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Lower uncarboxylated osteocalcin and higher sclerostin levels are significantly associated with coronary artery disease☆

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ABSTRACT

Systemic roles for bone-derived proteins have emerged from recent studies. In particular, the serum concentration of osteocalcin (OCN) or sclerostin was found to be associated with altered glucose metabolism or atherosclerosis. The aims of this study were to evaluate OCN and sclerostin levels in subjects who underwent coronary artery bypass graft (CABG) surgery compared with those in normal controls and to analyze their relationships with atherosclerosis.

This was an age- and sex-matched case–control study that included 61 male subjects who underwent CABG and 61 controls. Forty-six subjects (37.7%) with diabetes and 62 hypertensive subjects (50.8%) were included. Serum sclerostin, uncarboxylated OCN (ucOCN) and carboxylated OCN (cOCN) were measured. Coronary artery calcium (CAC) score was calculated according to Agatston's method, using a 64-slice multi-detector computed tomography scanner.

The levels of serum ucOCN were significantly lower and sclerostin concentrations were higher in the CABG group than in the controls ($p < 0.05$ for both), and these significances were maintained after adjusting for atherosclerotic risk factors in both diabetic and nondiabetic patients ($p < 0.05$ in both groups). However, there was no difference in cOCN levels between CABG patients and controls. The group with abnormal CAC scores (CAC scores ≥100) had significantly higher levels of serum sclerostin ($p < 0.05$). In multiple logistic regression analysis, both lower ucOCN and higher sclerostin levels were independently associated with CABG (odds ratio [OR] 0.43, 95% CI 0.22–0.84, $p < 0.05$ for log(ucOCN); and OR 2.09, 95% CI 1.08–4.05, $p < 0.05$ for log(sclerostin)).

In subjects with CAD who underwent CABG, the serum ucOCN level was decreased and the sclerostin level was increased compared with those in the controls, regardless of diabetic status. Longitudinal studies are warranted to establish the precise roles of ucOCN and sclerostin in the pathogenesis of atherosclerosis.

1. Introduction

Osteoporosis and atherosclerosis are representative clinical consequences of aging that occur in bone and vessels, respectively. Several epidemiologic studies have demonstrated the associations between these two conditions [1,2], and specific signaling pathways or molecular factors have been suggested to be common mediators of both diseases [3,4].

Bone remodeling is a coupled process involving both bone resorption by osteoclasts and bone formation by osteoblasts, and actively occurs throughout life [5]. In this way, the skeleton is a metabolically active organ, and growing evidence suggests that there is cross-talk between bone and other organs [6,7]. Osteocalcin (OCN), a bone matrix noncollagen protein mainly expressed by osteoblasts, is a Gla protein that contains three glutamic acid residues and is carboxylated via vitamin K-dependent posttranslational modification [8,9]. However, uncarboxylated OCN (ucOCN) that could not be carboxylated in vitamin K-deficient condition has reduced binding affinity for bone minerals and is secreted into circulation [10]. Carboxylated OCN (cOCN) secreted from osteoblasts can also be decarboxylated in the acidic environment present during active bone resorption, and released into circulation [11]. It is now becoming clear from several clinical and experimental studies that osteocalcin also plays an important role in the regulation of glucose and fat metabolism [12,13].

Beyond the possible connections between energy metabolism and OCN, the relationship between OCN and parameters related to atherosclerosis in humans has been explored in several recent studies, in which ucOCN levels were reported to be inversely associated with the abdominal aortic calcification score in men [14]. In addition, patients with atherosclerotic diseases showed lower OCN levels than the respective group without atherosclerosis [15,16].

☆ Conflicts of interest: None.

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Sclerostin, another bone-derived protein, is mainly produced in osteocytes and is a major antagonist of canonical Wnt signaling in bone metabolism [17]. Recently, a limited number of studies have reported that sclerostin concentrations are associated with atherosclerotic conditions other than bone metabolism: the expression of sclerostin was significantly increased in atherosclerotic cardiac valves, and diabetic patients with atherosclerosis had a higher sclerostin level compared with patients without atherosclerosis [18,19].

The potential roles of osteogenic factors in the pathogenesis of atherosclerosis have been frequently suggested, although these remain undefined [20,21]. The aims of this study were to evaluate circulating levels of both OCN and sclerostin in subjects with symptomatic, multivessel coronary artery disease (CAD) who underwent coronary artery bypass graft (CABG) surgery.

2. Materials and methods

2.1. Study design and study subjects

In this cross-sectional study, 61 male subjects who underwent CABG in Seoul National University Bundang Hospital (SNUBH), Seongnam, Korea, between May 2012 and December 2013 were enrolled as the CABG group. CABG was performed in symptomatic patients with coronary artery stenosis ≥75% in three major coronary arteries. Identical numbers of age- and sex-matched subjects were selected as a control group from asymptomatic patients who had undergone a cardiac evaluation by 64-slice multidetector computed tomography (MDCT) in the same study period, either as a routine physical examination or for cardiac evaluation because of risk factors for CAD. We defined diabetes mellitus (DM) as a fasting plasma glucose concentration of ≥126 mg/dL, a glycated hemoglobin (HbA1c) level ≥ 6.5%, or taking antidiabetic medication. Tests to confirm DM were repeated on a different day. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg, diastolic blood pressure (DBP) >90 mmHg, or taking antihypertensive medication. This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committees of SNUBH (SNUBH IRB#B-1203/147–006), and all subjects provided written informed consent.

