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Hypercholesterolemia, not metabolic syndrome, related to adhesion of monocytes to cultured endothelium in nondiabetic subjects

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Abstract

The interaction of leukocytes and endothelium plays an important role in the development of atherosclerosis. Previous studies found that adhesion of leukocytes to endothelium is greater in subjects with hypercholesterolemia. It is not clear if metabolic syndrome, a contributing risk factor of cardiovascular disease, is related to this adhesion. Therefore, we conducted a study, in which 48 nondiabetic subjects were enrolled, to determine the relationship between leukocyte adhesion and the components of metabolic syndrome. After a 12-hour overnight fast, subjects' fasting blood was obtained for measurement of lipoprotein concentrations and glucose and insulin levels. Results of the number of monocyte adhesion to human umbilical vein endothelial cells were divided into high monocyte adhesion group and low monocyte adhesion group (n = 24 in each group). Plasma concentrations of total cholesterol (245 ± 5 vs 229 ± 4 mg/dL, $P = .021$) and low-density lipoprotein cholesterol (LDL-C) (162 ± 4 vs 146 ± 3 mg/dL, $P = .003$) were both higher in the high monocyte adhesion group than in the low monocyte adhesion group. Monocyte adhesion was significantly correlated to plasma concentrations of LDL-C ($r = 0.407$, $P = .002$) but not to the total cholesterol ($r = 0.202$, $P = .085$). However, there was no difference in monocyte adhesion to endothelium between subjects with or without metabolic syndrome, based on the modified criteria from the Adult Treatment Panel III of the National Cholesterol Education Program. Insulin resistance index, presented as homeostasis model assessment insulin resistance, and glucose or insulin responses to oral glucose tolerance test were similar between groups. Our study demonstrated that monocyte adhesion to endothelium has a stronger relationship with the plasma concentration LDL-C than with characteristics of metabolic syndrome.

1. Introduction

The endothelium is a layer of cells spreading over the inner vascular surface with the function of preventing thrombogenesis. However, endothelial cells are continuously exposed to all components of blood in circulation. Some conditions, such as hypercholesterolemia and inflammation, will induce endothelial disorder and predispose individuals to atherosclerosis [1,2]. Leukocyte adhesion to the internal surface of vessels plays an important role in the development of inflammation and destruction of endothelial functions [3]. A previous study indicated that early hypercholesterolemia in cholesterol-fed rabbits can enhance leukocyte-endothelium interaction [4]. One of the most important processes is the activation of monocytes, including the up-regulation of adhesion molecules [5,6].

Diabetes is associated with inflammation and is a well-known risk factor of coronary heart disease [7-9]. Increased adhesion of leukocytes to endothelium has been reported in diabetic subjects [10,11]. Emerging evidence indicates that metabolic syndrome is not only associated with inflammation but also important in atherosclerosis and coronary heart disease [12-14]. Previous studies have shown that mononuclear cell adhesion to cultured endothelium was associated with degree of insulin resistance and soluble adhesion

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molecules in healthy subjects [15,16]. However, the relative contribution of cholesterol and metabolic syndrome in enhancing interaction between monocytes and cultured endothelium is still unclear. We, therefore, conducted a study to determine the relationship between leukocyte adhesion and the components of metabolic syndrome in a group of nondiabetic patients with hypercholesterolemia.

2. Methods

2.1. Patients and methods

This study was conducted in the Division of Endocrinology and Metabolism of Taichung Veterans General Hospital, Taiwan. Subjects with low-density lipoprotein cholesterol (LDL-C) between 130 and 210 mg/dL were enrolled. The exclusion criteria were (1) being younger than 20 or older than 75 years old, (2) plasma triglycerides more than 400 mg/dL, (3) a history of drug or alcohol abuse, (4) impaired hepatic function (the values of liver function tests more than the upper limit of normal range), (5) diabetic patients, (6) secondary hypercholesterolemia due to hypothyroidism or the nephritic syndrome, (7) current treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or fibric acid derivatives, and (8) current use of immunosuppressive drugs. This study was approved by a local institutional review board, and all participants gave written consent.

