Hyperhomocysteinemia Is Associated With the Presence of Left Atrial Thrombus in Stroke Patients With Nonvalvular Atrial Fibrillation

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Background and Purpose—Blood stasis is the fundamental mechanism leading to thrombus formation in the venous system. Homocysteine also poses a significant risk for venous thrombosis through its endothelial toxic and prothrombotic properties. In the present study, we hypothesized that high homocysteine might be associated with thrombus formation in another stasis-related condition, atrial fibrillation.

Methods—Forty-two consecutive patients with ischemic stroke caused by nonvalvular atrial fibrillation and admitted within the first day of symptom onset were included. Total fasting plasma homocysteine, serum folic acid, and vitamin B12 levels were measured. All patients were evaluated by transesophageal echocardiography for the presence of a left atrial (LA) thrombus. Homocysteine and vitamin levels were compared between groups with or without LA thrombus.

Results—Transesophageal echocardiography revealed LA thrombus in 20 patients. Mean homocysteine levels were significantly higher in patients with LA thrombus (20.75 versus 13.34 μmol/L, P<0.001). Multivariate logistic regression analysis showed that the effect of high homocysteine was independent of other clinical or echocardiographic variables known to increase LA thrombus (P=0.017). There was no difference in vitamin B12 levels between groups (P=0.118), whereas the mean folic acid level was significantly lower in patients with LA thrombus (P=0.004).

Conclusions—High plasma homocysteine conveys an independent risk for LA thrombus formation in patients with stroke caused by nonvalvular atrial fibrillation. This finding further supports the thrombogenic role of high homocysteine in conditions associated with blood stasis. (Stroke. 2003;34:909-912.)

Key Words: atrial fibrillation ■ homocyst(e)ine ■ stroke ■ thrombosis

Nonvalvular atrial fibrillation (AF) is a common problem in the elderly, occurring in 2% to 4% of the population >60 years of age. It is 1 of the most common causes of stroke, estimated to be responsible for 1 of every 10 ischemic strokes in this age group.1 Transesophageal echocardiography (TEE) reveals a thrombus lodged within the left atrial appendage (LAA) in up to 43% of patients with a recent embolic event.2 Even if not seen in TEE, a minute but still clinically important–sized thrombus trapped within the trabeculae of the LAA can frequently be identified at autopsy.3 The fundamental mechanism that leads to thrombus formation in AF is blood stasis in the LAA. Stagnant flow or stasis not only is unique to AF but also is a key feature of thrombus formation in the venous system.4 LA and venous thrombi also resemble each other microscopically. Therefore, factors in addition to stasis contributing to the formation of thrombus in the venous system might also operate in AF.

Homocysteine, an intermediate amino acid formed during the metabolism of dietary methionine, has been linked to a variety of vascular diseases. Large case-control and prospective studies have shown that elevated fasting and post–methionine-loading homocysteine levels are an independent risk factor for arterial and venous thrombotic events.5-7 Meta-analyses have found odds ratios (ORs) of 2.5 (95% CI, 1.8 to 3.5) to 2.95 (95% CI, 2.08 to 4.17) for venous thrombosis in patients with high homocysteine levels.6,7 Hyperhomocysteinemia is also a risk factor for recurrent venous thrombosis, increasing the risk >2-fold.8 Given that elevated homocysteine enhances venous thrombosis, we hypothesized that it may also convey a risk for the formation of LA thrombus in AF.

Methods

Over a 1-year period, 71 consecutive ischemic stroke patients with nonvalvular AF who were admitted within the first 24 hours after symptom onset were included. Baseline clinical characteristics—including age, sex, and history of hypertension, diabetes mellitus, and congestive heart failure—were recorded. A complete diagnostic evaluation was performed for each patient; this included blood chemistry, blood cell counts, erythrocyte sedimentation rate, ECG, coagulation panel (antiphospholipid and anticardiolipin antibodies, activated partial thromboplastin time, prothrombin time, international normalized ratio, protein C, protein S, antithrombin III, fibrinogen, protein C resistance, and factor V Leiden), carotid duplex...
Homocysteine was independent of other variables known to increase the risk of stroke. A multivariate logistic regression analysis was performed to show whether the effect of high homocysteine levels was independent of other variables known to increase the LA thrombus (variables with a value of $P<0.15$ were included in the model). The association between high homocysteine levels and LA thrombus was expressed by the use of ORs with 95% CIs. Spearman’s rank correlation coefficients were calculated to describe associations between folic acid, vitamin B12, and homocysteine levels. A value of $P<0.05$ was considered significant.

