Adhesion Molecules in Cerebrovascular Diseases Evidence for an Inflammatory Endothelial Activation in Cerebral Large- and Small-Vessel Disease

Klaus Fassbender, MD; Thomas Bertsch, MD; Orell Mielke, MD; Frank Mühler; Michael Hennerici, MD

Background and Purpose—Adhesion molecules mediate attachment and transendothelial migration of leukocytes as a critical step in pathogenesis of atherosclerosis. Their expression and release were comparatively investigated in patients with large- and small-vessel disease of the central nervous system.

Methods—With immunological methods, serum concentrations of endothelial-derived adhesion molecules (soluble endothelial-leukocyte adhesion molecule [sE-selectin], soluble vascular-leukocyte adhesion molecule-1, and soluble intercellular adhesion molecule-1 [sICAM-1]) were quantified in patients with obstructive disease of extracranial (n=89) and intracranial (n=20) large-vessel disease and patients with subcortical vascular encephalopathy (n=64), a cerebral small-vessel disease. As controls, age- and sex-matched subjects without obstructive cerebrovascular disease (n=67) were studied.

Results—We observed significantly increased serum concentrations of sE-selectin and sICAM-1 in patients with both obstructive disease of the large brain-supplying arteries and subcortical vascular encephalopathy. Interestingly, the highest levels were observed in intracranial macroangiopathy. Furthermore, concentrations of sICAM-1 and sE-selectin were significantly increased in current smokers but not in diabetic or hypertensive patients.

Conclusions—The observation of elevated release of endothelial-derived adhesion molecules in both patients with stenoses of the large brain-supplying arteries and patients with subcortical vascular encephalopathy indicates that inflammatory endothelial activation and adhesion of leukocytes play similarly important roles in cerebral large- and small-vessel disease. (Stroke. 1999;30:1647-1650.)

Key Words: angiopathy • cell adhesion molecules • cerebrovascular disorders

Infiltration of monocytes and lymphocytes into the vessel wall is considered a key event in the pathogenesis of atherosclerosis. By release of bioactive substances, eg, reactive oxygen metabolites, granular enzymes, or cytokines, these cells induce damage to endothelial cells, proliferation of smooth muscle cells, and enhanced release of matrix constituents.1

Normally, vascular endothelial cells have low adhesiveness for leukocytes; however, when stimulated they express adhesion molecules at their surface responsible for adhesion and activation of leukocytes as a precondition for transendothelial migration of leukocytes.2,3 Endothelial-leukocyte adhesion molecule (E-selectin) binds to an overlapping set of carbohydrate structures at leukocyte surfaces, whereas the members of the immunoglobulin gene superfamly of adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), interact with integrins at cellular surfaces.2,3 In contrast to ICAM-1, which can also be expressed on leukocytes, fibroblasts, or epithelial cells, E-selectin and VCAM-1 are exclusively expressed on endothelial cells.

Soluble isoforms of these adhesion molecules were demonstrated to be rapidly shed from surfaces of endothelial cells on cellular activation.4,5 Since they reflect activation of their originating cells, quantification of these molecules in circulation represents, apart from a recently described method based on binding of antibody-coated microbubbles to endothelial adhesion molecules,6 the only method to obtain information about endothelial inflammation and activation of the adhesion cascade in vivo.

Recently, upregulation of these molecules has been shown in atherosclerotic coronary7 and carotid8,9 arteries, and increased serum concentrations of their soluble isoforms were detected in human atherosclerosis,10,11 including carotid artery disease12,13 and ischemic stroke.14 However, expression and release of adhesion molecules have not been investigated...
in different cerebrovascular diseases, ie, in patients with intracranial stenoses or in patients with subcortical vascular encephalopathy (SVE), a small-vessel disease characterized clinically by progressive dementia, emotional lability, gait disorders, and incontinence and neuroradiologically by subcortical diffuse periventricular white matter lesion and lacunes.\textsuperscript{15-17}

It is unclear why many patients have large-vessel disease without apparent cerebral microangiopathy, whereas others suffer selectively of cerebral microangiopathy. To obtain further information about differences and similarities regarding possible inflammatory endothelial activation, which is currently thought to play a role in atherogenesis, soluble adhesion molecules that are derived from activated endothelium were analyzed in cerebral macroangiopathy and microangiopathy.

