Endothelial and Platelet Activation in Acute Ischemic Stroke and Its Etiological Subtypes

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Background and Purpose—Activation of endothelial cells and platelets is an important mediator of atherothrombosis. Markers of endothelial cell and platelet activation such as soluble adhesion molecules can be measured in plasma. We hypothesized that patients with acute ischemic stroke would have increased blood concentrations of soluble E-selectin and von Willebrand factor (vWF), primarily reflecting activation of endothelial cells, and increased concentrations of soluble P-selectin and platelet-derived microvesicles (PDM), primarily reflecting activation of platelets, compared with healthy controls. We also hypothesized that these markers would be differentially elevated in ischemic stroke caused by large- and small-artery atherothrombosis compared with cardiogenic embolism.

Methods—We conducted a case-control study of 200 hospital-referred cases of first-ever ischemic stroke and 205 randomly selected community controls stratified by age, sex, and postal code. Using established criteria, we classified cases of stroke by etiological subtype in a blinded fashion. The prevalence of vascular risk factors and blood concentrations of E-selectin, P-selectin, vWF antigen, and PDM were determined in stroke cases within 7 days and at 3 to 6 months after stroke and in controls.

Results—Mean blood concentrations of soluble E-selectin, P-selectin, and PDM within 7 days of stroke onset were all significantly higher in cases compared with controls. At 3 to 6 months after stroke, the mean blood concentrations of E-selectin and P-selectin fell significantly below that of controls, and PDM concentrations remained elevated. There was a strong, graded, and independent (of age, sex, and vascular risk factors) association between increasing blood concentrations of E-selectin during the acute phase and all etiological subtypes of ischemic stroke, particularly ischemic stroke caused by large-artery atherothrombosis. There was also a significant, graded, and independent association between increasing blood concentrations of vWF during the acute phase and ischemic stroke caused by large-artery atherothrombosis.

Conclusions—We have demonstrated significant associations between acute elevation of blood markers of endothelial cell and platelet activation and ischemic stroke and between acute elevation of blood markers of endothelial cell activation and ischemic stroke caused by large-artery atherothrombosis. Persistent elevated blood concentrations of PDM may be a marker of increased risk of ischemic stroke. (Stroke. 2003;34:1111-1119.)

Key Words: activation ▪ endothelium ▪ platelets ▪ selectins ▪ stroke, acute ▪ stroke, ischemic

A therothromboembolic ischemic stroke is thought to be the product of atherogenesis, plaque inflammation, plaque rupture, thrombosis (with or without embolism), and brain infarction.1

Selectins, including E-selectin and P-selectin, are transmembrane glycoproteins expressed on activated vascular endothelium (E- and P-selectin) and activated platelets (P-selectin). They are believed to play an important role in atherogenesis and plaque inflammation by attracting leukocytes from the circulation, “slowing them down,” and allowing them to roll along the endothelium.2–14 The leukocytes adhere to the endothelium through the actions of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and then migrate across the endothelium into the atherosclerotic plaque.2

When an active atherosclerotic plaque ruptures, circulating platelets are exposed to subendothelial collagen, fibronectin, and von Willebrand factor (vWF). vWF is a large, multimeric glycoprotein synthesized by endothelial cells and megakaryocytes that circulates in a noncovalent complex with factor VIII. Under conditions of high shear stress, platelets adhere to vWF via the platelet glycoprotein Ib-V-IX, stimulating platelet activation and ultimately thrombus formation.

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When arterial thrombosis causes ischemic neuronal death, tumor necrosis factor-α and interleukin-1β are thought to be released from damaged neurons and to induce further expression of E-selectin on vascular endothelial cells in the brain. E-selectin attracts a massive influx of leukocytes into the ischemic region and helps mediate reperfusion injury by facilitating the margination of leukocytes and subsequent migration into the ischemic area.15,16 The leukocytes are believed to liberate oxygen radicals and other neurotoxins in the ischemic brain and promote microvascular occlusion in the ischemic penumbra.17,18

Because the soluble form of selectins and other cell activation markers in the blood may play an important role in the pathophysiology of atherosclerotic ischemic stroke, we aimed to determine, by means of a prospective case-control study, (1) whether blood concentrations of specific markers of endothelial activation (E-selectin and vWF) and platelet activation (P-selectin and platelet-derived microparticles [PDM]) are elevated among patients within 7 days of onset of ischemic stroke compared with healthy controls of the same age and sex; (2) whether blood concentrations of E-selectin, vWF, P-selectin, and PDM decline at 3 months after ischemic stroke to approximate those of controls; and (3) whether there is a positive, strong, graded, and independent relationship between increasing blood concentrations of E-selectin, vWF, P-selectin, and PDM and all types of ischemic stroke and whether this relationship was restricted to atherosclerotic etiological subtypes of ischemic stroke.