### Results

Of the 71 patients with AF, 8 died before a TEE study could be performed, and 12 declined a TEE study. Nine additional patients were also excluded because the cause of their stroke was judged not to be AF: 6 had either severe internal carotid artery stenosis or occlusion ipsilateral to the acute hemispheric infarction; 1 had distal vertebral artery occlusion and a cerebellar infarction in the territory of the ipsilateral posterior inferior cerebellar artery; and 2 had hypertension and a lacunar infarction on MRI (small-vessel disease). The remaining 42 patients with nonvalvular AF as the cause of their stroke met the inclusion criteria. There were 18 female and 24 male patients; their mean age was 68.7 years. Except for 1 patient who presented with transient ischemic attack, all patients had a long-lasting deficit. Diffusion-weighted MRI revealed an acute infarction in each patient, including the one with transient ischemic attack. The cerebral lesions involved the territories of the middle cerebral artery in 3, the anterior cerebral artery in 3, the posterior cerebral artery in 3, the posterior inferior cerebellar artery in 2, and the anterior inferior cerebellar artery in 1 patient. Other baseline characteristics and echocardiography findings are presented in the Table.

A TEE was obtained within a mean of 9 days after stroke (range, 3 to 12 days). LA thrombus was identified in 20 of the 42 patients (47.6%). LA thrombus was limited to within the LAA in 13 of the 20 patients. In the remaining 7 patients, the thrombus extended beyond the limits of the LAA and protruded into the LA cavity. There was no difference between patients with or without LA thrombus with respect to age, sex, and history of hypertension, diabetes mellitus, and congestive heart failure (the Table). Likewise, LA diameter did not differ between groups, whereas SEC was more common in patients with LA thrombus. The mean±SE fasting plasma homocysteine level was higher in patients with than in those without LA thrombus (20.75 versus 13.34 μmol/L; $P<0.001$; the Figure). Homo-
Homocysteine and Left Atrial Thrombus in AF

Discussion

Homocysteine is a multipotent molecule exerting various modes of injury to the endothelium. It facilitates oxidative arterial injury, damages the vascular matrix, and induces vascular smooth muscle proliferation.\(^{10,11}\) Moreover, homocysteine alters the thrombotic properties of the endothelium by inhibiting the expression of thrombomodulin, activating protein C, enhancing the activity of factors 12 and 5, and augmenting platelet adhesion to the endothelial cells.\(^{12,13}\) The interference of homocysteine with the coagulation system creates a prothrombotic milieu. Virchow’s postulates for thrombogenesis require abnormalities of blood flow, vessel wall, and blood constituents. High homocysteine fulfills the 2 criteria of Virchow’s triad by its endothelial toxic and prothrombotic properties. Therefore, one can postulate that in conditions associated with abnormal blood flow such as stasis, high levels of homocysteine enhance thrombus formation. Indeed, elevated homocysteine has been linked closely to venous thrombosis, which is almost always associated with blood stasis.\(^{4}\) The present study is the first to question the thrombogenic role of homocysteine in another stasis-related condition, AF. In accordance with the venous thrombosis data, elevated homocysteine exhibited an association with the presence of LA thrombus; the mean fasting plasma homocysteine levels were significantly higher in patients with LA thrombus compared with that in patients with no thrombus. Moreover, this association was independent of other clinical and echocardiographic parameters known to be associated with LA thrombus formation. The only other study of homocysteine in patients with AF, by Friedman,\(^{14}\) revealed no difference in homocysteine levels among those with or without AF. However, homocysteine levels were significantly higher in a subset of AF patients with a history of stroke than in those who had AF but no stroke. Although Friedman did not stratify AF patients with respect to the presence of thrombus, his results are still in line with those of the present study because patients with AF and stroke are more likely to harbor an LA thrombus.\(^1\)

