ORIGINAL RESEARCH

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Adropin: A New Marker for Predicting Late Saphenous Vein Graft Disease after Coronary Artery Bypass Grafting

Abstract

Purpose: Saphenous vein graft disease (SVGD), defined as an occlusion of 50% or more of the SVG excluding distal anastomotic occlusion, is an important predictor of morbidity after coronary artery bypass grafting (CABG). Late graft occlusion is a serious complication that often limits the use of the saphenous vein as a coronary bypass graft. Late graft occlusion is particularly common in old, degenerated venous grafts with advanced atherosclerotic plaques. Adropin has been implicated in the homeostatic control of metabolism. The purpose of this study was to investigate whether serum adropin levels are associated with late SVGD following CABG.

Methods: Thirty-eight patients with SVGD involving at least one graft (occluded group; 14 females, 24 males) and 42 patients with a patent saphenous vein graft (patent group; 15 females, 27 males) were enrolled in this study. Venous blood samples were taken from all of the participants to measure plasma adropin levels using an enzyme-linked immunosorbent assay kit.

Results: The mean adropin level was significantly lower in the occluded group than in the patent group (3.2 ± 0.71 vs. 4.9 ± 1.51 ng/mL, $p < 0.001$). Multivariate regression analysis showed that the adropin level was the independent predictor of late saphenous vein graft occlusion.

Conclusions: Adropin levels are lower in patients with late saphenous vein graft occlusion and these reduced adropin levels, together with other factors, may lead to saphenous vein graft occlusion. Larger and prospective studies are needed to determine if adropin plays a role in the pathogenesis of SVGD.

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Coronary artery disease (CAD) is the major cause of mortality in developed countries [1]. Essential treatment modalities include pharmacotherapy, percutaneous transluminal coronary interventions and coronary artery bypass grafting (CABG) [2]. Although arterial graft conduits are increasingly used in bypass surgery, saphenous vein grafts (SVG) are still frequently used [3]. SVG occlusion is a frequent event and is responsible for the majority of re-interventions following CABG [4-5], affecting from 25% to over 50% of patients within 10 years [5]. The extent to which SVG occlusion affects the clinical outcome is unclear because of differences in definitions, patient selection and duration of follow-up among studies [6]. The lack of a universal definition also makes it difficult to compare studies, hindering attempts to identify factors associated with clinical outcomes.

Many blood-based protein biomarkers have an established role in coronary artery disease (CAD) management by providing information of crucial diagnostic or prognostic value. Adropin is a recently identified bioactive protein that is encoded by the energy homeostasis associated gene (*Enho*) and is expressed in the liver and brain [7]. Adropin appears to participate in the maintenance of energy homeostasis and insulin response, which is closely related to the development and progression of atherogenesis [7]. A recent study found that adropin is also expressed in coronary artery endothelial cells and plays a potential endothelial protective role in mice [8]. It is well known that endothelial dysfunction is a key early event in atherogenesis and is integral in the onset of CAD and acute coronary syndromes (ACS) [9]. Based on these findings, we hypothesized that adropin deficiency might be involved in the pathogenesis of SVG occlusion. The relationship between adropin concentrations and SVG occlusion has never been fully elucidated; therefore, serum adropin levels were determined in patients with SVG occlusion and were correlated with the presence of SVG occlusion.

Materials and Methods

Study population and definitions

The study population included 80 consecutive patients who underwent elective coronary angiography because of recurring postoperative symptoms. The patients were divided into two groups based on findings of their last control coronary angiography after CABG: 38 patients (14 females, 24 males, mean age 62.0 ± 9.7 years) with saphenous vein graft disease (SVGD), defined as occlusion of 50% or more of at least one SVG, excluding distal anastomotic occlusion (occluded group) and 42 patients (15 females, 27 males, mean age of 60.0 ± 12.0 years) with

patent SVGs (patent group). Transthoracic echocardiography was performed for measurement of ejection fraction in all patients using a 6 MHz transducer (VIVID 7; GE Medical Systems, Little Chalfont, United Kingdom).

