Role of Adiponectin in Insulin-Resistant Hypertension and Atherosclerosis

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Insulin resistance is one of the major risk factors associated with development of hypertension and atherosclerosis. Recent studies have shown that adiponectin, an adipocyte-derived hormone, may be involved in insulin resistance and development of atherosclerosis in diabetes patients. The aim of this study was to examine adiponectin levels in patients with essential hypertension to determine the relationships between adiponectin levels and insulin sensitivity and to examine the relationship of adiponectin with pulse wave velocity (PWV) in a general population based on the results of an epidemiological survey in Japan. In a clinical study, 20 normotensives (NT) and 30 non-treated essential hypertensives (EHT) were hospitalized, and euglycemic hyperinsulinemic glucose clamp (GC) was performed to evaluate insulin sensitivity defined as \( M \) value. EHT were divided into insulin-resistant EHT (EHT-R) and insulin-nonresistant EHT (EHT-N) according to the mean \( -1 \) SD of the \( M \) value of NT as a cut-off point. Fasting plasma glucose (FPG), immunoreactive insulin (IRI), and adiponectin concentrations were measured. There were no significant differences in body mass index (BMI) or FPG among the NT, EHT-N, and EHT-R groups. The \( M \) value and adiponectin concentration in EHT-R were significantly lower than those in the NT or EHT-N. The IRI level in the EHT-R was significantly higher than those in the other groups. A positive correlation between adiponectin concentration and \( M \) value was found in all subjects, and adiponectin concentration and \( M \) value were found to be significant determinants of each other in multiple regression analysis. In an epidemiological study, we studied 391 male inhabitants of rural communities in Hokkaido, Japan. Systolic blood pressure (SBP), BMI, FPG, IRI, and adiponectin were measured in all subjects early in the morning. Homeostasis model assessment (HOMA) values were calculated as an index of insulin sensitivity, and PWV was used as an index of atherosclerosis. A negative correlation between HOMA values and adiponectin concentration was found in all of the subjects. Multiple regression analysis revealed that adiponectin was a significant determinant for PWV in subjects less than 70 years of age. The results of the clinical study indicate that EHT-R had not only hyperinsulinemia but also a low concentration of adiponectin. The results of multiple regression analysis for determinants of degree of PWV using data obtained in the epidemiological study suggest that adiponectin plays a role in anti-atherosclerosis, partly through improvement of insulin resistance. (Hypertens Res 2003; 26: 705–710)

Key Words: adiponectin, hypertension, insulin resistance, pulse wave velocity, atherosclerosis

Introduction

Hypertension, diabetes mellitus, obesity, and dyslipidemia are regarded as risk factors for coronary artery disease (CAD). Insulin resistance and accompanying hyperinsulinemia have been reported to play important roles in the occurrence and maintenance of hypertension, dyslipidemia, and atherosclerosis (1, 2). The relationship between insulin resistance and cardiovascular disease has been examined in many cases.
epidemiological and clinical studies (3–7). We have demonstrated that insulin resistance and hyperinsulinemia are major risk factors for development of CAD, and that they increase the severity of coronary atherosclerosis in patients with or without impaired glucose tolerance (8).

Several studies have shown that there is an association between hyperinsulinemia and hypertension in humans (9–11). In a previous study, we reported that almost 40% of essential hypertensives were insulin-resistant (12). Hyperinsulinemia, which compensates for insulin resistance, is thought to cause and maintain high blood pressure through the following actions of insulin itself: 1) stimulation of the sympathetic nervous system and renin-angiotensin system; 2) promotion of sodium retention in the renal tubules; 3) a cell-proliferating effect associated with increases in intracellular pH and free Ca ions; and 4) an atherogenic effect mediated by the direct action of insulin via receptors for insulin-like growth factor. We have also shown that selective insulin resistance in muscle may cause hyperinsulinemia and that the consequent stimulation of mitogen-activated protein (MAP) kinase in the vasculature may induce atherosclerosis in vivo (13). However, the mechanism of insulin resistance is still obscure.

