Soluble Intercellular Adhesion Molecule-1 and Risk of Future Ischemic Stroke

A Nested Case-Control Study From the Bezafibrate Infarction Prevention (BIP) Study Cohort

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Background and Purpose—Inflammation is considered to be involved in the pathogenesis of ischemic stroke. Our purpose was to assess the role of soluble intercellular adhesion molecule-1 (sICAM-1) concentration, a marker of inflammation, in predicting future ischemic stroke among patients at risk because of chronic coronary heart disease.

Methods—We obtained baseline serum samples from patients with chronic coronary heart disease enrolled in the Bezafibrate Infarction Prevention trial (n = 3090), which assessed the efficacy of bezafibrate in secondary prevention. Using a prospective nested case-control design, we measured baseline sICAM-1 concentration in sera of patients who developed ischemic stroke during a mean follow-up of 8.2 years (cases, n = 134) and in age- and sex-matched controls without any subsequent cardiovascular events (n = 134).

Results—Baseline serum concentrations of sICAM-1 were significantly higher in cases compared with controls (379 versus 350 ng/mL, P < 0.05). sICAM-1 concentration at the highest quartile (> 394 ng/mL) was associated with significantly higher relative odds of ischemic stroke compared with the lower concentrations after adjustment for potential confounding variables (relative odds, 2.1; 95% CI, 1.1 to 4.3). After fibrinogen and total white blood cell count were added to the multivariable model, the relative odds were 2.1 (95% CI, 1.1 to 4.2) and 2.2 (95% CI, 1.1 to 4.8), respectively. The risk associated with raised concentrations of sICAM-1 seemed to be highest for large disabling strokes of cardioembolic origin.

Conclusions—Elevated concentrations of sICAM-1, a marker of inflammation, are associated with increased risk of ischemic stroke, independent of other traditional cerebrovascular risk factors and of plasma fibrinogen, among patients at increased risk because of manifest coronary heart disease. (Stroke. 2002;33:2182-2186.)

Key Words: cell adhesion molecules • inflammation • risk factors • stroke, ischemic

S oluble forms of cellular adhesion molecules may be useful markers of endothelial activation and local or systemic inflammation. Cellular adhesion molecules mediate the margination, adhesion, and transendothelial migration of circulating mononuclear cells from the bloodstream to the extravascular compartment, a critical step in the initiation and progression of atherosclerotic plaque.1,2

Plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) were found to predict carotid atherosclerosis.3,4 DeGraba and colleagues5 found increased local expression of ICAM-1 in high-grade regions of symptomatic compared with asymptomatic carotid plaques, suggesting that mediators of inflammation are involved in the conversion of carotid plaque to a symptomatic state. High levels of sICAM-1 were found both in patients with stenosis of the large brain-supplying arteries and in patients with subcortical vascular encephalopathy, suggesting that inflammatory endothelial activation and adhesion of leukocytes play similarly important roles in cerebral large- and small-vessel disease.6 Several studies found that sICAM-1 concentrations increase after acute stroke.7-9

Recent prospective studies indicate that sICAM-1 concentrations are elevated for many years before the development of clinical manifestation of coronary heart disease (CHD) or cardiovascular events in general.3,10,11 The magnitude of risk of future myocardial infarction associated with elevated levels of sICAM-1 rose with increasing length of follow-up, suggesting a time-dependent effect.10 There are no large
prospective studies that assess the role of sICAM-1 levels in the specific prediction of ischemic stroke. Our aims were 3-fold: to test among patients at increased risk because of manifest CHD whether high concentrations of sICAM-1 predict future ischemic stroke; to assess whether an observed association between sICAM-1 and risk of ischemic stroke varies over time; and finally to assess whether any observed association is dependent on other traditional cerebrovascular risk factors and on plasma fibrinogen.

Methods

Study Sample
We performed a prospective, nested, case-control study of sICAM-1 as a potential marker for future ischemic stroke among patients who participated in the Bezafibrate Infarction Prevention (BIP) study. The BIP study was a randomized, placebo-controlled clinical trial that evaluated the efficacy of the lipid-modifying drug bezafibrate versus placebo for the prevention of acute myocardial infarction and chronic CHD.12 CHD was defined as history of myocardial infarction >6 months but <5 years before enrollment in the study or history of angina pectoris confirmed by positive coronary angiography, nuclear scintigraphy, or a positive exercise test. Mean length of follow-up was 8.2 years (range, 6.7 to 9.6 years).