Subjects and Methods

Study Population

One hundred seventy-three patients presenting for follow-up of earlier cerebrovascular diseases were included in this study. Seventy-six percent of them had transient or persistent cerebral ischemia \( \geq 6 \) months ago. None of them had hemorrhagic stroke. Outcome of these ambulant patients was moderate or good (Rankin score \( \leq 3 \)). Eighty-five percent of the patients were treated with acetylsalicylic acid. Moreover, 67 subjects who were investigated for exclusion of possible obstructive disease of the large or small arteries but had none of these stenotic cerebrovascular diseases were studied as controls. Twenty-one percent of them had earlier transient or persistent cerebral ischemia \( \geq 6 \) months ago. Sixteen percent of these control subjects were treated with acetylsalicylic acid. The demographic characteristics and risk factor profiles of the different study groups are presented in the Table. History, clinical examination, extracranial and intracranial ultrasound, B-mode duplex, and CT or MR tomography were used in all patients and controls to provide information about the risk factor profile, type of cerebrovascular disease, history of stroke or transient ischemic attack, and presence of other cardiac or peripheral artery disease (typical signs and symptoms or a history of surgery for these conditions). None of them had an acute cerebral or myocardial ischemic event within the last 6 months. Exclusion criteria for patients and controls were presence of infections, other inflammatory or malignant diseases, conditions associated with tissue injury (eg, major surgical procedures), and immunosuppressive treatment.

Classification of Risk Factors

Diagnosis of hypertension was based on either antihypertensive treatment or blood pressure \( > 160 \) mm Hg (systolic value) and/or \( 95 \) mm Hg (diastolic value) during 2 separate examinations. Diagnosis of diabetes mellitus was based on the use of antidiabetic therapy or repeated pathological fasting values. Current smoking was assigned to persons who smoked at least 1 cigarette per day during the 3 preceding months.

Demographic Characteristics and Risk Factor Profile of the Study Populations

<table>
<thead>
<tr>
<th>Diagnostic Subpopulation</th>
<th>n</th>
<th>Median Age (Range), y</th>
<th>Sex (M/F)</th>
<th>Hypertension, %</th>
<th>Diabetes, %</th>
<th>Smoking, %</th>
<th>Stroke/TIA, %</th>
<th>Coronary Heart Disease, %</th>
<th>Peripheral Artery Disease, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>67</td>
<td>68 (45–92)</td>
<td>36/31</td>
<td>40</td>
<td>28</td>
<td>16</td>
<td>21</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Cerebrovascular diseases (total)</td>
<td>173</td>
<td>70 (46–89)</td>
<td>97/76</td>
<td>65</td>
<td>23</td>
<td>31</td>
<td>76</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Extracranial stenoses</td>
<td>89</td>
<td>70 (46–84)</td>
<td>55/34</td>
<td>66</td>
<td>26</td>
<td>40</td>
<td>83</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Intracranial stenoses</td>
<td>20</td>
<td>60 (47–88)</td>
<td>12 /8</td>
<td>50</td>
<td>15</td>
<td>55</td>
<td>70</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>SVE</td>
<td>64</td>
<td>73 (50–89)</td>
<td>30/34</td>
<td>69</td>
<td>22</td>
<td>13</td>
<td>69</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

TIA indicates transient ischemic attack.

Diagnosis of Microangiopathy

SVE was based on the International Statistical Classification of Diseases, 10th Revision criteria\textsuperscript{13} by combined information from neurological, neuropsychological, and neuroradiological examination. Thus, the presence of stepwise progressive disorders of memory and cognition, typical vascular risk factor profile, and typical subcortical diffuse white matter lesions or lacunes on CT scan or MRI scan was required for diagnosis apart from facultatively present disorders of gait or sphincter control and variable focal neurological signs and symptoms.\textsuperscript{13,17} In this study, patients with overlapping signs or symptoms for small- and large-vessel disease were excluded.