At the time of initial evaluation, physicians and clinical staff comprehensively reviewed medical records, assessed patients' angina symptoms and determined current Canadian Cardiovascular Society (CCS) angina class and medical regimen for CAD. Hypertension (HTN) was defined as systolic blood pressure of ≥ 140 mmHg, diastolic blood pressure of ≥ 90 mmHg or greater, a self-reported history of HTN or use of antihypertensive medications. Diabetes mellitus (DM) was defined as a fasting glucose of ≥ 126 mg/dL, HbA1c of $\geq 6.5\%$, a self-reported history of DM or use of hypoglycemic agents. Smoking was defined as current use and previous last use of up to 6 months. Body mass index (BMI) was calculated based on weight and height (weight/height^2 , kg/m^2). All patients provided written informed consent. Our local ethics committee approved the study.

Exclusion criteria

Subjects with any of the following were excluded from the study: acute or chronic inflammatory disease, myeloproliferative disease, malignancy, renal disease, liver disease, history of stroke, thyroid disease, immunological disease, hematocrit $< 30\%$ or $> 52\%$, platelet count $< 100,000/\text{mL}$, acute coronary syndrome within 48 h before hospitalization or severe valvular heart disease. Patients who had received revascularization after CABG were excluded. Patients who underwent CABG ≤ 1 year before enrolment were also excluded.

Measurement of adropin levels

Blood samples were obtained after an overnight fast (≥ 8 h) and before performing angiography. After resting for ≥ 20 min in a supine position, venous blood samples were drawn into EDTA-coated tubes, promptly centrifuged at 4°C and frozen at -80°C until required for adropin assays. Plasma adropin levels were measured using an enzyme-linked immunosorbent assay kit (catalog number EK-032-35; Phoenix Pharmaceuticals, Belmont, CA, USA) according to the manufacturer's instructions. The detection range of the assay was 0.01 – 100 ng/mL, the sensitivity was 0.3 ng/mL and the linear range was 0.01 – 100 ng/mL. The intra-assay (within-day) and interassay (between-day) coefficients of variations were $< 10\%$ and $< 15\%$, respectively.

Other laboratory data

Fasting plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured enzymatically using an autoanalyzer (Hitachi 911 autoanalyzer; Hitachi, Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol levels were determined using the Friedewald formula. Blood samples were also collected in an EDTA-coated tube to determine the complete blood count, including the platelet count and hemoglobin levels. A hematology analyzer was used for these measurements (Sysmex XE-2100, Sysmex, Kobe, Japan).

Coronary angiography

Coronary angiography (CA) (Siemens Axiom Artis zee 2011; Siemens Healthcare, Erlangen, Germany) was performed using the femoral approach according to standard clinical practice. All cine-angiograms were reviewed by two interventional cardiologists who were blinded to the study protocol. Operation notes and details were available for all patients. Inter- and intra-observer agreement was assessed using the intraclass correlation coefficients (ICCs). The ICCs for the inter- and intra-observer assessments of SVG occlusion ranged from 0.763 to 0.935, indicating very good agreement. If there was disagreement between the assessments, the patients' clinical data could be assessed or non-invasive stress tests could be performed. If tests were positive or the patient had angina, the graft was assumed to be occluded. The bypass grafts were examined in multiple projections and the degree of stenosis was determined in the projection that showed the most severe narrowing. The stumps of occluded (100% stenosis) grafts were selectively injected or visualized on aortograms in appropriate projections.

Statistical analysis

All statistical analyses were done using SPSS for Windows version 11.5 (SPSS, Chicago, IL, USA). Results are reported as the mean $\pm$ standard deviation (SD) or percent of, and categorical variables are presented as absolute numbers and percentage. Continuous variables were compared using Student unpaired *t* tests or Mann-Whitney nonparametric *U* tests. Categorical variables were compared using chi-square statistics or Fisher exact tests as appropriate. Observed unadjusted and adjusted measures of association were obtained using logistic regression models and are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Separate logistic regression analyses were performed to identify independent predictors of adropin levels using all clinical variables. These univariate predictors were entered into a second binary logistic regression

model to obtain the adjusted OR. The multivariate model consisted of adropin levels and the clinical variables. In all analyses, *p*-values of <0.05 were considered statistically significant.