Data on the occurrence of new cerebrovascular events were obtained during routine evaluations. Records from hospital or emergency department discharge, the primary care physician, or the neurologist were reviewed. Clinical data related to the new cerebrovascular event, results of brain CT scan, and other available ancillary tests for assessment of stroke classification were recorded on standardized forms. Data were centrally reviewed by the study stroke neurologist (D.T). Stroke was defined according to World Health Organization criteria as rapidly developing clinical signs of focal disturbance of cerebral function with symptoms lasting >24 hours or leading to death with no apparent cause other than that of vascular origin. Stroke type was differentiated by results of CT scan into ischemic stroke and intracerebral hemorrhage. Ischemic stroke subtypes were determined according to the Trial of Org 10172 in Acute Stroke Treatment classification; were based on neurological findings, history, results of CT scan, ECG, echocardiography, carotid duplex, and any other relevant diagnostic test available; and then were categorized into cardioembolic, noncardioembolic, or undetermined origin. Study physicians during follow-up assessed functional outcome after stroke by the modified Rankin Scale. Stroke severity was categorized as minor for patients in whom the modified Rankin score was 0 or 1 and as major for those with a score of ≥2 or 30-day case fatality.

For the purpose of the present study, we planned a prospective, nested case-control study. Cases (n = 134) were patients who developed an ischemic stroke and provided an adequate blood sample before randomization to the study. Fourteen of the patients also entered the bezafibrate versus placebo arm. Controls (n = 1100) were CHD patients who remained free of any recurrent coronary events or stroke (n = 576) or stroke, we computed the relative odds of ischemic stroke in patients who were free of any cardiovascular events by the end of the study (n = 310). To assess whether the effect of sICAM-1 (associated with an increase in concentration of 100 ng/mL) on risk of ischemic stroke varied over time, we stratified the analysis by years of follow-up, and to assess whether the risk varied by the characteristics and underlying mechanism, we stratified the analysis by severity and subtypes of the ischemic stroke.

Results

Baseline Characteristics
Cases and controls were well matched in terms of age and sex. Cases more often smoked, more often had diabetes mellitus, and had higher mean serum concentration of fibrinogen compared with controls (Table 1).

Serum concentrations of sICAM-1 in the study sample ranged from 29 to 794 ng/mL (mean±SD, 350±97 ng/mL; median, 335 ng/mL) and were normally distributed. The mean serum concentration of sICAM-1 was significantly higher in cases compared with controls (379 versus 350 ng/mL). The distribution of serum concentration of sICAM-1 in cases and controls by percentile levels is depicted in Figure 1. The median of differences in sICAM-1 concentrations between matched pairs was 33 ng/mL higher in cases as compared with matched controls, and the 75th percentile of difference in sICAM-1 was 114 ng/mL higher in cases.

To evaluate the combined role of sICAM-1 and fibrinogen concentrations as predictors of the risk of future ischemic stroke, we computed the relative odds of ischemic stroke in analyses in which patients were stratified into 9 groups according to tertiles of sICAM-1 and fibrinogen concentrations (Figure 2). The risk of ischemic stroke was low among patients with low concentrations of both sICAM-1 and fibrinogen. In contrast, the risk was highest in patients with high concentrations of both sICAM-1 and fibrinogen. However, even among patients with low levels of fibrinogen, the risk of ischemic stroke was 1.5-fold higher in those with sICAM-1 concentrations in the upper compared with the lower tertile.

To explore the risk of ischemic stroke associated with increasing sICAM-1 levels, we evaluated the risk of ischemic

Laboratory Procedures
Blood samples for the measurement of serum lipids, fibrinogen, blood chemistry, and other laboratory tests were assessed at baseline and thereafter at regular intervals. All laboratory analyses were performed in a single central laboratory using standard automated procedures with commercial kits. For the purpose of the present study, serum samples, which had been taken at baseline from each study participant and stored at −70°C, were thawed and assayed for sICAM-1 with a commercial enzyme-linked immunosorbent assay kit (R&D Diagnostics). A single laboratory technician blinded to the case or control status of each sample performed all the tests.

Statistical Analysis
Statistical analyses were done with SAS statistical software.13 To assess the significance of differences between means of continuous variables among the matched pairs, we used the paired t test procedure. McNemar’s test for paired samples was used to assess differences between rates.

To evaluate the combined role of sICAM-1 and fibrinogen concentrations as predictors of the risk of future ischemic stroke, we divided the patients into 9 groups according to tertiles of sICAM-1 and fibrinogen concentrations. Logistic regression was used to evaluate the risk of ischemic stroke in each of the 9 groups, with the group of patients in the lowest tertiles of both sICAM-1 and fibrinogen considered the reference group.

