Association Between Plasma Lipoprotein(a) and Endothelial Dysfunction in Normocholesterolemic and Non-Diabetic Patients With Angiographically Normal Coronary Arteries

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The present study was designed to examine whether elevated levels of lipoprotein(a) (Lp(a)) are related to the impairment of the endothelium-dependent vasoresponse to acetylcholine (ACh) in normocholesterolemic and non-diabetic human normal coronary arteries. ACh (30 μg) was injected into the left main coronary artery of 31 patients (serum low-density cholesterol <160 mg/dl and fasting plasma glucose <126 mg/dl) with angiographically normal coronary arteries, and the relation between diameter change and lipid levels was analyzed. The mean diameter change of all coronary segments examined (segments 6, 8, 11 and 13) was reduced by 14.6±26.5% in response to ACh, but increased by 23.3±6.0% in response to nitroglycerin, suggesting endothelial dysfunction in those arteries. The mean diameter change of the left anterior descending artery or left circumflex artery in each patient was negatively correlated only with the level of Lp(a). Stepwise multiple regression analysis also revealed that only Lp(a) among the lipids showed significant correlation with impaired vasodilation (p=0.033). These findings suggest that elevated levels of plasma Lp(a) might be a strong predictor of endothelial dysfunction in normocholesterolemic and non-diabetic subjects. (Circ J 2002; 66: 267–271)

Key Words: Acetylcholine; Endothelial dysfunction; Lipoprotein(a); Normocholesterolemia; Normoglycemia

Endothelial dysfunction, which can be proved by demonstrating loss of acetylcholine-induced vasodilation, is an early event of atherosclerosis in human coronary arteries1–2 and seems to accelerate the progression of the atherosclerotic process.3 In clinical studies, selective loss of endothelium-dependent vasodilation in response to acetylcholine (ACh) has been observed in not only angiographically atherosclerotic coronary arteries, but also in normal coronary arteries of hypercholesterolemic patients.4,5 Lipoprotein(a) (Lp(a)) has been associated with ischemic heart disease and myocardial infarction in many epidemiological studies6–10 and is now considered to be an independent risk factor for cardiovascular diseases. Lp(a) is a low-density lipoprotein (LDL) -like particle, so it could be atherogenic and impair endothelial function as does LDL.11 Recently, Sorensen et al have reported that flow-mediated dilation in femoral artery is inversely related to the Lp(a) level in children with familial hypercholesterolemia, suggesting a contribution by Lp(a) to endothelial dysfunction.12 A study by Tsurumi et al also revealed that Lp(a) correlated well with endothelial dysfunction in the human coronary artery as well as serum LDL cholesterol.13 Moreover, patients with diabetes mellitus (DM) showed endothelial dysfunction that was probably caused by hyperglycemia and hypertriglyceridemia.14–16

To confirm whether or not Lp(a) has an independent role in endothelial dysfunction, we examined normocholesterolemic and non-diabetic patients with angiographically normal coronary arteries.

Methods

Study Patients
The study group consisted of 31 patients (without any angiographically segmental stenoses or irregularities in the coronary arteries), who underwent diagnostic cardiac catheterization for chest pain or normal results of a noninvasive stress test. The patients with hypercholesterolemia (serum LDL cholesterol ≥160 mg/dl), hypertriglyceridemia (serum triglyceride ≥400 mg/dl), previous myocardial infarction, valvular heart disease, cardiomyopathies or DM (fasting plasma glucose ≥126 mg/dl) were excluded. Table 1 shows the profile of the patients: there were 14 male and 17 female patients, aged 59.4±9.2 years; 10 were smokers and 10 had a history of elevated blood pressure that resulted in the initiation of antihypertensive therapy by primary physicians. Patients had only been treated with calcium antagonists, not with angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. Because only normocholesterolemic and non-diabetic patients were selected, none had taken any lipid-lowering drugs, including HMG-CoA reductase inhibitors, or anti-diabetic drugs. All women were post menopausal, the result of bilateral oophorectomy in one patient (no.9 in Table 1) and aging in the others. None of the women were receiving estrogen therapy. None of the patients had a family history of early coronary artery disease. The institutional Ethics Committee approved the
study and written informed consent was obtained from all patients before cardiac catheterization.

Study Protocol
All drugs were withdrawn 48 h before cardiac catheterization except for sublingual nitroglycerin. After routine right-sided heart catheterization, control coronary angiography was performed by a standard percutaneous femoral approach using the Judkins technique. Five minutes after control angiography, an ACh-loading test was performed according to the method of Werns et al.17 in those patients who showed normal coronary arteries on the control angiography. Arterial blood pressure, heart rate and ST rate and volume of contrast material as used in the control procedures were independently performed by 2 angiographers and if there was a discrepancy, the value was determined by another angiographer. The response of the coronary arteries to ACh or nitroglycerin were expressed as % change in the diameter as compared with the baseline.

