Prospective Study of Serum Homocysteine and Risk of Ischemic Stroke Among Patients With Preexisting Coronary Heart Disease

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Background and Purpose—Substantial evidence is accumulating suggesting that hyperhomocysteinemia may be a risk factor for ischemic stroke. Results of prospective studies are, however, conflicting, and the role of hyperhomocysteinemia in patients with preexisting atherosclerotic vascular disease is not clear. Our aim was to assess prospectively the risk of incident ischemic stroke conferred by serum total homocysteine among patients with preexisting stable coronary heart disease (CHD).

Methods—We obtained baseline fasting serum samples from patients with chronic CHD enrolled in the Bezafibrate Infarction Prevention (n = 3090) secondary prevention study cohort. With a nested case-control design, we measured baseline total homocysteine concentration by a high-performance liquid chromatography–based method in sera (n = 160) of matched case-control pairs; patients who developed ischemic stroke during a mean follow-up of 8.2 years (cases) and age- and sex-matched controls without subsequent cardiovascular events.

Results—An increase of 1 natural log unit in homocysteine concentration was associated with a >3-fold increase in relative odds of incident ischemic stroke (3.3; 95% CI, 1.2 to 10.2). Homocysteine concentrations at the highest quartile (>17.4 μmol/L) were associated with significantly higher odds of ischemic stroke compared with the lowest quartile in matched-pair analysis (3.1; 95% CI, 1.1 to 9.8) and after multivariable adjustments (4.6; 95% CI, 1.3 to 18.9). Adding fibrinogen or soluble intercellular adhesion molecule-1 concentrations, markers of inflammation, to the model did not attenuate this association. The linear trends across the quartiles were significant for all models (P<0.05).

Conclusions—Serum total homocysteine concentration is a strong predictor for incident ischemic stroke among patients at increased risk because of chronic CHD. The graded association observed is independent of traditional risk factors or inflammatory markers and indicates the importance of serum homocysteine levels in patients with preexisting vascular disease. (Stroke. 2003;34:632-636.)

Key Words: atherosclerosis ■ homocyst(e)ine ■ risk factors ■ stroke, ischemic

Over the last decade, evidence has accumulated that elevated plasma or serum concentrations of the sulfur amino acid homocysteine are associated with an increased risk of atherosclerosis, cardiovascular disease, and ischemic stroke.1 Although this association is biologically plausible, study results are inconsistent. Case-control studies, the study methodology most often employed, found in general a strong association with ischemic stroke, but the few prospective studies available (cohort or nested case-control) offered only weak support2-5 or no support6-8 for such an association. In a recent meta-analysis, the summary odds ratios for cerebrovascular disease associated with total homocysteine concentration were lower in prospective study designs than in case-control studies.9 There is evidence that homocysteine is released from damaged tissues. Concentrations of total homocysteine vary with time in patients with acute vascular events.10-13 Such findings raise the possibility that the elevated total homocysteine concentrations observed in numerous case-control studies may be a consequence, rather than a possible cause, of an acute event. The role of hyperhomocysteinemia in patients with preexisting atherosclerotic vascular disease is not clear, and there are views that homocysteine serves as a mere marker of tissue damage and repair.14 No prospective studies have evaluated hyperhomocysteinemia as a potential risk factor for incident ischemic stroke in a cohort of patients with preexisting vascular disease. The dilemma of whether elevated total homocysteine concentration in patients with preexisting atherosclerotic vascular disease is a cause of...
vascular damage or merely its outcome was the stimulus to conduct the present prospective study among patients with stable coronary heart disease (CHD).

Subjects and Methods

Study Sample
We performed a prospective, nested case-control study of serum total homocysteine as a potential risk factor for incident ischemic stroke among patients with chronic CHD. Patients were participants in the Bezafibrate Infarction Prevention (BIP) Study, a multicenter, randomized, placebo-controlled clinical trial, conducted in 18 centers in Israel, that evaluated the efficacy of the lipid-modifying drug bezafibrate retard for secondary prevention in 3090 men and women with CHD.13 The trial was approved by the Helsinki Committee of each center and the central national Helsinki Committee.

