Endothelial Progenitor Cells During Cerebrovascular Disease

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Background and Purpose—Endothelial progenitor cells (EPCs) are associated with endothelial repair after ischemia in cardiac or peripheral circulation. There are no reports of EPCs with cerebrovascular disease. We present our experience with EPCs in patients with cerebrovascular disease.

Summary of Report—EPC counts differed significantly (P<0.001) between stroke patients (acute stroke: median 4.75 and range 0 to 33; stable stroke: median 7.25 and range 0 to 43) and control subjects (median 15.5 and range 4.3 to 50), independent of age. The level of EPCs was significantly correlated with the Framingham coronary risk score (FRS) (ρ = -0.349; P = 0.002).

Conclusions—Similar to cardiac experience, the low EPC levels may play a role in the pathophysiology of cerebrovascular disease. (Stroke. 2005;36:151-153.)

Key Words: cerebrovascular disorders • endothelial progenitor cells • stroke, acute

Endothelial dysfunction predisposes to atherosclerosis and thrombosis.1 It has been suggested that circulating endothelial progenitor cells (EPCs) may also be a marker of endothelial function and cardiovascular risk.2 EPC numbers are significantly decreased in subjects with elevated serum cholesterol, hypertension, and diabetes,2 and in smokers.3 EPCs appear to be mobilized in response to vascular trauma or tissue ischemia,4,5 prompted in part by cytokine release and vascular endothelial growth factor.5,6 This incorporation of EPCs has been clearly demonstrated at sites of induced carotid artery endothelial injury,7 as well as after experimental cerebral ischemia.8,9 Although there have been studies of EPCs in cardiovascular disease and there may be surrogate markers of vascular function, there are no studies on the relationship of EPCs with cerebrovascular disease. This study was undertaken to evaluate the effects of acute ischemic stroke, transient ischemic attack, and chronic cerebrovascular disease on EPC levels.

Materials and Methods

Three sets of subjects were recruited: (1) persons with acute ischemic stroke admitted to University of Alberta Hospital, a tertiary care medical center; (2) those with stable ischemic stroke or transient ischemic attacks >1 month after the event; and (3) healthy controls with no history of stroke or cardiovascular disease.

We measured EPCs according to the methods described elsewhere.6,10 Briefly, after collection of peripheral blood from patients (within 4 hours), mononuclear cells were separated using a Ficoll density gradient (Sigma Chemical Co). Plating was performed with 1 million cells cultured in each well of a 24-well fibronectin-coated plate. To confirm the endothelial progenitor cell lineage, indirect immunostaining was performed for mouse anti-human CD31, von Willebrand factor primary antibodies, and biotinylated rabbit anti-mouse IgG secondary antibody (Sero-tec, Raleigh, NC). Antibody detection was determined with ABC peroxidase elite kit (Vector Labs). EPC colonies were counted manually in each well of an individual sample after 7 days. An EPC colony consists of a central cluster of rounded cells with surrounding radiating thin, flat cells (Figure 1).6 To assess the reproducibility, colonies were counted by 2 different observers who were unaware of patients’ clinical profiles.

Statistical Analysis

Results were expressed as means±SD, median and range, and number (percentage). Nonparametric tests (Mann–Whitney U, Kruskal–Wallis H, and Spearman ρ correlation) were used when appropriate. Simple linear regression was used to examine the relationship between demographic and clinical factors with EPC. Variables with (P<0.20) were entered into a multiple linear regression model to identify the independent predictors of EPC level. SPSS software was used for data analysis.