We used logistic regression analyses conditioned on the matching variables (age, sex, and medical center) to estimate the odds ratios (ORs) and 95% CIs of experiencing an ischemic stroke according to quartiles of sICAM-1 serum level. We adjusted for traditional risk factors and potential confounding variables. Adjustments were performed for smoking status; for serum lipids concentrations at baseline; and finally for diabetes mellitus, current smoking, hypertension, and history of myocardial infarction. In separate models we added adjustments for plasma fibrinogen and white blood cell counts. Division to quartiles or tertiles was done according to sICAM-1 concentrations in a random sample of BIP study participants who were free of any cardiovascular events by the end of the study (n = 310). To assess whether the effect of sICAM-1 (associated with an increase in concentration of 100 ng/mL) on risk of ischemic stroke varied over time, we stratified the analysis by years of follow-up, and to assess whether the risk varied by the characteristics and underlying mechanism, we stratified the analysis by severity and subtypes of the ischemic stroke.
stroke according to sICAM-1 concentrations divided into quartiles (see Methods). The unadjusted analysis of the matched pairs revealed a significantly higher risk for ischemic stroke during follow-up in the highest quartile of sICAM-1 level compared with the first quartile (OR, 2.69; 95% CI, 1.34 to 5.71). Adjusting for smoking status and for serum lipids did not change the results materially (Table 2). A model that included traditional risk factors and potential confounding variables produced an OR of future ischemic stroke of 2.49 (95% CI, 1.11 to 5.87) in the highest quartile of sICAM-1 level compared with the lowest quartile. After further adjustment for plasma fibrinogen, the relative odds of ischemic stroke in the highest quartile of sICAM-1 level was 2.17, and adjusting for white blood cell count provided relative odds of 2.86 (Table 2).

Patients with sICAM-1 levels above the cutoff point of the 75th percentile (394 ng/dL) exhibited a relative odds of developing an ischemic stroke of 2.11 (95% CI, 1.22 to 3.78) versus levels below the 75th percentile in unadjusted matched-pair analysis. After multivariable adjustments, the relative odds was 2.13 (95% CI, 1.11 to 4.27). Further adjustments to plasma fibrinogen (OR, 2.05; 95% CI, 1.06 to 4.16) or to white blood cell counts (OR, 2.24; 95% CI, 1.11 to 4.75) again did not change the relative odds materially.

To assess whether the effect of sICAM-1 on risk of ischemic stroke varied over time, we stratified the analyses by years of follow-up. The relative odds associated with an increase in concentration of 100 ng/mL for the entire length of follow-up was 1.3 (95% CI, 1.1 to 1.7). The relative odds was 1.5 among events that occurred during the first 2 years of follow-up and 1.4, 1.3, and 1.2 for events occurring after follow-up periods of 3 to 4 years, 5 to 6 years, and >6 years, respectively, with wide overlap in the 95% CIs (Figure 3).

To assess whether the effect of sICAM-1 on risk of ischemic stroke varied with the characteristics and underlying mechanism of the ischemic stroke, we stratified the analyses by stroke severity and stroke subtype. The relative odds associated with an increase in sICAM-1 concentration of 100 ng/mL were 1.04 (95% CI, 0.8 to 1.4) for minor stroke and 2.3 (95% CI, 1.4 to 4.1) for major stroke. Relative odds were 1.3 (95% CI, 0.8 to 2.3) for noncardioembolic stroke, 1.2 (95% CI, 0.9 to 1.7) for stroke of undetermined origin, and 1.7 (95% CI, 1.0 to 3.3) for cardioembolic stroke.

**Discussion**

In this prospective nested case-control study, high concentrations of sICAM-1 were found to significantly predict future ischemic stroke. Patients with sICAM-1 concentrations above the 75th percentile had >2-fold-higher relative odds of subsequent ischemic stroke compared with patients with lower concentrations. This increased risk was independent of traditional cerebrovascular risk factors and persisted after adjustment to plasma fibrinogen. For comparison, ORs associated with diabetes mellitus in our model was 5.1, with current smoking 2.5, previous myocardial infarction 1.4, and history of high blood pressure 1.2.

This is the first large prospective study to demonstrate that high sICAM-1 levels specifically predict future ischemic stroke. It is evident from the results that the increase in risk was not equal throughout the range of sICAM-1 concentrations. Instead,
it seems that patients with sICAM-1 concentration above the 75th percentile were at particularly high risk.