Plasma Lipid Analysis
On the day of catheterization and after overnight fasting, blood samples for the determination of plasma lipid components were collected in tubes containing EDTA. Cholesterol and triglyceride in serum, and the LDL or high-density lipoprotein (HDL) fraction were measured enzymatically after centrifugation by the method described previously.19,20 Lip(a) was measured by latex immunoassay (Daiichi Pure Chemical, Tokyo), which is a reproducible method currently in clinical use.21 The concentrations of apolipoprotein (apo) A-I and apo B were determined by a
single radial immunodiffusion method described previously (Daiichi Pure Chemical, Tokyo).\(^{22}\)

**Statistical Analysis**

All values were expressed as mean ± standard deviation. Paired Student's t test was used to evaluate the effect of ACh on diameter change. Group Student's t test also was used to determine the effect of categorical risk factors on diameter change. Correlation between % diameter changes and lipid levels was analyzed by simple regression analysis. Magnitude of correlation between multivariates (lipid components, age, gender, history of hypertension, history of smoking and body mass index) and diameter changes was analyzed by multiple stepwise linear regression analysis (entry criteria F = 5). Although the distribution of Lp(a) values was similar to a skewed distribution, raw data, not log-transformed, were used for statistical analysis according to a previous report.\(^{13}\) Statistical significance was established at the p<0.05 level (Statview 5.0, SAS).

**Results**

**Vasoresponses to ACh and Nitroglycerin of Angiographically Normal Coronary Arteries**

The vasoresponse to ACh of angiographically normal coronary segments was heterogeneous, varying from dilation to constriction. The mean diameter of all segments examined in the 31 patients decreased by 14.6±26.5% from baseline and the diameter at segments 6, 8, 11, and 13 was decreased, respectively, by 15.5±28.1%, 18.7±26.7% (average of left anterior descending artery (LAD) 17.1±26.1%), 13.5±28.0% and 10.8±29.7% (average of left circumflex artery (LCx) 12.2±27.9%). There were no significant differences in diameter change between the proximal and distal segments in either of the 2 arteries (segment 6 vs 8, and segment 11 vs 13). In contrast, nitroglycerin dilated all segments examined (23.3±6.0%, p<0.001 vs changes induced by ACh).

**Relation Between Plasma Lipid Levels and Diameter Changes in Response to Acetylcholine**

The main lipid constituents measured in each patient are listed in Table 1. As shown in Fig 1, the means of the percent diameter change in response to ACh of the LAD,

![Fig 1. Correlation between plasma lipoprotein(a) (Lp(a)) levels and coronary artery diameter changes in response to acetylcholine (30μg) was measured in 31 patients. Plotted value indicates the mean diameter change of coronary segments 6 and 8 (A), 11 and 13 (B) or all (C) in each patient. Analysis was by a simple regression method. LAD, left anterior descending artery; LCx, left circumflex artery; LCA, left coronary artery.](image)

| Table 2 Univariate or Multivariate Analysis of Correlation Between Plasma Lypids, Conventional Risk Factors and Acetylcholine-Induced Vasodilation |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Univariate analysis | Multi variate analysis 1 | Multi variate analysis 2 |
|                 | r    | p value | Standardized coefficient | F value | p value | Standardized coefficient | F value | p value |
| LDL cholesterol | −0.028 | NS     | −0.014 | 0.005 | NS     | 0.243 | 1.634 | NS     |
| HDL cholesterol | 0.174 | NS     | 0.178 | 0.886 | NS     | −0.092 | 0.224 | NS     |
| LDL/HDL cholesterol | −0.041 | NS     | −0.027 | 0.020 | NS     | −0.197 | 1.047 | NS     |
| apo A-I         | 0.226 | NS     | 0.226 | 0.886 | NS     | −0.092 | 0.224 | NS     |
| apo B           | −0.137 | NS     | −0.137 | 0.886 | NS     | −0.197 | 1.047 | NS     |
| apo B/Apo A-I   | −0.215 | NS     | −0.215 | 0.886 | NS     | −0.197 | 1.047 | NS     |
| Total triglyceride | 0.178 | NS     | 0.178 | 0.218 | NS     | 0.130 | 0.450 | NS     |
| Lp(a)           | −0.407 | 0.022 | −0.397 | 0.024 | 0.033 | −0.397 | 0.024 | 0.033 |
| Age             | 0.019 | NS     | 0.019 | 0.295 | NS     | 0.106 | 0.106 | NS     |
| BMI             | 0.064 | NS     | 0.064 | 0.121 | 0.387 | −0.121 | 0.387 | NS     |
| Gender*         | −0.359 | NS     | −0.359 | 3.855 | NS     | −0.359 | 3.855 | NS     |
| Smoking*        | 0.020 | NS     | 0.020 | 0.011 | NS     | 0.020 | 0.011 | NS     |
| History of hypertension* | −0.307 | NS | −0.307 | 2.704 | NS | −0.307 | 2.704 | NS |
LCx or entire left coronary artery in each patient had a negative correlation with an increasing level of Lp(a) in all patients. On the other hand, the levels of none of the other lipids (total cholesterol, LDL cholesterol, HDL cholesterol, LDL to HDL cholesterol ratio, apo A-I, apo B or the apo B/A-I ratio) or other conventional risk factors (age, gender, history of smoking, or history of hypertension) correlated with the diameter changes (Table 2). Stepwise multiple regression analysis using these lipid components as independent variables revealed that only Lp(a) level correlated with impaired endothelium-dependent vasodilation (p=0.033). When considering apo A-I as an independent variable instead of HDL cholesterol or apo B instead of LDL cholesterol, the result was the same (p=0.033 for Lp(a) (Table 2). Conventional risk factors, such as gender and history of hypertension, had relative high F values, though these did not reach the significance level.

