Original Article

Plasma Level of Asymmetric Dimethylarginine (ADMA) as a Predictor of Carotid Intima-Media Thickness Progression: Six-Year Prospective Study Using Carotid Ultrasonography

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This study was designed to determine the relationship between plasma asymmetric dimethylarginine (ADMA) and the development of carotid atherosclerosis. Cross-sectional studies have revealed that plasma ADMA concentration is correlated with the intima-media thickness (IMT) of the carotid artery, but no prospective studies have appeared. Therefore we prospectively investigated whether or not plasma ADMA level can predict IMT progression. In a community-based cohort, we enrolled 712 subjects who were over 40 years old and who had no apparent cardiovascular diseases according to high-resolution carotid ultrasonography. Blood chemistries including ADMA were measured at baseline. In 575 subjects, IMT was remeasured 6 years later. The value of baseline ADMA for predicting IMT changes was investigated by multivariable analysis. At baseline, there was a significant (β=0.321; p<0.001) relationship between IMT and ADMA levels. Multiple linear regression analysis revealed that baseline ADMA (β=0.241; p<0.01) was the only predictor of IMT progression after adjustments for age, sex, baseline IMT, and four major risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) plus hyperuricacidemia. Plasma ADMA was a predictor of carotid IMT progression. (*Hypertens Res* 2008; 31: 1185–1189)

***Key Words*: atherosclerosis, nitric oxide, prospective study**

Introduction

Nitric oxide (NO), an endogenous vasodilator released from the endothelium, also possesses a variety of anti-atherosclerotic biological activities. Thus, NO is now recognized as the most potent endogenous molecule against atherosclerosis (*1–5*). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase. ADMA is produced by the methylation of arginine residues in intracellular proteins *via* arginine *N*-methyltransferase (*6–8*). Intra-arterial infusion of ADMA causes endothelial dysfunction in humans, and overexpression of ADMA induces atherosclerosis in animals (*9*).

Elevated plasma levels of ADMA were reported in patients with hypertension (*10–12*), chronic renal failure (*13*), and postprandial hypertriglyceridemia (*14*). It has been shown that ADMA is a strong predictor of cardiovascular events

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(*15–17*). In cross-sectional studies, we (*8*, *18*) and others (*19*) reported that plasma ADMA level is related to IMT, suggesting that ADMA has a role in the pathogenesis of atherosclerosis. However, no large-scale prospective studies have been done to evaluate whether or not plasma ADMA is a significant predictor of IMT progression. Accordingly, we measured plasma ADMA and employed high-resolution ultrasonography to determine common carotid IMT at baseline in 712 subjects of a community-based cohort, and followed the changes in IMT in most of these subjects after 6 years.

Methods

Study Subjects

A periodic epidemiological survey was performed in 1999 in a small farming community (a cohort of the Seven Countries Study) on the island of Kyushu, Japan. As reported previously, the demographic backgrounds of the subjects in this area are similar to those of the Japanese general population (*20*). The subjects' medical history, especially of cerebro-cardiovascular diseases, was ascertained in detail by a team of physicians. We randomly enrolled 712 subjects (305 men and 407 women) over the age of 40 years, who had no apparent cerebro-cardiovascular diseases, and examined IMT in all of them by means of high-resolution carotid ultrasonography. While the subject was seated, common carotid IMT was measured by duplex ultrasonography (SSA-380A; Toshiba, Tokyo, Japan) with a 10 MHz transducer. Longitudinal B-mode images at the diastolic phase of the cardiac cycle were recorded. The images were magnified and printed with a high-resolution line recorder (LSR-100A; Toshiba). IMT was measured using fine slide calipers at three levels of the lateral and medial walls 1 to 3 cm proximal to the carotid bifurcation. These six combined near- and far-wall measurements were averaged. Previous results from our laboratory showed that the mean plasma ADMA concentration was 0.51±0.10 μmol/L (*8*), whereas in the present study it was 0.50±0.13 μmol/L at baseline. And, in our laboratory, the inter-observer and intra-observer variabilities of IMT were 3.8% and 4.2%, respectively ($n=30$).