Similar observations were made for sICAM-1, other adhesion molecules, and inflammatory markers for future manifestations of CHD. In a separate case-control study nested within the BIP study cohort, we found that high concentrations of sICAM-1 also predict recurrent coronary events in patients with chronic CHD. In healthy male physicians included in the Physicians’ Health Study, elevated sICAM-1 levels at baseline predicted the occurrence of myocardial infarction years afterward. This association persisted after adjustment for other inflammatory markers, including C-reactive protein and fibrinogen. The risk of myocardial infarction associated with raised concentrations of sICAM-1 intriguingly seemed to increase with length of follow-up. In our study population, we did not find an increase in the risk of ischemic stroke with length of follow-up.

sICAM-1 concentrations are associated with traditional cardiovascular risk factors such as increasing age, smoking status, diabetes mellitus, and systolic blood pressure. They are also associated with plasma levels of fibrinogen, which has consistently been shown by us and others to be associated with increased risk of stroke. Fibrinogen is a clotting factor and a key determinant of plasma and blood viscosity, but it is also an acute phase protein and thus also involved in the process of inflammation. The risk of ischemic stroke was highest in patients with high concentrations of both sICAM-1 and fibrinogen. It is notable, however, that even among patients with low levels of fibrinogen, the risk of ischemic stroke was higher with increasing sICAM-1 concentrations. Furthermore, the increased risk of ischemic stroke conferred by high concentrations of sICAM-1 persisted after adjustments for traditional risk factors and potential confounders such as smoking status, diabetes, and high blood pressure as well as for concentrations of plasma fibrinogen.

Fibrinogen and sICAM-1 share some biological properties. Both may serve as ligands of the important β₂ integrin adhesion molecules (such as MAC-1), which play a key role in the adhesion of white blood cells to the endothelium. Furthermore, it has been suggested that fibrinogen binding to sICAM-1 enhances mononuclear cell adhesion and transendothelial migration. Therefore, it seems that fibrinogen and sICAM-1 participate in common pathogenic processes contributing to vascular injury.

sICAM-1, a member of the immunoglobulin superfamily, plays an important role in the adhesion of mononuclear cells to endothelial cells, an important step by itself in the progression of atherosclerotic plaques through invasion of circulating monocytes into the atheroma. High levels of sICAM-1 are associated with carotid artery atherosclerosis and with intracranial macroangiopathy.

Mediators of inflammation not only are instrumental in the formation of plaque but also may be involved in the rapid inflammatory endothelial activation and may therefore represent a common final pathway in which progression of atheromatous lesions leads to plaque fissuring and thrombosis. Entrance of mononuclear cells into the atheroma is an important step in the transformation of the stable plaque into an unstable plaque, promoting matrix metalloproteinases release and plaque rupture. Elevated sICAM-1 levels also may signify an inherent propensity toward plaque instability and rupture and hence the increased risk of ischemic stroke. Increased local expression of ICAM-1 was found in high-grade regions of symptomatice versus asymptomatic carotid plaques, suggesting that mediators of inflammation are indeed involved in the conversion of carotid plaque to a symptomatic state.

The findings in the present study indicate that ongoing inflammatory activity, as evidenced by elevated sICAM-1
levels, may serve as an aid in the prediction of future ischemic strokes in patients with already established CHD, a manifestation of symptomatic atherosclerosis. Recent reports have suggested that some of the beneficial effects of aspirin and statins are mediated by their anti-inflammatory effect, providing further evidence for the possible role of inflammation in atherosclerosis and thrombosis. The finding that the risk associated with elevated sICAM-1 seems to be highest for large disabling strokes from cardioembolic origin is intriguing but is based on analysis from relatively small subgroups and should be explored in future studies.

The main limitation of the present study is that the study relied on a single measurement of sICAM-1 in sera that were kept frozen for about 10 years. Protein degradation in the frozen samples, which is a potential concern, could not have led to any systematic bias, however, because samples from cases and controls were obtained at baseline and were handled identically throughout the collection, storage, and analytical phases of this analysis. Other inflammatory markers such as C-reactive protein, an important marker of inflammation, were not measured in the present study. Plasma fibrinogen levels, however, which are known to correlate with C-reactive protein and to predict stroke, were measured and did not materially affect the risk conferred by sICAM-1.

In conclusion, we provide evidence in the present study for the association between elevated sICAM-1 levels and risk of incident ischemic stroke in coronary patients. Our results indicate a role for sICAM-1 as an independent marker of stroke risk. However, they do not imply causality. More data from prospective cohort studies are needed to establish a causal relationship between sICAM-1 levels and progression or activation of the atherosclerotic plaque and to quantify the magnitude of risk associated with elevated sICAM-1 levels. sICAM-1 (and other adhesion molecules) may potentially serve as important additional targets for prevention of ischemic stroke among patients at increased risk.

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References
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