In the past, adipose tissue was thought to be a passive depot for storage of excess energy. However, recent studies have demonstrated that adipocytes synthesize and secrete biologically active molecules (14–17). These molecules, collectively known as “adipocytokines,” include tumor necrosis factor (TNF)-α, leptin, resistin, free fatty acid (FFA), and adiponectin. Our previous studies have shown that TNF-α and FFA are negatively related to insulin sensitivity in an insulin-resistant state (18, 19). These adipocytokines have also been found to affect insulin signaling and to contribute to the formation of atherosclerosis in the blood vessels (20). Adiponectin, an adipose-specific secretory protein, has been suggested to be involved in insulin resistance and development of atherosclerosis in obese and in diabetic individuals, although, interestingly, its concentration is decreased in both (21, 22). Moreover, recent studies have suggested that adiponectin has anti-inflammatory effects on the vascular wall (23, 24). We therefore performed clinical and epidemiological studies to elucidate the role of adiponectin in insulin resistance and atherosclerosis in patients with essential hypertension.

Methods and Results

Clinical Study

Twenty normotensives (NT) and 30 non-treated essential hypertensives (EHT) without diabetes mellitus were hospitalized and given a diet containing 120 mEq sodium and 75 mEq potassium per day without any medication. Subjects were screened for complications such as endocrine disturbances, cerebrovascular or cardiovascular diseases, and renal disease by routine physiological and laboratory examinations, and none showed any evidence of complications. Euglycemic hyperinsulinemic glucose clamp (GC) was performed to evaluate insulin sensitivity, which was defined as the M value. EHT were divided into insulin-resistant EHT (EHT-R) and insulin-nonresistant EHT (EHT-N) according to the mean ± 1 SD of the M value of NT as a cut-off point. Twelve of the 30 EHT were insulin-resistant, a ratio similar to that previously reported (40%). This division of the EHT group allowed us to evaluate the effect of insulin resistance on adiponectin directly in EHT independent of the influence of high blood pressure. Blood samples were collected before starting GC, and fasting plasma glucose (FPG), immunoreactive insulin concentration (IRI), and adiponectin were also measured. Adiponectin concentration was measured using the enzyme-linked immuorsorbent assay (ELISA) method (Otsuka Pharmaceuticals, Osaka, Japan).

There were no significant differences in age (NT: 45.6 ± 13.9 years; EHT-N: 46.4 ± 12.8 years; EHT-R: 46.3 ± 12.1 years), ratio of males/females (NT: 10/10; EHT-N: 9/9; EHT-R: 5/7), or body mass index (BMI) (NT: 24.5 ± 2.7 kg/m²; EHT-N: 24.7 ± 2.9 kg/m²; EHT-R: 25.3 ± 2.5 kg/m²) among the three groups. Mean blood pressure (MBP) was significantly higher in EHT (109.5 ± 16.9 mmHg, p < 0.01) than in NT (93.4 ± 10.7 mmHg), and there was no significant difference in MBP between EHT-N (111.5 ± 13.9 mmHg) and EHT-R (106.3 ± 21.0 mmHg). The M value in EHT-R (120.3 ± 13.9 mg/m²/min) was significantly lower than those in the other two groups (NT: 184.6 ± 46.5; EHT-N: 197.9 ± 41.2 mg/m²/min; p < 0.01). Although there were no significant differences in FPG levels among the three groups (NT: 89.0 ± 10.1; EHT-N: 86.1 ± 7.1; EHT-R: 88.1 ± 10.4 mg/dl), the insulin level in EHT-R (8.1 ± 4.8 mU/l) was significantly (p < 0.01) higher than the levels in NT (4.9 ± 2.3 mU/l) and EHT-N (4.0 ± 1.5 mU/l).

The adiponectin level in EHT-R (4.1 ± 0.4 µg/ml) was significantly (p < 0.05) lower than the levels in NT (5.7 ± 0.5 µg/ml) and EHT-N (6.0 ± 0.5 µg/ml), but no significant difference was found in the adiponectin levels between NT and EHT-N (Fig. 1).

There was a significant positive correlation between M value and adiponectin level. In multiple regression analysis, adiponectin level (β = 5.932, p < 0.05) and IRI (β = - 7.131, p < 0.01) were shown to be significant determinants of M value, indicating that adiponectin level is related to insulin resistance even when the insulin level has been adjusted. On the other hand, M value (β = 0.013, p < 0.05) and BMI (β = - 0.412, p < 0.01) were shown to be significant determinants of adiponectin levels, indicating that adiponectin level is strongly related to insulin resistance despite the influence of obesity.

