Adiponectin Reduces Atherosclerosis in Apolipoprotein E-Deficient Mice

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Background—Dysregulation of adipocyte-derived bioactive molecules plays an important role in the development of atherosclerosis. We previously reported that adiponectin, an adipocyte-specific plasma protein, accumulated in the injured artery from the plasma and suppressed endothelial inflammatory response and vascular smooth muscle cell proliferation, as well as macrophage-to-foam cell transformation in vitro. The current study investigated whether the increased plasma adiponectin could actually reduce atherosclerosis in vivo.

Methods and Results—Apolipoprotein E-deficient mice were treated with recombinant adenovirus expressing human adiponectin (Ad-APN) or β-galactosidase (Ad-βgal). The plasma adiponectin levels in Ad-APN–treated mice increased 48 times as much as those in Ad-βgal treated mice. On the 14th day after injection, the lesion formation in aortic sinus was inhibited in Ad-APN–treated mice by 30% compared with Ad-βgal–treated mice (P<0.05). In the lesions of Ad-APN–treated mice, the lipid droplets became smaller compared with Ad-βgal–treated mice (P<0.01). Immunohistochemical analyses demonstrated that the adenovirus-mediated adiponectin migrate to foam cells in the fatty streak lesions. The real-time quantitative polymerase chain reaction revealed that Ad-APN treatment significantly suppressed the mRNA levels of vascular cell adhesion molecule-1 by 29% and class A scavenger receptor by 34%, and tended to reduce levels of tumor necrosis factor-α without affecting those of CD36 in the aortic tissue.

Conclusions—These findings documented for the first time that elevated plasma adiponectin suppresses the development of atherosclerosis in vivo. (Circulation. 2002;106:2767-2770.)

Key Words: proteins ■ atherosclerosis ■ plasma ■ remodeling

Adipose tissue secretes a variety of bioactive molecules that directly contribute to the development of cardiovascular diseases.1–4 Adiponectin is an adipose-specific plasma protein that was identified by our group in human adipose tissue.4 Acrp30 or AdipoQ, independently cloned by 2 groups, is the mouse counterpart of adiponectin.5,6 Interestingly, low plasma adiponectin concentrations were observed in patients with obesity, coronary artery disease (CAD), and type 2 diabetes with macroangiopathy.7–9 Furthermore, the incidence of cardiovascular death was higher in patients with low plasma adiponectin compared with those with higher adiponectin levels.10 Immunohistochemical studies revealed that adiponectin from the plasma adhered to the injured artery.11,12 In cultured cells, human recombinant adiponectin suppressed the endothelial expression of adhesion molecules, the proliferation of vascular smooth muscle cells, and the transformation of macrophage to foam cells.8,12–14 These data suggest that adiponectin has anti-atherogenic properties.

In the present study, we investigated the therapeutic effects of adiponectin on the development of atherosclerosis in apolipoprotein E-deficient (apoE−/−) mice.

Method
Recombinant Adenovirus
Replication-defective recombinant adenovirus was constructed with Adenovirus Expression Vector Kit (Takara). Adenovirus expressing the full-length apM1 cDNA (Ad-APN) or β galactosidase gene (Ad-βgal) was propagated in 293 cells, purified by CsCl gradient centrifugations, and stored at −80°C until use.

Animal Protocol
Male apoE−/− mice (Jackson Laboratory, Bar Harbor, Me) were fed a normal chow. At the age of 12 weeks, Ad-APN or Ad-βgal was injected into tail vein (2.2×10⁹ pfu each). The mice were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg), and the hearts, which contained the aortic sinus and aortic arch, were harvested at the indicated time. This protocol was approved by the
Institutional Laboratory Animal Care and Use Committee of Osaka University.

**Plasma Data Analyses**

Blood samples were collected from mice ad libitum on the fifth day after adenovirus injection. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and glucose levels were measured with enzymatic kits (Wako), and insulin levels were assayed with EIA kit (Glazyme). Human or mouse adiponectin levels were determined from 3 cross-sections (100 pm) and high (C, bar=100 μm) magnification are shown. B, Quantification of lesion areas in each group. The average lesion area of each mouse was determined from 3 cross-sections (100 pm apart). D, The diameter of lipid droplets over 10 μm in Ad-βgal or Ad-APN treated mice (n=9 each). The values are mean±SEM.

