Original Article

Low-Density Lipoprotein Subfractions and the Prevalence of Silent Lacunar Infarction in Subjects with Essential Hypertension

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Recent lipid research has focused on low-density lipoprotein (LDL) subfractions as new markers for cardiovascular risk. However, the clinical significance of measurement of LDL subfractions in subjects with essential hypertension is yet to be established. We studied the association between the prevalence of silent lacunar infarction (SLI) and LDL subfractions in patients with essential hypertension. We performed brain MRI to detect SLI and measured LDL subfractions in 100 asymptomatic non-diabetic middle-aged subjects with essential hypertension (mean age, 62 years). We fractionated LDL into three parts, LDL-1, LDL-2, and LDL-3, with LDL-3 being the oxidized subfraction. Of the 100 study subjects, 24 (24%) had one or more SLIs, while the remaining 76 (76%) were considered as a non-SLI group. The LDL-3 levels were significantly higher in the SLI group than in the non-SLI group (8.3±4.4 mg/dl *vs.* 6.3±2.0 mg/dl, $p=0.006$). Multiple logistic regression analysis showed that LDL-3 levels alone were an independent predictor of SLI (odds ratio [OR]: 1.380; 95% confidence interval [CI]: 1.113–1.663; $p=0.003$). When subjects were divided into quartiles based on LDL-3 levels, the prevalence of SLI was significantly higher in the highest LDL-3 level group than in the lowest LDL-3 level group ($p=0.0036$). The present study suggests that LDL-3 levels are associated with the prevalence of SLI in subjects with essential hypertension. (*Hypertens Res* 2006; 29: 303–307)

***Key Words*: blood pressure, silent cerebral infarction, essential hypertension, lipoprotein, risk factors**

Introduction

With the development of MRI it has become possible to detect silent lacunar infarction (SLI), and therefore the accuracy of diagnosing asymptomatic lacunar stroke has improved. SLI is occasionally detected incidentally by MRI in patients who demonstrate no localized neurological symptoms of stroke (*1–3*). Lacunar stroke is the most common subtype of ischemic stroke in the Japanese population (*4–6*) and is a risk factor for the development of more severe cerebral infarction or insidious progressive brain damage and vascular dementia. In most cases of silent cerebral infarction, lacunar stroke is present in the basal ganglia and deep white matter of the brain, even in patients who appear otherwise healthy (*1*, *2*). An SLI is generally considered to be an important predictor of symptomatic cerebral infarction and therefore detection of these infarctions and reduction of the risk factors associated closely with SLI should lead to the prevention of subsequent overt ischemic stroke. Previous reports have shown that hypertension and advanced aging are associated with SLI (*1*, *2*).

It has been demonstrated recently that small dense low-density lipoprotein (LDL) is highly atherogenic and associated with coronary artery disease even in patients with low LDL cholesterol levels (*7*, *8*). Vascular dementia has also been shown to be linked with an elevation in the level of these

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Table 1. Characteristics of Subjects with and without Silent Lacunar Infarction

Variables	SLI (n=24)	Non-SLI (n=76)	p value
Age (years)	63±10	61±8	0.319
Male gender (%)	19 (79)	47 (62)	0.965
Systolic BP (mmHg)	132±10	132±9	0.494
Diastolic BP (mmHg)	81±7	78±10	0.278
BMI (kg/m^2)	24.9±2.7	24.3±3.1	0.494
Total cholesterol (mg/dl)	195±37	201±30	0.625
Triglyceride (mg/dl)	135±37	110±350	0.119
HDL cholesterol (mg/dl)	51±14	56±112	0.229
LDL cholesterol (mg/dl)	118±32	124±17	0.297
Fast plasma glucose (mg/dl)	102±11	101±10	0.691
Antihypertensive drugs (%)			
ACEI	3 (13)	10 (13)	0.933
ARB	5 (21)	12 (16)	0.547
CCB	22 (88)	54 (71)	0.074
LDL-1 (mg/dl)	77.6±20.6	86.1±15.0	0.106
LDL-2 (mg/dl)	31.7±15.3	32.0±9.7	0.922
LDL-3 (mg/dl)	8.3±4.4	6.3±2.0	0.006

Data are shown as the number (%) or mean±SD. SLI, silent lacunar infarction; BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.

small dense atherogenic LDL particles (*9*). However, the relationship between SLI and LDL subfractions, including small dense LDL, has not been investigated. LDL can be fractionated into three parts, LDL-1, LDL-2, and LDL-3, with LDL-3 being the oxidized subfraction. However, the clinical significance of LDL subfraction measurement in hypertensive subjects remains to be established, and thus the relationship between SLI and LDL subfractions has not been studied thoroughly.

