Higher Levels of Interleukin-6 Are Associated With Lower Echogenicity of Carotid Artery Plaques

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Background and Purpose—Echo-lucent carotid plaques can be fragile and vulnerable to rupture, representing a risk factor for ischemic stroke. Given the studies showing that elevated levels of circulating inflammatory markers are predictive of cardiovascular events, we sought to determine whether higher levels of serum interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP) are associated with lower echogenicity of carotid plaques.

Methods—The study comprised 246 patients who had carotid atherosclerotic plaques as evidenced by ultrasound. Using acoustic densitometry, we quantified the echogenicity of the largest plaque in each patient by integrated backscatter analysis. Serum IL-6 and hsCRP levels were determined in all patients.

Results—Both log-transformed IL-6 and hsCRP concentrations were negatively correlated with carotid plaque echogenicity ($r = -0.28$, $P < 0.001$, and $r = -0.14$, $P < 0.05$, respectively). When traditional atherosclerotic risk factors, plaque thickness, and medication use were controlled for, IL-6 levels were inversely associated with plaque echogenicity ($\beta = -0.21$, $P = 0.01$), whereas such an association was of borderline significance for hsCRP ($\beta = -0.12$, $P = 0.06$).

Conclusions—Higher IL-6 levels, in addition to hsCRP levels, appear to be associated with lower echogenicity of carotid plaques, suggesting a link between inflammation and potential risk of plaques. (Stroke. 2004;35:677-681.)

Key Words: atherosclerosis ■ carotid arteries ■ inflammation ■ interleukins ■ ultrasonography

Carotid plaque echogenicity, as assessed by B-mode ultrasound, is associated with its histological content. Particularly, echo-lucent plaques are thought to be lipid rich with increased density of macrophages, often containing a large lipid pool or hemorrhage.1-3 These properties are consistent with the features of rupture-prone plaques, which are commonly characterized by a large necrotic/lipid core with a thin fibrous cap infiltrated by inflammatory cells.4 Also, studies have shown that echo-lucent carotid plaques are associated with the risk for ischemic stroke.5-6 From these findings, we can assume that echo-lucent carotid plaques are fragile and susceptible to rupture, representing a risk factor for stroke.

From studies to date, inflammatory processes are thought to be involved in the pathogenesis of atherosclerotic plaques and their thrombotic complications.7 In particular, elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6) have been associated with the risk for cardiovascular disease (CVD).8-10 Nevertheless, the mechanism that links such inflammatory markers and CVD risk remains to be determined. If higher levels of high-sensitivity CRP (hsCRP) and IL-6 are associated with echo-lucent carotid plaques, they may help us understand the link between inflammation and the risk of atherosclerotic plaques.

Integrated backscatter (IBS) analysis is a quantitative method to evaluate echogenicity of atherosclerotic plaques. This analysis defines acoustic propagation properties through the estimation of native radiofrequency signals from the tissue, allowing objective evaluation of plaque echogenicity.11 Using IBS analysis, we examined the relationships of serum IL-6 and hsCRP levels with carotid plaque echogenicity.

Methods

Subjects

The subjects of this investigation were patients at the Department of Internal Medicine and Therapeutics at Osaka University Hospital who had undergone standard carotid ultrasound examination between October 2000 and September 2002. Because of the high prevalence of CVD and its risk factors, carotid ultrasound examinations were performed to screen for carotid atherosclerosis and stenosis or, in some cases, to assess vertebral artery circulation. Of note, under the current healthcare system in Japan, carotid ultrasound examination can be performed not only for patients with carotid stenosis but also for those with cardiovascular risk factors.

Because we focused on the echogenicity of plaques, the inclusion criterion for this study was the existence of carotid plaques ($\geq 1.3$ mm in thickness). Patients with smaller plaques were not included because such plaques could not be clearly separated from diffusely thickened intima-media complex. When carotid plaques

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were identified, the purpose and procedures for IBS analysis and inflammatory marker evaluation were explained to patients. After written informed consent was obtained, patients underwent IBS and blood sample testing.