Results

The demographic and clinical characteristics of the patients are shown in Table 1. There were no significant differences between the two groups. The mean time from CABG to the last coronary angiogram was similar in both groups (71.6 ± 45.5 vs. 67.5 ± 56.3 months, $p = 0.77$). The two groups did not differ in terms of medication taken before study enrollment, including the use of aspirin, β -blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, nitrates and statins. The laboratory variables are presented in Table 2. Total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, hemoglobin, fasting blood glucose and fibrinogen levels were not significantly different between the two groups. Angina class was significantly higher in the occluded group ($p = 0.001$).

The mean adropin level was significantly lower in the occluded group than in the patent group (3.2 ± 0.71 vs. 4.9 ± 1.51 ng/mL, $p < 0.001$).

Multivariate logistic regression was performed including all variables to assess the independent predictors of SVG occlusion. Lower serum levels of adropin was found to be the significant and independent predictor of SVG occlusion (OR = 0.558, 95% CI = 0.433–0.714, $p < 0.01$; Table 3. As demonstrated in Table 4, serum adropin levels were correlated with body mass index (BMI) ($p = 0.250$, $p = 0.007$). Serum adropin levels were also correlated with the use of statin and with smoking habits ($p = -0.455$, $p = 0.003$, $p = 0.386$, $p = 0.003$). There was no correlation between serum adropin levels with other parameters.

Discussion

This was the first study to determine the association between adropin levels and late SVG occlusion after CABG. Mean adropin levels were significantly lower in patients with an occluded SVG than in patients with a patent SVG. Additionally, multivariate logistic regression analysis revealed that only the adropin level was independently associated with late SVG occlusion.

CABG is a widely performed revascularization technique that improves the patient's quality of life unless graft occlusion occurs [10]. Graft occlusion is one of the main problems associated with CABG. Three consecutive phases of venous graft disease have been described: an initial phase comprising

TABLE 1. Demographic and clinical characteristics of the patients

Variable	Patients with SVGD (n=38)	Patients with patent SVGs (n=42)	p-value
Age, years	62 ± 9.7	60 ± 12.0	0.59
Females/males, <i>n</i>	14/24	15/27	0.6
BMI, kg/m ²	26 ± 5.1	25.3 ± 5.9	0.55
Waist circumference, cm	110 ± 7.1	108 ± 9.2	0.3
Time from surgery, months	71.6 ± 45.5	67.5 ± 56.3	0.77
Operation time, minutes	250.97 ± 50.93	260.41 ± 51.32	0.65
Hypertension, <i>n</i> (%)	21 (55.6)	21 (50)	0.76
Diabetes mellitus, <i>n</i> (%)	14 (38.9)	13 (31.8)	0.64
Smokers, <i>n</i> (%)	24 (63.1)	11 (27.3)	0.02
Hyperlipidemia, <i>n</i> (%)	23 (61.1)	25 (59.1)	0.89
Family history of CAD, <i>n</i> (%)	17 (44.4)	19 (45.4)	0.94
Angina class II-IV (CCS), <i>n</i> (%)	26 (68.4)	5 (11.9)	0.001
Pharmacotherapy, <i>n</i> (%)			
Acetylsalicylic acid	38 (100)	42 (100)	1
β-blocker	38 (100)	42 (100)	1
ACEI or ARB	27 (72.2)	25 (59.1)	0.39
Nitrate	19 (50)	23 (54.5)	0.76
Statin	33 (86.8)	39 (92.8)	0.65
CCB	4 (11.3)	6 (13.6)	0.81
Ejection fraction, %	47.8 ± 11.2	51.1 ± 8.5	0.29
CRP, mg/dL	5.1 ± 3.2	4.7 ± 3.9	0.34
SBP, mmHg	133.1 ± 10.4	135.3 ± 11.1	0.66
DBP, mmHg	83.2 ± 7.1	80.5 ± 8.7	0.61

