Adiponectin Produced in Coronary Circulation Regulates Coronary Flow Reserve in Nondiabetic Patients with Angiographically Normal Coronary Arteries

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Summary

Background: Epicardial adipose tissue expresses adiponectin protein, and its expression is significantly lower in patients with severe coronary artery disease (CAD) than in those without CAD. Transcoronary adiponectin levels are significantly decreased in nondiabetic but not in diabetic patients with CAD. Adiponectin is also an important adipocytokine that is linked to insulin resistance and reduces coronary microvascular function.

Hypothesis: Adiponectin may play a significant role in the localized coronary circulation. The present study examines the local dynamics of adiponectin in the coronary circulation in nondiabetic individuals with normal coronary arteries and the relationship between adiponectin and coronary microvascular function.

Methods: We examined 22 consecutive nondiabetic patients whose coronary arteries were angiographically normal. Plasma levels of adiponectin were measured in blood samples that were simultaneously collected from the orifice of the left coronary artery (LCA) and the great cardiac vein (GCV). To evaluate the function of the coronary microcirculation, we

measured coronary flow velocity at maximal hyperemia using a Doppler wire. Coronary flow reserve (CFR) was obtained from the ratio of hyperemia to the baseline coronary flow velocity.

Results: Plasma adiponectin levels in the GCV (median 6.95 $\mu\text{g/ml}$) were significantly higher than those in the LCA (median 6.60 $\mu\text{g/ml}$, $p < 0.0005$). The difference in plasma adiponectin levels between GCV and LCA significantly correlated with CFR ($R = 0.451$, $p < 0.05$).

Conclusions: Adiponectin is locally produced in the coronary circulation. This protein may participate in modulating the coronary circulation of nondiabetic patients with angiographically normal coronary arteries.

Key words: adiponectin, coronary circulation, microvascular function, coronary flow reserve.

Introduction

Adiponectin, an adipocyte-specific plasma protein isolated from human adipose tissues¹ suppresses the expression of adhesion molecules on endothelial cells,² the proliferation of vascular smooth muscle cells,³ and the transformation of macrophages into foam cells.² Systemic clinical hypoadiponectinemia is closely associated with obesity, type 2 diabetes, and coronary artery disease (CAD).⁴ Furuhashi *et al.* recently reported that transcardiac adiponectin levels are significantly decreased in nondiabetic patients with CAD, but not in diabetic patients with the same condition.⁵ These findings suggested that the transcardiac extraction of adiponectin, at least in part, prevents the progression of coronary atherosclerosis in nondiabetic patients with CAD, whereas impaired adiponectin utilization in diabetic patients is related to the development of coronary atherosclerosis.⁵ However, since epicardial adipose tissue expresses adiponectin protein and its expression is significantly lower in patients with severe CAD than in those without CAD,⁶ we investigated the transcardiac difference in adiponectin levels in nondiabetic individuals without CAD in the present study.

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Adiponectin is an important adipocytokine linked to insulin resistance⁷ and reduces coronary microvascular function.⁸ These findings imply that adiponectin plays a significant role in the coronary circulation; however, its relationship with coronary microvasculature function has not been evaluated. We therefore measured plasma levels of adiponectin at the inlet and outlet of the coronary circulation and evaluated its effect on the coronary microvascular function in nondiabetic individuals with angiographically normal coronary arteries.

Methods

We examined 22 consecutive nondiabetic patients (9 men and 13 women; mean age 59.7 ± 2.5 years; range 38–86) whose coronary arteries were angiographically normal. Patients with diabetes and/or insulin resistance were excluded from the present study because these conditions could affect local adiponectin dynamics in the coronary circulation. Patients with atypical chest pain ($n = 16$), microvascular angina ($n = 4$), or vasospastic angina ($n = 2$) were included. Plasma levels of adiponectin were measured in blood samples that were simultaneously collected from the orifice of the left coronary artery (LCA) and the great cardiac vein (GCV) after control coronary angiography. We measured plasma samples twice and used averaged data. If two values differed widely, the measurements were repeated and the two closest values were adopted in this study. The coefficient of variance for the adiponectin analyses at the mean plasma concentration level of $7.91 \mu\text{g/ml}$ was 4.01%. The lower range of plasma adiponectin levels measured using enzyme-linked immunosorbent assay (ELISA) kits was $1.9 \mu\text{g/ml}$. Written informed consent to participate in the study was obtained from all patients, and the Ethics Committee of our institution approved the study protocol. The difference in plasma adiponectin levels between the GCV and LCA was defined as plasma adiponectin level at the GCV minus that at the LCA. Positive values suggested that adiponectin is produced in the coronary circulation.

