%), 11 (55.0%), 5 (25%), and 1 (5.0%) in the CAD group and 2 (10%), 9 (45.0%), 7 (45%), and 2 (10%) in the NO-CAD group, respectively. No differences in diabetes treatment were found between the two groups.

Classical risk factors and Lp(a)

The characteristics of diabetic patients according to the two groups (NO-CAD and CAD) are shown in Table 1. LDL cholesterol levels were significantly higher and HDL cholesterol levels lower in the CAD group in comparison to the NO-CAD group ($P = 0.033$ and $P = 0.005$, respectively). Patients in the CAD group were also more frequently affected by PAD than those in the NO-CAD group ($P = 0.035$). No differences were noted regarding the other classical risk factors between the two groups. The proportion of patients with Lp(a) values >30 mg/dl was similar in the CAD and in the NO-CAD group.

Glycemic control and microangiopathy

Table 1 shows that A1C did not differ between the two study groups at recruit-

ment. Patients in the CAD group were more frequently affected by diabetic nephropathy than those in the NO-CAD group (*P* = 0.001). No differences were noted for diabetic retinopathy.

Osteoprotegerin, adiponectin, and hs-CRP
Plasma OPG values were significantly higher in the NO-CAD group in comparison to healthy nondiabetic individuals (10.4 ± 5.9 vs. 6.0 ± 3.8 pmol/l, *P* = 0.002). They were also higher in the CAD group in comparison to the NO-CAD group (13.0 ± 3.9 vs. 10.4 ± 5.9 pmol/l, *P* = 0.01). The proportion of individuals with OPG values above median was greater in the CAD group compared with the NO-CAD group (70 vs. 25%, *P* = 0.004). Differences in hs-CRP and adiponectin levels between the two groups did not reach statistical significance (Table 1).

Multivariate analysis
The following variables associated with CAD at *P* ≤ 0.05 in univariate analysis were entered in a multiple logistic regression analysis: nephropathy, OPG, LDL, and HDL cholesterol levels. Even though PAD was associated with CAD in univariate analysis (*P* = 0.031), it was present in only four individuals, all in the CAD groups. It was thus not entered into the model (20). Analysis showed that OPG values above median (OR 8.31 [95% CI 1.18–58.68]; *P* = 0.034) and nephropathy (OR 21.98, [1.24–388.36]; *P* = 0.035) were significant independent predictors of asymptomatic CAD in type 2 diabetic patients.

CONCLUSIONS — Since all diabetic patients should benefit from an intensive control of cardiovascular risk factors, screening for silent myocardial ischemia in this population is essentially designed to identify asymptomatic subjects who might present coronary lesions susceptible to benefit from revascularization. Even though stress-MPI has been shown to be effective in detecting CAD in asymptomatic diabetic patients (21), it cannot be generalized to the whole diabetic population, especially in regard to its cost ($600 at our institution). Some new factors, such as adiponectin and even more recently OPG, have been shown to be associated with CAD (14,22). If they are confirmed to be powerful predictors of CAD, they might represent useful and

cost-effective means to select which subset of patients require stress testing (duplicate dosages of OPG in our lab cost $19). OPG and adiponectin, in addition to classical risk factors, inflammatory markers, and Lp(a) are potential predictors of silent CAD in asymptomatic type 2 diabetic patients. We showed that OPG is an independent predictor of asymptomatic CAD in addition to diabetic nephropathy.

OPG could be produced by cells of the cardiovascular system, including coronary artery smooth muscle cells and endothelial cells (23,24) and may operate in vascular physiopathology regulating vascular calcification, apoptosis, and immune defense (24), suggesting that alterations of OPG serum levels may be associated with CAD. Increased OPG serum levels have recently been reported in diabetic individuals (11,25), which is in agreement with our observations and are associated with increased cardiovascular mortality (11). OPG serum levels increase with aging (15,25) and in patients with renal failure (26). In our study, we assessed OPG serum levels in age-matched groups, and serum creatinine did not differ between our CAD and NO-CAD groups. The higher levels of serum OPG in patients with significant CAD were thus not affected by age and/or renal failure. It has also been suggested that OPG levels could be linked to a microinflammatory state (12,27). However, in our study, we did not observe any relationship between hs-CRP and OPG.

Inflammation is a key event in diabetes-induced atherogenesis (28). However, because of the intraindividual variability in inflammatory markers such as hs-CRP (29), this relationship can be difficult to show up in case-control studies. Indeed, in the present work, we found a trend toward an association between CAD and hs-CRP that did not reach the level of statistical significance. Recently, it has been shown that adipose tissue could account in part for the proinflammatory milieu due to secretion of a large number of adipokines (30). Among them, adiponectin appears as an anti-inflammatory molecule and is decreased in patients with CAD (22). Hotta et al. (31) found prominently lower levels of plasma adiponectin in type 2 diabetic patients with CAD than in patients without CAD. In the present study, we did not find such an association. However, Hotta et al. included diabetic pa-

tients who suffered from myocardial infarction without giving any details regarding the time period between the acute event and the inclusion whereas in our study, patients with history and/or symptoms of coronary events were excluded. Since adiponectin levels have been reported to be decreased in acute coronary syndromes (32), this might have influenced the results of Hotta et al. and could explain the discordance with our results.

The present study suffers from several limitations, the major one being the small number of patients. These results must thus be confirmed in a larger population. A substantial overlap in OPG values was noted between patients with and without CAD. However, this is an intrinsic problem of biological markers of prognostic significance, including hs-CRP. Finally, since early CAD may not be associated with inducible ischemia on stress-MPI and the sensitivity of radionuclide imaging is 60–70% in single-vessel disease, CAD cannot be excluded in the NO-CAD group. However, if anything, this would have diluted the potential impact of OPG in predicting silent CAD.

Based on our results, we conclude that a larger study needs to be done to evaluate whether OPG, in addition to other risk factors, could contribute effectively to the identification of asymptomatic diabetic patients who require further evaluation for silent CAD.

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