We conducted this study in order to elucidate whether the LDL-3 subfraction, as measured by a recently developed, novel high performance liquid chromatography (HPLC) method (*10*), was associated with the prevalence of SLI detected by MRI.

Methods

Subjects

We studied 100 asymptomatic subjects with essential hypertension, aged 42 to 81 years, who were recruited from our outpatient office. Essential hypertension was defined as an office systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg. The blood pressure of all patients was controlled at a level below 140/90 mmHg with antihypertensive agents for more than 1 month prior to the study. Subjects with secondary hypertension, or subjects with diabetes mellitus, cancers, or liver, renal or infectious diseases were excluded from the study. We also excluded subjects who had received lipid-lowering medications, and habitual smokers. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). No cervical bruits were audible in any of the subjects. The study was approved by an institutional ethical committee and the subjects gave informed consent.

Brain Magnetic Resonance Imaging

A brain MRI was performed in all 100 subjects with a superconditioning magnet using a main field strength of 1.5 T (Toshiba MRT 200 FXII; Toshiba, Tokyo, Japan). The brain was imaged in the axial plane at 8 mm slice thickness. T_1-weighted images were obtained by using a short spin-echo pulse sequence with a repetition time of 500 ms and an echo time of 13 ms. T_2-weighted images were obtained by using a long spin-echo pulse sequence with a repetition time of 4,000 ms and echo times of 60 and 112 ms. The matrix was 256 × 224 pixels. A lacuna was defined as a high-intensity area, 5–15 mm in diameter, identified on a T_2-weighted image, coinciding with a low-intensity area of a penetrating artery on a T_1-weighted image, without significant occlusive lesions of the cerebral artery or internal carotid artery being evident on magnetic resonance angiography. All of the MRI were evaluated independently in a blinded fashion by one observer.

Clinical and Laboratory Data

Blood pressure was measured in the sitting position with an automated sphygmomanometer (BP203RV-II; Nippon Colin Co, Ltd., Komaki Japan) after the patients had rested for 5

Table 2. Multiple Logistic Regression Analysis of Variables for Silent Lacunar Infarction

Variables	OR	95% CI	*p* value
Age (years)	1.008	(0.937–1.083)	0.831
Systolic BP (mmHg)	1.008	(0.942–1.078)	0.819
Diastolic BP (mmHg)	1.025	(0.958–1.097)	0.468
Triglyceride (mg/dl)	1.000	(0.989–1.011)	0.980
HDL cholesterol (mg/dl)	0.986	(0.934–1.041)	0.612
LDL cholesterol (mg/dl)	1.004	(0.975–1.033)	0.807
Fast plasma glucose (mg/dl)	1.010	(0.959–1.964)	0.714
LDL-3 (mg/dl)	1.380	(1.113–1.663)	0.003

OR, odds ratio; CI, confidence interval; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

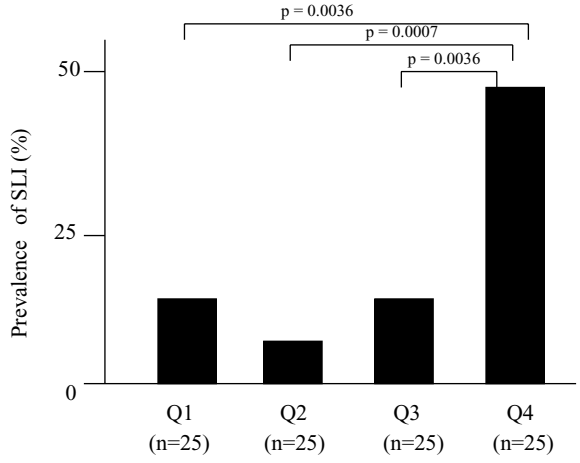

Fig. 1. *Prevalence of silent lacunar infarction in quartiles of LDL-3 levels. We divided the study subjects into quartiles of LDL-3 levels. Q4 (the highest LDL-3 level) had a significantly higher prevalence of silent lacunar infarction than Q1, Q2, or Q3.*

min. Blood samples were drawn from the antecubital vein in the early morning after an overnight fast of more than 12 h. Serum total cholesterol and triglyceride levels were determined by enzymatic methods and high-density lipoprotein (HDL) cholesterol levels by a precipitation method in an autoanalyzer. LDL cholesterol was calculated using the Friedwald formula (*11*). Fasting plasma glucose concentration was measured by an enzymatic method.

