Correlation of NO Metabolites and 8-Iso-Prostaglandin F$_{2\alpha}$ With Periventricular Hyperintensity Severity

Hiroshi Shibata, Toru Nabika, Hidehiko Moriyama, Junichi Masuda, Shotai Kobayashi

Objective—Oxidative stress and NO are thought to play important roles in arteriosclerosis pathogenesis, a major cause of white matter lesions in the brain. Therefore, we examined whether NO metabolites (NOx) and 8-iso-prostaglandin F$_{2\alpha}$ (IsoP) levels in vivo correlated with the severity of periventricular hyperintensity (PVH) to evaluate potential roles of oxidative stress and NO in white matter lesions.

Methods and Results—Participants (687 males and 528 females) of a health-screening examination were recruited into the study. The plasma NOx and urinary IsoP levels were measured using the Griess method and ELISA, respectively. PVH was diagnosed on the basis of MRIs. In nonparametric univariate trend analyses, plasma NOx as well as aging, presence of hypertension and of lacunes, mean blood pressure, and high-density lipoprotein cholesterol showed highly significant monotone correlation with PVH severity ($P<0.01$). By the multivariate ordinal regression analysis, the plasma NOx ($P=0.002$) and urinary IsoP ($P=0.01$) levels were found to be independent factors influencing the severity of PVH together with aging ($P<0.001$), presence of hypertension ($P<0.001$) and lacunes ($P<0.001$), and mean blood pressure ($P=0.001$).

Conclusions—Oxidative stress and NO have a close correlation with PVH severity. (Arterioscler Thromb Vasc Biol. 2004;24:1659-1663.)

Key Words: lacunar infarction ■ nitric oxide ■ oxidative stress ■ white matter ■ small-vessel disease

Cerebral white matter lesions (WMLs), including periventricular hyperintensity (PVH), are often found in the elderly during MRI examinations.$^{1-3}$ The pathogenesis of WMLs is based on histopathologic changes such as subependymal gliosis, demyelination, small cavitations, and loss of axons deep in the white matter.$^{1,2,4}$ Although the clinical significance of mild WMLs is controversial, the severe form is thought to relate closely to vascular dementia.$^1$

Aging and hypertension are 2 established risk factors for WMLs.$^{1,3}$ However, because not all hypertensive elderly people experience severe WMLs, other genetic or environmental factors may influence the risk of developing this cerebrovascular lesion.$^5$ It is hypothesized that a major cause of WMLs is the occlusion and narrowing of peripheral penetrating arteries because of hyalinosis and fibrinoid necrosis, resulting in chronic ischemia or multiple minute infarctions.$^{1,2,4}$ Considering this hypothesis, oxidative stress is a good candidate for an additional risk factor for WMLs.$^6$ Evidence has indicated that excess oxidative stress is a risk factor not only for atherosclerosis in larger arteries but also for arteriosclerosis and remodeling of smaller arteries.$^{7-9}$ Consistent with this hypothesis, Schmidt et al showed in their cross-sectional study that the plasma concentration of antioxidant α-tocopherol was correlated inversely with WML severity, although they did not adjust α-tocopherol levels with total cholesterol concentrations.$^{10,11}$

Further, they also indicated in a separate study that a genotype of the paraoxonase 1 gene significantly influenced WML progression.$^{12}$ Because paraoxonase 1 was proposed to hydrolyze oxidized lipids in vivo,$^{13}$ this result further suggested the importance of oxidative stress in the pathogenesis and progression of WMLs.

Recently, 8-iso-prostaglandin F$_{2\alpha}$ (IsoP), an end product of oxidized arachidonate, was proposed to be a good biological marker for oxidative stress in vivo.$^{14,15}$ Its biological characteristics, such as chemical stability in urine, are suitable for large-scale epidemiological studies.$^{15,16}$ Therefore, in this study, we examined whether the urinary IsoP level was correlated with the severity of PVH under a cross-sectional study design.

In addition to IsoP, we studied the plasma NO metabolites (NOx) concentration as a marker for the systemic level of NO. NO is a potent vasodilator thought to have a close connection with oxidative stress.$^{17,18}$ The active NO level was reported to decrease under enhanced oxidative stress either through oxidation of NO itself or through inhibition of NOS activity by oxidative stress.$^{18,19}$ Therefore, we hypothesized that the NO level in vivo might be a good candidate for a direct contributor to the vascular biology influencing WMLs under oxidative stress.
We report here that urinary IsoP and plasma NOx levels were correlated significantly with the severity of PVH in a multivariate analysis, which suggested that the increased oxidative stress and the decreased NO level in vivo were additional independent risk factors for WML progression.