2.2. Biochemical analysis

After overnight fasting for at least 12 h, serum was collected in the morning on the day of CABG surgery. Serum concentrations of total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, blood urea nitrogen (BUN), creatinine, HbA1c, and fasting plasma glucose (FPG) were measured by standard biochemical methods. Serum sclerostin was measured using a commercially available ELISA kit (Biomedica, Vienna, Austria) according to the manufacturer's instructions. The detection limit was 3.2 pmol/L and the intra- and inter-assay variability were 5.2% and 6.5%, respectively. The concentrations of ucOCN and cOCN were measured using an enzyme-linked immunoassay kit (Takara Bio Inc., Shiga, Japan). The detection limit was 0.25 ng/mL and the assay showed 5% cross-reactivity with cOCN in ucOCN assays, whereas the detection limit was 0.5 ng/mL with no cross-reactivity in cOCN assays. The intra- and inter-assay variability were 4.7% and 6.7% in ucOCN assays, and 2.1% and 2.4% in cOCN assays, respectively.

2.3. Cardiac CT angiography

Cardiac CT angiography was performed with a 64-slice MDCT scanner using the standard scanning protocol described previously to evaluate coronary artery calcium (CAC) status and the percentage stenosis of coronary arteries in subjects with risk factors for CAD [22]. Subjects with a heart rate >70 beats per minute received 10–30 mg of intravenous esmolol (Jeil Pharm, Seoul, Korea) before MDCT imaging. The CAC scores were calculated according to Agaston's method [23], and were categorized into two groups: <100 and ≥100.

2.4. Statistical analysis

The distributions of the ucOCN, cOCN and sclerostin concentrations were skewed (Kolmogorov–Smirnov $Z = 2.988$, 2.056, and 4.390, respectively; $p < 0.05$), and the values of these parameters were normalized by logarithmic transformation for all analyses. The Student *t* test and analysis of covariance (ANCOVA) with the covariates of age, body mass index (BMI), SBP, DBP, total cholesterol, creatinine, and statin or anti-diabetic medications were used to compare variables between the CABG and control groups. Data are shown as mean ± standard deviation (SD) or median (interquartile range) for the Student *t* test, and estimated marginal mean ± standard error (SE) for ANCOVA. A logistic regression model was used to evaluate the association of ucOCN, cOCN and sclerostin levels with CABG; and age, BMI, DM status, hypertension, SBP, DBP, HbA1c, total cholesterol, creatinine, and statin therapy were used as independent variables in a multivariate model. Data were analyzed using SPSS for Windows version 19.0 (IBM Corporation, Armonk, NY) and $p < 0.05$ was considered significant.

3. Results

3.1. Characteristics of study populations

The baseline characteristics of the CABG patients and age- and sex-matched controls are presented in Table 1. Because the control group was selected to be age matched subjects at a 1:1 ratio, there was no significant difference in age between the groups. Regarding the conventional risk factors, the CABG group had a lower BMI than controls ($p < 0.05$). After adjusting for age and BMI, the CABG group showed impaired glucose tolerance, indicated by higher FPG and HbA1c, compared with the control group (114.8 ± 44.0 mg/dL vs. 97.8 ± 21.5 mg/dL for FPG and 6.99 ± 1.69 mg/dL vs. 5.93 ± 0.86 mg/dL for HbA1c, both $p < 0.05$). Furthermore, the levels of total cholesterol and HDL-cholesterol were lower in the CABG group. These differences might be caused by the higher percentage of current statin users in the CABG group (88.5% in the CABG group vs. 41.0% in the control group, $p < 0.001$). The prevalence of type 2 DM and hypertension was higher in the CABG group than in controls ($p < 0.001$ for both). There were no patients

Table 1
Baseline characteristics of study participants.

	CABG patients	Controls	*p*-Value
N	61	61	
Age, years	62.1 ± 9.4	59.2 ± 7.7	0.062
BMI, kg/m2	23.8 ± 2.3	24.9 ± 2.9	0.033[a]
SBP, mmHg	126.2 ± 16.7	127.5 ± 13.0	0.543[b]
DBP, mmHg	72.7 ± 9.6	79.3 ± 9.5	0.003[b]
FPG, mg/dL	114.8 ± 44.0	97.8 ± 21.5	0.006[b]
HbA1c, %	6.99 ± 1.69	5.93 ± 0.86	<0.001[b]
Calcium, mg/dL	8.8 ± 0.6	8.7 ± 0.6	0.315[b]
Phosphorus, mg/dL	3.5 ± 0.6	3.4 ± 0.7	0.497[b]
Total cholesterol, mg/dL	174.7 ± 49.2	204.9 ± 35.7	0.001[b]
Triglyceride, mg/dL	162.4 ± 150.7	142.1 ± 72.5	0.073[b]
HDL-cholesterol, mg/dL	42.0 ± 18.6	50.3 ± 12.9	0.002[b]
LDL-cholesterol, mg/dL	98.6 ± 37.7	112.9 ± 27.7	0.063[b]
BUN, mg/dL	22.2 ± 14.3	15.2 ± 4.2	0.002[b]
Creatinine, mg/dL	1.3 ± 1.4	1.1 ± 0.1	0.159[b]
Type 2 DM, *N* (%)	35 (57.4%)	11 (18.0%)	<0.001
Hypertension, *N* (%)	43 (70.5%)	19 (31.3%)	<0.001
Statin user, *N* (%)	54 (88.5%)	25 (41.0%)	<0.001

CABG, coronary artery bypass graft; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-cholesterol, high-density lipoprotein-cholesterol; HDL-cholesterol; LDL-cholesterol. High-density lipoprotein-cholesterol; BUN, blood urea nitrogen; DM, diabetes mellitus.

[a] *p*-Values for age adjusted.

[b] *p*-Values for age and BMI adjusted.