An Association between Plasma Homocysteine Concentrations and Ischemic Stroke in Elderly Japanese

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The present study was performed to clarify the relation between plasma homocysteine and ischemic stroke. We studied the relationship between ischemic stroke and the known risk factors for atherosclerosis including plasma homocysteine in 91 in-patients (80.3 ± 6.8 years) in a medical ward. Those diagnosed with transient ischemic attack, cerebral infarction were placed in the disease group. Blood was drawn from in-patients in a fasting state for determination of plasma homocysteine. Plasma homocysteine concentrations were determined using a high-performance liquid chromatography assay. The odds ratio of ischemic stroke was higher in the second (10.0 – $13.9 \mu\text{mol/l}$) and third highest plasma homocysteine concentration groups ($\geq 14.0 \mu\text{mol/l}$) than in the first group ($< 10.0 \mu\text{mol/l}$) by 5.18 and 4.42-fold, respectively. Logistic regression analysis using ischemic stroke as an object variable, adjusted by various risk factors including the plasma homocysteine concentration showed that the odds ratio on combining the second and third groups was 5.80 (95% confidence interval (CI): 1.50–22.5) compared with the first group. The findings confirmed that the association between plasma homocysteine concentration and ischemic stroke in Western populations is also present among the elderly Japanese. *J Atheroscler Thromb*, 2002; 9: 121–125.

Key words: Atherosclerosis, Risk factor, Age, Gender, Elderly

Introduction

Homocysteine is a sulfhydryl amino acid; its precursor, the essential amino acid methionine, is derived from dietary protein. Almost 25 years ago, McCully (1) reported that a child dying of homocystinuria and cystathioninuria secondary to abnormal cobalamin metabolism exhibited widespread, severe arteriosclerosis analogous to the lesions seen in cases of homocystinuria caused by cystathionine β -synthase deficiency.

The association between the plasma homocysteine concentration and atherosclerosis has recently become the subject of a number of clinical studies, which have

consistently linked moderate hyperhomocysteinemia to occlusive vascular disease (2). Boushery *et al.* (3) conducted a meta-analysis of 27 studies, and reported that an increase in homocysteine of $5 \mu\text{mol/l}$ increases the odds ratio of coronary heart diseases 1.6-fold for males and 1.8-fold for females, and the odds ratio of cerebral vascular diseases 1.5-fold. Matsui *et al.* (4) reported that high levels of plasma homocysteine are correlated with silent brain infarction in Japanese rural communities. There have, however, been few studies on the relation between plasma homocysteine concentrations and ischemic stroke in Japan.

This study was performed to clarify the relation between plasma homocysteine and ischemic stroke in a Japanese rural area.

Subjects and Methods

Subjects

The subjects were in-patients aged 70 years or older

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treated consecutively between July and December, 2000. All gave informed consent. Those who had congestive heart failure, chronic renal failure (creatinine ≥ 1.8 mg/dl) or nutritional disorders (acute body weight loss) that would affect blood pressure, lipid metabolism or glucose metabolism were excluded from the study.

Case definitions

Stroke was defined as a clinical syndrome characterized by rapidly developing clinical symptoms and/or signs of focal and at times global loss of brain function, leading to early death, and with no apparent cause other than that of vascular origin. Ischemic stroke was defined as a stroke with either a normal CT brain scan or evidence of a recent infarct in the clinically relevant area of the brain on a CT scan. Patients with cerebral hemorrhage or cerebral venous thrombosis were not included. Control subjects were selected from in-patients aged 70 years or older without a history of ischemic stroke.

Determination of plasma total homocysteine concentrations

Blood was drawn from the in-patients while they fasted within 2 days of admission for determination of plasma homocysteine. Plasma homocysteine concentrations were determined using a high-performance liquid chromatography assay (BML, Ltd., Tokyo). Subjects with concentrations below $10.0 \mu\text{mol/l}$ were placed in the first group, between 10.0 and $13.9 \mu\text{mol/l}$ in the second group and greater than $14.0 \mu\text{mol/l}$ in the third group.

Other risk factors

We measured blood pressure in the right upper arm of patients in a sedentary position using a standard sphygmomanometer. Arterial hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher (5), or the need for antihypertensive medication. A cigarette smoker was defined as having ever smoked. In the blood biochemistry analyses, total cholesterol (TC; cholesterol oxidase enzymic method, Determiner L TC II), triglyceride (TG; glycerol-3-phosphate oxidase enzymic method, Determiner L TG II), HDL-cholesterol (HDL-C; polyethylene glycol-modified enzymic method, Determiner HDL-C), uric acid (uricase-peroxidase method, Ekdia L 'eiken' UA) and blood sugar (hexokinase UV method, Glu-UL) were measured upon fasting within 2 days after admission. Diabetes mellitus was defined as a diagnosis of such at the first examination based on the 75 g oral glucose tolerance test or the need for previous treatment for diabetes mellitus.

