Reciprocal Association of C-Reactive Protein With Adiponectin in Blood Stream and Adipose Tissue

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Background—High-sensitive C-reactive protein (hs-CRP) is a well-known risk factor for coronary artery disease (CAD). Recently, we have demonstrated that adiponectin served as an antiatherogenic plasma protein which was secreted specifically from adipocytes. The present study investigated the association between adiponectin and CRP in the bloodstream and adipose tissue.

Methods and Results—We studied a total of 101 male patients, 71 of whom had angiographically documented coronary atherosclerosis. As a control group, 30 patients with normal coronary angiogram were included. The plasma hs-CRP levels were negatively correlated with the plasma adiponectin levels ($r = -0.29$, $P<0.01$). The plasma adiponectin concentrations were significantly lower and the hs-CRP levels were significantly higher in the CAD patients compared with control subjects. The mRNA levels of CRP and adiponectin were analyzed by quantitative real-time polymerase chain reaction method. We found that the CRP mRNA was expressed in human adipose tissue. A significant inverse correlation was observed between the CRP and adiponectin mRNA levels in human adipose tissue ($r = -0.89$, $P<0.01$). In addition, the CRP mRNA level of white adipose tissue in adiponectin deficient mice was higher than that of wild-type mice.

Conclusions—The reciprocal association of adiponectin and CRP levels in both human plasma and adipose tissue might participate in the development of atherosclerosis. (Circulation. 2003;107:671-674.)

Key Words: coronary disease ■ risk factors ■ inflammation ■ proteins

C-reactive protein (CRP) is a well-known systemic marker for inflammation. Previous prospective studies indicate that a chronic low-grade inflammation is involved in the pathogenesis of atherosclerosis, and elevated high-sensitive CRP (hs-CRP) level is a risk factor for coronary artery disease (CAD). Plasma hs-CRP levels were also strongly associated with obesity and obesity-related diseases, including insulin resistance, diabetes mellitus, and hyperlipidemia. Although a recent report indicated that the plasma hs-CRP level decreased during weight reduction, the precise interaction of CRP with obesity has not been fully elucidated.

Adipose tissue secretes various bioactive substances, conceptualized as adipocytokines, including leptin, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and adiponectin, that may directly contribute to obesity-like metabolic and vascular diseases. Adiponectin is an adipocyte-specific plasma protein that we identified in a human adipose tissue cDNA library. We have reported that physiological concentrations of human recombinant adiponectin suppressed TNF-$\alpha$–induced endothelial adhesion molecule expression, macrophage-to-foam cell transformation, and TNF-$\alpha$ expression in macrophage and adipose tissue. Recently, we have reported that adiponectin-deficient mice exhibit severe diet-induced insulin resistance and enhanced neointimal thickening after vascular injury. Clinically, hypoadiponectinemia was observed in patients with obesity, type 2 diabetes, and CAD. These findings suggest that adiponectin has antiinflammatory properties and acts as an endogenous modulator of obesity-related diseases. Atherosclerosis can be considered a hyperresponsive healing process of the arterial wall. Fat tissue is an important secretory organ of the innate immune system. Therefore, we hypothesized that adiponectin and CRP might affect each other and contribute to the development of atherosclerosis. In the present study, we investigated the relation between adiponectin and CRP in both human plasma and adipose tissue.
Methods

Measurement of CRP and Adiponectin Levels in CAD Patients

Consecutive male patients (n=101, age 36 to 75 years) were enrolled from the inpatients who underwent coronary angiography in Osaka University Hospital. The criteria for CAD were a 75% or greater organic stenosis of at least 1 segment of a major coronary artery confirmed by quantitative coronary angiography by 2 perpendicular projections. Of these 101 patients, 71 had CAD. As a control group, 30 patients without CAD were included. All subjects enrolled in this study were Japanese and gave written informed consent. The Ethics Committee of Osaka University approved this study. The hs-CRP and adiponectin concentrations were determined with an ultrasensitive CRP test with a coefficient of variation below 5% (N Latex CRP; Dade Behring Co Ltd). The adiponectin concentrations were determined by ELISA with a coefficient of variation below 5% (Otsuka Pharmaceutical Co Ltd).14 Because the distributions of CRP and adiponectin were skewed, logarithmically transformed values were used for statistical analysis.

Quantitation of Adiponectin and CRP mRNA Levels

Human subcutaneous adipose tissue samples were obtained from 8 male Japanese subjects who suffered from gallbladder stones and underwent the elective surgical removal of gallbladder. These patients had no symptoms and no systemic signs of inflammation determined by the routine CRP assay before surgery. All subjects provided written informed consent. The Ethics Committee of Osaka University approved this study.

Adiponectin knockout (KO) male mice (12 weeks old) were generated as described previously.12 White adipose tissues were obtained from wild-type (WT) male or adiponectin KO male mice. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

Total RNA was prepared by RNA-TRIZOL extraction (Gibco) and treated with DNase I (Takara). cDNA was produced using Taqman reverse transcription (Perkin Elmer) kits. Real-time polymerase chain reaction was performed on ABI-Prism 7700 using SYBR Green I as a double stranded DNA specific dye according to manufacturer’s instruction (PE-Applied Biosystems).13 Primers were 5'-GGTTTTCCCAAAGAGTCGGATACT-3' and 5'-TCCAGAGTGGCTACAGAGGGAATCC-3' for mouse CRP, 5'-AGGTTGGATGGCAGGC-3' and 5'-CCAAAAAGGTTGGCTACAGAGGGAATCC-3' for human CRP, 5'-TGGTTTCCCAAAGAGTCGGATACT-3' and 5'-AGGTTGGATGGCAGGC-3' for mouse adiponectin. The primers for rodent GAPDH were obtained from Perkin Elmer.