During the study period, IBS examinations were performed on 332 patients. However, patients with the following criteria were excluded: (1) calcified plaques with acoustic shadow (n=45) or occluded carotid artery (n=8) because it was technically impossible to determine their echogenicity reliably; (2) carotid endarterectomy (n=6); and (3) acute inflammatory diseases (n=3), vasculitis/collagen diseases (n=5), malignant neoplasm (n=12), or recent (<3 months) CVD events (n=7) because levels of inflammatory markers could be modified in such patients.

After exclusion of patients with any of the above criteria, the study sample comprised 246 patients (mean±SD age, 65.7±7.8 years), including 80 patients with a history of stroke/transient ischemic attack (TIA) (25 atherothrombotic infarctions, 26 lacunar infarctions, 5 cardioembolic infarctions, 4 cerebral hemorrhages, 9 other or unclassified strokes, and 11 TIs based on our criteria12). In stroke/TIA patients, the average interval between the events and IBS/blood testing was 60 months. Although the prevalence of stroke/TIA patients, the average interval between the events and IBS/blood testing was 60 months. Therefore, the prevalence of stroke/TIA patients was generally well controlled by medication (Table 1).

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Age, y</th>
<th>65.7±7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, %</td>
<td>65</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.3±2.5</td>
</tr>
<tr>
<td>Hypertension/medical treatment/ACEI or ARB use, %</td>
<td>79/68/30</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>136±16</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78±11</td>
</tr>
<tr>
<td>Diabetes mellitus/medical treatment, %</td>
<td>22/13</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L (mg/dL)</td>
<td>5.8±1.5 (104±27)</td>
</tr>
<tr>
<td>Hyperlipidemia/medical treatment/statin use, %</td>
<td>76/40/33</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (mg/dL)</td>
<td>5.7±0.8 (211±31)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (mg/dL)</td>
<td>1.6±0.8 (138±72)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L (mg/dL)</td>
<td>1.5±0.4 (56±16)</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>23</td>
</tr>
<tr>
<td>History of CVD, %</td>
<td>46</td>
</tr>
<tr>
<td>Stroke/transient ischemic attack, %</td>
<td>28/4</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>19</td>
</tr>
<tr>
<td>ASO, %</td>
<td>4</td>
</tr>
<tr>
<td>Aspirin use, %</td>
<td>23</td>
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<tr>
<td>Inflammatory markers</td>
<td></td>
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<tr>
<td>hsCRP, mg/dL</td>
<td>0.15±0.23 (0.07)*</td>
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<tr>
<td>IL-6, pg/mL</td>
<td>2.75±2.60 (2.07)*</td>
</tr>
<tr>
<td>Ultrasound parameters</td>
<td></td>
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<tr>
<td>IBS index</td>
<td>48±17</td>
</tr>
<tr>
<td>Plaque thickness, mm</td>
<td>2.48±1.03</td>
</tr>
</tbody>
</table>

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker; and ASO, arteriosclerosis obliterans.

*Median.

Figure 1. IBS analysis of carotid plaques. Through acoustic densitometry, IBS values are obtained from the plaque (pl), vessel lumen (lm), and adventitia (ad) at the same depth, where IBS index is defined as (pl−lm)/(ad−lm)×100.

measured on the longitudinal B-mode or color Doppler images perpendicular to the vascular wall.

Subsequently, with the use of acoustic densitometry, longitudinal IBS images of the largest plaque were recorded onto optical disks and used for the echogenicity evaluation. The acoustic densitometry system is capable of providing 2-dimensional IBS images in which the gray level is displayed proportionally to the integrated backscattered power. The IBS value is internally calibrated in decibels, having a dynamic range of 0 to 64 dB in the SONOS 5500 system.11 For all patients, IBS images were acquired with the same time gain compensation setting and gain control values. IBS values were obtained from outline of the plaque (pl), vessel lumen (lm), and adventitia (ad) at the same depth of the plaque (Figure 1). Because reproducibility of carotid plaque echogenicity was better when 2 reference structures (vessel lumen and adventitia) were used than when 1 reference was used,13 we defined IBS index as (pl−lm)/(ad−lm)×100. Accordingly, a lower IBS index corresponds to lower echogenicity. In case of echo-lucent plaques, color Doppler images were used to help identify the blood-plaque boundaries.