Methods
A total of 1215 consecutive participants (687 males and 528 females) who voluntarily visited the Shimane Institute of Health Science for a health screening examination between 1995 and 2000 were recruited into the study. In the interview, participants were asked about history of smoking, and medication for hypertension, diabetes mellitus, and hypercholesterolemia. A smoking index (SI; cigarettes per day/years) was calculated on the basis of the interview, and smoking status was categorized into 3 groups accordingly (non-smoker SI=0; mild smoker SI<200; heavy smoker SI≥200).3

Plasma was collected after overnight fasting to measure total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels. The low-density lipoprotein cholesterol level was calculated using Friedman formula. Subjects with a serum TG concentration ≥4.52 mmol/L (11 males and 5 females) were excluded because of the formula requirement.20 Nitrite/nitrate (NOx) was measured in plasma by the Griess method using commercial kits (Cayman Chemical). Urine was collected on site and was kept frozen at −20°C until analyzed. Urinary IsoP was measured by an enzyme immunoassay using commercial kits (Cayman Chemical). IsoP concentrations in urine kept frozen at −20°C were stable for at least 6 months and after 5 rounds of freeze-thawing (data not shown). To avoid variance among different lots, kits from the same lot were used for measurement. IsoP levels were standardized with urinary creatinine.

PVH severity was rated as follows: 0, absent; 1, a small localized area of PVH at the frontal horn; 2, thin PVH surrounding the lateral ventricle; 3, thick PVH surrounding the lateral ventricle; and 4, marked diffuse PVH extending into the white matter.3,21 White matter hyperintensity (WMH) was rated with Fazekas criteria as described previously.10 Diagnostic criteria for lacunar infarction were described previously.22,23 Spotty areas <3 mm in diameter were diagnosed as état criblé. Diagnosis was based on T2-weighted (repetition time [TR] 2000 ms, echo time [TE] 24 ms) and proton density–weighted (TR 5000 ms, TE 96 ms) MRIs (0.2 T; Siemens) of transverse slices 7-mm thick.23 Typical images of PVH, WMH, and lacunar infarction are shown in Figure 1.

Because TG, IsoP, and NOx levels were skewed toward lower values, log-transformed values were used in the analysis. Monotone trends of various factors along with PVH severity were evaluated with the nonparametric Jonckheere–Terpstra test or with an ordinal measure of association γ. Independent effects of each factor on PVH severity were evaluated with the ordinal regression analysis. All statistical analyses were performed using SPSS (version 11.0; SPSS Inc.) and Statview (version 5.0; SAS Institute). All subjects agreed to participate in the study, which was approved by the local ethics committee.

Results
Demographic data on the population studied are listed in Table 1. A nonparametric trend analysis indicated highly significant monotone correlation of age, HDL-C, and mean blood pressure (MBP) with PVH severity. Presence of hypertension and lacunes showed a strong association with the severity as well. In addition, gender, body mass index (BMI), TG, and smoking status correlated significantly with PVH severity.

Figure 2 shows plasma NOx and urinary IsoP levels in the population studied. The monotone decrease of plasma NOx with the increase in PVH severity was significant (P=0.009). Although urinary IsoP showed a similar tendency to increase with a severity of 0 to 2, the monotone trend of changes did not reach a significant level (P=0.19). In contrast to PVH, neither plasma NOx nor urinary IsoP were associated significantly with lacune presence (Figure 2).

Because 9 factors were correlated significantly with PVH severity (Table 1), we performed a multivariate analysis including these factors as well as plasma NOx and urinary IsoP to test whether they have independent effects on severity. The results of the ordinal regression analysis are shown in Table 2. In addition to age, MBP, and the presence of hypertension and lacunes, plasma NOx and urinary IsoP levels showed independent effects on severity in the population studied. The other 5 factors (ie, male sex, BMI, HDL-C, TG [log-transformed], and smoking status), were excluded as independent factors through the analysis.