Methods

The Institutional Review Board of the Royal Perth Hospital approved this study, including the manner in which the controls were enrolled, and each study participant provided informed consent.

Cases

Consecutive patients presenting to the Royal Perth Hospital between March 1996 and June 1998 with first-ever ischemic stroke were approached for consent to participate in our study. Ischemic stroke was defined as a clinical stroke syndrome with either a normal CT brain scan or evidence of a recent infarct in the clinically relevant area of the brain on a CT or MRI brain scan performed within 3 weeks of the event or at autopsy.19,20

At baseline, demographic data (age, sex), history of conventional vascular risk factors (hypertension, diabetes, hypercholesterolemia, current smoker), and history of previous vascular events (myocardial infarction, angina, claudication, amputation) were obtained. All patients underwent a CT brain scan. Echocardiography and extracranial duplex ultrasound were performed at the discretion of the clinician. Within 7 days of stroke, an overnight fasting blood sample was obtained for measurement of blood concentrations of E-selectin, vWF, P-selectin, and PDM. A second blood sample was taken 3 to 6 months after stroke to measure E-selectin, vWF, P-selectin, and PDM concentrations in the convalescent state in patients who agreed to return for review.

On the basis of clinical evaluation and results of imaging studies, the study neurologist (G.J.H.), who remained blinded to the results of laboratory assays, classified all strokes into 4 major etiological subtypes (large-artery disease, small-artery disease, cardioembolic, other) according to predefined criteria.20,21

Controls

Control subjects were randomly selected from the Western Australian electoral roll and stratified by 5-year age group, sex, and postal code. A letter of invitation to participate, together with a stamped and self-addressed envelope, was sent to potential controls. Nonresponders were reached by telephone. Controls who agreed to participate in the study were required to fast for a minimum of 8 hours before their appointment and were given the option of attending the hospital outpatient clinic or being visited at home by the study nurse. Baseline demographic data (age, sex), history of conventional vascular risk factors, and history of previous vascular events were obtained for each control. A fasting blood sample was obtained for measurement of blood concentrations of E-selectin, vWF, P-selectin, and PDM.

Laboratory Analysis

All samples were collected and processed using a standardized protocol. Blood concentrations of E-selectin and P-selectin were measured with a commercially available sandwich enzyme-linked immunosorbent assay technique (ELISA; Medisystems Diagnostics). Blood concentrations of vWF were measured with a commercially available ELISA technique (Gradipore). PDMs were enumerated on a Coulter EPICS® flow cytometer (Beckman Coulter). Diluted citrated whole blood was incubated with FITC glycoprotein IIIa (Becton Dickinson) as a marker antibody, and fluorescent particles <1 μm were defined as PDMs. Fluorescent beads were included as an internal standard to allow quantification. Twenty normal subjects gave a range 50 to 150×10^3/L. All tests were performed by laboratory personnel who remained blinded to case or control status of the study participant.

Statistical Analysis

All analyses were based on available data. The few subjects for whom certain variables were missing were excluded from the analyses relevant to those variables.

| TABLE 1. Baseline Demographics, Conventional Vascular Risk Factors, and History of Previous Vascular Events in Cases and Controls |
|-----------------|-----------------|-----------------|-----------------|
|                 | Controls (n = 205) | Cases (n = 200) | OR (95% CI) |
| Age, (mean ± SD), y | 67.0±11.8 | 66.2±12.4 | . . . | 0.52 |
| Female sex, n (%)  | 74 (36.1) | 71 (35.5) | 1.0 (0.6–1.5) | 0.90 |
| Caucasian, n (%)   | 189 (92.2) | 176 (88.0) | 0.6 (0.3–1.2) | 0.16 |
| Hypertension, n (%) | 68 (33.2) | 109 (54.5) | 2.4 (1.6–3.6) | <0.001 |
| Diabetes mellitus, n (%) | 22 (10.7) | 53 (26.5) | 3.0 (1.7–5.2) | <0.001 |
| Current smoker, n (%) | 36 (17.7) | 70 (35.0) | 2.5 (1.6–4.0) | <0.001 |
| Hypercholesterolemia, n (%) | 45 (22.0) | 48 (24.0) | 1.1 (0.7–1.8) | 0.62 |
| Previous arterial event, n (%) | 26 (12.7) | 56 (28.0) | 2.7 (1.6–4.5) | <0.001 |
| Previous VTE, n (%) | 6 (2.9) | 4 (2.0) | 0.8 (0.2–2.4) | 0.54 |

