Novel Modulator for Endothelial Adhesion Molecules
Adipocyte-Derived Plasma Protein Adiponectin

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Background—Among the many adipocyte-derived endocrine factors, we recently found an adipocyte-specific secretory protein, adiponectin, which was decreased in obesity. Although obesity is associated with increased cardiovascular mortality and morbidity, the molecular basis for the link between obesity and vascular disease has not been fully clarified. The present study investigated whether adiponectin could modulate endothelial function and relate to coronary disease.

Methods and Results—For the in vitro study, human aortic endothelial cells (HAECs) were preincubated for 18 hours with the indicated amount of adiponectin, then exposed to tumor necrosis factor-α (TNF-α) (10 U/mL) or vehicle for the times indicated. The adhesion of human monocytic cell line THP-1 cells to HAECs was determined by adhesion assay. The surface expression of vascular cell adhesion molecule-1 (VCAM-1), endothelial-leukocyte adhesion molecule-1 (E-selectin), and intracellular adhesion molecule-1 (ICAM-1) was measured by cell ELISA. Physiological concentrations of adiponectin dose-dependently inhibited TNF-α–induced THP-1 adhesion and expression of VCAM-1, E-selectin, and ICAM-1 on HAECs. For the in vivo study, the concentrations of adiponectin in human plasma were determined by a sandwich ELISA system that we recently developed. Plasma adiponectin concentrations were significantly lower in patients with coronary artery disease than those in age- and body mass index–adjusted control subjects.

Conclusions—These observations suggest that adiponectin modulates endothelial inflammatory response and that the measurement of plasma adiponectin levels may be helpful in assessment of CAD risk. (Circulation. 1999;100:2473-2476.)

Key Words: endothelium ■ cell adhesion molecules ■ coronary disease ■ adiponectin

Obesity, the most common nutritional disorder in the industrial countries, is associated with increased cardiovascular mortality and morbidity.1–3 Adipose tissue expresses various secretory proteins, including leptin, tumor necrosis factor-α (TNF-α), and plasminogen activator inhibitor type 1, which may contribute to the development of Cardiovascular diseases.4–7 TNF-α overproduction in adipose tissue is a possible cause not only of the development of insulin resistance6 but also of the development of atherosclerosis through an inflammatory process in obese subjects. However, the molecular basis for the link between obesity and vascular disease has not been fully clarified. From an extensive search of the human adipose tissue cDNA library, we isolated an adipocyte-specific cDNA, apM1.4,9 The cDNA encoded a 244-amino-acid protein homologous to collagen VIII and X and complement factor C1q. Adiponectin, the gene product of apM1, was found to be a secretory protein abundant in human plasma.10 Plasma adiponectin level was decreased in obesity,10 suggesting that dysregulation of adiponectin may be relevant to obesity-linked disorders. Although adiponectin is the most abundant gene product in adipose tissue9 and accounts for 0.01% of total plasma protein,10 its physiological functions have not been elucidated.

At the early stages of atherosclerosis, endothelial cell activation by various inflammatory stimuli, including TNF-α, results in the synthesis of adhesion molecules and increases the adherence of monocytes.11 This monocyte adhesion to the arterial endothelium is considered crucial for the development of vascular diseases.11 We hypothesized that circulating adiponectin might modulate vascular wall functions like other adipocyte-derived secretory proteins.

In this study, we investigated the propositions that physiological concentrations of adiponectin suppressed TNF-α–mediated expression of adhesion molecules in human aortic
endothelial cells (HAECs) and that plasma adiponectin levels were markedly low in patients with coronary artery disease (CAD).

**Methods**

**Cell Culture**

HAECs (Clonetics) were maintained in MCDB131 medium (Clonetics) supplemented with 5% FCS, and cells from passages 4 through 6 were used for experiments. HAECs (confluent in type I collagen-coated plate) were incubated for 18 hours in medium 199 (Gibco) containing 0.5% FCS and 3% BSA with the indicated amount of adiponectin, then exposed to human recombinant TNF-α (R&D systems) at a final concentration of 10 U/mL or vehicle for the times indicated. Human recombinant adiponectin was prepared as previously described.10

**Adhesion Assay**

The adhesion of the THP-1 human monocytic cell line to HAECs was determined by previously described.12 FITC-labeled THP-1 cells (5×10^5 cells/well) were incubated with HAECs in 24-well plates for 10 minutes at 37°C. The plates were sealed, inverted, and centrifuged (250g) for 5 minutes to separate nonadherent monocytes. Adherent cells were lysed with 50 mmol/L Tris (pH 8.4)/0.1% SDS, and the fluorescence was measured. Statistical significance was determined by a paired t test.

**Cell ELISA**

HAECs were incubated at room temperature with anti-human vascular cell adhesion molecule-1 (VCAM-1), anti–endothelial-leukocyte adhesion molecule-1 (E-selectin), or anti–intracellular adhesion molecule-1 (ICAM-1) monoclonal antibody (DAKO) at 1:1000 dilution in medium 199 containing 0.5% BSA for 1 hour and then with horseradish peroxidase–conjugated goat anti-mouse IgG (Cappel) at 1:1000 dilution in the same medium. Color formation with o-phenylenediamine dihydrochloride was measured at 492 nm.