Novel LDL Subfraction Assay

Human plasma lipoproteins were separated by a modified HPLC method using a diethylaminoethyl-type anion exchange gel column with stepwise elution (*10*). This method fractionates LDL into three parts, designated as LDL-1, LDL-2, and LDL-3, by their elution order. Of these LDL subfractions, LDL-3 is the most strongly retained fraction and shows the fastest migration in the anodic direction, and thus have a property reflecting the most negative net charges of the molecules. We confirmed the accuracy and reproducibility of this assay method with a within-day precision of $<3.6\%$ and a between-day precision of $<4.9\%$. In this method, there was no need for lipoprotein extraction prior to the assay.

Statistical Analysis

Data are expressed as the mean±SD. Statistical analyses were performed using Stat View 5.0 software (SAS Japan Ltd., Tokyo, Japan). Student's *t*-test was used to detect differences in the mean value of factors between groups with and without SLI. The χ^2 test or Fisher's exact test was used to detect the differences between two groups in the prevalence of SLI or of risk factors. The Kruskel-Wallis test and Mann-Whitney *U* test with Bonferroni correction were used to detect the difference among the four groups in the prevalence of SLI. Multiple logistic regression analysis was used to determine the independent predictors of SLI. *p* values <0.05 were considered to be statistically significant.

Results

Clinical Characteristics of the Study Subjects

Among the 100 subjects, 24 (24%) had one or more SLIs (SLI group) while no SLI was observed in the remaining 76 (76%) (non-SLI group). Table 1 compares clinical characteristics between the SLI group and non-SLI group. Age, sex, BMI, systolic and diastolic blood pressures, serum levels of total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, plasma glucose levels and the use of antihypertensive drugs were similar between the two groups. Although the levels of LDL-1 and LDL-2 were similar between the two groups, LDL-3 levels were significantly higher in the SLI group than in the non-SLI group (8.3±4.4 mg/dl *vs.* 6.3±2.0 mg/dl, $p=0.006$).

LDL-3 and Prevalence of Silent Lacunar Infarction

Table 2 shows the results of multiple logistic regression anal-

Table 3. Multiple Logistic Regression Analysis of Variables for White Matter and Basal Ganglia Infarction

Variables	White matter infarction ($n=15$)			Basal ganglia infarction ($n=19$)		
	OR	95% CI	p value	OR	95% CI	p value
Age (years)	1.047	(0.967–1.133)	0.256	1.067	(0.928–1.228)	0.360
Systolic BP (mmHg)	0.955	(0.886–1.029)	0.223	1.331	(0.946–1.871)	0.100
Diastolic BP (mmHg)	1.015	(0.943–1.093)	0.685	1.161	(0.968–1.393)	0.107
Triglyceride (mg/dl)	0.998	(0.987–1.009)	0.727	0.997	(0.970–1.025)	0.858
HDL cholesterol (mg/dl)	0.981	(0.927–1.038)	0.510	1.132	(0.943–1.358)	0.183
LDL cholesterol (mg/dl)	1.019	(0.989–1.049)	0.213	0.938	(0.858–1.024)	0.153
Fast plasma glucose (mg/dl)	1.016	(0.946–1.072)	0.551	0.864	(0.712–1.048)	0.139
LDL-3 (mg/dl)	0.998	(0.818–1.216)	0.981	5.698	(1.297–25.038)	0.021

OR, odds ratio; CI, confidence interval; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

ysis to determine independent predictors of SLI. Age, systolic and diastolic blood pressures, the levels of total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and glucose, and the LDL subfractions were used as independent variables. Only the LDL-3 level was found to be an independent predictor of SLI (odds ratio [OR]: 1.380; 95% confidence interval [CI]: 1.113–1.663; $p=0.003$). To assess precisely the association between the LDL-3 levels and the prevalence of SLI, we divided the study subjects into quartiles based on their LDL-3 levels. The circulating LDL-3 levels (mean±SD) in each quartile were 3.6±0.6 mg/dl in Q1, 5.3±0.5 mg/dl in Q2, 6.7±0.4 mg/dl in Q3, and 11.4±3.4 mg/dl in Q4. The prevalence of SLI was 56% in Q4 (the highest LDL-3 level quartile), and 16% in Q1 (the lowest LDL-3 level quartile). Q4 showed significantly higher prevalence of SLI than Q1 ($p=0.0036$), Q2 ($p=0.0007$), or Q3 ($p=0.0036$) (Fig. 1).

