Homocysteine and Pulsatility Index of Cerebral Arteries

Mi-Hye Lim, MD; Young I. Cho, PhD; Seul-Ki Jeong, MD

Background and Purpose—A pulsatility index (PI) represents vascular resistance distal to an examined artery. The purpose of the present study was to evaluate an association between plasma total homocysteine (tHcy) and PIs of the cerebral arteries in patients with ischemic stroke.

Methods—Consecutive patients with ischemic stroke referred to a neurovascular ultrasound laboratory were evaluated from March 2007 to February 2008. PI was defined as (peak systolic velocity−end-diastolic velocity)/mean flow velocity as recommended. Transcranial Doppler was examined in both middle cerebral arteries and vertebral arteries, and basilar arteries. All patients with ischemic stroke were subdivided according to the presence of proximal internal carotid arterial steno-occlusion (ICS).

Results—The numbers of patients enrolled for the present analysis as ischemic stroke without and with ICS were 272 and 92, respectively. PIs measured in the cerebral arteries did not show a significant difference in the two groups, in spite of the fact that mean flow velocities of both basilar arteries and vertebral arteries were significantly elevated in the patients with ICS. Plasma tHcy was found to be independently associated with graded increases of PIs in all cerebral arteries in the patients without ICS, even adjusted for the potential confounders. However, there was no association between tHcy and PI in the patients with ICS.

Conclusion—Plasma tHcy was directly associated with increased cerebral arterial resistance. But in clinical situations when the cerebral arterial hemodynamics were altered as in the patients with ICS, the effect of tHcy on arterial remodeling could be obscured. (Stroke. 2009;40:3216-3220.)

Key Words: homocysteine ■ pulsatility index ■ ischemic stroke ■ vascular resistance ■ hemodynamics ■ transcranial Doppler

Homocysteine (Hcy) is a naturally occurring amino acid from a methyl donor, methionine.1 Although Hcy is essential for an intracellular metabolism, it has been reported that it reduces nitric oxide (NO) bioavailability by stimulating the formation of reactive oxygen species2,3 and increasing matrix metalloproteinase activity, resulting in both an alteration of vascular elastin/collagen ratio and a reduced compliance of arterial walls.4 Hcy eventually causes a reduction of vessel radius by thickening arterial wall.5

Even though in vivo experimental studies consistently showed the adverse effects of Hcy on arterial walls, clinical studies did not. Some observational studies have reported a significant association between plasma total homocysteine (tHcy) and increased vascular outcomes.6,7 But others reported negative results.8,9 Clinical trials have focused on tHcy reduction by folate or multivitamin treatment and subsequently measured changes in vascular events, finding somewhat diverse results.10,11

Meta-analyses for the clinical trials began to divide the effectiveness of the multivitamin treatments for ischemic stroke and cardiovascular disease (CVD).12,13 The diverse effects of tHcy on ischemic stroke and CVD may be closely related with the vascular biology of these organs.14 Such diverse effects can be attributed to the fact that the surrounding tissues of small arteries which can indirectly affect the vascular resistance are totally different: brain parenchyma for cerebral arteries but muscle for coronary arteries. Most clinical studies, however, have just focused on an association between tHcy and vascular events, not paying much attention to pathophysiologic effect of tHcy on vascular system.

The objective of the present study was to investigate whether or not plasma tHcy was associated with pulsatility index (PI) in intracranial cerebral arteries by transcranial Doppler (TCD) examination. PI was known to be an independent risk factor for cerebral infarction15 and reflects downstream arterial resistance.16 The authors examined PIs in both middle cerebral arteries (MCA) and vertebral arteries (VA), as well as basilar artery (BA) in ischemic stroke patients, and analyzed relations between intracranial arterial PIs and tHcy separately according to the presence of proximal internal carotid arterial steno-occlusion (ICS), adjusting for potential confounders.