Abbreviations: ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker; BMI = body mass index, CAD = coronary artery disease, CCB = calcium channel blocker, CCS = Canadian Cardiovascular Society, CRP = C-reactive protein, DBP = diastolic blood pressure, SBP = systolic blood pressure and SVG = saphenous vein graft

thrombotic occlusion, especially during the first month post-surgery; an intermediate phase of neointimal hyperplasia, for a period of up to 1 year; and, a final phase comprising atherosclerotic disease, after 1 year. Late vein graft occlusion is considered to be part of the atherosclerotic process [11]. Accordingly, this study was performed in patients who were followed up for a long time after CABG with a SVG: the mean time from surgery was 71.6 ± 45.5 months in the occluded SVG group and 67.5 ± 56.3 months in the patent SVG group.

Adropin is a newly identified protein that exerts a protective role on endothelial cells and has been referred to as a novel regulator of these cells [12]. Lovren *et al* [8] demonstrated that adropin could enhance the expression of endothelial nitric oxide synthase (eNOS) in the endothelium via activation of vascular endothelial growth factor receptor (EGFR) 2 phosphatidylinositol 3-kinase Akt and EGFR 2 extracellular signal-regulated kinase 1/2 pathways. As eNOS is responsible for the production of vascular nitric oxide (NO) [13], adropin defi-

TABLE 2. Laboratory data

Variable	Patients with SVG occlusion (<i>n</i> = 38)	Patients with patent SVGs (<i>n</i> = 42)	<i>p</i> -value
Hemoglobin, g/dL	13.4 ± 1.6	13.0 ± 1.8	0.48
Hematocrit, %	41 ± 6.6	41 ± 5.3	0.67
White blood cell count, ×10 ³ /mL	12.0 ± 3.5	12.6 ± 3.9	0.58
Platelet count, ×10 ³ /mL	248 ± 70.8	250.3 ± 66.3	0.82
Red cell distribution width, %	13.0 ± 2.1	13.5 ± 1.4	0.62
Red blood cell count, ×10 ⁶ /mL	4.2 ± 0.6	4.1 ± 0.3	0.71
Mean corpuscular volume, fL	82.0 ± 5.1	84.0 ± 5.6	0.59
Mean platelet volume, fL	10.3 ± 1.2	10.4 ± 1.4	0.60
Total cholesterol, mg/dL	190 ± 35	195 ± 20	0.6
LDL cholesterol, mg/dL	102 ± 20	108 ± 32	0.36
HDL cholesterol, mg/dL	36 ± 8	40 ± 6	0.11
Triglyceride, mg/dL	149 ± 37	154 ± 57	0.53
Fasting glucose, mg/dL	90.2 ± 11.1	92.6 ± 17.1	0.2
Adropin, ng/mL	3.2 ± 0.71	4.9 ± 1.51	< 0.001

Abbreviations: LDL = low-density lipoprotein; HDL = high-density lipoprotein

ciency is associated with reduced nitric oxide bioavailability in the endothelium.

Loss of NO bioavailability is a cardinal feature of endothelial dysfunction that precedes the development of overt atherosclerosis and is an independent predictor of the development of SVG occlusion. The reduced circulating adropin concentrations are correlated with metabolic disorders that are associated with insulin resistance and obesity. These metabolic disorders are related to the progression of coronary atherosclerosis and increased incidence of cardiovascular events such as SVG occlusion [7] and acute coronary syndromes [14].

In present study, serum adropin levels were found to be negatively correlated with body mass index and positively correlated with statin using and smoking habits. For obese patients, these findings are as expected. Statin use and smoking habits may effect these results by reducing adropin levels.

Wu *et al.* [13] reported at the adropin level was significantly associated with the severity of CAD in patients with diabetes mellitus. Our results, lower adropin levels in the occluded SVG group in comparison with the patent SVG group, support these earlier studies.