VTE indicates venous thromboembolism.
Means or proportions for baseline demographics, vascular risk factors, and blood levels of E-selectin, vWF, P-selectin, and PDM were calculated for cases and controls. The distributions of values of the markers were not perfectly normal but were skewed slightly to the right. Because the log-transformed data were also not normally distributed according to the Shapiro-Wilk test, we performed both nonparametric tests (Wilcoxon test) and Student’s t tests (the results of which were consistent) and presented means and probability values from the t tests. Because the sample size was not small, according to the central limit theorem, the mean follows a normal distribution regardless of the shape of the parent population.

The significance of any relationship between blood concentrations of E-selectin, vWF, P-selectin, and PDM and the timing of blood sampling during long-term follow-up was examined by Pearson’s correlation coefficient.

Logistic regression was used to examine the association of blood concentrations of E-selectin, vWF, P-selectin, and PDM during the acute phase (independent variables) and ischemic stroke (dependent variable) after the samples were divided into quartiles defined by the distribution of the complete cohort. Adjusted estimates were obtained with a separate model that controlled for age, sex, conventional vascular risk factors, and history of previous vascular events. Statistical significance for all analyses was taken as a 2-sided value of P = 0.05.

Results

Table 1 lists the demographics and vascular risk factors of the 200 consecutive ischemic stroke patients and 205 controls. There was a significantly higher prevalence of conventional vascular risk factors among cases compared with controls.

**TABLE 2.** E-Selectin, vWF, P-Selectin, and PDM Levels at Baseline and 3 to 6 Months After Ischemic Stroke in Cases versus Controls

<table>
<thead>
<tr>
<th>Stroke Cases (n = 90)</th>
<th>Controls (n = 205)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Follow-Up (3–6 mo)</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>Mean 35.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>32.3–39.5</td>
</tr>
<tr>
<td>vWF antigen, %</td>
<td>Mean 183.8</td>
</tr>
<tr>
<td>Soluble P-selectin, ng/mL</td>
<td>Mean 322.2</td>
</tr>
<tr>
<td>PDM (×10^{9}L)</td>
<td>Mean 180.8</td>
</tr>
</tbody>
</table>

VTE, indicates venous thromboembolism.

**TABLE 3.** Association Between Quartiles of E-Selectin Measured at Baseline, Ischemic Stroke, and Etiological Subtypes of Ischemic Stroke Presented as ORs, 95% CIs, and Probability Value for Trend of Association*

<table>
<thead>
<tr>
<th>Quartile 1 (≤15.7 mg/L)</th>
<th>Quartile 2 (15.8–25.7 mg/L)</th>
<th>Quartile 3 (25.8–35.7 mg/L)</th>
<th>Quartile 4 (≥35.7 mg/L)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stroke (n = 202)</td>
<td>1.0</td>
<td>1.8 (0.9–3.3)</td>
<td>3.4 (1.8–6.5)</td>
<td>7.9 (4.0–15.6)</td>
</tr>
<tr>
<td>Large artery (n = 62)</td>
<td>1.0</td>
<td>1.6 (0.5–4.8)</td>
<td>4.8 (1.7–13.3)</td>
<td>12.4 (4.3–36.2)</td>
</tr>
<tr>
<td>Cardioembolic (n = 45)</td>
<td>1.0</td>
<td>1.6 (0.5–5.3)</td>
<td>5.3 (1.8–15.7)</td>
<td>7.5 (2.3–24.4)</td>
</tr>
<tr>
<td>Small artery (n = 67)</td>
<td>1.0</td>
<td>1.2 (0.5–3.0)</td>
<td>1.9 (0.8–4.5)</td>
<td>7.3 (3.0–17.7)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, conventional vascular risk factors, and history of arterial vascular events. Reliable estimates of odds of “other” stroke could not be obtained because of the small number of cases in this group (n=28).
E-selectin, von Willebrand Factor, P-Selectin, and PDM Concentrations in Acute Cases, Convalescent Cases, and Controls
Among the 90 patients in whom repeated blood samples were obtained 3 to 6 months after stroke, the mean blood concentrations of E-selectin and P-selectin were significantly lower than those of controls and were significantly lower than corresponding measurements at baseline (Table 2). Blood concentrations of PDM were elevated at baseline and remained elevated 3 to 6 months after stroke compared with controls.