We repeated the survey 6 years later in 575 subjects; the follow-up rate was 80.8%. The investigators were blinded to the clinical characteristics and baseline IMT values of the individuals. Changes in IMT were calculated as the values of follow-up IMT divided by baseline IMT, and expressed as percentages. Of the 712 subjects, 59 refused the re-examination, 43 had died (16 deaths from cancer, 9 from cerebrovascular diseases, 4 from lung diseases, 2 from traffic accidents, 2 from cardio-renal failure, and 10 from unknown etiologies), follow-up was lost for 23, 7 had moved, and 5 were hospitalized. Finally, complete data sets were available from 575 subjects.

Informed consent was obtained from all 575 subjects. The Kurume University Ethics Committee approved the study.

Study Protocol

The subjects' medical history as well as their alcohol intake and smoking habits were ascertained by a questionnaire. Alcohol intake and smoking habits were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) as an index of obesity. Blood pressure (BP) was measured in the right arm twice with a mercury sphygmomanometer after the subject had rested in the supine position for more than 5 min. The second BP with the fifth-phase diastolic pressure was used for analysis.

Blood was drawn from the antecubital vein for determination of fasting plasma glucose (FPG), glycosylated hemoglobin A1c (HbA1c), lipid profiles (total cholesterol, high-density lipoprotein-cholesterol [HDL-c], and triglycerides), immunoreactive insulin (IRI), creatinine, uric acid, and ADMA levels in the morning after a 12-h fast. Fasting blood samples were centrifuged immediately after collection. Plasma concentrations of ADMA levels were measured by the high-performance liquid chromatography (HPLC) method as previously described (*8*). Serum total cholesterol, HDL-c, triglycerides, and creatinine were measured by the enzymatic assay method, and HbA1c was measured by ion-exchange HPLC. All blood chemistry analyses were performed at a commercial laboratory.

Hypertension was defined as BP ≥140/90 mmHg or current treatment with antihypertensive medication. Hypercholesterolemia was defined as total cholesterol ≥5.69 mmol/L and/or current treatment with lipid-lowering drugs. Diabetes was defined as HbA1c ≥6.0% and/or current treatment with an oral hypoglycemic agent or insulin injections. Hyperuricacidemia was defined as serum uric acid ≥416.4 μmol/L and/or current treatment with uric acid–lowering drugs. We defined hypertension, hypercholesterolemia, diabetes, and smoking as four major risk factors for atherosclerosis.

Statistical Analysis

Results are presented as means±SD. Because of skewed distributions, triglycerides and FPG were log-transformed before data analysis; mean values, as well as upper and lower 95% confidence limits, were exponentiated and presented as geometric means±SD, where the SD was approximated as the difference in the exponentiated confidence limits divided by 3.92, the value of SD in a 95% confidence interval for normally distributed data. Dummy variables were sex, smoking habit, alcohol intake, and medication for hypertension, diabetes, hyperlipidemia, and hyperuricacidemia.

The mean parameters stratified by tertiles of ADMA levels were compared using analysis of variance. The χ^2 test was used for categorical parameters to test differences among groups. To investigate factors for IMT progression after 6

Table 1. Baseline Characteristics of 575 Subjects Stratified by Tertiles of ADMA Levels

