Asymmetric Dimethylarginine in Cerebral Small Vessel Disease

Usman Khan, MRCP; Ahamad Hassan, MRCP; Patrick Vallance, FRCP; Hugh S. Markus, FRCP

Background and Purpose—Endothelial dysfunction may play a causal role in cerebral small vessel disease (SVD). Asymmetric dimethylarginine (ADMA), a circulating endogenous inhibitor of nitric oxide, has been implicated in endothelial dysfunction, particularly in hyperhomocysteinemia, a known risk factor for SVD. We determined if ADMA was elevated in SVD, correlated with disease severity, and interacted with homocysteine.

Methods—ADMA and homocysteine levels were determined in 47 consecutive symptomatic SVD patients and 38 controls. SVD was graded by leukoariosis severity and number of lacunar infarcts.

Results—Mean (and SD) ADMA was higher in SVD patients compared with controls (0.814 [0.145] versus 0.747 [0.184] μmol/L; P=0.014) after controlling for age, gender, vascular risk factors, and creatinine clearance. Additionally controlling for homocysteine had only a small effect on this relationship (P=0.055). Mean homocysteine was higher in SVD cases compared with controls (15.14 [5.59] versus 12.49 [4.15] μmol/L; P=0.035). Leukoariosis grade correlated positively with ADMA (P=0.026) and homocysteine (P=0.003). Lacunar grade correlated with homocysteine (P=0.017), but not ADMA.

Conclusions—ADMA is independently associated with SVD and correlates with leukoariosis severity. (Stroke. 2007;38:000-000.)

Key Words: ADMA ■ endothelial dysfunction ■ homocysteine ■ lacunar ■ leukoariosis ■ stroke

Cerebral small vessel disease (SVD) causes both lacunar infarction and more diffuse subcortical ischemic change referred to as leukoariosis. The pathogenesis of the underlying arteriopathy is uncertain but studies have implicated both endothelial dysfunction and hyperhomocysteinemia, particularly when lacunar infarction is accompanied by confluent leukoariosis.1,2

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase.3 Elevated levels have been reported in cardiovascular disease,4 and it may play a role in mediating endothelial dysfunction seen in hyperhomocysteinemia.5 ADMA is synthesized through arginine methylation by protein arginine methyltransferase, type 1. Protein arginine methyltransferase type 2 generates symmetric dimethylarginine, which has no endothelial nitric oxide synthase inhibitory action. Furthermore, symmetric dimethylarginine is largely eliminated through renal excretion, whereas most ADMA is metabolized by dimethylarginine dimethylaminohydrolase.

We compared ADMA levels between SVD patients and controls to test 2 hypotheses. First, that SVD is associated with elevated ADMA levels that correlate with SVD severity. Second, that there is a significant interaction between ADMA and homocysteine.

C

Methods

Study Population

Forty-seven white patients with SVD, defined using modified TOAST criteria,6 were recruited from inpatient and outpatient facilities. All had brain imaging, imaging of the extracranial carotid arteries, and ECG. Echocardiography was performed in 20 (43%). Thirty-eight white community controls free of symptomatic cerebrovascular disease were recruited by sampling family doctor lists from the same geographic regions as the patients. Sampling was stratified to provide similar distributions of age, gender, hypertension, hypercholesterolemia, and smoking history to patients. Local research ethics committees approved the protocol. Written informed consent was obtained from all participants.

Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or current treatment with antihypertensive drugs. Diabetes mellitus was defined as a previous diagnosis of type I or type II diabetes, or at least 2 random glucose readings of >11.1 mmol/L or fasting blood glucose readings of >7.0 mmol/L. Hypercholesterolemia was defined as a serum cholesterol >5.2 mmol/L, or current statin therapy. A positive smoking history was recorded in those who had ever smoked.

Assays

All assays were performed blinded to the case identity. In patients, all samples were collected at least 3 months after the most recent stroke to exclude transient elevation in biochemical markers. Plasma ADMA and symmetric dimethylarginine were analyzed by high-

DOI: 10.1161/01.STR.0000254500.27412.ac
pressure liquid chromatography. Serum homocysteine was measured as described previously. Because ADMA is handled by the kidneys, creatinine clearance was determined in cases and controls.

Grading of SVD
MRI was available in 41 cases (87.2%) and CT only in 6 cases (12.8%). Leukoariosis on MRI or CT was graded using a modified Fazekas scale into absent (0), mild (1), early confluent (2), and confluent (3). This method has been previously validated, and a good correlation was found between grading on CT and MRI.1 Lacunar infarcts (5 to 15 mm) were scored as absent (0), 1 to 2 lesions (1), 3 to 5 lesions (2), and >5 lesions (3). Because only 2 patients had lacunar infarction grade 3, for analysis patients were grouped into 2 categories: lacunar infarct grades 0 to 1 and 2 to 3.

Statistical Analysis
All biochemical markers underwent logarithmic transformation to normalize distributions before analysis. Multivariate logistic regression was used to control for age, gender, vascular risk factors, and creatinine clearance. ADMA levels were split into tertiles to enable logistic regression analysis, and odds ratios (ORs) and 95% CIs were calculated. Linear regression analysis was used to assess associations between ADMA, homocysteine, and SVD severity scores.

Results
Subject Characteristics (Table 1)
Age, gender, or vascular risk factors were well-matched between patients and controls.

