Homocysteine-Lowering Treatment With Folic Acid, Cobalamin, and Pyridoxine Does Not Reduce Blood Markers of Inflammation, Endothelial Dysfunction, or Hypercoagulability in Patients With Previous Transient Ischemic Attack or Stroke

A Randomized Substudy of the VITATOPS Trial

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Background and Purpose—Epidemiological and laboratory studies suggest that increasing concentrations of plasma homocysteine (total homocysteine \([tHcy]\)) accelerate cardiovascular disease by promoting vascular inflammation, endothelial dysfunction, and hypercoagulability.

Methods—We conducted a randomized controlled trial in 285 patients with recent transient ischemic attack or stroke to examine the effect of lowering \(tHcy\) with folic acid 2 mg, vitamin \(B_12\) 0.5 mg, and vitamin \(B_6\) 25 mg compared with placebo on laboratory markers of vascular inflammation, endothelial dysfunction, and hypercoagulability.

Results—At 6 months after randomization, there was no significant difference in blood concentrations of markers of vascular inflammation (high-sensitivity C-reactive protein \([P=0.32]\); soluble CD40L \([P=0.33]\); IL-6 \([P=0.77]\)), endothelial dysfunction (vascular cell adhesion molecule-1 \([P=0.27]\); intercellular adhesion molecule-1 \([P=0.08]\); von Willebrand factor \([P=0.92]\)), and hypercoagulability (P-selectin \([P=0.33]\); prothrombin fragment 1 and 2 \([P=0.81]\); D-dimer \([P=0.88]\)) among patients assigned vitamin therapy compared with placebo despite a 3.7-\(\mu\)mol/L (95% CI, 2.7 to 4.7) reduction in total homocysteine \((tHcy)\).

Conclusions—Lowering \(tHcy\) by 3.7-\(\mu\)mol/L with folic acid-based multivitamin therapy does not significantly reduce blood concentrations of the biomarkers of inflammation, endothelial dysfunction, or hypercoagulability measured in our study. The possible explanations for our findings are: (1) these biomarkers are not sensitive to the effects of lowering \(tHcy\) (eg, multiple risk factor interventions may be required); (2) elevated \(tHcy\) causes cardiovascular disease by mechanisms other than the biomarkers measured; or (3) elevated \(tHcy\) is a noncausal marker of increased vascular risk.

Key Words: cardiovascular diseases ■ homocyst(e)ine ■ inflammation

A raised plasma concentration of total homocysteine \((tHcy)\) has been proposed as an important causal risk factor for cardiovascular disease.\(^1\)\(^,\)\(^2\) Its deleterious effect is believed to be mediated by an increase in vascular inflammation, endothelial dysfunction, and/or hypercoagulability.\(^1\)\(^,\)\(^3\)

The aim of this study was to determine the effect of lowering \(tHcy\) by means of the combination of folic acid 2 mg/d, cobalamin 0.5 mg/d, and pyridoxine 25 mg/d on soluble circulating blood markers of vascular inflammation, endothelial dysfunction, and hypercoagulability, which have been linked previously with cardiovascular disease.

Materials and Methods

The protocol was approved by the Institutional Research Ethics Committee of the Royal Perth Hospital in Perth, Australia. Each patient gave written informed consent.

We randomized 285 consecutive consenting patients with a stroke or transient ischemic attack (TIA) in the previous 7 months to receive either folic acid 2 mg/d, cobalamin 0.5 mg/d, and pyridoxine 25...
Outcomes at 6 Months After Randomization

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Folic Acid + Vitamin B₁₂ + Vitamin B₉ (n=143)</th>
<th>Placebo (n=142)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with blood tests at 6 mo, no. (%)</td>
<td>125 (87)</td>
<td>125 (88)</td>
<td>0.87</td>
</tr>
<tr>
<td>tHcy, mean (SD), μmol/L</td>
<td>9.1 (2.5)</td>
<td>12.8 (5.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Inflammatory Markers**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Folic Acid + Vitamin B₁₂ + Vitamin B₉</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP, mg/L</td>
<td>2.14 (0.16, 31.7)</td>
<td>2.40 (0.16, 37.5)</td>
<td>0.32</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.58 (0.09, 13.3)</td>
<td>2.66 (0.14, 16.41)</td>
<td>0.77</td>
</tr>
<tr>
<td>sCD40L, ng/mL</td>
<td>0.55 (0.04, 5.20)</td>
<td>0.56 (0.11, 19.23)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Endothelial Markers**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Folic Acid + Vitamin B₁₂ + Vitamin B₉</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM, ng/mL</td>
<td>977 (506, 4324)</td>
<td>977 (476, 2594)</td>
<td>0.27</td>
</tr>
<tr>
<td>ICAM, ng/mL</td>
<td>453 (134, 1536)</td>
<td>514 (140, 1544)</td>
<td>0.08</td>
</tr>
<tr>
<td>vWF, %</td>
<td>175 (0, 246)</td>
<td>171 (0, 244)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Hypercoagulability Markers**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Folic Acid + Vitamin B₁₂ + Vitamin B₉</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin, ng/mL</td>
<td>142.0 (22.3, 1404.9)</td>
<td>152.8 (11.5, 998.9)</td>
<td>0.33</td>
</tr>
<tr>
<td>PF1+2, nmol/L</td>
<td>0.80 (0.07, 16.1)</td>
<td>0.79 (0.10, 32.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>DD, mg/L</td>
<td>0.38 (0.09, 21.0)</td>
<td>0.37 (0.05, 21.00)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Medians (min, max) are reported unless otherwise specified.