Association Between E-Selectin, von Willebrand Factor, P-Selectin, and PDM Concentrations and All Stroke
There was a strong, graded, and independent association between increasing blood concentrations of E-selectin and vWF during the acute phase and ischemic stroke (Tables 3 and 4) but not between P-selectin or PDM (Tables 5 and 6) and ischemic stroke.

E-selectin, von Willebrand Factor, P-Selectin, and PDM Concentrations in Etiological Subtypes of Ischemic Stroke and Controls
Among the 202 cases of ischemic stroke, 62 (31%) were adjudicated to be caused by large-artery atherothrombembolism, 67 (33%) by small-artery disease, 45 (22%) by cardiogenic embolism, and 28 (14%) by other miscellaneous or unidentified causes.

Blood Concentrations as Quartiles
There was a strong, graded, and independent (of age, sex, and vascular risk factors) association between increasing blood concentrations of E-selectin, and less so of vWF, during the acute phase and each of the 3 etiological subtypes of ischemic stroke, particularly large-artery atherothrombembolism (Tables 3 and 4). There was no such significant association with blood markers of platelet activation (Tables 5 and 6).

Discussion
The principal findings were that within 7 days of ischemic stroke, mean blood concentrations of E-selectin (a marker of endothelial activation) and P-selectin and PDM (markers of platelet activation) were all significantly higher compared with controls. Blood vWF concentrations were also higher in acute cases compared with controls, but not significantly.

There was a strong, graded, and independent association between increasing blood concentrations of markers of endothelial activation (E-selectin and vWF) during the acute phase and both ischemic stroke of all types and ischemic stroke caused by large-artery atherothrombembolism. At 3 to 6 months after stroke, the mean blood concentrations of E-selectin and P-selectin fell significantly below those of controls, and PDM concentrations remained elevated.

The strengths of our study are that it was prospectively designed and executed, the controls were selected at random from the same community and age and sex groups as the cases, the controls were carefully characterized, and the sample size exceeded most, if not all, previous similar studies.

The weaknesses of our study are that evidence of recent infection and information on use of medications (eg, aspirin, heparin, statins) were not sought among cases and controls, the diagnostic criteria used to distinguish etiological subtypes of ischemic stroke may not be valid or reliable, repeated blood measurements were not performed in the controls,

**TABLE 4. Association Between Quartiles of vWF Antigen Measured at Baseline, Ischemic Stroke, and Etiological Subtypes of Ischemic Stroke. Presented as ORs, 95% CIs, and Probability Value for Trend of Association**

<table>
<thead>
<tr>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stroke</td>
<td>1.0</td>
<td>1.7 (0.9–3.2)</td>
<td>2.3 (1.2–4.4)</td>
<td>2.8 (1.5–5.2)</td>
</tr>
<tr>
<td>Large artery</td>
<td>1.0</td>
<td>1.5 (0.5–4.2)</td>
<td>2.8 (1.1–7.3)</td>
<td>3.8 (1.5–9.9)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>1.0</td>
<td>2.8 (0.9–9.2)</td>
<td>3.2 (1.0–10.5)</td>
<td>3.0 (0.9–10.1)</td>
</tr>
<tr>
<td>Small artery</td>
<td>1.0</td>
<td>2.0 (0.8–4.8)</td>
<td>1.9 (0.8–4.8)</td>
<td>2.4 (1.0–5.8)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, conventional vascular risk factors, and history of arterial vascular events. Reliable estimates of odds of “other” stroke could not be obtained because of the small number of cases in this group (n=28).