**Results**

Plasma adiponectin levels in Ad-APN–treated mice increased to a level 48 times higher than the level of endogenous acrp30 in Ad-βgal treated mice; however, no significant difference was observed between 2 groups in cholesterol, glucose, and insulin levels (Table). In 12-week-old non-treated apoE−/− mice, the atherosclerotic lesion area in aortic sinus and the diameter of lipid droplets in fatty streaks were 2.98×10^4 ±0.41 μm² and 17.8±0.2 μm, respectively. After 14 days of adenovirus injection, the lesion area of Ad-APN–treated mice was significantly reduced by 30% compared with Ad-βgal treated mice (5.20×10^4 ±0.62 versus 3.66×10^4±0.35 μm², P<0.05) (Figure 1A and 1B). In the lesions of Ad-APN–treated mice, the diameter of lipid droplets in fatty streaks was significantly reduced relative to Ad-βgal–treated mice (22.4±0.2 versus 18.0±0.2 μm, P<0.01) (Figure 1C and 1D).
To assess the role of elevated plasma adiponectin in atherosclerotic lesion formation, the localization of adenovirus-derived human adiponectin, macrophages, and smooth muscle cells were analyzed immunohistochemically on the fifth day after adenovirus injection. Adenovirus-derived human adiponectin abundantly adhered to Mac-1 positive and α-actin negative cells in the fatty streak lesions (Figure 2A).

To further assess the mechanism of lesion reduction, the mRNA levels of VCAM-1, SR-A, TNF-α, and CD36 were quantified. Ad-APN treatment significantly suppressed the mRNA levels of VCAM-1 (100±10.3 versus 70.9±4.8%, P<0.05) and SR-A (100±10.0 versus 65.8±9.2%, P<0.05, and tended to reduce those of TNF-α (100±33.6 versus 63.1±15.2%) without affecting those of CD36 in the aortic tissue (100±4.5 versus 100.2±13.0%) (Figure 2B).

**Discussion**

In the present study, we demonstrated that adenovirus-mediated increase of plasma adiponectin significantly suppressed the progression of atherosclerotic lesions in apoE−/− mice. These mice develop hyperlipidemia and vascular lesions similar to human atherosclerosis. Even on a normal chow, the initial foam cell lesions in the aortic root progressed from 12- to 14-week-old mice as previously reported. Adenovirus-derived adiponectin accumulated in the fatty streak lesions composed of macrophages and foam cells in apoE−/− mice. Ad-APN treatment significantly suppressed the expression of VCAM-1 and SR-A and reduced the lipid accumulation in macrophages in atherosclerotic lesions of apoE−/− mice, although no difference was observed in plasma cholesterol, glucose, and insulin levels between Ad-APN and Ad-βgal treatment.

We previously reported that human recombinant adiponectin suppressed nuclear factor-κB inducible gene expression including VCAM-1 in human aortic endothelial cells and SR-A expression in human monocyte-derived macrophages. The recombinant protein did not affect CD36 expression in macrophages. Adiponectin treatment dose-dependently decreased the uptake of modified low-density lipoprotein.

In the present study, the Ad-APN treatment actually inhibited the expression of VCAM-1 and SR-A without affecting that of CD36 in vivo. VCAM-1 and SR-A play a pivotal role in the development of atherosclerosis. The expression of VCAM-1 localized over the surface of endothelial cells in lesion-prone sites, and the targeted disruption of SR-A reduced the size of atherosclerotic lesions in apoE−/− mice. Therefore, our findings suggest that the elevated plasma adiponectin protected endothelial cells from hypercholesterolemia-induced vascular injury and suppressed the uptake of modified low-density lipoprotein into foam cells in apoE−/− mice.

The hypo-adiponectinemia was observed in patients with CAD and was associated with the incidence of cardiovascular death. This study documented for the first time that the overexpression of adiponectin actually reduced atherosclerosis through attenuating endothelial inflammatory response and macrophage to foam cell transformation in vivo. Therefore, in future studies, this model of adiponectin gene transfection will be useful to determine the therapeutic level of plasma adiponectin for preventing atherosclerosis.

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**References**

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