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Smokers show reduced circulating adiponectin levels and adiponectin mRNA expression in peripheral blood mononuclear cells

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ABSTRACT

Objectives: Circulating adiponectin levels in cigarette smokers are lower than those in nonsmokers. We have previously shown that adiponectin is expressed in human monocytes. The aim of this study was to further investigate the effect of smoking on adiponectin expression in peripheral blood mononuclear cells (PBMCs).
Methods: A group of 77 cigarette smokers and 51 nonsmokers were consecutively enrolled in this study. The participants' body weight, blood pressure, and metabolic parameters, including plasma glucose and plasma adiponectin levels, were recorded. The RNA from the PBMCs was assessed with real-time polymerase chain reaction (PCR) to determine the levels of adiponectin mRNA.
Results: Of the 77 smokers, 67 (87.0%) were male. Their mean (standard deviation) age was 43.17 (11.47) years, and they smoked 24.56 (12.53) cigarettes/day. The duration of smoking was 23.73 (11.69) years. Both circulating adiponectin levels ($p = 0.0262$) and adiponectin mRNA levels in PBMCs ($p < 0.0001$) of smokers were significantly lower than those in nonsmokers. Both circulating adiponectin levels and adiponectin mRNA levels were negatively correlated with the number of cigarettes smoked per day ($p < 0.01$). In multiple linear regression analysis, smoking was an independent factor affecting adiponectin mRNA expression in PBMCs ($p < 0.0001$).
Conclusions: Circulating adiponectin levels and adiponectin expression in PBMCs were lower in smokers; this finding suggested that attenuation of both systemic and local actions of adiponectin might contribute to the atherosclerotic process in cigarette smokers.

1. Introduction

Cigarette smoking is a strong risk factor for various atherosclerotic diseases [1]. Several lines of evidence have demonstrated the detrimental effects of smoking on arterial stiffness and pulse-pressure amplification [2,3]. In contrast, cessation of smoking has beneficial effects, including extension of life span [4], lowering of arterial pressure and heart rate [5], and reduction of the occurrence of stroke and coronary heart disease [6,7]. Although several mechanisms have been suggested to explain the improvement in arterial stiffness and atherosclerosis in subjects who quit smoking, the reasons for the improvement in cardiovascular outcomes after smoking cessation are not completely understood [3,8].

The atherosclerotic process is initiated by the adhesion of activated monocytes to the impaired endothelium, which is followed by monocyte migration into the subendothelium, differentiation to macrophages, and subsequent formation of foam cells [9]. Several studies have shown that cigarette smoking increases the plasma levels of intercellular adhesion molecule-1 (ICAM-1), which is a marker of endothelial dysfunction [10–12]. A study also showed that cigarette smoking induces the expression of adhesion molecules, such as CD11b, on the surface of monocytes and causes

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the adhesion of these monocytes to endothelial cells [9], which may initiate the process of atherosclerosis.

Adiponectin, one of the well-characterized adipokines, has been shown to possess insulin-sensitizing and antiatherogenic properties [13]. Previous studies have shown that the effects of adipose-derived adiponectin on monocyte/macrophage cells are produced primarily through inhibition of the expression of endothelial cell adhesion molecules. For example, adiponectin dose-dependently inhibits the expression of adhesion molecule-1 (VCAM-1), E-selectin, and ICAM-1 in THP-1 (human monocytic leukemia) cells as well as the tumor necrosis factor (TNF)-alpha-induced adhesion of THP-1 cells to human aortic endothelial cells (HAECs) [14]. More recently, adiponectin mRNA expression has been detected in several other cell types, including primary hepatic sinusoidal endothelial cells, stellate cells, and macrophages, in mice with acute liver failure [15]. We recently demonstrated that *de novo*-synthesized adiponectin in human monocytes inhibits fibronectin-mediated adhesion, which is dependent on the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) and downregulation of integrin expression [16]. This finding suggests a potential active role of monocyte-secreted adiponectin in the pathogenesis of atherosclerosis.

The plasma concentration of adiponectin has been found to decrease in a dose-dependent manner in smokers [17,18] and increase after smoking cessation [19–21]. However, the effect of cigarette smoking on adiponectin expression in monocytes has not been studied. The purpose of this study was to investigate the potential interrelationships between smoking, circulating levels of adiponectin, and adiponectin expression in the peripheral blood mononuclear cells (PBMCs).