**TABLE 5. Association Between Quartiles of P-Selectin Measured at Baseline, Ischemic Stroke, and Etiological Subtypes of Ischemic Stroke. Presented as ORs, 95% CIs, and Probability Value for Trend of Association**

<table>
<thead>
<tr>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stroke</td>
<td>1.0</td>
<td>1.5 (0.8–2.7)</td>
<td>0.9 (0.5–1.7)</td>
<td>1.9 (1.0–3.5)</td>
</tr>
<tr>
<td>Large artery</td>
<td>1.0</td>
<td>2.4 (1.0–6.1)</td>
<td>1.3 (0.5–3.3)</td>
<td>2.7 (1.0–7.2)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>1.0</td>
<td>1.4 (0.5–3.7)</td>
<td>0.6 (0.2–1.7)</td>
<td>1.7 (0.6–4.6)</td>
</tr>
<tr>
<td>Small artery</td>
<td>1.0</td>
<td>1.4 (0.6–3.6)</td>
<td>1.4 (0.6–3.3)</td>
<td>2.4 (1.0–5.9)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, conventional vascular risk factors, and history of arterial vascular events. Reliable estimates of odds of “other” stroke could not be obtained because of the small number of cases in this group (n=28).
follow-up of the cases was incomplete, and the reasons for incomplete follow-up of cases (e.g., death, recurrent stroke, unwillingness) were not determined. We expected that \( \approx 10\% \) of our cases (who were hospitalized, had survived a few days after stroke, and were well enough to provide informed consent) may have died during the first 3 to 6 months, and \( \approx 5\% \) may have experienced a recurrent stroke in addition. In addition, we did not adjust for potential regression-dilution bias associated with a single blood measurement among the controls and the potential confounding of time and repeated blood samples among the cases. Furthermore, some of our measures, e.g., vWF and soluble P-selectin, are not specific markers of endothelial and platelet activation, respectively. That is, elevated blood levels of vWF may reflect either endothelial cell or platelet activation, and elevated levels of P-selectin may arise as a result of endothelial cell activation.

Our findings of elevated blood markers of endothelial and platelet activation during the first 7 days after ischemic stroke are consistent with several previous studies that have examined E-selectin, vWF, and P-selectin in acute stroke, but not with all studies. Other studies involving smaller sample sizes have found that P-selectin is not increased in acute stroke and that vWF remains elevated 3 months after stroke.

Possible reasons for the acute increase in blood markers of both endothelial and platelet activation after ischemic stroke are previous recent infection, inflammation and activation of the symptomatic atherosclerotic plaque, atherothrombosis after plaque eruption, and ischemic neuronal damage causing expression of factors that promote a cascade of events that involve these markers. We hypothesized that exploring the association between elevated blood levels of soluble E-selectin, vWF, soluble P-selectin, and PDM and specific etiological subtypes of ischemic stroke may help to decipher the most likely explanation. Our finding of a strong, graded, and independent association between increasing blood concentrations of markers of endothelial activation during the acute phase (E-selectin and less so vWF) and ischemic stroke caused by cardiogenic embolism. This is unlikely to represent ischemic neuronal damage (causing expression of factors that promote a cascade of events that involve these markers) because the magnitude of the increase in blood markers and the significance of the associations were similar for ischemic strokes caused by small-artery disease, which are invariably much smaller infarcts than those caused by cardiogenic embolism.

We were also surprised by the lack of a significant association between increasing blood concentrations of markers of platelet activation during the acute phase (P-selectin and PDF) and ischemic stroke of all types and any etiological subtype of ischemic stroke, particularly large-artery atherothromboembolism. This may reflect the small sample size because there was a nonsignificant trend of increasing odds of ischemic stroke resulting from large- and small-artery disease and increasing concentrations of P-selectin and PDM.

Finally, we did not expect that blood concentrations of markers of endothelial activation (E-selectin and vWF) would fall significantly below those of the controls 3 to 6 months after stroke and that PDM concentrations would remain significantly elevated. The precipitous fall in E-selectin and vWF may reflect to some extent the widespread (but unquantified) use of cholesterol-lowering agents (e.g., statins) and antithrombotic therapies (e.g., aspirin) in the poststroke period. The persistently elevated PDM concentrations may be due to chance or an epiphenomena because it was an unanticipated posthoc finding. However, it is possible that elevated concentrations of PDM may be a marker of increased risk of stroke.

The implication of our results for clinicians is that there is no practical rationale for measuring E-selectin, vWF, P-selectin, or PDM in patients with ischemic stroke. However, the implication for researchers is that unanswered questions remain about the independent prognostic significance of elevated concentrations of E-selectin, vWF, P-selectin, or PDM for recurrent stroke and survival free of handicap and about the elevated concentrations of PDM as a marker of increased risk of stroke.

**Acknowledgments**

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References

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