D-dimer indicates D-dimer; hsCRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; PF1+2, prothrombin fragment 1 and 2; sCD40L, soluble CD40L; SD, standard deviation; tHcy, total homocysteine; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor.

mg/d or placebo using a computer-generated randomization sequence.

A venous blood sample was collected after an overnight fast from all patients before commencing randomized study treatment to measure blood glucose, creatinine, cholesterol, and tHcy, and again at 6 months to measure tHcy levels and blood markers of inflammation, endothelial dysfunction, and hypercoagulability.

Venous blood was collected and processed using a standardized protocol. Fasting tHcy was measured by high-performance liquid chromatography. Commercially available enzyme-linked immunosorbent assays were used to measure high-sensitivity C-reactive protein (Dade Behring Diagnostics, Marburg, Germany), IL-6 (R&D Systems, Minneapolis, Minn), soluble CD40L (Bender MedSystems, Vienna, Austria), vascular cell adhesion molecule-1 (Bender MedSystems), intercellular adhesion molecule-1 (Bender MedSystems), soluble P-selectin (Bender MedSystems), and prothrombin fragment 1 and 2 (Dade Behring Diagnostics). Von Willebrand factor and D-dimer were tested using latex immunoassay on the STA-R automated coagulation analyzer (Diagnostica Stago).

Baseline demographics, stroke type, and the prevalence of conventional cardiovascular risk factors in the 2 randomized treatment groups were compared using parametric statistics for continuous data and nonparametric statistics for categorical data. The distributions of blood markers of inflammation, endothelial dysfunction, and hypercoagulability at 6 months were skewed; therefore, the significance of differences between the 2 randomized treatment groups were examined using a Wilcoxon rank-sum test. Statistical significance was concluded with a 2-sided \( P < 0.05 \).

**Results**

Two-hundred eighty-five patients were randomly assigned to receive either folic acid 2.0 mg, cobalamin 0.5 mg, and pyridoxine 25 mg, once daily (n=143), or placebo, once daily (n=142). The mean time between the onset of stroke or TIA and randomization was 87.4 days (SD: 133.3) for patients allocated to placebo and 66.8 days (SD: 84.4) for patients allocated to multivitamins (\( P = 0.18 \)).

Patients in the 2 treatment groups were well-matched at baseline with regard to demographic variables, conventional vascular risk factors, and baseline laboratory values, including fasting cholesterol, glucose, creatinine, and tHcy. A significantly greater proportion of patients randomized to placebo compared with vitamins had experienced a TIA as their qualifying event (19% versus 10%; \( P = 0.03 \)).

As previously reported, mean tHcy levels at 6 months were 9.1 μmol/L in patients randomized to vitamins compared with 12.8 μmol/L in those randomized to placebo (difference=-3.7 μmol/L; 95% CI, 2.7 to 4.7 μmol/L; \( P < 0.001 \)).

There was no significant difference between the 2 randomized treatment groups for any of the inflammatory, endothelial dysfunction, or hypercoagulability markers examined (Table).

**Discussion**

The results of this randomized trial suggest that treatment with folic acid, cobalamin, and pyridoxine does not significantly alter blood concentrations of putative blood markers of inflammation, endothelial cell dysfunction, or hypercoagulability at 6 months, despite significantly reducing blood concentrations of tHcy by a mean of 3.7 μmol/L.

We believe that our results are valid and unlikely to reflect poor compliance or an inadequate dose or duration of vitamin treatment, because random allocation to vitamin therapy was associated with an expected almost 30% reduction in tHcy at 6 months. Although it is possible that the concentrations of biomarkers at baseline (before randomization) may have been different in the vitamin and placebo groups, we think this is unlikely because the 2 treatment groups were otherwise well-matched at baseline with regard to demographic variables, conventional vascular risk factors, and baseline laboratory values, including fasting cholesterol, glucose, creatinine, and tHcy. The only difference was a greater proportion of patients with TIA in the group randomized to placebo compared with vitamin therapy (19% versus 10%; \( P = 0.03 \)).
We also believe that our results do not reflect random error because of lack of statistical power. Our data are consistent with those of 2 recent studies.\textsuperscript{5,6}

The possible explanations for these findings are that these biomarkers are not sensitive to the effects of lowering tHcy (eg, multiple risk factor interventions may be required) and elevated tHcy causes cardiovascular disease by mechanisms other than the biomarkers measured, or elevated tHcy is a noncausal marker of increased vascular risk.

References
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