The results of our clinical study indicate that EHT-R had not only hyperinsulinemia but also a low concentration of adiponectin, and that adiponectin may be related to insulin resistance in EHT without obesity or diabetes mellitus.
We conducted a longitudinal epidemiological study of cardiovascular diseases in two rural communities in Hokkaido, Japan. A total of 391 male inhabitants aged 65 ± 11 years were randomly selected for the studies. Systolic blood pressure (SBP), BMI, FPG, IRI, and adiponectin were measured in all subjects early in the morning. Homeostasis model assessment (HOMA) values were calculated as an index of insulin sensitivity, and pulse wave velocity (PWV) was measured as an index of atherosclerosis. Subjects who showed an ankle-brachial pressure index (API) of less than 0.9 were considered to have arteriosclerosis obliterans and were excluded from the study.

There was a significant negative correlation between adiponectin and HOMA value in all subjects ($r = -0.274, p < 0.001$, Fig. 2). Adiponectin was not detected as a significant determinant when HOMA was entered into the analysis. We also performed multiple regression analysis, with the result that adiponectin, age, BMI, and HOMA were found to be significant determinants. These results also indicated that adiponectin level is still significantly related to insulin resistance despite the influence of obesity and lipids.

The concentration of adiponectin and PWV were increased with age (data not shown). Aging itself may facilitate atherosclerosis in blood vessels, and our subjects over 70 years old showed complete atherosclerotic changes. Therefore, because the purpose of this study was to examine the effects of adiponectin on the development of atherosclerosis, we performed multiple regression analysis using data obtained from subjects less than 70 years of age. Multiple regression analysis showed that adiponectin and age were significant determinants of PWV ($\beta = -14.97, p < 0.01$), but when HOMA was added to the analysis, adiponectin did not appear as a determinant, although it did show a slight determinant tendency ($\beta = -10.23, p = 0.076$). This result indicates that adiponectin may be involved in atherosclerosis, and that its involvement may be partly mediated by insulin resistance.

**Discussion**

Adiponectin (also known as Acrp30, adipoQ, GBP28, and apM1) (25–28) is an adipocyte-secreted protein consisting of 247 amino acids and exists abundantly in plasma (21). Adiponectin possesses a signal sequence at the NH$_2$-terminal end and is composed of a domain of collagen repeats and a COOH-terminal globular domain (27, 29). Several recent animal studies have suggested that adiponectin plays a role in modulation of glucose tolerance and insulin sensitivity (30–33). Plasma adiponectin concentrations are decreased in patients with type 2 diabetes (34, 35). Despite the fact that adiponectin is an adipose-specific protein, adiponectin concentration in the blood decreases and insulin resistance increases as an individual becomes more obese.

Conversely, body weight reduction increases the plasma concentration of adiponectin (36). In the present study, only the EHT-R had hypoadiponectinemia, and no significant differences were found between the NT and EHT-N. Also, no significant differences were found in BMI among the three groups. Theses results indicate that high blood pressure is not a determinant of adiponectin production and secretion and that insulin resistance is strongly related to adiponectin concentration without influence of obesity. These results are consistent with results of GC studies in humans and monkeys showing that plasma concentrations of adiponectin were significantly correlated with the degree of insulin sensitivity (30). It has also been shown that supplementation of recombinant adiponectin resulted in improvement in insulin resistance according to some studies.
resistance in diabetic mice (33).

Several studies have suggested that insulin resistance can be improved by treatment with adiponectin. Yamauchi et al. (33) reported that adiponectin-treated KKAY mice showed increased expression levels of enzymes involved in β-oxidation and uncoupling protein (UCP) 2 in skeletal muscle. Also, acyl-CoA oxidase (ACO) activities and FFA combustion were increased in the skeletal muscle of mice treated with adiponectin, and these increases led to a decrease in tissue triglyceride content, which was associated with decreases in serum FFA and triglyceride levels. Increased tissue triglyceride content has been reported to interfere with insulin-stimulated activation of phosphatidylinositol-3-kinase and subsequent translocation of glucose-transporter protein 4 (GLUT4) and uptake of glucose, leading to insulin resistance (37). Thus, a decrease in triglyceride content in skeletal muscle might contribute to improvement of insulin signal transduction. We previously reported that fructose-fed rats, an insulin-resistant hypertensive animal model, showed increased intramuscular triglyceride compared with that in control rats, and that the triglyceride level in muscle showed a significant negative correlation with M value (38). We did not examine lipid parameters in the present study, but there might be some relationship between adiponectin and metabolism of lipids in patients with insulin-resistant essential hypertension. Further study is needed to examine this possibility.