A logistic regression analysis indicated that only 2 factors, age and the presence of hypertension, showed a significant association with the presence of lacunes, excluding NOx and...
IsoP from factors associated independently with lacunar infarction (data not shown).

**Discussion**

First, we showed that the plasma NOx level correlated significantly with PVH severity in this cross-sectional study. In general, one cannot infer a causal relationship between the factors analyzed under a cross-sectional study design. However, because it was not likely that PVH limited to the brain white matter influenced systemic plasma NOx levels, the present result may suggest that the plasma NOx decrease was related causally to PVH progression. The plasma NOx concentration is thought to represent the level of endogenous NO production when measured after overnight fasting. Reduced NO levels in peripheral circulation

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**TABLE 1. Demographic Data for the Population Studied**

<table>
<thead>
<tr>
<th>PVH</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>≥3</th>
<th>( P_{1}^{*} )</th>
<th>( P_{2}^{†} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>431</td>
<td>559</td>
<td>195</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>55.0 [54.3, 55.6]</td>
<td>58.1 [57.5, 58.7]</td>
<td>61.1 [60.0, 62.2]</td>
<td>69.9 [65.2, 74.5]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>52.9</td>
<td>57.2</td>
<td>61.5</td>
<td>57.1</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 [22.9, 23.4]</td>
<td>23.6 [23.4, 23.9]</td>
<td>23.3 [22.9, 23.7]</td>
<td>24.6 [23.3, 26.0]</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>90.3 [89.1, 91.5]</td>
<td>94.1 [93.0, 95.2]</td>
<td>97.2 [95.3, 99.1]</td>
<td>101.4 [91.5, 111.1]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>6.0 [5.8, 6.2]</td>
<td>6.0 [5.8, 6.2]</td>
<td>6.4 [5.3, 7.4]</td>
<td>6.4 [5.3, 7.4]</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>T-chol (mmol/L)</td>
<td>5.46 [5.38, 5.53]</td>
<td>5.48 [5.40, 5.56]</td>
<td>5.53 [5.30, 5.56]</td>
<td>5.72 [5.33, 6.08]</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.62 [1.58, 1.67]</td>
<td>1.57 [1.52, 1.62]</td>
<td>1.49 [1.43, 1.55]</td>
<td>1.55 [1.34, 1.75]</td>
<td>0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.26 [3.18, 3.34]</td>
<td>3.31 [3.26, 3.39]</td>
<td>3.31 [3.21, 3.44]</td>
<td>3.52 [3.18, 3.85]</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.10 [1.08, 1.16]</td>
<td>1.16 [1.13, 1.21]</td>
<td>1.21 [1.13, 1.30]</td>
<td>1.30 [1.03, 1.63]</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.75 [0.74, 0.77]</td>
<td>0.75 [0.74, 0.77]</td>
<td>0.78 [0.75, 0.80]</td>
<td>0.84 [0.67, 1.00]</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>HT (%)</td>
<td>20.2</td>
<td>33.5</td>
<td>53.3</td>
<td>71.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM (%)</td>
<td>10.0</td>
<td>11.6</td>
<td>13.3</td>
<td>21.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>No</td>
<td>63.8</td>
<td>57.4</td>
<td>57.9</td>
<td>50.0</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>6.3</td>
<td>4.7</td>
<td>5.1</td>
<td>0.0   </td>
<td>  </td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>29.9</td>
<td>37.9</td>
<td>69.9</td>
<td>50.0</td>
<td>  </td>
</tr>
<tr>
<td>Lacunes (+) (%)</td>
<td>1.4</td>
<td>9.1</td>
<td>27.7</td>
<td>78.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Determined by ANOVA or \( \chi^2 \)-test. † Determined by tests for monotone trends using either the Jonckheere-Terpstra test or an ordinal measure of association gamma.

BMI indicates body-mass index; MBP, mean blood pressure; FBG, fasting blood glucose; T-chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HT, hypertension; DM, diabetes mellitus.

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**Figure 2.** Correlation of plasma NOx and urinary IsoP levels with the severity of PVH and lacunar infarction. Plasma NOx and urinary IsoP levels were measured by the Griess method and ELISA, respectively. PVH severity was rated as described in Methods. Monotone trends of changes in NOx and IsoP were examined with the nonparametric Jonckheere-Terpstra test. Columns and lines indicate means and SEMs.
might exacerbate stenosis because of the remodeling and then hypoperfusion in the white matter.