LDL-3 and Location of Silent Lacunar Infarction

Of the 24 subjects in the SLI group, 5 (21%) had SLI located only in the white matter of the brain, 9 (38%) had SLI only in the basal ganglia, and the remaining 10 (42%) had SLI in both locations. In the multiple logistic regression analysis to identify parameters capable of predicting the location of the SLI, we found that the LDL-3 level was an independent predictor of the basal ganglia infarction (OR: 5.698; 95% CI: 1.297–25.038; $p=0.021$) but not of the white matter infarction. Other variables could predict neither white matter infarction nor basal ganglia infarction (Table 3).

Discussion

The novel findings of our study were that circulating LDL-3 levels were higher in subjects with SLI than in those without SLI, and that the LDL-3 levels could be an independent predictor of the prevalence of SLI—particularly SLI located in the basal ganglia—in subjects with essential hypertension.

SLI is now thought to be a predisposing condition for clinically overt stroke. One prospective study demonstrated that SLI was a strong predictor of subsequent clinically overt stroke, with an OR of greater than 10 (*12*). Previous studies demonstrated that SLI was detected in 40% of healthy elderly subjects recruited from the Japanese community (*13*). In our study, SLI was seen in 24% of subjects aged 42 to 81 years old, which prevalence was slightly less than those reported for this age group in the previous studies (*2*, *3*, *14*). In addition, although it is well known that the prevalence of SLI is associated with age and blood pressure, our results did not show either of these associations. These discrepancies between our results and previous ones might have been caused by the fact that our study included younger subjects with well-controlled blood pressure and excluded subjects having diabetes or a smoking habit.

Recent lipid research has focused on LDL subfractions as new markers for cardiovascular risk. The increased prevalence of small dense LDL particles has been reported to be a leading cause of coronary artery disease in Japanese patients (*7*, *8*). However, unlike coronary artery disease, the effect of LDL subfractions on stroke has not yet been investigated thoroughly. LDL particles are heterogeneous with respect to size and density of lipid composition, with small dense LDL particles being highly atherogenic as a result of their increased ability to penetrate into the arterial wall and also their lower binding affinity for the LDL receptor, prolonged plasma half-life, and lower resistance to oxidative stress (*15*). LDL particle size has been measured by gradient gel electrophoresis using non-denaturing polyacrylamide according to the method of Krauss and Burke (*16*). However, this procedure requires a long assay time that involves overnight electrophoresis, staining, and de-staining. In addition, this assay does not provide a quantitative determination of small dense LDL. Analytical centrifugation is another technique for quantification of small dense LDL (*17*). However, this method is also too laborious for general clinical use as it requires special equipment and a long running time. In contrast, LDL-3 measurement by the novel HPLC method we used in this study can be achieved simply within a short time. At present, there are no clinical data regarding the association between LDL-3 levels and small dense LDL measured by conventional assay methods. However, we consider LDL-3 to be an oxidized

LDL subfraction for the following reasons: 1) LDL-3 migrated fastest in the anodic direction; 2) LDL-3 exhibited properties characteristic of the most negative net charges of the molecules; 3) malondialdehide, which induces lipid peroxidation, reacted to the most fragmented ApoB in LDL-3, but not to that in LDL-1 or LDL-2; 4) when LDL was isolated from plasma by ultracentrifugation and then was oxidized *in vivo* by copper sulfate, it migrated at the position of LDL-3; and 5) we found that LDL-3 caused oxidative stress–induced injury to cultured endothelial cells (unpublished data). Therefore, our results suggest that oxidative modification of LDL may be linked to the development of SLI.

The pathogenesis of SLI has been considered to differ between the white matter infarction and the basal ganglia infarction. Recent studies have shown that the relative risk factors are different between the white matter infarction and the basal ganglia infarction (*18*). Thus high blood pressure was a predominant risk factor for white matter infarction, but basal ganglia infarction was associated with systemic atherosclerosis such as coronary artery disease and carotid artery stenosis. Therefore, it is hypothesized that lipid peroxidation, which plays one of the major pathogenetic roles in atherosclerosis, may lead to the prevalence of basal ganglia infarction. Our finding that circulating LDL-3 levels were associated with basal ganglia infarction may support this hypothesis.

Our study employed a relatively small cohort of patients and was a cross-sectional investigation. In addition, there may be racial and demographic differences in the prevalence of cardiovascular and cerebrovascular diseases. Stroke, particularly lacunar stroke, is more common in Japan than in Western countries, and therefore the relevance of our findings in Japanese subjects should be investigated further in a larger prospective study on subjects of different ethnicity.

In conclusion, LDL-3, or the oxidized LDL subfraction, appears to be associated with the prevalence of silent lacunar infarction in patients with essential hypertension. LDL-3 may be a new therapeutic target for protecting hypertensive patients from ischemic stroke.

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