Statistical analysis

The mean values were statistically analyzed using SPSS 10.0 J (Statistical Package for Social Science, Inc., Chicago, IL) (6), and compared by the chi-square test or

Mann-Whitney U test. Spearman's rank correlation was also used to analyze the relation between age and plasma homocysteine concentrations. A value of $p < 0.05$ was considered significant. The relation between ischemic stroke and plasma homocysteine concentrations, gender (female: 0, male: 1), age, body mass index [BMI: bodyweight (kg)/height (m)²], history of cigarette smoking, hypertension, lipid metabolism (TC, TG, HDL-C), glucose metabolism and uric acid was examined by logistic regression analysis.

Results

Characteristics of Subjects

The breakdown of all 91 subjects was 44 patients with ischemic stroke (age 81 ± 7.0 , 54.5% male) and 47 control subjects without a history of ischemic stroke (age 79 ± 6.5 , 31.9% male). Table 1 shows background factors classified by the presence of ischemic stroke. The prevalence of males and homocysteine concentrations were higher in the groups with ischemic stroke. There were no inter-group differences in age, BMI, history of smoking, hypertension, TC, TG, HDL-C, uric acid, creatinine, albumin and diabetes mellitus.

Relation between age and the plasma homocysteine concentration (Fig. 1)

A significant relationship between age and the plasma homocysteine concentration was shown in patients without ischemic stroke ($r = 0.507$) and all patients ($r = 0.410$).

Table 1. Comparison of demographic characteristics in patients with or without Ischemic stroke.

	Ischemic stroke		<i>p</i> -value
	Yes <i>N</i> =44	No <i>N</i> =47	
Gender, male, <i>N</i> (%)	24 (54.5)	15 (31.9)	0.036
Age (years)	81 ± 7.0	79 ± 6.5	0.158
Body mass index (kg/m ²)	21.5 ± 3.5	21.4 ± 3.3	0.984
History of smoking, <i>N</i> (%)	16 (36.4)	16 (34.0)	0.830
Hypertension, <i>N</i> (%)	29 (65.9)	32 (68.1)	1.000
Systolic blood pressure (mmHg)	135 ± 17	138 ± 20	0.525
Diastolic blood pressure (mmHg)	71 ± 12	76 ± 10	0.080
Antihypertensive drug use, <i>N</i> (%)	25 (56.8)	23 (48.9)	0.530
Total cholesterol (mg/dl)	177 ± 29	181 ± 42	0.416
Triglyceride (mg/dl)	93 ± 44	97 ± 52	0.937
HDL-cholesterol (mg/dl)	50 ± 17	54 ± 17	0.241
Uric acid (mg/dl)	5.7 ± 1.8	5.2 ± 2.3	0.123
Creatinine (mg/dl)	0.81 ± 0.36	0.77 ± 0.32	0.818
Albumin (mg/dl)	3.9 ± 0.43	3.9 ± 0.41	0.601
Diabetes mellitus, <i>N</i> (%)	10 (22.7)	13 (27.7)	0.636
Fasting blood sugar (mg/dl)	118 ± 33	125 ± 48	0.980
Homocysteine ($\mu\text{mol/l}$)	14.6 ± 5.6	12.9 ± 6.6	0.023

Values are the mean $\pm$ standard deviation.

Fig. 1. Relation between age and the plasma homocysteine concentration.

Table 2. Comparison of plasma homocysteine in patients with or without ischemic stroke.

Homocysteine ($\mu\text{mol/l}$)	Ischemic stroke		Unadjusted Odds ratio (95% CI)
	Yes N=44	No N=47	
<10.0	5 (11.4)	18 (38.3)	1.0*
10.0~13.9	23 (52.3)	16 (34.0)	5.18 (1.59-16.9)
≥ 14	16 (36.4)	13 (27.7)	4.42 (1.29-15.2)
≥ 10.0	39 (88.6)	29 (61.7)	4.84 (1.61-14.6)

*Reference group. CI: confidence interval.

Relation between the plasma homocysteine concentration and ischemic stroke (Table 2)

The odds ratio of ischemic stroke was higher in the second and third highest plasma homocysteine concentration groups than in the first group by 5.18 and 4.42-fold, respectively.

Relation between plasma homocysteine concentrations and ischemic stroke as determined by logistic regression analysis

Table 3 shows the results of logistic regression analysis using the presence of ischemic stroke as an object variable, adjusted by various risk factors including the plasma homocysteine concentration. On combining the second and third highest homocysteine groups, the odds ratio compared with the first group was 4.41 (95% confidence interval (CI): 1.44-13.6) controlling for age alone and 5.80 (95% CI: 1.50-22.5) for all other demographic variables.

Discussion

We demonstrated in a cross-sectional study that there was a significant relation between ischemic stroke and plasma homocysteine concentrations. In the group with the second highest homocysteine concentration, <14 $\mu\text{mol/l}$, which has so far been deemed as within the normal range in healthy adults (7), the odds ratio of ischemic stroke was significantly higher than in the first group, even after adjustment for known risk factors.