To evaluate the function of the coronary microcirculation, we injected papaverine (10 mg) into the LCA over a period of 90 s and measured coronary flow velocity at maximal hyperemia using a 15-MHz Doppler wire (FloWire, 0.014 inch, Volcano Therapeutics, Inc., Rancho Cordova, Calif., USA). Coronary flow reserve (CFR) was obtained from the ratio of hyperemia to the baseline coronary flow velocity. Since the QT interval was excessively prolonged in one patient who received < 10 mg of papaverine, we could not obtain a complete dataset from this patient. Therefore, we obtained CFR values from 21 patients. Baseline characteristics of the participants are expressed as means $\pm$ standard error (SE). The HOMA index was calculated as fasting plasma glucose (mg/dl) $\times$ fasting plasma insulin level ($\mu\text{U/ml}$)/405 and plasma adiponectin values are expressed as medians and means $\pm$ SE. Because the distribution of plasma adiponectin levels was skewed, we used a nonparametric analysis for this comparison. Differences in plasma adiponectin levels between the LCA and GCV were assessed using the Wilcoxon rank sum test. Linear regression analyses

compared correlations between differences in plasma adiponectin levels through the coronary circulation and the CFR. A p value of < 0.05 was considered statistically significant.

Results

The baseline characteristics of body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, fasting plasma glucose values and the HOMA index in the participants were $24.4 \pm 0.83 \text{ kg/m}^2$, $131.1 \pm 3.3 \text{ mmHg}$, $76.1 \pm 2.2 \text{ mmHg}$, $208.3 \pm 5.9 \text{ mg/dl}$, $92.8 \pm 2.7 \text{ mg/dl}$, and 1.47 ± 0.14 , respectively. Figure 1 shows the plasma adiponectin levels at the LCA and the GCV of the individual patients. The plasma adiponectin levels in the GCV (median $6.95 \mu\text{g/ml}$) were significantly higher than those in the LCA (median $6.60 \mu\text{g/ml}$, $p < 0.0005$). The mean values of adiponectin at the LCA and GCV were 8.54 ± 1.01 and $9.18 \pm 1.07 \mu\text{g/ml}$, respectively. The median and mean transcortical increases in plasma adiponectin levels between LCA and GCV were 0.35 and $0.64 \mu\text{g/ml}$, respectively. The plasma level of adiponectin in the GCV was higher than that in the LCA in 20 of 22 patients (91%). This suggested that adiponectin is produced in the coronary circulation of nondiabetic patients with angiographically normal coronary arteries. The difference in plasma adiponectin levels between GCV and LCA significantly correlated with CFR ($R = 0.451$, $p < 0.05$) (Fig. 2), suggesting that adiponectin modulates the coronary microvascular function in nondiabetic patients.

Discussion

The present study showed that adiponectin is produced in the coronary circulation and that it modulates the coronary microvascular function in nondiabetic patients with angiographically normal coronary arteries.

The mRNA of adiponectin was originally detected solely in adipose tissue.¹ Therefore, the production site of adiponectin

FIG. 1 Plasma adiponectin concentration in the left coronary artery (LCA) and the great cardiac vein (GCV) in individual patients. Plasma adiponectin level is significantly higher in GCV than in LCA (median 6.95 vs. $6.60 \mu\text{g/ml}$, $p < 0.0005$).