**Northern Blot Analysis**

HAECs were pretreated with 50 μg/mL of adiponectin and exposed to TNF-α for 4 hours. Total RNA (20 μg per lane) was extracted, electrophoresed, and transferred to a nylon membrane. The membranes were hybridized with human VCAM-1, E-selectin, or ICAM-1 cDNA probes labeled with [α-32 P]dCTP.

**Measurement of Plasma Adiponectin Concentration**

Consecutive CAD patients were recruited from inpatients admitted to Osaka University Hospital. The criterion for CAD was a ≥75% organic stenosis of ≥1 segment of a major coronary artery confirmed by coronary angiogram. The control subjects were selected from the inpatients of Osaka University Hospital and matched with the CAD patients for age and body mass index (BMI). They had no evidence of vascular diseases, including angina pectoris. All subjects enrolled
in this study were Japanese and gave written informed consent. The Ethics Committee of Osaka University approved this study.

The concentrations of adiponectin in human plasma were determined as described.\textsuperscript{10} The significance of the differences between the groups was determined by the Mann-Whitney test. Linear regression analysis was used to evaluate the relationship between the variables.

### Results

#### Adiponectin Inhibits TNF-α–Induced Monocyte Adhesion and Expression of Adhesion Molecules

We investigated the effects of adiponectin on monocyte adhesion to HAECs. Adiponectin treatment alone had no significant effect on the adhesion of human mononuclear cell line THP-1 cells to HAECs (Figure 1A). TNF-α treatment (10 U/mL for 6 hours) enhanced adhesion of THP-1 cells to HAECs by 2-fold (Figure 1A). When HAECs were preincubated with adiponectin for 18 hours before a 6-hour coinoculation with TNF-α, THP-1 cell adhesion was dose-dependently suppressed by adiponectin treatment (Figure 1A). However, THP-1 cell adhesion to TNF-α-stimulated HAECs was not suppressed when adiponectin was added at the same time as THP-1 cells (data not shown), suggesting that adiponectin does not occupy the binding sites of THP-1 cells on HAECs.

TNF-α treatment increased the surface expression of VCAM-1, E-selectin, and ICAM-1 on HAECs by 2- to 5-fold (Figure 1, B through D). Dose-dependent suppression of TNF-α-induced surface expression of VCAM-1, E-selectin, and ICAM-1 was observed when HAECs were pretreated with adiponectin (Figure 1, B through D). Adiponectin treatment alone did not lead to any significant changes in the surface expression of these molecules in the absence of TNF-α (Figure 1, B through D). The suppressive effects of adiponectin on cell-surface expression of these adhesion molecules were accompanied by changes in steady-state mRNA levels analyzed by Northern blot (Figure 1, B through D, inset).

#### Plasma Adiponectin Concentrations in Patients With CAD

Plasma adiponectin levels were significantly lower in patients with CAD than in age- and BMI-adjusted control subjects both in men (3.4±1.8 versus 7.4±3.5 μg/mL) (P<0.01) and in women (4.3±1.5 versus 9.3±6.8 μg/mL) (P<0.05) (Figure 2). Although atherogenic lipid profiles were observed in the CAD group compared with the control (data not shown), the decreased plasma adiponectin level may be another coronary risk factor.

#### Discussion

In the present study, we demonstrated that physiological concentrations of adiponectin (5 to 25 μg/mL) had significant inhibitory effects on TNF-α–induced monocyte adhesion and adhesion molecule expression in a dose-dependent manner in vitro. Atherosclerosis has been regarded as an inflammatory disease. The initial atherosclerotic lesion consists of monocytes/macrophages and T lymphocytes.\textsuperscript{11} At this stage, adhesion molecules on arterial endothelial cells are responsible for the accumulation of these hemocytes. VCAM-1, E-selectin, and ICAM-1 have been detected in human atherosclerotic lesions.\textsuperscript{13} The expression of adhesion molecule plays an important role in the regulation of inflammatory reactions in various types of cells.\textsuperscript{14,15} If the excess inflammatory response could be neutralized by adiponectin, it might be possible to prevent the process of atherogenesis. Our findings indicate that adiponectin acts as an endogenous modulator of endothelial cell function, although further investigation is needed to elucidate the intracellular signals by which adiponectin suppresses adhesion molecules.

Recently, we developed an ELISA system to quantify the plasma adiponectin concentration.\textsuperscript{10} Paradoxically, the plasma adiponectin levels in obese subjects were significantly lower than those in nonobese subjects,\textsuperscript{10} although adiponectin is secreted only from adipose tissue. Because obesity is frequently accompanied by vascular diseases, we postulated that the dysregulation of adiponectin may be associated with vascular disease in obese subjects. In this study, plasma adiponectin levels were significantly lower in patients with CAD than in age- and BMI-adjusted control subjects. This finding suggests that the decreased plasma adiponectin level may relate to the development of CAD.

Adiponectin is an adipocyte-specific plasma protein, which acts as an endogenous regulator of endothelial cells in response to inflammatory stimuli. Our observations raise the possibility that the measurement of plasma adiponectin levels may be helpful in assessment of CAD risk.

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