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 either positive coronary angiography, nuclear scintigraphy, or exercise test. Mean length of follow-up was 8.2 years (range, 6.7 to 9.6 years).

Routine visits to the clinics were scheduled every 4 months throughout the study for clinical evaluation. During these visits, data on adverse events, hospitalizations, and study outcomes were obtained. Data on the occurrence of incident cerebrovascular events were systematically obtained during these evaluations. Records from hospital or emergency department discharge, primary care physician, or 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 (TOAST) classification on the basis of neurological findings, history, and results of CT scan, ECG, echocardiography, carotid duplex, and any other relevant diagnostic test that was available, and then categorized into cardioembolic, atherothrombotic, and of undetermined origin. Study physicians during follow-up assessed functional outcome after a stroke by the modified Rankin Scale. Stroke severity was categorized as minor for cases in which the modified Rankin Scale score was 0 or 1 and as major for score of ≥2 or 30-day case fatality.

To assess the risk of incident ischemic stroke associated with mild to moderate total homocysteine prospectively, we performed a nested case-control study within the BIP Study cohort. Case subjects (n=80) were patients who developed an ischemic stroke and for whom an adequate blood sample before randomization to the study was available. Each case was paired with a control subject matched for age, sex, and bezafibrate versus placebo arm. Controls (n=80) were patients who remained free of any recurrent coronary events or stroke throughout the follow-up period and were alive at the end of study.

Laboratory Procedures
Blood samples, drawn after ≥12 hours of fasting, were collected in the 18 participating medical centers with the use of standardized equipment and procedures and were transferred in cooled containers to a central laboratory. Blood lipids, fibrinogen, blood chemistry, and other laboratory tests were assessed at baseline. All laboratory analyses were performed in a single central laboratory with the use of 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 total homocysteine concentrations by the high-performance liquid chromatography–based method of Jacobsen et al.16 A laboratory technician, who was blinded to the case or control status of each sample, performed all the tests.

Statistical Analysis
To assess the significance of differences between means of continuous variables, among the matched pairs we used the paired t test procedure. The McNemar test for paired samples was used to assess differences between rates. Because total homocysteine concentrations were positively skewed, we used log-transformed values. Alternatively, we divided total homocysteine concentrations into quartiles according to concentrations in a random sample of BIP Study participants who were free from any cardiovascular events by the end of the study (n=168). We used logistic regression analyses conditioned on the matching variables (age, sex, and BIP Study medication [bezafibrate versus placebo]) to estimate the odds ratios (and 95% CIs) of experiencing an incident ischemic stroke. We conducted several models adjusting for smoking and traditional stroke risk factors. We also conducted logistic regression models adjusting, in addition, for the inflammatory markers soluble intercellular adhesion molecule-1 and fibrinogen, which both predict stroke in our cohort.17,18 To assess whether the effect of serum total homocysteine on risk of ischemic stroke varied by ischemic stroke subtype, we stratified the analysis accordingly. All analyses were performed with the use of SAS statistical software.19

Results

Baseline Characteristics
Cases and controls were well matched in terms of age, sex, and bezafibrate use. Cases smoked cigarettes more often, had higher mean systolic blood pressure, and had diabetes mellitus more often than controls (Table 1). The median concentration of total homocysteine was 16.4 μmol/L (interquartile range, 12.7 to 21.5) among cases versus 14.3 μmol/L (interquartile range, 12.0 to 17.8) among controls. Concentrations

