Inflammation, Homocysteine, and Vitamin B6 Status After Ischemic Stroke

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Background and Purpose—Epidemiological studies have described an association between low vitamin B6 (measured as pyridoxal 5'-phosphate [PLP]) and vascular disease. As homocysteine (tHcy) is frequently elevated in individuals with low PLP, these findings have been considered to be mediated via tHcy. However, we and others have found that the relationship remained after accounting for tHcy, suggesting that other mechanisms may be involved.

Methods—Consecutive cases with new ischemic stroke were compared with matched controls. Fasting tHcy, PLP, and CRP were measured.

Results—The adjusted odds ratio of low PLP in the highest compared with the lowest CRP quartile was 16.6 (2, 139.9, P=0.01). Age, CRP, supplemental vitamin use, and albumin were independent predictors of PLP (P<0.05 for all). No relationship was observed between CRP and tHcy.

Conclusion—The relationship between inflammation and low B6 status may partially explain the findings of previous epidemiological studies. (Stroke. 2004;35:12-15.)

Key Words: homocyst(e)ine ■ inflammation ■ nutrition ■ pyridoxine ■ stroke

Following early descriptions of atherosclerosis in pyridoxine-deficient monkeys,1 epidemiological studies have reported an association between low vitamin B6 (measured as pyridoxal 5'-phosphate [PLP]) and vascular disease.2 As homocysteine (tHcy) is frequently elevated in individuals with low PLP, these findings have often been considered to be mediated via tHcy. However, we and others have found that the relationship remained after accounting for tHcy, suggesting that other mechanisms may be involved.

Other explanations for the association between B6 and vascular disease include a possible causal influence on atherosclerosis or thrombosis. Supporting this hypothesis, experimental studies have indicated that PLP may influence platelet adhesion and cholesterol metabolism. Alternatively, low PLP may be a marker of acute or chronic inflammation, which may promote atherosclerosis. Markers of inflammation, such as C-reactive protein (CRP) and interleukin-6, have been strongly associated with the incidence and outcome of stroke and coronary artery disease (CAD). Recent data also suggest that inflammation may contribute to low B6 status in population-based cohorts and in patients with rheumatoid arthritis. Furthermore, as B6 status is an important determinant of tHcy, it is possible that inflammation may indirectly influence tHcy via alterations in B6 metabolism.

We recently reported a strong association between low PLP and ischemic stroke, which was independent of tHcy and other vascular risk factors. To further investigate this finding, we examined the relationship of inflammation, tHcy, and PLP in patients with stroke and controls.

Subjects and Methods

Subjects
Cases were unselected consecutive patients with recent (<30 days) ischemic stroke (neurological deficit >24 hours, confirmed by CT or MRI), admitted to a single acute care hospital. Controls were matched for age and supplemental vitamin use and recruited from a large, primary care practice (>18,000 patients) serving the hospital catchment area. Exclusion criteria (cases and controls) were (1) prespecified nonatherosclerotic stroke syndromes (vasculitis, endocarditis, fibromuscular dysplasia, migraine, venous infarction); (2) pregnancy; (3) diseases/medications affecting folate/tHcy (cirrhosis, leukemia, psoriasis, renal dialysis, phenytoin, carbamazepine, methotrexate); (4) inability to obtain consent; and (5) insufficient stored plasma for CRP measurement. Controls were also excluded if there was a history of stroke, transient ischemic attack, or carotid endarterectomy.

Stroke subtype was assigned using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria by a blinded experienced stroke neurologist. As our intent was to examine the relationship between PLP and inflammation following stroke, we did not exclude patients with stroke-related infection, in whom such a link may be most apparent. Cases with fever and leukocytosis, together with positive cultures (blood/urine/sputum), infiltrate on chest x-ray, or leukocytosis on urine microscopy, were considered to have proven or probable infection. The study was approved by the hospital Institutional Review Board.

Biochemical Measurements
Plasma for tHcy and CRP was frozen (−20°C) for later measurement. Fasting (>8 hours) tHcy was measured by fluorescence
Clinical Characteristics of Study Sample

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=156)</th>
<th>Controls (n=118)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean</td>
<td>69.2</td>
<td>68.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Male sex, % (n)</td>
<td>55.8 (87)</td>
<td>54.2 (64)</td>
<td>0.8</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
<td>74.4 (116)</td>
<td>59 (69)</td>
<td>0.007</td>
</tr>
<tr>
<td>Current smoking, % (n)</td>
<td>24.8 (37)</td>
<td>12.3 (14)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes, % (n)</td>
<td>25 (39)</td>
<td>4.3 (5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Atrial fibrillation, % (n)</td>
<td>21.8 (34)</td>
<td>6.9 (8)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Neuroimaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI, % (n)</td>
<td>85.3 (133)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CT, % (n)</td>
<td>96.8 (151)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Vitamin use, % (n)</td>
<td>40.9 (61)</td>
<td>41.4 (46)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

|                      |              |                 |     |
| TOAST                |              |                 |     |
| Large artery, % (n)  | 34.6 (54)    |                 |     |
| Small artery, % (n)  | 8.3 (13)     |                 |     |
| Cardioembolic, % (n)| 39.7 (62)    |                 |     |
| Other/undetermined, % (n) | 17.4 (27)    |                 |     |
| Albumin, mg/dL (mean, SD) | 3.4 (0.48) | 3.9 (0.4) | <0.0001 |
| tHcy,* (µmol/L)      | 10.1         | 10.33           | 0.58 |
| PLP,* (mmol/L)       | 30.9         | 62.4            | <0.0001 |
| CRP,* (mg/dL)        | 0.87         | 0.16            | <0.0001 |

*Geometric mean.

polarization immune assay. PLP was measured by the tyrosine decarboxylation method. High-sensitivity CRP was measured by immunonephelometry.