2. Methods

2.1. Subjects

The smokers were recruited from the smoking cessation clinic at National Taiwan University Hospital, Taipei, Taiwan. The inclusion criteria were as follows: (1) subjects who smoked 10 or more cigarettes per day; (2) subjects who smoked less than 10 cigarettes per day but had a Fagerström test for nicotine dependence (FTND) score [22] of 5 points or higher; (3) subjects aged 18 years or older. Thus, 77 subjects were recruited from September 2007 to September 2010. The 51 nonsmokers in the study were volunteers who were comparable with smokers in terms of demographic variables. This study was approved by the institutional ethics committee, and written informed consent was obtained from each participant.

2.2. Data collection and regular physical check-up

We recorded the demographic characteristics and smoking history, including the duration of smoking, the amount of cigarettes smoked per day, smoking index (number of cigarettes smoked per day × number of years for which the participant had been smoking), and number of attempts to quit smoking that lasted longer than 24 h in the past year. Based on the medical history, the presence of chronic diseases such as hypertension (systolic pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg), type 2 diabetes (fasting plasma glucose ≥ 126 mg/dl, 2 h plasma glucose ≥ 200 mg/dl during an oral glucose tolerance test, or a random plasma glucose ≥ 200 mg/dl with classic symptoms of hyperglycemia according to the American Diabetes Association criteria), dyslipidemia (total cholesterol ≥ 240 mg/dl, low-density lipoprotein cholesterol ≥ 160 mg/dl, triglyceride ≥ 200 mg/dl, or high-density lipoprotein cholesterol < 35 mg/dl), cardiovascular disease, obesity, and liver or renal disease among the study participants was identified and recorded. All smokers completed the Fagerström test for nicotine dependence (FTND). Data on height, weight, pulse rate, and blood pressure were also collected. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. This present study defined obesity as BMI ≥ 27 kg/m^2.

2.3. Blood sampling and adiponectin measurement

Random non-fasting blood samples were drawn. Plasma adiponectin levels were determined by using a radioimmunoassay method (LINCO Research, Inc., St. Charles, MO) as previously described [23].

2.4. Isolation of human peripheral mononuclear cells and quantitative real-time polymerase chain reaction analysis

Human PBMCs were isolated by centrifugation on a Ficoll-sodium metrizoate density gradient (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer's instructions. Total RNA was extracted by REzol (PROtech Technology, Sparks, NV) according to the manufacturer's instructions and then used for cDNA synthesis with a high-capacity RNA-to-cDNA Kit (Applied Biosystem, Carlsbad, CA). cDNAs were amplified in a 20-μl reaction mixture containing TaqMan gene expression master mix (Applied Biosystem, Carlsbad, CA), which was used according to the manufacturer's instructions. Quantitative-polymerase chain reaction (PCR) was performed with ABI 7000 real-time PCR system with primers for measuring adiponectin (forward: 5′-AGA AAG GAG ATC CAG GTC TTA TTG GT-3′, reverse: 5′-AAC GTA AGT CTC CAA TCC CAC ACT-3′). The cDNA concentration in each sample was normalized using transcripts for GAPDH (Hs99999905_m1, NM_002046.3 from ABI Biosystems).

2.5. Statistical analyses

The estimated frequency and percentage were described for categorical data variables, and the estimated mean and standard deviation (SD) were reported for non-categorical variables. The p values of categorical variables in the contingency table were computed by Fisher's exact test. Assumptions of normality and equal variances and no serious collinearity were checked to perform the appropriate statistical hypothesis tests. Independent 2-sample t test and the Wilcoxon rank-sum test were performed to compare noncategorical variables between smokers and nonsmokers. The linear strength of association between 2 noncategorical variables was estimated by the Pearson correlation coefficient (r), and the null hypothesis of zero correlation (that is, standardized slope in this setting) was tested by performing simple linear regression analysis. To adjust for the variation in personal baseline demographic information, multiple linear regression analysis was also utilized. The statistical software SAS version 9 (SAS Institute Inc., Cary, NC) was used for data management and data analysis. The statistical significance level was set as 0.05, and all statistical tests were 2-tailed.

3. Results

3.1. Circulating adiponectin and adiponectin mRNA levels in the PBMCs of the study subjects

Of the 77 smokers, 67 (87.0%) were male. Their mean (SD) age was 43.17 (11.47) years, and they smoked 24.56 (12.53) cigarettes/day. The mean duration of smoking was 23.73 (11.69) years. The smokers' dependence on nicotine was severe, as attested by their high mean FTND score of 6.30 (2.33). Gender distribution, BMI, and prevalence of obesity and cardiovascular diseases