| TABLE 1. Baseline Characteristics of Study Participants |
|-------------|---------------|-------------|
| Age, y       | 61.2±6.3      | 61.3±6.4    |
| Men          | 76 (95%)      | 76 (95%)    |
| Bezafibrate treatment | 44 (55%) | 43 (54%)    |
| Diabetes mellitus | 4 (5%)  | 13 (17%)    |
| Hypertension | 29 (38%)      | 35 (46%)    |
| Current smoking | 6 (8%)   | 17 (21%)    |
| Previous myocardial infarction | 60 (77%) | 68 (87%)    |
| Angina pectoris | 48 (62%) | 49 (63%)    |
| New York Heart Association class ≥2 | 22 (28%) | 23 (29%)    |
| Antiplatlet use | 48 (60%) | 49 (61%)    |
| Diastolic blood pressure, mm Hg | 80.8±10.2 | 83.2±10.6 |
| Systolic blood pressure, mm Hg | 133.0±16.8 | 138.4±18.1 |
| Body mass index, kg/m² | 26.9±2.9 | 26.8±3.0 |
| Total cholesterol, mg/dL | 212.2±17.3 | 214.0±17.3 |
| HDL cholesterol, mg/dL | 32.6±5.7 | 33.5±5.4 |
| LDL cholesterol, mg/dL | 147.6±16.0 | 150.7±16.7 |
| Triglycerides, mg/dL | 157.6±52.8 | 148.7±51.9 |
| Serum creatinine | 1.15±0.15 | 1.16±0.16 |
| Fibrinogen, mg/dL | 336.2±77.7 | 378.4±80.3 |
| Soluble ICAM-1, ng/mL | 345.6±90.8 | 367.4±105.8 |

Continuous variables are expressed as mean±SD, categorical variables as number (%). ICAM-1 indicates intercellular adhesion molecule-1.
of total homocysteine were higher in two thirds of the matched pairs in the case subjects. The median of differences in total homocysteine concentrations between matched pairs was 2.1 μmol/L higher in cases versus matched controls, and the 75th percentile of difference in total homocysteine was 5.3 μmol/L higher in cases.

The relative odds for incident ischemic stroke associated with the log-transformed concentration of total homocysteine (per 1-unit change) were 3.30 (95% CI, 1.20 to 10.19) and changed little after adjustment for conventional risk factors (odds ratio, 3.41; 95% CI, 1.08 to 12.30). To explore the risk of ischemic stroke associated with increasing total homocysteine levels, we evaluated the risk of ischemic stroke according to total homocysteine concentrations divided into quartiles (Table 2). The unadjusted analysis of the matched pairs demonstrated a significantly higher risk for incident ischemic stroke during follow-up in the upper quartile of total homocysteine level compared with the first quartile (odds ratio, 3.07; 95% CI, 1.07 to 9.80). Adjustment for smoking status did not change the results materially. A model that included traditional risk factors and potential confounding variables produced a graded association with increasing total homocysteine levels, with an odds ratio of 4.62 (95% CI, 1.32 to 15.53) in the upper quartile of total homocysteine level compared with the lowest quartile. Further adjustment for plasma fibrinogen or for soluble intercellular adhesion molecule-1, an inflammatory marker, did not attenuate this association (Table 2). The linear trends across the quartiles were significant for all models (P<0.05).

The relative odds associated with a 5-μmol/L increase of total homocysteine were 2.00 (95% CI, 1.04 to 6.12) for cardioembolic stroke, 1.16 (95% CI, 0.68 to 2.14) for atherothrombotic stroke (large- and small-vessel disease), and 1.09 (95% CI, 0.80 to 1.38) for ischemic stroke of undetermined origin.

<table>
<thead>
<tr>
<th>Table 2. Crude and Multivariable Adjusted Relative Odds for Incident Ischemic Stroke According to Serum Total Homocysteine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Log (per 1 unit)</td>
</tr>
<tr>
<td>Model a: unadjusted analysis</td>
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<tr>
<td>Model b: smoking</td>
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<tr>
<td>Model c: multivariable analysis</td>
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<tr>
<td>Model d: plasma fibrinogen</td>
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<tr>
<td>Model e: soluble adhesion molecule-1</td>
</tr>
<tr>
<td>All analyses are matched for age, sex, and BIP study medication (bezafibrate vs placebo). Model b, adjusted for current smoking; model c, adjusted for diabetes mellitus, current smoking, hypertension, and previous myocardial infarction; model d, adding fibrinogen concentration to the multivariable model; model e, adding soluble intercellular adhesion molecule-1 (sCAM-1) to the multivariable model.</td>
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Discussion

Our findings in this prospective, nested case-control study are consistent with a strong predictive role of total homocysteine levels, independent of traditional risk factors or inflammatory markers, for incident ischemic stroke in patients at increased risk due to preexisting CHD.