FIG. 2 Relationship between adiponectin production in coronary circulation and coronary flow reserve (CFR). Closed circles, closed triangles, and closed squares represent data from patients with chest pain syndrome, vasospastic angina, and microvascular angina, respectively. Differences in plasma adiponectin levels between the great cardiac vein (GCV) and the left coronary artery (LCA) significantly correlated with CFR ($R = 0.451$, $p < 0.05$).

in the coronary circulation is believed to be adipose tissue in the myocardium and the epicardial fat pad and around the coronary vessels. Indeed, fat is normally present on the epicardial surface of the ventricle and around the coronary blood vessels.^{9, 10} Iacobellis *et al.* recently showed that epicardial adipose tissue expresses adiponectin protein and that its expression is significantly less in patients with severe CAD than in those without CAD.⁶ Epicardial adipose tissue is also a source of inflammatory factors such as interleukin-1 β , interleukin-6, monocyte chemoattractant protein-1, and tumor necrosis factor- α .¹¹ These findings suggest that epicardial adipose tissue is involved in a localized cytokine-inflammatory pathway in the coronary circulation. Further investigations are required to confirm the production site of adiponectin in the coronary circulation. Furuhashi *et al.* considered that atherosclerotic plaque might consume adiponectin which would prevent its progression in nondiabetic patients with CAD and subsequently decrease the plasma adiponectin level throughout the coronary circulation.⁵ Adiponectin can be immunohistochemically stained in the walls of injured but not intact rat blood vessels.¹² However, if adiponectin is not utilized in the coronary atherosclerotic plaque of diabetic patients with more advanced CAD; then the plasma adiponectin level would not change throughout the coronary circulation.⁵ The Furuhashi study included patients with CAD; half of whom had a previous myocardial infarction. In contrast, our patients had angiographically normal coronary arteries and none had had a myocardial infarction. Since the difference between LCA and GCV is the summation of changes in the production and consumption of adiponectin in the coronary circulation, some adiponectin could be recruited to the wall of the angiographically normal coronary artery. This is because being angiographically normal may not mean that the artery is anatomically intact and diffuse, or mild coronary atherosclerosis may be involved that recruits adiponectin into the arterial walls. However, the increase in the adiponectin level across the coronary circulation

suggests that somewhat more adiponectin is produced than consumed in the coronary circulation in angiographically normal coronary arteries.

Microvascular dysfunction of the coronary artery is characterized by an inadequate vasodilator response to metabolic and pharmacologic stimuli. The present study included patients with angiographically normal coronary arteries who presented with chest pain syndrome, microvascular angina, and vasospastic angina; however, the responses to injected papaverine among these three subgroups did not differ. We determined coronary microvascular function in the present study using papaverine. Since it is an endothelium-independent vasodilator, the mechanisms involved in the relationship between the increased adiponectin level in the coronary circulation and CFR seem to depend on an endothelium-independent effect.¹³ Ouchi *et al.* reported a positive correlation between plasma adiponectin levels and the forearm vasodilator response to reactive hyperemia in hypertensive individuals, as well as impaired endothelium-dependent vasorelaxation in adiponectin-KO mice.¹⁴ They concluded that adiponectin exerts vasodilatory effects in an endothelium-dependent manner.¹⁴ In addition, hypoadiponectinemia is associated with impaired microvascular reserve of the extremities in obese, dyslipidemic, and prediabetic monkeys, but the involved mechanisms have not been described.¹⁵ Adiponectin might modulate coronary microvascular function, because it is an important adipocytokine linked to insulin resistance⁶ that influences this function. Insulin exerts a vasodilatory effect through both endothelium-dependent and -independent mechanisms.¹⁶ Although the present study did not clarify the mechanism(s) of adiponectin, it may depend on a direct effect upon smooth muscle cells and/or the smooth muscle cytosolic calcium level. The published results and the present findings together suggest that adiponectin contributes, at least in part, to regulation of the coronary as well as the systemic circulation.

Conclusions

The adipocyte-specific plasma protein, adiponectin, is locally produced in the coronary circulation and may play a part in modulating the coronary circulation in nondiabetic patients with angiographically normal coronary arteries. Although the production site of adiponectin in the coronary circulation remains unknown, epicardial adipose tissue is one possible candidate site within the heart. Further investigations are required to define the site of adiponectin production in the coronary circulation.

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