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Associations of atherosclerotic risk factors with oxidized low-density lipoprotein evaluated by LOX-1 ligand activity in healthy men

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ABSTRACT

Background: The aim of this study was to determine the relationships of risk factors for atherosclerosis with oxidized low-density lipoprotein (OxLDL) evaluated by a new enzyme immunoassay for measurement of LOX-1 (lectin-like OxLDL receptor) ligand.
Methods: Subjects were 236 healthy men aged 33–62 years. LOX-1 ligand containing apoB (LAB) was measured by an enzyme-immunoassay using immobilized recombinant LOX-1 and anti-ApoB monoclonal antibody.
Results: In simple regression analysis, log-converted LAB showed significant positive correlations with history of smoking, waist circumference, diastolic blood pressure, LDL cholesterol, log-converted triglycerides, uric acid and white blood cell count and showed a significant negative correlation with HDL cholesterol. In multiple regression analysis using history of smoking, history of drinking, waist circumference, diastolic blood pressure, HDL cholesterol, log-converted triglycerides and uric acid as explanatory variables, log-converted LAB showed significant correlations only with history of smoking and log-converted triglycerides. Log-converted LAB was significantly higher in heavy smokers (≥20 cigarettes per day) than in nonsmokers and light smokers (<20 cigarettes per day), while no difference in log-converted LAB was found between nonsmokers and light smokers. Log-converted LAB was significantly higher in subjects with hypertriglyceridemia (≥150 mg/dl), large waist circumference (≥85 cm), high diastolic blood pressure (≥85 mm Hg), or metabolic syndrome defined by the NCEP-ATP III criteria than in subjects without each risk factor or metabolic syndrome.
Conclusions: Hypertriglyceridemia and smoking are determinants of LOX-1 ligand activity in healthy men and are thus thought to be crucial risk factors for initiation of atherosclerotic progression through generation of OxLDL.

1. Introduction

Major risk factors for atherosclerosis are known to be advancing age, cigarette smoking, hypertension, diabetes mellitus and dyslipidemia such as hyper-LDL-cholesterolemia, hyper-triglyceridemia, and hypo-HDL-cholesterolemia. Among these risk factors, elevated LDL cholesterol is perhaps most important for coronary heart disease. Formation of modified LDL, primarily oxidized LDL (OxLDL), has been shown to be important for the etiology of atherosclerosis [1, 2]. In the past decade, methods for measurement of OxLDL levels by using anti-OxLDL monoclonal antibodies have been developed, and high OxLDL has been demonstrated to be associated with coronary heart disease [3–5].

A lectin-like 52-kD receptor for OxLDL (LOX-1) is a scavenger receptor present primarily on vascular endothelial cells and binds and regulates OxLDL, which causes endothelial dysfunction and triggers atherosclerosis [6]. Proinflammatory, pro-oxidative and mechanical stimuli have been reported to induce expression of LOX-1 [7, 8]. A receptor-based assay to determine levels of LOX-1 containing apoB (LAB) using recombinant LOX-1-coated plates and anti-apoB antibody has been developed, and the levels of OxLDL evaluated by this method reflect ligand activity of LOX-1 [9]. Since OxLDL interacts with cells via LOX-1, LAB is thought to reflect biological activity of OxLDL more directly than the total amount of OxLDL measured by using anti-OxLDL monoclonal antibodies. LAB has recently been shown to be elevated in animal models of atherosclerosis such as apoE-deficient mouse and hereditary hyperlipidemic rabbits [9, 10]. However, there has been limited information on associations between LOX-1 ligand

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activity and atherosclerosis in humans. Our recent study has shown that LAB is associated with high non-HDL cholesterol and incidence of cardiovascular disease including stroke and coronary heart disease [11]. The purpose of this study was to determine the relationships between LOX-1-bound OxLDL and risk factors for atherosclerosis such as obesity, smoking, hypertension, dyslipidemia, diabetes, hyperuricemia and metabolic syndrome using blood samples from healthy men.

2. Methods

2.1. Subjects

The subjects were 236 men aged 33 to 62 years (mean ± SE: 43.6 ± 7.8 years) who were employed at a printing company. No subjects were engaged in factory work using organic materials or other harmful chemicals, and none had a history of cardiovascular or other serious diseases. The protocol of this study was approved by the Ethics Committee of Hyogo College of Medicine. Histories of cigarette smoking, alcohol drinking, physical activity, illness, and drug therapy were surveyed by questionnaires. Persons receiving therapy for any diseases were excluded from the subjects of this study. In smokers, current smoking status was classified into 1–10, 11–20, 21–30 and ≥31 cigarettes consumed per day. Alcohol drinking was evaluated by frequency of drinking, which was classified into never, 1–2 days per month, 1–2 days per week, 3–4 days per week and almost every day. Physical activity was evaluated by habitual exercise (30 min or longer), which was classified into never, 1–3 times per month, 1–2 times per week, and 3 or more times per week.