We also indicated that the urinary IsoP level was correlated positively with PVH severity in a multivariate analysis. The positive correlation of IsoP with the severity of PVH suggested a role for oxidative stress in PVH progression. Because this correlation was not evident in a univariate analysis, IsoP levels seemed influenced by other confounding factors. In fact, a weak but significant correlation was observed between IsoP and smoking status (Spearman \( r = 0.16; P < 0.001 \)). Another potential confounding factor was medication for hypertension or hypercholesterolemia. Some drugs, such as angiotensin II receptor antagonists and statins, are known to have substantial effects on oxidative stress. These drugs might add further noise to the results, arguing that mild forms of PVH had not been caused by ischemia, arguing that mild forms of PVH had no pathophysiological significance. PVH was interpreted to reflect a normal structure through a histopathologic evaluation. How-ever, the evaluation was based on relatively small sample sizes, leaving a possibility that some “pathological” PVH cases were mixed with normal cases. In fact, a histopathologic study indicated arteriosclerotic lesions in milder forms of PVH, which were hypothesized to be related causally to the lesion by some authors. In addition, the present study indicated that even for the milder forms, several parameters correlated with the severity of PVH (Tables 1 and 2), suggesting some pathophysiological background underlying PVH progression. This view was further supported by our recent prospective study showing that the PVH severity was a good predictor of symptomatic strokes.

In contrast to PVH, neither NOx nor IsoP levels influenced the presence of lacunar infarction. This result was rather unexpected because lacunar infarction and WMLs were thought to share the same etiologic background. Our observation may be interpreted as follows. (1) In the lacunar infarctions, the NO level or oxidative stress has a minor etiologic role when compared with WMLs. Although WMLs are a chronically progressing diffuse change, lacunar infarctions may be more accidental and acute changes. Thus, even if the same etiologies are shared, some additional factors may contribute more to lacunar pathogenesis. (2) Lacunar infarction stratification in the present study was not adequate to detect effects of NO or oxidative stress. Although we stratified our population by the presence or absence of lacunes, it may be necessary to use other stratifications such as lacune size or number.

There are some limitations to this study. First, IsoP levels were determined in spot urine samples by ELISA in this study instead of using a 24-hour collection of urine and gas chromatography (GC)/mass spectrometry (MS) measurements. Second, plasma NOx levels after overnight fasting might still be under some influence of dietary NOx. These limitations were a consequence of the study design. The population used here consisted of otherwise healthy subjects attending a health examination voluntarily. Therefore, it was impractical to collect urine samples frequently enough for a 24-hour collection. Second, plasma NOx levels after overnight fasting might still be under some influence of dietary NOx. These limitations were a consequence of the study design. The population used here consisted of otherwise healthy subjects attending a health examination voluntarily. Therefore, it was impractical to collect urine samples frequently enough for a 24-hour collection.

<table>
<thead>
<tr>
<th>TABLE 2. Parameters Independently Correlated With PVH Severity by the Ordinary Regression Analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β</strong></td>
</tr>
<tr>
<td>Age (≥10 years)</td>
</tr>
<tr>
<td>HT (+)</td>
</tr>
<tr>
<td>Lacunes (+)</td>
</tr>
<tr>
<td>MBP (≥100 mm Hg)</td>
</tr>
<tr>
<td>log(NOx)</td>
</tr>
<tr>
<td>log(IsoP/creatinine)</td>
</tr>
</tbody>
</table>

Smoking status, sex, HDL-C, TG, and BMI were excluded by the analysis. HT indicates hypertension; MBP, mean blood pressure.
for 24 hours or to control dietary NOx using diets with low NOx contents before plasma collection. In addition, GC/MS is difficult to apply to a large number of samples. In spite of these limitations, IsoP levels measured by ELISA as well as the NOx levels after overnight fasting were applied successfully to recent clinical and epidemiological studies. Another limitation was, as discussed above, a lack of information on antihyperpertensive and antihypercholesterolemic medications in the cohort. It was difficult to obtain precise information on what type of medications they received in the interview. However, as pointed out in the recent study, these limitations might reduce the signal-to-noise ratio of the study, producing false-negative results.

In summary, the present study provided new epidemiological evidence implying possible roles for oxidative stress and NO in WML progression. The results warrant further pathophysiological and epidemiological study.

Acknowledgments

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References


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