CD34+/CD144+ Circulating Endothelial Cells as an Indicator of Carotid Atherosclerosis

Takamichi Sugimoto, MD, PhD,*† Naohisa Hosomi, MD, PhD,* Tomohisa Nezu, MD,* Tetsuya Takahashi, MD, PhD,* Shiro Aoki, MD, PhD,* Ikuko Takeda, MD, PhD,* Tomoya Mukai, MD,* Kazuhide Ochi, MD, PhD,* Takeshi Kitamura, MD, PhD,† Toshiho Ohtsuki, MD, PhD,‡ and Masayasu Matsumoto, MD, PhD*

Background: The relationships between the number of circulating endothelial cells (CECs) or endothelial progenitor cells (EPCs) and indicators of carotid atherosclerosis, such as the intima-media thickness (IMT) and plaque score are not well characterized in patients with chronic ischemic stroke. The objective of this study was to investigate these relationships in patients with chronic ischemic stroke and in patients with risk factors for stroke. *Methods:* A total of 58 patients (69.6 ± 10.0 years, 21 females) with chronic ischemic stroke or with risk factors for stroke were included in this study. IMT was measured using an IntimaScope, and the numbers of CECs and EPCs were measured using flow cytometry. CECs and EPCs were defined as CD34+/CD144+ and CD34+/CD133+ cells, respectively. *Results:* The number of CECs in patients with large artery atherosclerosis was higher than that in patients with cardioembolism or small vessel occlusion ($P < .05$). In contrast, there were no significant differences in the number of EPCs between groups. A positive correlation was also observed between the plaque score and the number of CECs ($r^2 = .139$, $P < .05$, n = 36). Moreover, the number of CECs in patients with moderate and severe atherosclerosis (.32 ± .11/μL, n = 22) was higher than that in patients with no plaque and mild atherosclerosis (.25 ± .07/μL, n = 34, $P < .05$). *Conclusions:* The number of CECs was high in patients with large artery atherosclerosis who experienced chronic ischemic stroke. And this number may reflect severity of carotid atherosclerosis. **Key Words:** Atherosclerosis—carotid intima-media thickness—carotid ultrasound—circulating endothelial cells—circulating endothelial progenitor cells.

Circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs) are 2 populations of endothelioid cells present in the blood.[1] CECs have mainly been studied in the fields of oncology and immunology,[2,3] and EPCs were discovered in and isolated from the peripheral blood of human adults.[4] Blood biomarkers

From the *Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical and Health Sciences, Hiroshima; †Department of Neurology, Chugoku Rosai Hospital, Kure; and ‡Stroke Center, Kindai University Hospital, Osaka-Sayama, Japan.

Received August 26, 2014; revision received October 1, 2014; accepted October 2, 2014.

This study was supported in part by research grants from the Ministry of Health, Labour and Welfare, Japan, the Japan Science and Technology Agency, the Smoking Research Foundation, the Tsuchiya Foundation, and the Japan Heart Foundation.

The authors declare no conflicts of interest.

Address correspondence to Naohisa Hosomi, MD, PhD, Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical and Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail: nhosomi@hiroshima-u.ac.jp.

1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2014.10.001

have been studied in patients with ischemic stroke,[5-7] and several studies have reported that the number of EPCs is related to certain indicators of atherosclerosis, such as intima-media thickness (IMT) in healthy adults[8] and brachial artery flow-mediated dilatation (FMD) in patients with cardiovascular risk factors.[9]

Ultrasonography that enables quantification of arterial wall thickness and the size of atherosclerotic lesions in the carotid arteries is commonly used as a clinical tool for evaluating atherosclerosis because carotid arteriosclerosis is regarded as an indicator of generalized atherosclerosis.[10,11] Persson et al[12] showed that ultrasonographic and microscopic IMT measurements were closely correlated. Increased carotid arterial wall thickness is also related to the prevalence of myocardial infarction, angina pectoris, cerebrovascular disease, and peripheral vascular disease and is associated with risks of stroke and myocardial infarction.[13-17]

To our knowledge, 2 studies have evaluated the relationship of CECs or EPCs with indicators of carotid atherosclerosis, such as IMT or the plaque score, in acute ischemic stroke with an ischemic response or inflammation.[18,19] This ischemic response or inflammation may influence the number of CECs or EPCs in the blood. The relationship of the number of CECs or EPCs with indicators of carotid atherosclerosis has not been clarified in patients with chronic ischemic stroke. The objective of this study was to investigate the relationship between the number of CECs or EPCs, as determined using flow cytometry, and indicators of carotid atherosclerosis in patients with chronic ischemic stroke and in patients with risk factors for stroke.