All blood samples were drawn after overnight fasting. The definitions of the 5 components of the metabolic syndrome, including waist circumference, blood pressure (BP), the fasting plasma concentrations of high-density lipoprotein cholesterol (HDL-C), triglycerides, and glucose, were accessed based on the Third Report of the National Cholesterol Education Program (NCEP) [17], except for a modified criteria of waist with circumference of more than 90 cm in men and more than 80 cm in women to categorize the presence of central obesity [18]. The 75-g oral glucose tolerance test (OGTT) was performed for excluding diabetes. We also collected the plasma concentrations of glucose and insulin at 0, 30, 60, 90, and 120 minutes during OGTT. Fasting plasma concentrations of glucose and insulin were used for a quantitative evaluation of insulin resistance according to the homeostasis model assessment, which was described by Matthews et al [19]. The homeostasis model assessment insulin resistance (HOMA IR) index = (fasting insulin [μIU/mL] $\times$ fasting glucose [mmol/L])/22.5. All data of glucose and insulin during OGTT were used for calculating the area under the curve for glucose (Glu-AUC) and the area under the curve for insulin (Ins-AUC). Glucose levels were measured enzymatically using the glucose oxidase–peroxidase method using commercial kits (WAKO, Tokyo, Japan). Plasma total cholesterol and triglyceride concentrations were assayed by enzymatic analysis using commercial kits (WAKO) [20,21]. The HDL-C level was determined in the supernatant of plasma after magnesium chloride–phosphotungstic precipitation of apolipoprotein B–containing lipoproteins [22]. Low-density lipoprotein cholesterol level was calculated according to the method of Friedewald et al [23].

We collected the additional fasting blood samples of the subjects about 10 mL into the tube with 0.1 mol/L sodium citrate, and purified the mononuclear cells by Ficoll-Hypaque (density 1.077; Biochrom AG, Berlin, Germany) gradient centrifugation (900*g*, 30 minutes). Primary human umbilical vein endothelial cells had been prepared in 24-well plates, and about 5×10^5 mononuclear cells were added to attach for 30 minutes at 37°C. Afterwards, nonadherent cells were removed by washing twice, and the remaining cells were detached from plates by treatment with trypsin-EDTA. The cell suspensions were washed and labeled with CD45-conjugated phycoerythrin for 30 minutes. After further washing, the cell suspensions were assayed by flow cytometry (FACScan; Becton Dickinson, Mountain View, Calif). With this method, it is easy to discriminate fluorochrome-labeled monocytes from endothelial cells. Computer analysis was performed using Lysis II software (Becton Dickinson) [24].

All descriptive data were presented as mean $\pm$ SEM. Statistical analyses were conducted by a nonparametric Mann-Whitney *U* test to compare the components of metabolic syndrome, the numbers of monocyte adhesions, HOMA IR, Glu-AUC, and Ins-AUC. The χ^2 test was used to assess the differences based on sex and patients with metabolic syndrome in the 2 groups. The relationship between monocyte adhesion and cholesterol was determined by Spearman correlation. Statistical analysis was performed using a Macintosh computer with StatView IV software (Abacus Concepts, Berkeley, Calif).

3. Results

A total of 48 subjects completed all of the assessments in the study. For analysis of the relationship between monocyte

Table 1
Clinical characteristics of the study subjects

	High monocyte adhesion	Low monocyte adhesion	*P*
n	24	24	
Sex (M/F)	16:8	15:9	.763
Age (y)	58 ± 2	56 ± 2	.457
Total cholesterol (mg/dL)	245 ± 5	229 ± 4	.021*
LDL-C (mg/dL)	162 ± 4	146 ± 3	.003*
Waist (cm)	88.8 ± 1.4	90.0 ± 2.0	.620
Waist-hip ratio	0.88 ± 0.01	0.89 ± 0.01	.293
Body mass index (kg/m^2)	25.8 ± 0.6	26.0 ± 0.7	.789
Systolic BP (mm Hg)	118 ± 3	117 ± 4	1.000
Diastolic BP (mm Hg)	70 ± 2	70 ± 2	.790
HDL-C (mg/dL)	50 ± 2	52 ± 3	.828
Triglyceride (mg/dL)	164 ± 17	162 ± 18	.861
Fasting glucose (mg/dL)	94 ± 2	94 ± 2	.893
Metabolic syndromes (persons)	7	6	.745

M indicates male; F, female.
* $P < .05$.