Characteristics	Group 1 (n=192)	Group 2 (n=192)	Group 3 (n=191)	p value
Asymmetric dimethylarginine (μmol/L)	0.36±0.06	0.48±0.03	0.64±0.10	<0.001
Age (years)	60.1±9.9	61.1±10.2	64.5±10.7	<0.001
Sex (% men)	31.8	39.1	49.7	<0.001
Body mass index (kg/m^2)	23.1±3.2	23.3±3.3	22.9±2.9	0.504
Systolic blood pressure (mmHg)	131.4±19.7	133.9±19.1	136.4±19.3	0.042
Diastolic blood pressure (mmHg)	79.9±10.1	81.0±11.1	80.2±11.8	0.587
Total cholesterol (mmol/L)	5.15±0.89	5.22±0.81	5.09±0.89	0.348
HDL-cholesterol (mmol/L)	1.44±0.31	1.45±0.36	1.43±0.34	0.889
Triglycerides (mmol/L)*	1.10±0.02	1.07±0.22	1.13±0.02	0.752
Fasting plasma glucose (mmol/L)*	5.26±0.06	5.38±0.10	5.40±0.07	0.617
Hemoglobin A1c (%)	5.2±0.6	5.2±0.7	5.2±0.9	0.843
Creatinine (μmol/L)	72.6±13.8	72.6±14.0	76.8±17.3	0.008
Uric acid (μmol/L)	287.2±79.5	290.6±75.9	308.7±96.9	0.029
Intima-media thickness (mm)	0.65±0.17	0.70±0.19	0.74±0.21	<0.001
Smoking status (%)	12.5	15.6	16.7	0.481
Alcohol intake (%)	18.7	22.9	27.2	0.147
Hypertensive medication (%)	20.3	22.4	23.0	0.797
Diabetic medication (%)	3.1	3.6	5.2	0.552
Hyperlipidemic medication (%)	5.7	3.7	4.2	0.600

*Log-transformed values were used for the calculation of means and exponentiated geometric means are presented. Values are means±SD or %, unless otherwise indicated. ADMA, asymmetric dimethylarginine; HDL, high-density lipoprotein.

years, multiple linear regression analyses were performed with age, sex, baseline IMT, and the four major risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) plus hyperuricacidemia as covariates to adjust for confounders.

Statistical significance was defined as $p<0.05$. All statistical analyses were performed with the use of SAS statistical software (SAS Institute, Cary, USA) (*21*).

Results

Table 1 shows the baseline characteristics of the 575 subjects stratified by tertiles of ADMA level. At baseline, there were significant cross-sectional relationships between ADMA and age ($p<0.001$), sex ($p<0.001$), systolic BP ($p<0.05$), creatinine ($p<0.01$), uric acid ($p<0.05$), and baseline IMT ($p<0.001$). Multiple linear regression analysis revealed that baseline ADMA ($\beta=0.241$, $p<0.01$; $r^2=0.193$) was the only predictor of IMT progression after 6 years after adjustments for age, sex, baseline IMT, and the four major risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) plus hyperuricacidemia. Because only 5 subjects had had a cardiovascular event in the intervening 6 years, we did not perform the statistical analysis.

Figure 1 shows percentage changes in IMT after 6 years according to the tertiles of baseline ADMA levels. There was a significant relationship ($p=0.025$) between baseline ADMA and IMT progression by analysis of covariance adjusted for age, sex, baseline IMT, and the four major risk factors plus hyperuricacidemia.

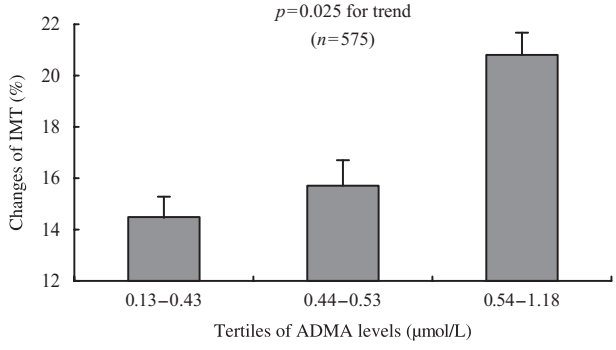

Fig. 1. *Percentage changes in IMT were calculated by tertiles of baseline ADMA by adjusting for age, sex, baseline IMT, and four major risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) plus hyperuricacidemia. Percentage changes in IMT were calculated as the values of follow-up IMT divided by baseline IMT × 100. There was a significant ($p=0.025$) relationship between baseline plasma ADMA level and IMT progression analyzed by analysis of covariance adjusted for age, sex, baseline IMT, and the major risk factors plus hyperuricacidemia.*