Subjects and Methods

This study was approved by the Ethics Committee of Hiroshima University Hospital. All of the participants provided written informed consent. We examined patients who had experienced ischemic stroke at least 6 months before the onset of the study and patients with one or more risk factors for stroke at our hospital from December 2011 to June 2012. All patients were more than 20 years old. Three subtypes of ischemic stroke were classified using the Trial of Org 10172 in Acute Stroke Treatment criteria: large artery atherosclerosis, cardioembolism, and small vessel occlusion.[20] Patients who had experienced stroke of other determined etiology or stroke of undetermined etiology were excluded from this study. The risk factors for stroke were defined as hypertension, diabetes mellitus, dyslipidemia, and atrial fibrillation, and hypertension, diabetes mellitus, and dyslipidemia were diagnosed by physicians. Patients were designated as hypertensive if they were taking antihypertensive agents and had a systolic blood pressure of 140 mm Hg or more and/or a diastolic blood pressure of 90 mm Hg or more. Patients were diagnosed with diabetes mellitus if they were treated with oral hypoglycemic agents or insulin and/or if their serum fasting blood glucose level was 126 mg/dL or more or hemoglobin A1c by the National Glycohemoglobin Standardization Program was 6.5% or more. Patients were diagnosed with dyslipidemia if they were taking antidyslipidemia medication or presented with a serum low-density lipoprotein cholesterol level of 3.62 mmol/L or more, a triglyceride level of 3.879 mmol/L or more and/or a high-density lipoprotein cholesterol level of less than 1.034 mmol/L. Patients with malignancy or immunological disorders, including vasculitis, were excluded. Patient disability was evaluated using the modified Rankin Scale.

Patient blood samples were collected within 1 month of carotid ultrasonography. The blood parameters were as follows: the platelet count, hemoglobin, fibrinogen, C-reactive protein, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, hemoglobin A1c, blood urea nitrogen, creatinine, the estimated glomerular filtration rate, and the numbers of CECs and EPCs. The estimated glomerular filtration rate (mL/minute/1.73 m^2) was calculated using the following equation: $194 \times \text{serum creatinine}^{(-1.094)} \times \text{age}^{(-.287)} \times .739$ (if female).[21]

Ultrasound examinations of carotid arteries were performed using an SSA-770A imaging system (Toshiba, Tokyo, Japan) with a 5-11 MHz linear array transducer. Seven investigators (A.H., I.T., T.M., T.O., M.N., T.N., and T.N.) obtained the ultrasonographic images. The maximum and mean IMTs of the common carotid artery (CCA) were measured by 1 investigator (T.S.) using an IntimaScope (Media Cross, Tokyo, Japan) at the R wave of the electrocardiogram. The maximum IMT of CCA was defined as the maximum IMT value at the far wall. The mean IMT of CCA was defined as the average of IMT measurements along a 20-mm-long section, including the maximum IMT, and was measured 3 times on each side. The severity of carotid atherosclerosis in each patient was evaluated using the plaque score, as follows: none (IMT <1.1 mm), mild (plaque score ≤ 5), moderate ($5 <$ plaque score ≤ 10), and severe (plaque score >10).[22] Patients who had plaque scores that were obviously more than 10 but who could not be assigned an exact score because of calcification, occlusion, or image quality were designated as having severe atherosclerosis.

Fluorescence-activated cell analysis was performed to determine the numbers of CECs and EPCs using a single platform and a lyse-no-wash procedure, as previously reported.[23-26] We defined CECs as CD34+/CD144+ cells and EPCs as CD34+/CD133+ cells. Briefly, 4 tubes containing 200 μL peripheral venous whole blood with ethylenediamine tetraacetic acid-2Na (VP-NA052K; Terumo, Tokyo, Japan) were incubated in 10 μL FcR Blocking Reagent (130-059-901; Miltenyi Biotec, Bergisch Gladbach, Germany) for 10 minutes on ice. Thereafter,