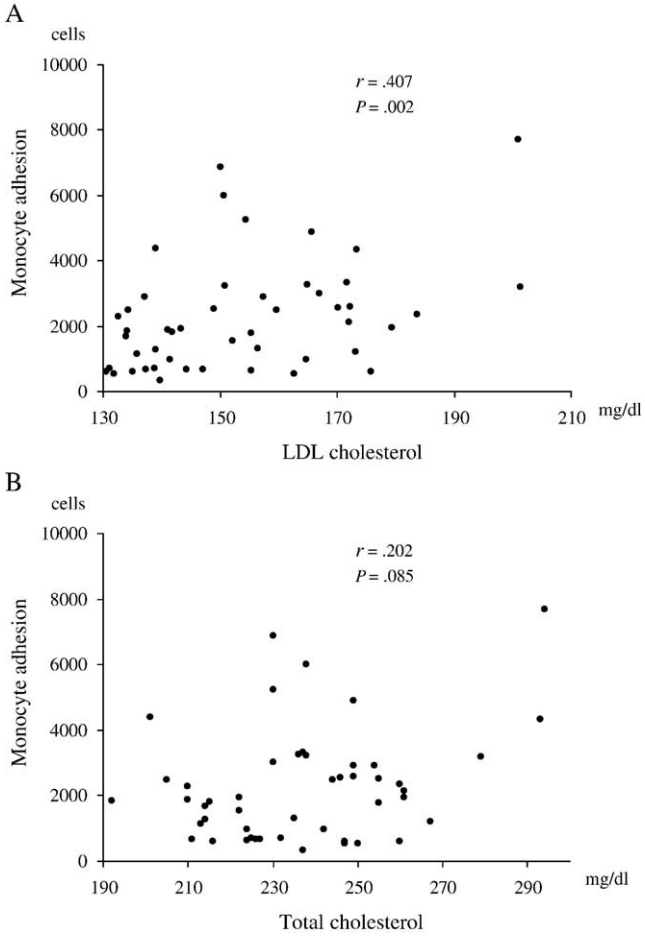

Fig. 1. (A) Correlation between monocyte adhesion and plasma concentrations of LDL-C (r = 0.407, P = .002). (B) Correlation between monocyte adhesion and plasma concentrations of total cholesterol (r = 0.202, P = .085).

adhesion and metabolic syndrome, these patients were divided into 2 equal-number subgroups by median of monocyte count that adhered to endothelium. A significantly larger number of monocytes adhered to endothelium in the high monocyte adhesion group than in the low monocyte adhesion group (3523 ± 322 vs 1031 ± 101 cells, respectively; P < .001). There were no significant differences based on sex and age between groups (Table 1).

Table 2
The relationship between cell counts of monocyte adhesion (cells) and the presence of each metabolic syndrome component

	Yes	No	P
Waist circumference Men >90 cm Women >80 cm	1821 ± 241	2815 ± 436	.063
Triglycerides ≥150 mg/dL	2195 ± 469	2326 ± 283	.443
HDL-C Men <40 mg/dL Women <50 mg/dL	1937 ± 293	2402 ± 320	.719
BP ≥130/≥85 mm Hg	1925 ± 283	2452 ± 341	.585
Fasting glucose ≥110 mg/dL	3550 ± 1696	2160 ± 225	.654
Metabolic syndrome (≥3 components)	1875 ± 228	2426 ± 320	.523

Table 3
The insulin resistance index of the study subjects

	High monocyte adhesion	Low monocyte adhesion	P
HOMA IR ([μIU/mL] · [mmol/L])	2.0 ± 0.2	2.1 ± 0.3	.984
Glu-AUC (mmol/L per hour)	17.7 ± 0.7	17.1 ± 0.9	.682
Ins-AUC (pmol/L per h)	1082.8 ± 121.0	785.6 ± 111.1	.098

Fasting plasma concentrations of total cholesterol were significantly higher in subjects of the high monocyte adhesion group than in those of the low monocyte adhesion group (245 ± 5 vs 229 ± 4 mg/dL, respectively; P = .021). Fasting plasma concentrations of LDL-C were also higher in the high monocyte adhesion group (162 ± 4 vs 146 ± 3 mg/dL, P = .003) (Table 1). The monocyte adhesion counts showed a significant correlation to the plasma concentrations of LDL-C (r = 0.407, P = .002) but not to the total cholesterol (r = 0.202, P = .085) (Fig. 1).