In the present study, statins were used at similar rate in two groups and there were no significant differences in HDL cho-

lesterol, LDL cholesterol or total cholesterol levels between the two groups. In our study, age, BMI, statin using and smoking were independent risk factors, together with adropin levels, for SVG occlusion. Serum adropin levels were found to be correlated with body mass index, statin use and smoking habits.

Adropin is not currently used as a marker for any disease, so our study is a pioneering work. According to our results, serum adropin level may be used as a novel marker for evaluation and follow up in patients with SVG occlusion or atherosclerosis.

Some limitations of this study warrant mention, particularly the small number of patients. Additionally, some risk factors for SVG occlusion, such as oxidized-LDL, homocysteine, high-sensitivity C-reactive protein and interleukin-6, were not measured and thus it cannot be ruled out that the basic pathomechanisms of late saphenous vein graft occlusion include the activation of monocytes/macrophages caused by high homocysteine level and the associated oxidative stress. For this reason, some uncertainty concerning the independent value of adropin exists.

TABLE 3. Multivariate logistic regression analysis for the predictors of SVG occlusion.

Variables	OR (95% CI)	P value
Age (year)	1.006 (0.969–1.151)	0.002
Male	0.709(0.311–1.513)	0.418
BMI (kg/m ²)	0.851 (0.713–0.980)	0.004
SBP (mm/Hg)	0.985 (0.991–1.109)	0.263
DBP (mmHg)	0.963 (0.929–0.999)	0.141
TC (mg/dl)	1.313 (0.968–1.837)	0.179
TG (mg/dl)	1.025 (0.769–1.573)	0.810
LDL-c (mg/dl)	1.219 (0.631–1.802)	0.277
HDL-c (mg/dl)	0.414 (0.083–1.061)	0.290
FBG (mg/dl)	0.819(0.583–1.250)	0.249
CRP (mg/L)	1.388 (1.115–1.728)	0.103
LVEF	1.107 (1.063–1.153)	0.123
Smoking habits	1.446 (0.648–2.227)	0.003
On statins	1.889 (0.775–4.605)	0.018
On ACEI/ARB	0.791 (0.352–1.778)	0.571
Adropin (per ng/ml)	0.558 (0.433–0.714)	< 0.001

Abbreviations: All values are presented as mean $\pm$ SD, median value (interquartile range) or n (%). BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol, TG = triglycerides, LDL-c = low-density lipoprotein cholesterol, HDL-c = high density lipoprotein cholesterol, FBG = fasting glucose, CRP = C-reactive protein, LVEF = left ventricular ejection fraction and ACEI/ARB = (on the use of) angiotensin converting enzyme inhibitor/angiotensin receptor blocker.

Conclusions

Our results showed that the mean adropin level was lower in patients with late SVG occlusion than inpatients with a patent SVG. Low adropin levels, along with other factors such as smoking, age, BMI, may lead to SVG occlusion. Circulating adropin level may be a novel marker for coronary atherosclerosis and SVG occlusion. Randomized, controlled studies on this subject are needed.

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There was no external support for the conduct of this study.

TABLE 4. Correlations between adropin and other clinical characteristics in patients with SVG occlusion

Variables	Correlation coefficient	p-value
Age (year)	0.085	0.225
BMI (kg/m ²)	0.250	0.007
SBP (mm/Hg)	0.045	0.809
DBP (mmHg)	0.063	0.550
TC (mg/dl)	0.072	0.510
TG (mg/dl)	0.302	0.141
LDL-c (mg/dl)	0.101	0.312
HDL-c (mg/dl)	- 0.108	0.193
FBG (mg/dl)	0.110	0.301
CRP (mg/L)	0.111	0.285
LVEF	- 0.057	0.371
On statins	- 0.455	0.003
Smoking habits	0.386	0.003

Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol, TG = triglycerides, LDL-c = low-density lipoprotein cholesterol, HDL-c = high density lipoprotein cholesterol, FBG = fasting glucose, CRP = C-reactive protein, LVEF = left ventricular ejection fraction and ACEI/ARB = (on the use of) angiotensin converting enzyme inhibitor/angiotensin receptor blocker.

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