Subjects and Methods

Study Population
All patients with ischemic stroke referred to the Neurovascular Ultrasound Laboratory in stroke center of Chonbuk National Uni-
by guest on April 12, 2017 http://stroke.ahajournals.org/ Downloaded from

Assessments and Measurements

The data in the present study collected from the consecutive patients including prior history of CVD, type 2 diabetes mellitus (DM), hypertension, dyslipidemia, and medication usage. Smoking status was classified into 3 categories: current smokers, ex-smokers, and nonsmokers. Patients with a persistent elevation of blood pressure (ie, \( \geq 140/90 \) mm Hg) or taking antihypertensive medications were classified as hypertensive according to the criteria defined by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.\(^{19}\) Subjects with type 2 DM were those who reported having been told by a physician that diabetes was present or those with fasting blood glucose of greater than 7.0 mmol/L more than 2 times according to the criteria defined by the American Diabetic Association.\(^{20}\) Dyslipidemia defined as having a total cholesterol greater than 6.2 mmol/L, triglyceride greater than 2.3 mmol/L, or LDL cholesterol greater than 4.1 mmol/L as recommended.\(^{21}\)

Plasma tHcy was measured by fluorescence polarization immunoassay in the third day after the onset of acute ischemic stroke (AxSYM, Abbott Laboratories). All other laboratory data used in the present analysis were measured on the Hitachi analyzer was carried out by duplicate measurements made on the Hitachi analyzer (Hitachi High-Technologies Corporation). The level of free T4 (FT4) in serum was determined by a Hitachi 7600-110 analyzer (Hitachi High-Technologies Corporation). Daily quality control of the above measurements was performed with a commercially available control material (Bio-Rad Laboratories).

Transcranial Doppler Examination

The blood flow velocities at both right and left middle cerebral arteries (MCA), the basilar artery (BA), and both right and left vertebral arteries (VA) were measured using SONAR/tek 5.18B with 2-MHz probe. For consecutive patients referred to the Neurovascular Ultrasound Laboratory of CNU Hospital with the diagnosis of subacute (3 to 14 days from) ischemic stroke, the complete diagnostic spectral TCD examination was conducted.\(^{22}\) All TCD measurements were performed by 2 trained technicians, and the study reports were prepared by 2 reviewers (M.H.L. and S.K.J.) who were blinded to each other’s report. When the two reviewers did not agree, a case conference was held to discuss the discrepancy and the final conclusion on agreement was made. Pulsatility index (PI) was used as a reflection of impedance to flow distal to the point of occlusion; SUE, stroke of undetermined etiology; SDE, stroke of other determined etiology; tHcy, total homocysteine.

\( P \) values by independent \( t \) test, \( \chi^2 \) test, or \( t \) test for trend as appropriate.

Sampling.\(^{23}\) PI was automatically calculated by the TCD instrument using the following definition (peak systolic velocity--end diastolic velocity)/mean flow velocity (MFV). TCD was measured along the full segments of each cerebral artery as recommended.\(^{22}\) In the present study, however, only the PIs at the most proximal segment of each artery (ie, M1 proximal segment for MCA, at 50 to 60 mm depth; proximal BA at 75 to 80 mm) were selected, because the PIs at the most proximal segment of each cerebral artery were thought to represent the arterial resistance of the relevant distal arterial beds.

Statistical Analysis

The descriptive data for the major characteristics were expressed as a mean±SD or percentage as appropriately. An independent \( t \) test was used to determine the statistical differences in the continuous variables, and a \( \chi^2 \) test for trend for categorical variables. A general linear model was used for all intracranial arteries to evaluate the linear relationship between adjusted PIs and tHcy level categories (tertiles) in the 2 groups (ie, patients with and without ICS) separately, because the presence of ICS might potentially modify the relation between PI and tHcy. Bonferroni tests were applied to correct for multiple comparisons. Interaction terms like categorizations (stroke with or without ICS)×tertiles of tHcy for PI in each intracranial artery were created, and their significance was assessed. All statistical analyses were conducted using SPSS software version 16.0 (SPSS).

Results

In total, 549 patients were referred to the Neurovascular Ultrasound Laboratory in CNU Hospital for the aforemen-
tioned 1-year period. Among them, 158 patients (28.8%) were stroke free. In the 391 stroke patients (71.2%), 27 patients (4.9%) were diagnosed as having an ischemic stroke attributable to cardiac embolism, who were excluded from the present study. Finally 272 patients (49.5%) were categorized as ischemic stroke without ICS and 92 patients (16.8%) as ischemic stroke with ICS, respectively, and a total of 364 patients were included in the present analysis.