Diagnosis of Extracranial and Intracranial Stenoses of Brain-Supplying Large Vessels

Examination of stenoses in the extracranial and intracranial brain-supplying arteries (Multi-Dop L ultrasound device, DWL, and Acuson 128XP) was performed according to standard criteria.\textsuperscript{18} A degree of stenosis of \( > 50\% \) had to be present for inclusion in the subgroup with extracranial stenoses. The subgroup of patients with extracranial stenoses had Doppler sonography more than once. Intracranial stenosis was defined as focal increase of cerebral blood flow velocity of \( \geq 140 \) cm/s in any of the following large intracranial vessels: anterior, middle, or posterior cerebral artery or basilar artery, assessed by transcranial Doppler examination.

Blood Sampling and Quantification of Soluble Adhesion Molecules

Blood was allowed to clot at room temperature for 1 hour; after centrifugation, the serum was stored at \(-80\) °C until used. Concentrations of circulating E-selectin, ICAM-1, and VCAM-1 were determined with quantitative enzyme-linked immunoassays (R&D Systems, Europe [sE-selectin], and Bender MedSystems [sICAM, sVCAM]). Briefly, a monoclonal antibody specific for the antigens of interest was coated onto a 96-well microtiter plate. In a single-step reaction, samples were incubated in the microtiter plate together with a second horseradish peroxidase–linked monoclonal antibody specific for a different epitope of these antigens. After it was washed, the bound enzyme–antibody conjugate was measured enzymatically with tetramethylbenzidine as the substrate. Adsorbence was measured at 450 nm on an MR 4100 spectrophotometer (Dynatech) with 630 nm used as the reference wavelength. A standard curve was established with the use of recombinant antigens. The intra-assay (interassay) coefficients of variation for soluble E-selectin (sE-selectin), soluble ICAM-1 (sICAM-1), and soluble VCAM-1 (sVCAM-1) were \( < 5.1\% \) (9.2\%), 4.2\% (7.7\%), and 3.2\% (5.3\%), respectively. The lower limits of detection of sE-selectin, sICAM-1, and sVCAM-1 were 2.0, 3.3, and 0.9 ng/mL, respectively.

Statistical Analysis

Results are expressed as mean\( \pm \)SEM. For conservative statistical analysis, the Mann-Whitney \( U \) test was used with a Bonferroni correction.
Adhesion Molecules in Cerebrovascular Diseases

Results

Adhesion Molecules in Patients With Cerebrovascular Diseases and Control Subjects

Compared with controls, patients with cerebrovascular diseases exhibited significantly increased concentrations of sE-selectin and sICAM-1 (Figures 1 and 2), whereas concentrations of VCAM-1 did not significantly differ. Both in controls subjects ($r=0.43$, $P<0.01$) and in patients with cerebrovascular diseases ($r=0.40$, $P<0.01$), sE-selectin and sICAM-1 significantly correlated with each other.

Relation to Subtype of Cerebrovascular Disease

Increased levels of sE-selectin and sICAM-1 were observed in both patients with stenoses of the large extracranial and intracranial brain-supplying arteries and patients with SVE (Figures 1 and 2). Interestingly, maximal concentrations of sE-selectin and sICAM-1 were observed in patients with intracranial macroangiopathy. Patients with stable or progressive (>20% increase within 1 year) stenotic carotid artery disease did not significantly differ in regard to concentrations of sE-selectin (55.29$\pm$3.78 versus 55.65$\pm$6.89 ng/mL; $P=0.77$), sICAM-1 (278.95$\pm$13.49 versus 271.56$\pm$25.62 ng/mL; $P=0.90$), or sVCAM-1 (541.84$\pm$29.80 versus 592.25$\pm$52.48 ng/mL; $P=0.79$).