In AF, the fibrillating LA creates a milieu for blood stasis, most prominently in the LAA. This can be qualitatively detected on echocardiography as SEC, a phenomenon characterized by smokelike echoes swirling in the LA, resulting from a stasis-induced, increased erythrocyte aggregation.\(^{15}\) Likewise, LAA outflow velocity measurements serve as a quantitative marker for blood stasis in LAA. Both the presence of SEC and reduced LAA peak outflow velocity (<20 cm/s) have been shown to convey an independent risk for thrombus formation in LAA.\(^{16}\) In addition to stasis, various markers of hypercoagulable state such as factor 8, fibrinogen, d-dimer, prothrombin fragment 1.2, and von Willebrand factor have been shown to increase in AF.\(^{17-20}\) The presence of a prothrombotic state is important because it ties Virchow’s criteria to thrombus formation in AF. However, this is not sufficient to establish a causal relationship because LA thrombus in AF might be a trigger rather than a result of elevated hemostatic markers. Homocysteine is different than conventional hemostatic factors because it is neither a direct contributor to the coagulation pathway such as coagulation factors nor a byproduct of the thrombotic (such as fibrinopeptide A and prothrombin fragment 1.2) or fibrinolytic (such as d-dimer) systems. Unlike most other hemostatic factors, homocysteine levels do not increase in the acute phase of thrombotic episodes such as acute coronary events and stroke.\(^{21,22}\) Therefore, the independent association between high homocysteine and LA thrombus demonstrated in the present study is highly suggestive of a causal relationship. However, it is not possible to be certain about a causal relationship without confirming such an association in a prospective design.

The present study is the first to demonstrate a relationship between LA thrombus in AF and lower folic acid levels. Folic
acid plays a key role as a cofactor in the process of the remethylation of homocysteine to form methionine.\textsuperscript{23} It has previously been shown that as folic acid levels decrease, homocysteine levels increase.\textsuperscript{24} Subnormal levels of folic acid are associated with moderately elevated homocysteine levels.\textsuperscript{25,26} Accordingly, homocysteine levels showed an inverse but modest correlation with serum levels of folic acid in the present study. This association suggests that the homocysteine elevations observed in our cohort might have been caused in part by low folic acid levels.

Some limitations may apply to the present study. First, LAA outflow velocities were not measured and therefore were not included in the logistic regression analysis. However, the presence of SEC and the increased LA diameter are also strong markers of LA stasis and were included in the analyses. The persistence of a significant association between high homocysteine and LA thrombus after adjustment for these factors strongly suggests that the effect of homocysteine on the LA thrombus is independent of the degree of LA stasis. Second, homocysteine levels increase after stroke; thus, measurements obtained after stroke may not reflect the levels before stroke.\textsuperscript{22} It has previously been shown that homocysteine levels in stroke patients and in control subjects are also strong markers of LA stasis and were included in the analyses. The presence of SEC and the increased LA diameter are also strong markers of LA stasis and were included in the analyses. The persistence of a significant association between high homocysteine and LA thrombus after adjustment for these factors strongly suggests that the effect of homocysteine on the LA thrombus is independent of the degree of LA stasis.

Accurate identification and proper management of risk factors predisposing to thrombus formation in the LA are critical to minimize the embolic complications of AF. Such attempts might be more important in patients who cannot continuously and effectively use warfarin because of contraindications. The link between high homocysteine levels and LA thrombus established in the present study raises the question of whether homocysteine-lowering therapies could be an appropriate candidate for this task. It is possible to reduce plasma homocysteine levels with folic acid therapy at a dosage between 0.5 and 5 mg/d by \(\approx 25\%\), even in people who are not vitamin deficient.\textsuperscript{28} The efficacy of such a measure in reducing the stroke rate in AF remains to be tested in future studies.

References

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