Results

Of the 88 subjects in the sample, 56 (64%) were male and 32 (36%) were female, with the mean age of 63 years (SD 13.26). The relationship between the levels of EPCs in patients with acute stroke, with stable ischemic cerebrovascular disease, and of controls is shown in Figure 2. EPC counts differed significantly (β = -0.074; P = 0.003) between stroke patients (acute stroke: median 4.75 and range...
0 to 33; stable stroke: median 7.25 and range 0 to 43) and control subjects (median 15.5 and range 4.3 to 50). There was no significant difference in EPC counts between stable vascular disease and acute stroke ($P=0.166$). There was a significant inverse relationship between EPC levels and Framingham risk score ($\beta=-0.349$, $P=0.002$). In univariate regression analysis, acute stroke status ($\beta=-17.593$; $P<0.001$), stable stroke status ($\beta=-13.53$; $P<0.001$), Framingham coronary risk score ($\beta=-0.399$; $P=0.011$), age ($\beta=-0.261$; $P=0.014$), fasting glucose ($\beta=-1.346$; $P=0.078$), and hypertension ($\beta=-6.83$; $P=0.018$) were significant predictors of EPC levels. Multiple linear regression models identified age and type of subject as important predictors of EPC levels (Table).

**Discussion**

This is the first study evaluating the relationship between cerebrovascular disease and progenitor endothelial function. A number of observations are evident. The number of circulating EPCs was significantly lower in patients with cerebrovascular disease than in control subjects. EPCs were not significantly lower in patients with acute stroke ($P=0.164$) compared with patients with stable vascular disease. No significant increase in the EPC levels was observed in the weeks after acute and stable stroke. There was a significant inverse relationship of EPCs with Framingham risk factor score. We believe that the low EPC counts in our patient population, similar to what has been observed in

Figure 1. EPC colonies from a patient with transient ischemic attack. The colonies are well-formed after 7 days of culture.

Figure 2. EPC levels in patients with acute and stable stroke are compared with controls. Box plot showing median (line), interquartile range (boxes), and 5% to 95% percentile (whiskers), and outliers (dots) and extremes (stars) EPC.

The discovery that endothelial function may be regulated by circulating EPCs opens a new avenue for exploring vascular behavior to established risk factors. The first evidence for EPCs in adult circulation emerged when mononuclear cells from healthy human volunteers were shown to acquire an endothelial cell-like phenotype in vitro and incorporate into capillaries in vivo.\footnote{Asahara T, Takahashi T, Masuda H. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. \textit{EMBO J}. 1999;18:3964–3972.} These cells may serve several functions, including restoration of endothelial lining, and an important role in new vessel formation.\footnote{Libby P. Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis and stabilization. \textit{Am J Cardiol}. 2000;86:3J–9J.} EPCs increase with exogenous stimuli such as ischemia through activation of cytokines and likely increase the activity of matrix metalloproteinase-9.\footnote{Hattori K, Heissig B, Wu Y. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1 stem cells from bone-marrow microenvironment. \textit{Nat Med}. 2002;8:841–849.} Such factors may also play a role in homing and differentiation of EPCs on endothelial sites where they are required.\footnote{Asahara T, Murohara T, Sullivan A. Isolation of putative endothelial progenitor cells for angiogenesis. \textit{Science}. 1997;275:964–967.} Finally, infusion of EPCs improves blood flow in peripheral ischemia and cardiac function after myocardial ischemia.\footnote{Kawamoto A, Gwon HC, Iwaguro H. Therapeutic potential of \textit{ex vivo} expanded endothelial progenitor cells for myocardial ischemia. \textit{Circulation}. 2001;103:634–637.}

In conclusion, our study shows, for the first time to our knowledge, the effects of acute and chronic cerebrovascular disease on the levels of circulating EPCs. Compared with controls, the levels are significantly lower after an acute stroke and in patients with stable cerebrovascular disease that may indicate vascular dysfunction. Future studies would clarify the relationship of EPCs to the subsequent risk of recurrent stroke.

\textbf{Significant Predictors of EPC Levels From Multiple Linear Regression Analysis}

<table>
<thead>
<tr>
<th>Factors</th>
<th>Coefficients (β)</th>
<th>Standard Error of β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable vs control</td>
<td>−12.662</td>
<td>3.416</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute vs control</td>
<td>−16.134</td>
<td>4.284</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>−0.195</td>
<td>0.097</td>
<td>0.048</td>
</tr>
</tbody>
</table>

References

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