Age, plasma tHcy, and the proportion of type 2 DM were significantly higher in the stroke with ICS, but FT4 levels and the proportion of women were lower in that group (see Table 1). Stroke laterality according to the presence of ICS was not different, but in the case with stroke with ICS, occurrences of ipsilateral stroke to the side of carotid steno-occlusion were significantly higher. For example, 64% patients had right-sided strokes with right-sided carotid stenoses, whereas 74.2% patients had left-sided strokes with left-sided carotid stenoses with probability values >0.007 for both cases. The stroke classifications according to the TOAST criteria showed a significantly higher proportion (82.6%) of LAA in the stroke with ICS compared with the stroke without ICS (55.9% with \( P \) for trend=0.001).

TCD accessibilities were somewhat lower in both MCAs than in BA and both VAs as indicated in Table 2. PIs were not significantly different in all intracranial arteries according to the presence of ICS, but the mean values of MFVs at BA and both VAs were significantly higher in the stroke with ICS than without ICS. The mean values of MFVs at MCAs were greater than those at VA or BA for the case of patients without ICS. Compared with the patients without ICS, the MFV at BA in patients with ICS was found to increase by 16.6%, and those at both the right and left MCAs increased by 7.4 and 9.9%, respectively. For the case of patients with ICS, a sum of the increases in MFVs at both the right and left MCAs (17.3%) was nearly the same as the increase at BA (16.6%).

In the stroke patients without ICS, the higher tertiles of tHcy were independently associated with the graded increases of PIs in all intracranial arteries (probability values <0.05), adjusting for the vascular risk factors, hematocrit, FT4 levels, and TOAST classification (as shown in Table 3). But in the stroke with ICS, such an association was not observed in all arteries. Interaction terms between tHcy and the categorization according to the presence of ICS were significant for both the left MCA and BA (\( P \) for interaction=0.007 and 0.024, respectively).

Discussion

The present study found that an increased plasma tHcy was independently associated with increased PIs in all intracranial arteries in patients with ischemic stroke, when there was no combined internal carotid arterial steno-occlusion (ICS). The present finding adds a further scientific evidence of the adverse biological effects of tHcy on intracranial cerebral arteries and ischemic stroke.14 To the authors’ knowledge, it is the first report to show an association between tHcy and PIs of cerebral arteries in patients with ischemic stroke.

The significant interaction terms in some cerebral arteries indicated that the stroke groups could be divided according to the presence of ICS as depicted in the present study. But, the remaining question is why the associations between tHcy and PIs were only evident in the stroke patients without ICS. It is not clear whether or not tHcy exerts its adverse effects only in that particular group. The mean value of tHcy was, however, significantly higher for the case of ischemic stroke patients with ICS than for those without ICS (as shown in Table 1). The proportion of LAA, the subtype which was known to be closely related with an elevated tHcy, was also

### Table 2. Pulsatility Indexes, Mean Flow Velocities (MFV), and Ultrasound Accessibilities in the Intracranial Arteries