All examinations were done by 1 sonographer (H.Y.) who was blinded to patients’ clinical details. Before this study, we examined the reproducibility of the IBS index for 43 randomly selected plaques without severe calcification; IBS analyses were performed twice by the same examiner (H.Y.) and subsequently by 2 examiners (H.Y. and S.Y.).
Measurement of Serum Inflammatory Markers

After IBS examinations, blood was drawn with minimally traumatic venipuncture for measurement of serum inflammatory markers. Then, blood was centrifugated at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at −70°C. Serum IL-6 was measured by enzyme-linked immunosorbent assay (High Sensitivity Quantikine kit, R&D System). The detectable limit for IL-6 was 0.10 pg/mL. Circulating levels of hsCRP were measured by latex turbidimetric immunoassay with a sensitivity of 0.01 mg/dL (Shionogi Biomedical Laboratory Inc).

Evaluation of Atherosclerotic Risk Factors

Supine blood pressure was evaluated before the IBS examination. Levels of fasting blood glucose, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined from the blood sample taken for inflammatory marker evaluation. Information on patients’ medical histories and medication use was obtained from the clinical records with the IBS data masked. Hypertension was defined by casual blood pressure ≥140/90 mm Hg or current use of antihypertensive agents. Diabetes mellitus was defined by fasting blood glucose ≥7.0 mmol/L or use of glucose-lowering agents. Hyperlipidemia was defined by fasting serum total cholesterol >5.7 mmol/L, triglycerides >1.7 mmol/L, or use of cholesterol-lowering agents. Smoking status was categorically evaluated from self-reports, with a smoker defined as currently smoking ≥10 cigarettes per day for ≥1 year.

Statistical Analyses

All analyses were performed with SPSS 9.0J (SPSS Japan Inc). Because distributions of hsCRP and IL-6 levels appeared to be left skewed, they were normalized by log transformation. Thereafter, relationships between IBS index and continuous variables were examined by Pearson’s correlation analysis. For categorical variables, differences in IBS index were examined by unpaired t test. Subsequently, multiple linear regression analyses were performed to examine associations between IBS index and inflammatory markers by controlling for traditional atherosclerotic risk factors, plaque thickness, medication use, and log-transformed hsCRP. The magnitude of the IBS index was compared across the tertiles of IL-6 (Figure 2 and Table 4). IBS index was lower in patients in the highest tertile than in those in the middle or lowest tertile. Moreover, the differences persisted after adjustment for traditional atherosclerotic risk factors, plaque thickness, medication use, and log-transformed hsCRP.

Results

The associations of IBS index with atherosclerotic risk factors, lipid measures, and plaque thickness are shown in Table 2. A measure of plaque echogenicity, IBS index was positively correlated with HDL cholesterol and negatively associated with plaque thickness. Also, IBS index was lower in men than in women and had a trend for negative correlation with triglycerides. Additionally, although IBS index was similar by history of CVD, it was lower in patients with than in those without ischemic stroke/TIA (44.9±17.6 versus 49.6±17.0, P=0.046)

Table 3 shows the associations between IBS index and log-transformed concentration of IL-6 or hsCRP. By univariate analysis, IBS index was found to be negatively correlated with IL-6. When traditional atherosclerotic risk factors and CVD history were controlled for, IBS index remained negatively associated with IL-6. Moreover, the association was only slightly attenuated when plaque thickness and medication use were also controlled for. In addition to IL-6, IBS index had a negative weak association with hsCRP. However, the association was only of borderline significance when traditional atherosclerotic risk factors, as well as plaque thickness and medication use, were controlled for.