Some studies have reported, particularly in Western countries, a relation between ischemic stroke and plasma homocysteine concentrations. The British Regional Heart Study cohort reported that the homocysteine level in 107 stroke patients was 13.7 $\mu\text{mol/l}$ (12.7-14.8), a significantly higher level than the 11.9 $\mu\text{mol/l}$ (11.3-12.6) in 118 control subjects, and the odds ratio increased with the increase in homocysteine (8). Kittner *et al.* (9) defined a homocysteine concentration higher than 7.3 $\mu\text{mol/l}$ as abnormal in young females 15 to 44 years old and reported that the odds ratio for ischemic stroke increases 1.6-fold (1.1-2.5) on such occasions. Eikelboom *et al.* (10) reported that an increase in homocysteine was a strong and independent risk factor for ischemic stroke (adjusted odds ratio 2.7, 95% CI 1.4 to 5.1 for a 5 $\mu\text{mol/l}$ increase in fasting plasma homocysteine from 10 to 15 $\mu\text{mol/l}$). These findings suggest that homocysteine is a strong and independent risk factor for stroke. However, one study reported a marginal association with incident stroke,

Table 3. Odds ratio of plasma homocysteine in patients with ischemic stroke determined with logistic regression models.

Homocysteine ($\mu\text{mol/l}$)	Odds ratio (95% CI)			
	Model A	Model B	Model C	Model D
<10.0	1.0*	1.0*	1.0*	1.0*
≥ 10.0	4.41 (1.44-13.6)	3.69 (1.14-11.9)	4.58 (1.32-15.9)	5.80 (1.50-22.5)
p-value	0.010	0.029	0.017	0.011

*Reference group. CI: confidence interval.

Model A was adjusted for age; Model B, age, gender and albumin; Model C, age, gender, albumin, creatinine, hypertension and smoking; Model D, age, gender, albumin, creatinine, hypertension, diabetes mellitus, smoking, body mass index, total-cholesterol, triglyceride, HDL cholesterol and uric acid.

essentially confined to events occurring before the age of 60 years (11), and another study reported no association with either incident MI or stroke (12). We also observed that the association between homocysteine concentrations and ischemic stroke was independent of known risk factors for atherosclerotic disease; 21.7% in those with $<10 \mu\text{mol/l}$ homocysteine, 59.0% in those with $10\text{--}13.9 \mu\text{mol/l}$, and 55.2% in those with $\geq 14 \mu\text{mol/l}$.

As for the rise in homocysteine and the mechanism of onset of atherosclerosis, it was reported that endothelium cell injury induced by homocysteine accelerated the proliferation of smooth muscle cells (13), thrombosis formation by promotion of prothrombin activity (13), and more recently promotion of LDL oxidation (14) and the mechanism mediated by active oxygen (15). It has been suggested that homocysteine influences endothelial factors involved in maintaining the balance between coagulation and fibrinolysis. Such an elevated homocysteine concentration increased endogenous factor V activity (16) and reduced antithrombin levels (17). Bots *et al.* (18) reported that an elevated homocysteine level was positively associated with factor VII activity.

Many studies have reported on the definition of a high plasma homocysteine value. McCully *et al.* (19) define the normal value for homocysteine as $6\text{--}10 \mu\text{mol/l}$ for females and $8\text{--}12 \mu\text{mol/l}$ for males, Joosten *et al.* (20) reported an elevated homocysteine concentration that exceeded $13.9 \mu\text{mol/l}$, which is higher than the mean value plus 2SD of healthy young controls, and the Framingham Heart Study (21) suggested a value of more than $14 \mu\text{mol/l}$ in subjects with normal folic acid, Vitamin B₆ and B₁₂. According to the present findings, there are differences in ischemic stroke above $10.0 \mu\text{mol/l}$, the value previously considered normal, and it seems necessary to re-consider the ideal homocysteine value.

There are several limitations to this study. One is that the subjects were in-patients who have various diseases. When the fact that the mean age of the hospitalized subjects is 80 years was considered, the possibility that dietary and renal disturbances affected the result of this study must be considered. In the ischemic stroke group, creatinine was slightly elevated. Therefore, in examining ischemic stroke and its relation to homocysteine and various risk factors, the values were adjusted for age, albumin and creatinine to exclude these effects. We can not deny that the control might include subclinical ischemic stroke (silent lacunar stroke) because the in-patients were aged 70 years or older. Homocysteine assays were performed within 2 days of admission, during which time homocysteine may be lower than during the convalescent phase (22). However both these factors are likely to reduce the difference between cases and controls and thereby bias the results of the study toward the null.

The present study of the association between the plasma homocysteine value and ischemic stroke in a

series of in-patients revealed that plasma homocysteine was a significant independent risk factor for ischemic stroke after adjustment for other risk factors. Such a tendency was also observed in homocysteine values falling within the normal range. We, therefore, suggest that plasma homocysteine is an important risk factor for ischemic stroke also in elderly Japanese and that further study is warranted to confirm its normal values.

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