Discussion

Methodological Considerations

In this study, we evaluated changes in IMT by high-resolution carotid ultrasonography but did not measure plaques. It may have been preferable to measure changes in plaques rather than in IMT. However, it is very difficult to accurately estimate changes in plaques because of their complex morphology. In contrast, the evaluation of IMT is relatively simple and accurate. Furthermore, IMT, an indicator of subclinical atherosclerosis, has been shown to be a strong risk factor for cardiovascular events (*22*).

The mean plasma ADMA level in this study was 0.50 μmol/L, which was similar to that in our previous report (*8*) and in a report on subjects in eastern Finland (*16*). In the present study, we used column-switching HPLC to measure ADMA (*7*, *8*). Recently, Krempl *et al*. (*17*) developed a new method using ELISA to measure ADMA. They reported 0.59 μmol/L in control subjects; this value was very similar to that in our study. Thus the reported value of plasma ADMA in the present study was considered accurate for the community cohort, and our measurements were believed to be stable and reliable. It is well known that renal function affects plasma ADMA levels (*13*, *15*, *19*) and that ADMA levels are elevated in patients with renal dysfunction (*23*). In this study, as shown in Table 1, there was a positive relationship between ADMA and creatinine. Thus we confirmed that renal function affects plasma levels of ADMA even in a population of normal creatinine levels. In addition, cross-sectional studies (*8*, *11*, *14*) have revealed positive associations between plasma ADMA levels and atherosclerotic risk factors. Indeed, our study revealed positive associations between plasma ADMA at baseline and age, male gender, systolic BP, and uric acid level (Table 1). Thus we confirmed the previous findings in this large cohort population.

Predictors for IMT Progression

A positive association between plasma ADMA level and IMT has been shown in several cross-sectional studies (*8*, *18*, *23*). The present study confirms this relationship, as shown in Table 1. Because age, male gender, and the four major risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) plus hyperuricacidemia are well-known as significant factors in IMT progression, we adjusted for them to determine the factors in IMT progression. Consequently, multiple linear regression analysis showed that the plasma level of ADMA was the only predictor of IMT progression (β=0.241, $p<0.01$; r^2=0.193). Although one small prospective study (*24*) reported ADMA's value for predicting IMT progression in patients with renal failure who were on chronic hemodialysis, ours was the first large prospective study in a community-based cohort without apparent cardiovascular diseases.

Our data may suggest that elevated plasma ADMA is not just a marker of atherosclerosis, but rather may play a causal role in the pathogenesis of human IMT progression. Thus, in clinical practice, plasma ADMA levels may be useful not only for risk stratification but may also serve as a surrogate marker for interventional trials (*24*, *25*).

Study Limitations

Our study has several limitations. First, we did not measure total plaque area. Second, we only examined subjects older than 40 years. It would be interesting to determine whether or not plasma ADMA concentration is a predictor of progression of IMT of the carotid artery in younger subjects. Third, although it is shown that elevated ADMA is associated with endothelial dysfunction in humans (*7*, *9*), it may be interesting to examine whether or not ADMA is a predictor of endothelial dysfunction in our follow-up study. However, it is very difficult to measure endothelial function even with the simplest methods, such as flow-mediated vasodilation in 575 subjects. Fourth, several pharmacological interventions have beneficial effects in preventing IMT progression (*26*, *27*). However, because we have no data on the subjects' use of medications after the baseline examination, we can not comment on whether or not such interventions had any beneficial effects on IMT progression or ADMA concentration in this study. Finally, because elevated ADMA may be associated with increased oxidative stress, it would have been interesting to measure oxidative stress markers in this study. Further studies are needed to address this issue.

In conclusion, to our knowledge, this is the first epidemiological report in a community cohort to show that ADMA plasma level may be a predictor of carotid IMT progression.

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