<table>
<thead>
<tr>
<th></th>
<th>Stroke Without ICS (n=272)</th>
<th>Stroke With ICS (n=92)</th>
<th>Difference of MFVs, %</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rt MCA, accessibilities, %</td>
<td>240 (88.2)</td>
<td>80 (87.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility indexes</td>
<td>0.965±0.272</td>
<td>0.962±0.256</td>
<td>7.4</td>
<td>0.186</td>
</tr>
<tr>
<td>MFV, cm/sec</td>
<td>66.5±27.8</td>
<td>71.4±30.2</td>
<td>74.2</td>
<td>0.024</td>
</tr>
<tr>
<td>Lt MCA, accessibilities, %</td>
<td>235 (86.4)</td>
<td>82 (89.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility indexes</td>
<td>0.967±0.249</td>
<td>0.979±0.229</td>
<td>16.6</td>
<td>0.001</td>
</tr>
<tr>
<td>MFV, cm/sec</td>
<td>67.5±28.8</td>
<td>74.2±34.3</td>
<td>9.9</td>
<td>0.084</td>
</tr>
<tr>
<td>BA, accessibilities, %</td>
<td>271 (99.6)</td>
<td>92 (100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility indexes</td>
<td>0.960±0.234</td>
<td>0.978±0.204</td>
<td>19.8</td>
<td>0.005</td>
</tr>
<tr>
<td>MFV, cm/sec</td>
<td>−42.8±18.4</td>
<td>−49.9±19.8</td>
<td>16.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Rt VA, accessibilities, %</td>
<td>266 (97.8)</td>
<td>87 (94.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility indexes</td>
<td>0.939±0.220</td>
<td>0.990±0.204</td>
<td>22.4</td>
<td>0.001</td>
</tr>
<tr>
<td>MFV, cm/sec</td>
<td>−37.9±16.3</td>
<td>−46.4±22.2</td>
<td>19.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Lt VA, accessibilities, %</td>
<td>272 (100.0)</td>
<td>90 (97.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility indexes</td>
<td>0.936±0.241</td>
<td>0.975±0.212</td>
<td>19.8</td>
<td>0.001</td>
</tr>
<tr>
<td>MFV, cm/sec</td>
<td>−34.6±13.7</td>
<td>−44.8±22.4</td>
<td>22.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean ± SD was expressed, unless noted otherwise. ICS indicates internal carotid steno-occlusion; TCD, transcranial Doppler; MCA, middle cerebral artery; BA, basilar artery; VA, vertebral artery. 

\( P \) values by independent \( t \) test.
significantly higher in the stroke with ICS. These findings indicate the possibilities of some kind of pathophysiologic roles of tHcy in the stroke with ICS in the present design, an observation that was consistent with previous studies.\(^{24,25}\)

The findings in the present study showing totally different patterns of the association between PIs and tHcy among the stroke patients based on the presence of ICS should be considered carefully for the possible obscuring factors. First of all, the PI could be already influenced by the proximal arterial steno-occlusive lesions caused important hemodynamic changes to maintain adequate cerebral blood flow (as depicted in Table 2). The significantly increased MFVs in BA (16.6%), and the nearly same amounts of the increases in total MFVs at both right and left MCAs (17.3%) in the stroke patients with ICS strongly support that the increased blood flow in the posterior circulatory beds attributable to ICS might roughly compensate the equivalent amount of flow which was reduced at the carotid arteries across the circle of Willis. That kind of positive hemodynamic changes seemed to modify (even reverse) the adverse flow effect of atherosclerotic properties induced by tHcy.

PI was useful in revealing the adverse biological effects of tHcy on intracranial cerebral arteries and ischemic stroke because it eliminated a need for angular corrections\(^{22}\) and reflected a downstream peripheral resistance which could be directly related with adverse effects of tHcy.\(^{5}\) Compared to arteries embedded in muscle (like coronary arteries), an increased resistance in distal small arteries in brain parenchyma seemed to affect more the proximal arterial circulation.

In addition to the conventional vascular risk factors, variables such as hematocrit and FT4 levels were adjusted in the present study. The additional adjustment of these variables seemed to be meaningful because these factors were known to be correlated with whole blood circulatory status. The slightly lower FT4 levels in the stroke with ICS might suggest the role of subclinical hypothyroidism for atherosclerosis, even it should be elucidated further.\(^{26}\)