Discussion

In the present study, we have found that elevated serum IL-6 levels are associated with lower echogenicity of carotid plaques as quantified by IBS analysis. Also, plaque echogenicity was lower in patients with higher IL-6 levels than in those with lower levels after adjustment for other putative factors, including hsCRP. To the best of our knowledge, this is the first study to demonstrate associations between IL-6 levels and echogenicity of carotid plaques.

For evaluation of carotid plaque echogenicity, we have defined the IBS index, which is derived from the IBS value of plaques in reference to vessel lumen and adventitia (Figure 1). Namely, a lower IBS index implies lower echogenicity of plaques. Studies using B-mode methods have shown associations between echo-lucent carotid plaques and lower HDL cholesterol, advanced stenoses, male sex, and increased levels of triglyceride-rich lipoprotein. In the present study, lower IBS index was associated with lower HDL cholesterol

| TABLE 3. Associations of IBS Index With Inflammatory Markers |
|------------------|------------------|
|                  | IL-6*            | hsCRP*           |
|                  | r or β           | P                | r or β           | P                |
| Univariate       | −0.28            | <0.001           | −0.14            | 0.03             |
| Multivariate†    | −0.29            | <0.001           | −0.11            | 0.09             |
| Multivariate‡    | −0.21            | 0.002            | −0.12            | 0.06             |
| Multivariate§    | −0.21            | 0.003            | −0.12            | 0.06             |

*hsCRP and IL-6 were analyzed as log-transformed values. †When controlling for age, sex, body mass index, hypertension, diabetes mellitus, smoking status, history of CVD, total cholesterol, triglycerides, HDL cholesterol, and hyperlipidemia medication. ‡When additionally controlling for plaque thickness. §When additionally controlling for use of statin, aspirin, and angiotensin-converting enzyme inhibitors/angiotensin II type I receptor blockers.
and greater plaque thickness (Table 2). Also, the IBS index was lower in men and had a trend toward negative correlation with triglycerides. Thus, carotid plaque echogenicity as assessed by B-mode analysis appears to have associations with atherosclerotic risk factors and plaque size similar to those found by B-mode methods. Particularly, IBS index was lower in patients with than in those without ischemic stroke/TIA, which is consistent with the risk of echo-lucent plaques for ischemic cerebrovascular diseases.

In addition to commonly known atherosclerotic risk factors, circulating inflammatory markers such as hsCRP and IL-6 represent novel predictors of CVD. In the present study, IBS index was negatively associated with IL-6, and the association remained significant even after adjustment for age, sex, body mass index, hypertension, diabetes mellitus, smoking status, history of CVD, total cholesterol, triglycerides, and HDL cholesterol. However, whether it is derived from carotid echo-lucent plaques or from diffuse systemic atherosclerosis cannot be determined by the data presented. Additionally, IL-6 could facilitate the formation of unstable plaques through the stimulation of mononuclear cells, proinflammatory cytokines, and matrix metalloproteinases. Thus, the link between higher IL-6 and lower IBS index may offer a clue to the understanding of the risk of atherosclerotic plaques.

This study has some limitations. First, because this study is cross-sectionally designed, we cannot determine the causal relationships between higher IL-6 levels and lower plaque echogenicity. Second, patient selection bias can exist because we excluded a relatively large portion of the original patients (86 of 332), predominantly because of the technological limitations of ultrasound. Third, although associations between IBS index and inflammatory markers were virtually unmodified by medication use (Table 3 and 4), IL-6 and hsCRP levels have been shown to be modified by statins, aspirin, angiotensin-converting enzyme inhibitors, and angiotensin II type 1 receptor blockers. Future studies are needed to examine the effect of such medications on carotid plaque echogenicity. Larger prospective studies, probably using other methods combined with ultrasound, are necessary to establish the associations between IL-6, hsCRP, and carotid plaque echogenicity.

In conclusion, we have demonstrated an association between higher IL-6 and lower plaque echogenicity. The finding can broaden our understanding of the link between inflammation and the risk of atherosclerotic plaques.

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