The results from prospective studies assessing total homocysteine concentrations and risk of cerebrovascular disease differed substantially from those for case-control studies, and overall lower odds ratios associated with total homocysteine concentration were found.9 Levels of total homocysteine rise after an acute vascular event such as myocardial infarction or stroke,10–13 and it is not clear whether elevated total homocysteine in such patients is a true risk factor or a marker of tissue damage.14 Available prospective studies assessed total homocysteine among low-risk subjects mainly from population-based cohorts. Our cohort included patients with preexisting atherosclerotic vascular disease, associated with higher total homocysteine concentrations, yet we found that total homocysteine concentrations are a powerful predictor for incident ischemic stroke over a mean follow-up of 8 years. These findings are in accordance with a strong graded association found between total homocysteine and overall mortality in patients with angiographically confirmed coronary artery disease.20,21

Although cases, as expected, smoked cigarettes and had diabetes mellitus more often, factors that may be associated with hyperhomocysteinemia, relative odds were not diminished after adjustment for these factors. Relative odds were also not materially affected by adjustment for markers of inflammation, such as soluble intercellular adhesion molecule-1 and plasma fibrinogen, although these 2 markers are strong predictors of ischemic stroke in our cohort.16,17 These findings are consistent with the notion that whereas elevated levels of both inflammatory markers and total homocysteine represent independent risk factors for vascular disease, the relationship between total homocysteine, inflammation, and atherosclerosis cannot be explained through a direct link between processes.21,22

It is well recognized that ischemic stroke is pathologically and etiologically heterogeneous and that risk factors for one etiologic subtype may not be risk factors for other subtypes of stroke. Recent studies have shown that acute hyperhomocysteinemia causes endothelial dysfunction, which might affect cerebrovascular reactivity and promote atheroma development.23,24 Raised total homocysteine concentrations are associated with carotid artery wall thickening and stenosis25 and are associated with ischemic events in patients with significant carotid stenosis.26 Eikelboom and colleagues27 have
found in a case-control study that hyperhomocysteinemia is associated in particular with stroke due to large-vessel atherosclerosis, and an association with white matter lesions was found in the cross-sectional Rotterdam Scan Study. In the present study of patients with preexisting atherosclerotic vascular disease, the strongest associations identified were with cardioembolic stroke.

The interplay between inherited and/or acquired factors and iatrogenic factors and pathophysiologic conditions interferes with total homocysteine metabolism. Experimental evidence suggests that the atherogenic propensity associated with hyperhomocysteinemia is caused by endothelial dysfunction and thrombus formation. A number of mechanisms have been proposed to link total homocysteine to vascular damage, stroke, and cardiovascular disease. These include impairment of endothelial functions, endothelial desquamation, oxidation of LDL, increased monocyte adhesion to the vessel wall, impaired vascular response to nitric oxide, and thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction.

Several aspects of our study design that might affect the validity of the findings should be addressed. First, the exposure (total homocysteine) was measured blinded to the case-control status of the patients. Incident ischemic strokes were determined before and without knowledge of total homocysteine concentrations, and therefore any misclassification is likely to be random. Similarly, inclusion of patients with silent brain infarcts in the control group, which we could not adjust for, might have led to attenuation of the associations. The use of a single total homocysteine measurement to classify persons may have underestimated the strength of any associations because of regression dilution. These shortcomings may result in an underestimation of the strength of any risk association. Finally, our study lacked information on some determinants of total homocysteine levels, notably vitamin status. The latter, however, does not detract from our findings on total homocysteine concentrations and risk of incident ischemic stroke.

Hyperhomocysteinemia as a risk factor for ischemic stroke may have profound public health implications because total homocysteine can be lowered inexpensively and easily by modification of dietary patterns and vitamin supplements. Recently, Bazzano and colleagues found that dietary intake of folate from food sources is independently and inversely related to the risk of stroke and cardiovascular disease in a representative sample of the noninstitutionalized US population. In the present study we provide evidence consistent with a strong graded association in patients with preexisting atherosclerotic vascular disease between total homocysteine concentration and incident ischemic stroke. The results of ongoing clinical trials to determine the effect of multivitamin therapy on stroke and other serious vascular events in patients with vascular disease are awaited. If causally related, our findings suggest that patients with preexisting atherosclerotic vascular disease may particularly benefit from interventions that lower total homocysteine concentrations.

References


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