The present study had some limitations. First, the sampling time for tHcy was slightly different (earlier) from TCD examination so that there could be some intervening effect of time span between them. But tHcy was reported to fluctuate after vascular events in some studies,\(^{27}\) and TCD was also variable after 24 hours of vascular accidents.\(^{28}\) Hence, to improve the consistency in data, the authors sampled tHcy and measured TCD separately. Second, the present study was performed for consecutive patients as a cross-sectional design so that a causal relationship between tHcy and increased PIs could not be defined. The findings in the present study should be reevaluated with a longitudinal or interventional design for the pathophysiologic roles of tHcy on vascular remodeling by considering possibly altered hemodynamics. Third, information about an intake of folic acid or nutritional supplements was not obtained in the present study; However, folic acid fortification was not obligatory in South Korea, and all patients were evaluated for both neuroimaging and tHcy initially in their initial strokes, and according to the results, vitamin supplement was considered and initiated for the relevant patients. Lastly, the cerebral arterial status distal to the selected PIs for the present study was not described thoroughly. The distal arterial status could be variable and influence the proximal PIs itself. However, the results adjusted with TOAST classification as in Table 3 showed the robust associations between tHcy and cerebral arterial PIs. The subtype of LAA for the patients with no proximal ICS (55.9%) seemed to largely have the intracranial atherosclerosis because extracranial steno-occlusion (stroke with ICS) was already excluded.

In summary, plasma tHcy was associated with graded increases of PIs in cerebral arteries in patients with ischemic stroke, reflecting its direct influence on increased cerebral arterial resistance. But in clinical situations where the cerebral arterial hemodynamics was altered, the effect of tHcy on arterial remodeling (atherosclerosis) could be obscured in the patients with ICS.

### Table 3. Adjusted Mean Values of Pulsatility Indexes According to the Tertiles of tHcy

<table>
<thead>
<tr>
<th>Intracranial Arteries</th>
<th>Stroke</th>
<th>1st, &lt; 9.72</th>
<th>2nd, 9.72 to 12.7</th>
<th>3rd, ≥ 12.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right MCA</td>
<td>Without ICS</td>
<td>0.871 ± 0.046</td>
<td>0.988 ± 0.047</td>
<td>1.042 ± 0.047*</td>
</tr>
<tr>
<td>With ICS</td>
<td>1.003 ± 0.068</td>
<td>0.942 ± 0.052</td>
<td>0.953 ± 0.053</td>
<td></td>
</tr>
<tr>
<td>Left MCA</td>
<td>Without ICS</td>
<td>0.911 ± 0.025</td>
<td>0.989 ± 0.025*</td>
<td>1.010 ± 0.025*</td>
</tr>
<tr>
<td>With ICS</td>
<td>1.012 ± 0.048</td>
<td>0.957 ± 0.040</td>
<td>0.987 ± 0.039</td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>Without ICS</td>
<td>0.890 ± 0.023</td>
<td>0.986 ± 0.023*</td>
<td>1.020 ± 0.024†</td>
</tr>
<tr>
<td>With ICS</td>
<td>1.002 ± 0.041</td>
<td>0.973 ± 0.031</td>
<td>0.971 ± 0.032</td>
<td></td>
</tr>
<tr>
<td>Right VA</td>
<td>Without ICS</td>
<td>0.883 ± 0.023</td>
<td>0.961 ± 0.022*</td>
<td>0.978 ± 0.023*</td>
</tr>
<tr>
<td>With ICS</td>
<td>0.940 ± 0.048</td>
<td>1.008 ± 0.036</td>
<td>1.014 ± 0.035</td>
<td></td>
</tr>
<tr>
<td>Left VA</td>
<td>Without ICS</td>
<td>0.881 ± 0.025</td>
<td>0.959 ± 0.024</td>
<td>0.983 ± 0.025*</td>
</tr>
<tr>
<td>With ICS</td>
<td>0.957 ± 0.043</td>
<td>0.973 ± 0.034</td>
<td>0.980 ± 0.034</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted by age, sex, hypertension, type 2 DM, smoking, dyslipidemia, hematocrit, free T4 levels, and TOAST classifications. *P<0.05, †P<0.01; Compared with the 1st tertile of tHcy (Bonferroni adjustment for multiple comparisons).
Sources of Funding
This article is supported by Research Institute of Clinical Medicine, Chonbuk National University Hospital.

Disclosures
None.

References
Homocysteine and Pulsatility Index of Cerebral Arteries
Mi-Hye Lim, Young I. Cho and Seul-Ki Jeong

Stroke. 2009;40:3216-3220; originally published online July 23, 2009;
doi: 10.1161/STROKEAHA.109.558403
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 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/40/10/3216

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/