There was no significant difference in any of the 5 components of the metabolic syndrome, including the waist circumference (P = .620), BP (systolic BP, P = 1.000; diastolic BP, P = .790), the fasting plasma concentrations of HDL-C (P = .828), triglycerides (P = .861), and glucose (P = .893) between groups (Table 1). In addition, there was no significant difference in the number of subjects with metabolic syndrome, defined as the presence of 3 or more modified criteria of metabolic syndrome by the Adult Treatment Panel III of the NCEP, between groups (P = .745). Furthermore, we analyzed the difference in the number of monocyte adhesions between the subjects with or without the presence of each criterion of metabolic syndrome, and there was no statistically significant difference (Table 2).

The insulin resistance, estimated by HOMA IR, was relatively comparable between the high and low monocyte adhesion groups (2.0 ± 0.2 vs 2.1 ± 0.3 [μIU/mL] · [mmol/L], respectively; P = .984). There were no significant differences in Glu-AUC (17.7 ± 0.7 vs 17.1 ± 0.9 mmol/L per hour, P = .682) and Ins-AUC (1082.8 ± 121.0 vs 785.6 ± 111.1 pmol/L per hour, P = .098) during OGTT between these 2 groups (Table 3).

4. Discussion

Endothelial dysfunction is considered as one of the important causes of cardiovascular disease. The impairment of endothelial function is related to a high concentration of cholesterol, particularly LDL-C [25,26]. The change in endothelium of abnormal lipoprotein can occur before the development of atheroma, and the effect is not restricted to the site of atheroma [1]. In our study, higher plasma concentrations of total cholesterol and LDL-C were found in subjects with greater adhesion of monocytes to cultured endothelium. The adhesion of leukocytes to endothelium has been observed in the process of endothelial dysfunction and atherosclerosis [26]. The exact mechanism of mono-

cyte-endothelial interaction is unclear, but it may be involved in the effect of oxidation of lipoprotein, up-regulation of angiotensin II on monocytes, and the presentation of adhesion molecules [16,27-32].

Metabolic syndrome is also thought to increase the risk for cardiovascular disease [33-35]. However, its association with the increased adhesion of monocytes and endothelium remains unknown. In this study, we were unable to demonstrate the relationship between adhesion of monocytes to cultured endothelium and metabolic syndrome, either with individual components or metabolic syndrome as a whole. This indicated that monocyte adhesion to cultured endothelium is regulated mainly by the influence of hypercholesterolemia.

Hyperglycemia per se may influence the binding of monocytes to endothelium, and the expression of adhesion molecules may be more frequent in the effect of diabetes than in hyperlipidemia [36]. Therefore, we excluded the subjects with diabetes. Thomas et al [37] demonstrated that endothelial dysfunction is related to the level of plasma glucose in the healthy population. In their study, however, LDL-C and total cholesterol were higher in the high-glucose group. In our study, the interaction of monocytes with endothelium was not related to the plasma glucose concentration or the Glu-AUC.

In accordance with our finding, Chen et al [16] demonstrated that monocyte adhesion is not related to body mass index, triglyceride, or HDL-C. However, they did find that monocyte adhesion was associated with insulin resistance, not LDL-C concentration. These discrepancies may be explained by differences in populations and the relatively low cholesterol levels in their study population. Our findings seem to suggest that LDL-C is a greater determinant than insulin resistance and any component of metabolic syndrome in regulating interaction between monocytes and endothelium. We compared the number of subjects with metabolic syndrome (presence of 3 or more risk factors as defined by the criteria of the NCEP of the Adult Treatment Panel III) in both groups, but no difference was found ($P = .523$).

According to the NCEP, the primary goal of therapy for hypercholesterolemia is controlling LDL-C. It is probably more important than dealing with metabolic syndrome [17]. In view of the interaction of monocytes and endothelium, our findings support this recommendation.

In conclusion, monocyte adhesion to cultured endothelium is related to the plasma concentration LDL-C rather than the presence of metabolic syndrome or its individual components.

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