On the other hand, hypoadiponectinemia is closely associated with the incidence of CAD (39). Hotta et al. (40) analyzed plasma adiponectin concentrations in age- and BMI-matched nondiabetic and type 2 diabetic subjects with and without CAD. They found that plasma adiponectin concentrations in diabetic patients with CAD were lower than those in diabetic patients without CAD, and they suggested that the decreased plasma adiponectin concentrations in diabetic patients might be an indicator of macroangiopathy. Zoccali et al. (41) reported that the degree of hypoadiponectinemia was correlated with the incidence of fatal heart ischemia in subjects with chronic heart failure. In our study, adiponectin level was found to be significantly correlated to PWV in male subjects less than 70 years of age; however, multiple regression analysis revealed that adiponectin showed only a slight tendency to act as a determinant of PWV when HOMA was added to the analysis. Although further studies will be needed to resolve this matter, our results indicated that adiponectin may be involved in atherosclerosis directly or may be involved indirectly via insulin resistance. These findings indicate that adiponectin has an antiatherogenic property and that hypoadiponectinemia is involved in the pathophysiology of atherosclerosis.

In addition, recent studies have suggested that adiponectin has anti-inflammatory and anti-atherosclerotic effects. In tissue-culture experiments, adiponectin suppressed TNF-α-induced mRNA expression of several adhesion molecules, including vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and E-selectin, in vascular endothelial cells and suppressed monocyte attachment to endothelial cells (42, 43). Kubota et al. (24) generated adiponectin-deficient mice and investigated whether adiponectin directly conferred protective effects against atherosclerosis in vivo. Adiponectin-deficient mice (adipo-/-) showed 2-fold greater neointimal formation in response to external vascular cuff injury than did wild-type mice. Although we did not examine some of the inflammatory parameters, such as TNF-α or high-sensitive C-reactive protein (CRP), in the present study, these previous studies have demonstrated that adiponectin may be an insulin-sensitizing hormone and that hypoadiponectinemia may be involved in the pathophysiology of atherosclerosis.

In contrast to the concentration of many other adipocyte-derived hormones, such as leptin, TNF-α, FFA, and plasminogen-activator inhibitor 1, adiponectin concentrations are reduced in obese animals and humans. The mechanisms by which adiponectin production is regulated have not yet been elucidated. Adiponectin expression and secretion are increased by the addition of ionomycin, a calcium ionophore, in primary human preadipocytes (44). β-Adrenergic agonists, activators of adenylyl cyclase, TNF-α, and glucocorticoids have also been reported to inhibit adiponectin gene expression and secretion (45–48). Nishizawa et al. (49) reported that the plasma concentration of adiponectin is lower in men than in women, but that there is no difference in the concentrations in premenopausal and postmenopausal women. They also demonstrated that testosterone reduced the secretion of adiponectin in 3T3-L1 adipocytes into a culture medium. It has been shown that thiazolidinedione agonists of peroxisome proliferator-activated receptor gamma (PPARγ) increase both adiponectin gene expression and circulating adiponectin levels in animals and humans (50).

However, further studies are needed to examine the regulation of adiponectin using antihypertensive drugs such as α1 blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor antagonists. Obesity is one of the strong determinants of the regulation of adiponectin, and our results demonstrated that M value and BMI were significant determinants for adiponectin in this clinical study. Although patients with hypertension and high BMI show lower adiponectin concentration, no significant differences in BMI were found between EHT-R and EHT-N in the present study. This result may indicate that insulin resistance independently affects the regulation of adiponectin.

As in our previous study, insulin resistance and hyperinsulinemia were closely related to the occurrence and maintenance of hypertension. In this study, there was a negative correlation between M value and blood pressure in the EHT group (data not shown). These results indicated that insulin resistance and hyperinsulinemia might play roles not only in the occurrence and maintenance of hypertension but also in the development of atherosclerosis, with the latter effect occurring, at least in part, via the reduction of adiponectin.

In summary, adiponectin may play a role in anti-athero-
sclerosis either directly or indirectly through its improvement of insulin resistance. Although its receptor and regulatory mechanism have not been clarified, examination of this new “adipocytokine” may provide new insights into the development of atherosclerosis in essential hypertensive patients with insulin resistance.

References