Statistic Analysis

Data are presented as mean±SEM. Differences were analyzed by Student’s unpaired t test. The association between CRP and adiponectin was analyzed by linear regression analysis. P<0.05 was accepted as statistically significant. All calculations were performed by using a standard statistical package (JMP for Macintosh, version 4.0).

Results

Plasma Adiponectin Levels Negatively Correlated With hs-CRP Levels

We first investigated the relationship between plasma hs-CRP and adiponectin levels in consecutive patients who underwent coronary angiograms. As shown in Figure 1A, a significant negative correlation was observed between plasma adiponectin and hs-CRP levels (r=-0.29, P<0.01). Plasma adiponectin concentrations were significantly lower (log adiponectin: 0.65±0.03 versus 0.76±0.04 μg/mL) and the hs-CRP levels were significantly higher (log hs-CRP: 3.00±0.07 versus 2.75±0.09 μg/l) in CAD patients compared with control subjects (Figure 1B). No significant difference was observed in age (57.6±1.5 versus 61.5±1.1 years) or body mass index (23.6±0.6 versus 24.3±0.4 kg/m2) between control and CAD subjects. There were no significant differences in the percentage of smokers and diabetic patients between control and CAD subjects, although the percentage of the subjects receiving statins or aspirin was higher in CAD group than that in control group (data not shown).

Inverse Correlation Between CRP and Adiponectin mRNA Levels in Adipose Tissue

We next examined whether CRP mRNA was expressed in human adipose tissue. The quantitative real-time polymerase chain reaction revealed that the human fat tissue expressed CRP mRNA. Interestingly, an inverse correlation was found between CRP and adiponectin mRNA levels in human adipose tissue (r=-0.89, P<0.01; Figure 2A).

Finally, we examined CRP mRNA expression in white adipose tissue in adiponectin KO mice. The adiponectin mRNA in adipose tissue was not expressed in KO mice.
of the regulation of the CRP expression. Previous studies have indicated that adiponectin might influence IL-6 and CRP production through modulating the TNF-α action. Therefore, adiponectin may directly or indirectly affect the CRP levels in plasma and adipose tissue through modulating the inflammatory cascades. Further study will be needed to clarify the mechanism of the regulation of the CRP expression.

(Figure 2B), thereby confirming our hypothesis. The CRP mRNA levels of adipose tissue in adiponectin KO mice were higher than those in wild-type (WT) mice (Figure 2B). No significant differences were observed in body weight and various metabolic profiles between WT and KO mice, as previously described.14

Discussion

In the present study, we found for the first time that human adipose tissue expressed CRP, and an inverse relationship was observed between CRP and adiponectin in both plasma and adipose tissue. A low-level chronic inflammatory state is highly associated with obesity-related disorders.1-5 We have demonstrated that adiponectin served as an anti-inflammatory molecule for vascular walls as well as adipose tissue and that hypoadiponectinemia was observed in obesity-linked diseases.9,12,13 Therefore, hypoadiponectinemia can be responsible for a low-grade systemic chronic inflammation state, which is closely related to an increased hs-CRP level.

Adipose tissue expresses a variety of adipocytokines, which participate in the immune and inflammation system. In the current study, human adipose tissue expressed CRP mRNA, which was negatively correlated with adiponectin expression in adipose tissue. The CRP mRNA level in adipose tissue was increased in adiponectin KO mice. These data suggested that the expression of CRP was negatively regulated by adiponectin in adipose tissue, although further mRNA study in a larger number might be needed to elucidate the precise link between CRP and adiponectin. Recent reports indicated that interleukin-6 (IL-6) was one of important adipocytokines linking obesity and CAD and was a main regulator of hepatic CRP production.2,17 Another adipocytokine, TNF-α, was reported to regulate the hepatic CRP synthesis and induce IL-6 expression.17 Because adiponectin could counteract the proinflammatory effects of TNF-α in vascular cellular components and adipose tissue,9,11-13 adiponectin might influence IL-6 and CRP production through modulating the TNF-α action. Therefore, adiponectin may directly or indirectly affect the CRP levels in plasma and adipose tissue through modulating the inflammatory cascades. Further study will be needed to clarify the mechanism of the regulation of the CRP expression.

The principal source of CRP production has been assumed to be the liver.2 Recent data indicated that CRP was detected in arterial walls and that its expression was upregulated in atherosclerotic lesions, suggesting that vascular walls are one of the main CRP producers.2 In addition, plasma hs-CRP levels were positively associated with total body fat mass6 and decreased during weight reduction.15 These results proposed that adipose tissue acts as an important factor in modulating circulating hs-CRP levels. Taken together, the increased CRP expression in adipose tissue may partially account for the elevation of plasma hs-CRP.

Among adipocytokines, CRP and adiponectin have opposite properties against insulin resistance and atherosclerosis. Our observations suggest that the dysregulated elevation of CRP and reduction of adiponectin in adipose tissue and plasma may participate in the development of atherosclerosis.

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References


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