Biochemical Markers in SVD Patients and Controls (Table 2)
ADMA and homocysteine were increased in SVD patients compared with controls on univariate analysis and multivariate analysis. In contrast, symmetric dimethylarginine levels did not differ. The OR for SVD increased with increasing tertile of ADMA. After controlling for age, OR (95% CI) for the middle compared with the lowest tertile was 3.94 (1.22 to 12.78; \( P = 0.022 \)), and for the upper compared with the lowest tertile 4.80 (1.36 to 16.96; \( P = 0.015 \)). After additionally controlling for gender, vascular risk factors, and creatinine clearance, the ORs were 6.40 (1.20 to 34.16; \( P = 0.030 \)) and 10.04 (1.70 to 59.27; \( P = 0.011 \)), respectively.

**Associations Between ADMA and Homocysteine**
There was no correlation between ADMA and homocysteine \( (R=0.114, P=0.329) \). The positive association between AMDA and SVD on multivariate analysis \( (P=0.014) \) was only slightly attenuated after additionally controlling for homocysteine \( (P=0.055) \). Similarly, the association between homocysteine and SVD \( (P=0.035) \) was attenuated slightly after additionally controlling for ADMA \( (P=0.055) \).

**Differences in ADMA and Homocysteine Between Cerebral SVD Subtypes (Figure)**
Leukoariosis grade correlated positively with both ADMA \( (R=0.324, P=0.026) \) and homocysteine \( (R=0.433, P=0.003) \). There was no correlation between the lacunar grade and ADMA \( (R=0.029, P=0.849) \), but a positive correlation with homocysteine \( (R=0.362, P=0.017) \).

**Discussion**
We found ADMA was elevated in SVD after controlling for vascular risk factors and correlated with leukoariosis severity but not lacunar grade. Homocysteine was also elevated in SVD, and in contrast was correlated with both leukoariosis severity and lacunar grade. These findings may be consistent with ADMA mediating smaller perforator damage, contributing to leukoariosis, and homocysteine acting on both smaller and larger perforators additionally contributing to lacunes. A role for ADMA in mediating endothelial dysfunction in hyperhomocystinemia has been proposed, but we failed to demonstrate significant interaction between these two biomarkers.

The association of ADMA with atherosclerosis may offer an alternative explanation for the elevation of ADMA seen in SVD. Undetected atherosclerosis elsewhere in the vascular tree could result in high ADMA levels. However, several factors argue against this. SVD patients and controls were well-matched for vascular risk factors. Thirty-nine of 47 SVD patients had no evidence of plaque on extracranial vessel imaging. Eight patients had minor plaque (≤30% stenosis). None of the patients who had echocardiography had evidence of a cardiac or aortic atherosclerosis. Of the 47 SVD patients, only 1 had a history of myocardial infarction and 2 had symptomatic peripheral vascular disease.

Our findings demonstrate an association between SVD and ADMA and are consistent with, but do not prove, a

---

### Table 1. Demographics of Cerebral SVD and Control Groups

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Controls</th>
<th>SVD</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.5 (8.2)</td>
<td>65.3 (10.4)</td>
<td>0.543</td>
</tr>
<tr>
<td>Male</td>
<td>20 (52.6)</td>
<td>31 (66.0)</td>
<td>0.212</td>
</tr>
<tr>
<td>Hypertension</td>
<td>30 (78.9)</td>
<td>37 (78.7)</td>
<td>0.980</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0 (0)</td>
<td>4 (8.5)</td>
<td>0.999</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>24 (63.2)</td>
<td>32 (68.1)</td>
<td>0.634</td>
</tr>
<tr>
<td>Smoking</td>
<td>28 (73.7)</td>
<td>38 (80.9)</td>
<td>0.430</td>
</tr>
</tbody>
</table>

**Mean (SD) for continuous variables and numbers (%) for categorical variables.**

---

### Table 2. ADMA, SDMA, and homocysteine in SVD Cases and Controls

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Controls</th>
<th>SVD</th>
<th>Univariate ( P ) Value</th>
<th>Multivariate ( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA (( \mu \text{mol/L} ))</td>
<td>0.747 (0.185)</td>
<td>0.814 (0.145)</td>
<td>0.040</td>
<td>0.014</td>
</tr>
<tr>
<td>SDMA (( \mu \text{mol/L} ))</td>
<td>0.859 (0.260)</td>
<td>0.881 (0.190)</td>
<td>0.388</td>
<td>0.206</td>
</tr>
<tr>
<td>Homocysteine (( \mu \text{mol/L} ))</td>
<td>12.49 (4.15)</td>
<td>15.14 (5.59)</td>
<td>0.029</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Mean (SD) provided. Multivariate analysis controlled for age, gender, vascular risk factors, and creatinine clearance.**

---

Downloaded from http://stroke.ahajournals.org/ by guest on April 19, 2017
role in disease pathogenesis. Our findings need to be replicated in a larger sample and in a prospective study to examine causality.

**Sources of Funding**
This work is supported by a Stroke Association grant (Prog 3).

**Disclosures**
None.

**References**
Asymmetric Dimethylarginine in Cerebral Small Vessel Disease
Usman Khan, Ahamad Hassan, Patrick Vallance and Hugh S. Markus

Stroke. published online January 4, 2007;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2007/01/04/01.STR.0000254500.27412.ac.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/