Statistical Analysis

Univariate (2-sided t tests, chi-squared tests, Pearson correlation) and multivariable (linear regression) analyses were performed, with calculation of odds ratios and 95% confidence intervals by standard formulas. Log transformations were applied for continuous variables (tHcy, PLP, and CRP) for which the distributions were skewed. Analysis of variance and Dunnet’s test were performed for comparisons of CRP and PLP between control and case groups stratified by infection. For determination of CRP quartiles, individual values across both groups were combined, and quartile-specific thresholds were calculated.

Results

Clinical Characteristics

A total of 274 subjects (156 cases, 118 controls) were included. These represented a subgroup of a larger study population, characteristics of which have previously been described (Table). Briefly, no difference in age, sex, or supplemental vitamin use was present. Seventy-six percent of cases had phlebotomy within 5 days of symptom onset (median, 3 days; interquartile range, 2 to 5 days; range, 0.5 to 32 days).

CRP and PLP in Cases and Controls

Mean PLP was lower and CRP higher in cases compared with controls (P<0.0001) (Table). To control for the potential confounding influence of stroke-related infection on CRP, cases were stratified according to the presence or absence of infection at the time of phlebotomy. A clear trend of increasing CRP was present when compared between controls, stroke cases without infection, and stroke cases with infection (P<0.0001). An identical but inverse trend was present for PLP (P<0.0001) (Figure 1), consistent with a dose-response relationship between the intensity of inflammatory stimulus, CRP, and PLP.

PLP Distribution Across CRP Quartiles

When examined across increasing quartiles of the CRP distribution, mean PLP showed a strong inverse relationship with CRP in both case and control groups (P=0.001 for trend in each group) (Figure 2). The adjusted OR of low PLP increased in a graded fashion across increasing CRP quartiles, becoming statistically significant for comparisons of the 3rd (OR, 9.04 [1.05, 77.5]; P=0.045) and highest quartiles (OR, 16.6 [2, 139.9]; P=0.01).

Predictors of PLP and tHcy

In cases, PLP was associated with CRP (r = -0.3, P<0.0001), age (r = -0.22, P=0.006), albumin (r = 0.29, P=0.0006), NIHSS (r = -0.17, P=0.03), tHcy (r = -0.15, P=0.07), and vitamin supplement use (P<0.0001). Among controls, CRP (r = -0.29, P=0.002), tHcy (r = -0.24, P=0.009), and vitamin supplement use (P<0.0001) were also associated with PLP.

Among cases, CRP (P=0.003), age (P=0.008), albumin (the major PLP-binding plasma protein, P=0.03) and supplemental vitamin use (P=0.005) remained as independent predictors of PLP in the regression model (r²=0.27). Among controls, CRP (P=0.02), vitamin use (P<0.0001), and tHcy (P=0.06) were independent predictors of PLP.

Predictors of tHcy in the larger study sample have been previously described. No correlation was observed between tHcy and CRP on univariate or multivariable analysis.

Discussion

We previously reported that low B6 status was associated with incident ischemic stroke, independently of other vascular risk factors and tHcy. In this study, we investigated the influence of inflammation on B6 status and tHcy, controlling for albumin and supplemental vitamin use. We found a strong, dose-dependent inverse relationship between CRP and PLP in patients with stroke and ambulatory controls, which remained after adjusting for other factors that influence PLP. The findings in controls support the validity of those in stroke patients and suggest that low PLP is related to chronic inflammation in addition to the acute phase response. In contrast, we found no association between CRP and tHcy, despite a significant correlation between tHcy and PLP.

Animal studies have reported widespread atherosclerotic changes in rhesus monkeys and dogs fed pyridoxine-deficient diets. Epidemiological studies have reported that low B6 status was associated with carotid stenosis, CAD, and stroke. As elevated tHcy is associated with low PLP, this association has often been interpreted to be mediated primarily via tHcy. However, several studies have found that the association remains after controlling for tHcy, suggesting that other mechanisms may also contribute.

One possible explanation is that PLP may be a marker of inflammatory status and vascular disease. Inflammatory markers such as CRP strongly predict the risk of carotid...
stenosis, first stroke, and poststroke mortality.\textsuperscript{10,11} In community-living elderly subjects in the United States and Britain, low B6 status was inversely correlated with inflammatory markers such as CRP and α1-antichymotrypsin.\textsuperscript{12,13} PLP also correlates inversely with markers of inflammation such as tumor necrosis factor-α and the erythrocyte sedimentation rate in rheumatoid arthritis, a prototypic inflammatory disorder.\textsuperscript{14}

Although we did not measure dietary B6 intake, this limitation is addressed in part by the inclusion of information on vitamin supplement use, which is a major determinant of B6 status. We cannot exclude the possibility that the timing of
sampling may have contributed to some of the variability in tHcy, as levels have been reported to increase gradually after stroke (about 10% in the first week). However, tHcy sampling was relatively uniform (90% collected <7 days) and the onset-to-phlebotomy interval was not a predictor of tHcy on multivariate analysis. Therefore, we consider it unlikely that the timing of phlebotomy had a significant effect on tHcy levels. Although we excluded controls with a history of clinical cerebrovascular disease, we cannot exclude the possibility that some controls may have had subclinical craniocervical atherosclerosis.

We and others found no relationship between tHcy and CRP, suggesting that tHcy predicts vascular risk independently of an inflammation-related mechanism. In contrast, our findings support previous observations of a relationship between B6 status and inflammation, not previously described in acute stroke. These results may partially explain the association between low B6 and vascular disease previously reported in other studies.

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