2.2. Measurements of atherosclerotic risk factors

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Systolic and diastolic blood pressure levels were measured in a sitting position after at least 5 min of rest. Waist circumference was measured in a standing position, not at the smallest circumference of the torso but at the navel level according to the recommendation of the definition of the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome [12].

Serum total cholesterol, HDL cholesterol, triglycerides and fasting blood sugar and uric acid were automatically measured by conventional methods in blood samples obtained under fasting conditions. White blood cells were counted with a Coulter Counter. LDL cholesterol concentration was estimated by using the Friedewald formula as (total cholesterol) − (HDL cholesterol) − (triglycerides/5). The estimated LDL cholesterol concentrations were used for analysis only when concentrations of triglycerides in subjects were less than 400 mg/dl. Only two men showed triglycerides ≥400 mg/dl and were excluded from subjects for analysis of LDL cholesterol.

2.3. Measurement of LAB

LAB was measured with a sandwich enzyme immunoassay described previously [9, 11, 13]. In brief, recombinant LOX-1 was immobilized on plates, and the plates were incubated with plasma samples or standard solutions of OxLDL. After washing with phosphate-buffered saline (PBS), the plates were further incubated with chicken anti-ApoB monoclonal antibody (HUC20). After washing with PBS, the plates were incubated with peroxidase-conjugated goat anti-chicken IgG. After washing with PBS, peroxidase activity was determined by using a TMB Peroxidase EIA Substrate Kit (Bio-Rad Laboratories, Hercules, CA).

2.4. Criteria of risk factors and definition of metabolic syndrome

Criteria of risk factors were as follows: abdominal visceral fat accumulation (waist circumference ≥85 cm), high blood pressure (systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg), dyslipidemia (triglycerides ≥150 mg/dl and/or HDL cholesterol <40 mg/dl), and diabetes mellitus (fasting blood sugar ≥110 mg/dl). Metabolic syndrome was defined according to the diagnostic criteria of metabolic syndrome by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) [14]: that is, any three or more of the above risk factors.

2.5. Statistical analysis

Results are shown as means ± SDs or SEs. The prevalence of each risk factor and percentage of smokers or drinkers were compared using the chi-square test for independence. In univariate analysis, comparison of means in two groups was performed with Student's *t*-test, and comparison of means in three or more groups was performed with analysis of variance and subsequent Scheffé's F-test. In multivariate analysis, mean values were compared using analysis of covariance and then Student's *t*-test after Bonferroni correction. Pearson's coefficients and standardized partial correlation coefficients were calculated in simple and multiple regression analyses, respectively. Blood LAB and triglyceride levels were used for analysis after log conversion. A $p<0.05$ was considered statistically significant.

3. Results

Profiles of subjects are presented in Table 1. About one third of the subjects were smokers. LAB levels have a wide range (19–4520 ng/ml) (Table 1). Fig. 1 displays histograms for LAB and log-converted LAB, and LAB showed a normal distribution only after log conversion (Kolmogorov–Smirnov test: $p<0.001$ for LAB and $p=0.200$ for log-converted LAB). Log-converted LAB was used for further analysis in this study.

Table 2 displays results of regression analysis for the relationships between log-converted LAB and variables related to atherosclerotic risk. In the simple regression analysis, among the variables tested, history of smoking, BMI, waist circumference, diastolic blood pressure, total cholesterol, LDL cholesterol, log-converted triglycerides, uric acid and white blood cell count showed significant positive correlations with log-converted LAB, while there was a significant

Table 1
Profiles of subjects.

Variables	Means ± SDs (ranges) or %
Age (year)	43.6 ± 7.8 (33–62)
Body mass index (kg/m^2)	22.87 ± 3.13 (17.1–32.9)
% of smokers	33.1
% of drinkers	70.3
Waist circumference (cm)	82.6 ± 8.5 (64.5–107.4)
Systolic BP (mm Hg)	119.9 ± 12.8 (90–156)
Diastolic BP (mm Hg)	73.6 ± 10.1 (51–108)
Total cholesterol (mg/dl)	204.6 ± 33.6 (131–330)
HDL cholesterol (mg/dl)	60.8 ± 14.9 (33–121)
Triglycerides (mg/dl)	109.3 ± 85.0 (23–869)
LDL cholesterol (mg/dl)	122.7 ± 29.8 (59–240)
Fasting blood glucose (mg/dl)	94.5 ± 8.9 (74–127)
Uric acid (mg/dl)	5.75 ± 1.18 (2.8–9.7)
White blood cell (/μl)	6645 ± 1987 (2930–14,480)
LAB (ng/ml)	282.5 ± 477.5 (19–4520)
Log(LAB)	2.22 ± 0.41 (1.28–3.66)
% of large waist circumference	37.7
% of high blood pressure	28.0
% of low HDL cholesterol	5.1
% of high triglycerides	19.5
% of high fasting blood sugar	5.9
% of metabolic syndrome	6.4

Means with SDs of variables and their ranges in parentheses and percentages of smokers, drinkers and subjects with each risk factor are shown. LAB, LOX-1 ligand containing apoB; BP, Blood pressure.