Discussion

The main aim of this study was to clarify whether Lp(a) cause deterioration of endothelium-dependent vasodilation even in normocholesterolemic and non-diabetic subjects. After highlighting that Lp(a) was independently associated with the coronary vasomotor response to ACh, a large study of patients suggested that a high Lp(a) level selectively impairs receptor-mediated endothelial dilatation. Although these 2 reports imply an association of Lp(a) with endothelial dysfunction, the serum cholesterol level was relatively high (169±41 mg/dl LDL cholesterol) in the latter study. Moreover, endothelial dysfunction associated with Lp(a) has been recognized in children with familial hypercholesterolemia. Therefore, we excluded patients with DM even if there was a normal lipid profile, because endothelium-dependent relaxation is often impaired by hypertriglyceridemia and hyperglycemia. Our findings reveal that Lp(a) alone in normocholesterolemic and normoglycemia is also associated with endothelial dysfunction and that other lipid constituents are not. This confirms evidence against the independence of Lp(a) in untoward effects and also indicates that high level of Lp(a) alone is sufficient for endothelial dysfunction.

Regarding the mechanism of endothelial dysfunction by Lp(a), Rubanyi et al reported that oxidation of Lp(a) or LDL caused significant suppression of ACh-dependent relaxation of the ex vivo aortic ring from transgenic mice. Others have reported that hypercholesterolemia causes impairment of endothelium-dependent vasomotor function and its normalization restores that function. Recently, Kugiyama et al showed that the level of remnant lipoproteins had a significant correlation with abnormal coronary vasomotor responses to ACh, and they also suggested that this phenomenon might be caused by the oxidatively modified remnant in the arterial intima. To minimize this influence of remnant lipoprotein, patients with hypertriglyceridemia and DM was excluded from the present study. Because Lp(a) is a LDL-like particle, it may have the same mechanism producing the untoward effect on endothelial function, possibly after accumulating in the intima. Another study is needed to elucidate whether a high level of Lp(a) alone, probably coexisting with oxidized Lp(a), is enough to cause endothelial dysfunction.

We did not find that conventional coronary risk factors, such as age, total or LDL cholesterol, HDL cholesterol, related apolipoproteins or a history of hypertension or smoking, were significantly correlated with endothelial dysfunction. Although all the reports on attenuation of endothelium-dependent vasodilation do not support a correlation with these risk factors, this discrepancy could be because the present patients were selected by LDL cholesterol value and in the present study, we were not able to address this issue. We need to study more patients and be able to measure circulating oxidized Lp(a).

Study Limitations

Although we studied only patients with normal coronary arteries, considering the age distribution in the study population, there may have been early atherosclerosis in some of these patients, which could not be detected by angiography as has been previously suggested. To clarify this issue, further study will be needed in a large population including younger subjects. Moreover, we could not investigate the relation of vasomotor response and Lp(a) in a hypercholesterolemic or diabetic group, because of a shortage of cases. Thus, we could not conclude whether hypercholesterolemia or hyperglycemia and hypertriglyceridemia would act synergistically with a high level of Lp(a) to produce endothelial dysfunction.

Conclusions

We demonstrated that the Lp(a) level was only related to impaired endothelium-dependent vasodilatation in normocholesterolemic and non-diabetic patients with angiographically normal coronary arteries. This finding indicates that Lp(a) could be causal in endothelial dysfunction, even in such patients.

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References

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