Relation to Demographic Characteristics

Levels of sE-selectin ($r=-0.15$; $P=NS$), sICAM-1 ($r=-0.12$; $P=NS$), or sVCAM-1 ($r=0.03$; $P=NS$) did not correlate with age. Moreover, sE-selectin (55.89$\pm$2.92 versus 45.8$\pm$1.86 ng/mL; $P=0.08$), sICAM-1 (280.90$\pm$69.21 versus 269.64$\pm$7.87 ng/mL; $P=0.23$), and sVCAM-1 (688.07$\pm$60.68 versus 645.79$\pm$25.77 ng/mL; $P=0.61$) did not differ in regard to sex.

Relation to Conventional Vascular Risk Factors

Patients with cerebrovascular diseases who were current smokers ($n=59$) had significantly increased serum levels of sICAM-1 (297.31$\pm$11.02 versus 266.19$\pm$5.63 ng/mL; $P=0.01$) and sE-selectin (56.85$\pm$5.33 versus 49.05$\pm$2.16 ng/mL; $P=0.05$) compared with nonsmokers ($n=114$). In contrast, adhesion molecules in cerebrovascular disease patients did not differ in regard to presence of hypertension or diabetes mellitus.

Discussion

This first comparative study on markers of inflammatory endothelial activation in obstructive disease in different segments of the cerebral vasculature demonstrates that release of adhesion molecules is significantly increased to a similar degree in stenotic disease of large cerebral vessels and SVE. Although macroangiopathic and microangiopathic cerebrovascular diseases differ in clinical symptomatology and pathophysiology, this study indicates that both are similarly associated with inflammatory endothelial activation. Such inflammatory endothelial activation may therefore represent a common final pathway in which etiologically different cerebrovascular diseases may converge.

In small arteries, arterioles, and capillaries, adherence of leukocytes and their transendothelial migration mediated by ICAM-1 or E-selectin may be responsible for vascular injury by release of reactive oxygen metabolites, granular enzymes, or toxic or growth-promoting cytokines. In SVE, leukocyte-induced injury of arterioles or capillaries may play a pathogenetic role, since blood-brain barrier disruption and intraparenchymal severe protein extravasation shown pathohistologically have been considered important factors in development of macroangiopathic periventricular white matter lesions. The cause for the inflammatory endothelial activation in patients with cerebral large- and small-vessel disease observed in this study is unclear. Conventional vascular risk factors could chronically irritate the endothelium, leading to leukocyte adhesion and focal leukocyte recruitment. We separately analyzed the possible contribution of vascular risk factors on markers of endothelial activation. We found an association between concentrations of sICAM-1 and sE-selectin and smoking known to be associated with stenotic cerebrovascular disease. However, the observation that...
smoking was rare in SVE despite the marked endothelial activation in this disease together with the absence of associations between endothelial activation indicators and further vascular risk factors argues against a major direct role of these risk factors in endothelial activation in these stenotic cerebrovascular diseases. Other factors currently thought to contribute to endothelial activation are hemodynamic shear stress, excessive load with low density lipoprotein, or chronic infections of the vasculature. These noxious stimuli could activate endothelial cells, either directly or indirectly, via stimulation of local mononuclear phagocytes as principal producers of proinflammatory cytokines involved in upregulation of endothelial adhesion molecules. 

In contrast, it cannot be excluded that treatment with acetylsalicylic acid could have reduced expression and shedding of adhesion molecules as a result of its anti-inflammatory properties. If this were true, concentrations of soluble adhesion molecules would have been underestimated in patients with cerebrovascular diseases. Previous studies showed that deficiency in inflammatory cell adhesion molecules (eg, ICAM-1, CD18, P-selectin) protects against experimental atherosclerosis in mice and that treatment with antibodies against adhesion molecules prevents recruitment of mononuclear phagocytes in aortic intima in hypercholesterolemic rats.

In conclusion, this study indicates a strong inflammatory activation of the endothelium in obstructive disease of both the large and the small cerebral vessels. On the basis of the similar abnormalities of these markers of inflammatory endothelial activation in extracranial or intracranial stenoses and SVE, we speculate that anti-inflammatory and antiadhesion strategies could be effective in reduction of progression in cerebral large- and small-vessel disease.

References

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