L-folinic acid versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients

Rapid Communication

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L-folinic acid versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients.

Background. The hyperhomocysteinemia found in most hemodialysis patients is refractory to combined oral B-vitamin supplementation featuring supraphysiological doses of folic acid (FA). We evaluated whether a high-dose L-folinic acid-based regimen provided improved total homocysteine (tHcy)-lowering efficacy in chronic hemodialysis patients, as suggested by a recent uncontrolled report.

Methods. We block-randomized 48 chronic, stable hemodialysis patients based on their screening predialysis tHcy levels, sex, and dialysis center into two groups of 24 subjects treated for 12 weeks with oral FA at 15 mg/day or an equimolar amount (20 mg/day) of oral L-folinic acid (FNA) [L-5-formyltetrahydrofolate]. All 48 subjects also received 50 mg/day of oral vitamin B6 and 1.0 mg/day of oral vitamin B12.

Results. The mean percentage (%) reductions (with 95% CIs) in predialysis tHcy were not significantly different [FNA = 22.1% (11.8 to 31.4%), FA = 20.7% (11.7 to 30.5%), P = 0.950 by paired t test]. Final on-treatment values (mean with 95% CI) were as follows: FNA, 15.9 μmol/L (14.0 to 18.0); FA, 16.9 μmol/L (14.8 to 18.8). Moreover, in those subjects with baseline tHcy levels ≥14 μmol/L, neither treatment resulted in “normalization” of tHcy levels (that is, final on-treatment values <12 μmol/L) among a significantly different or clinically meaningful number of patients [FNA = 2 out of 22 (9.1%); FA = 2 out of 24 (8.3%); Fisher’s exact test of between groups difference, P = 1.000].

Conclusions. Relative to high-dose FA, high-dose oral L-folinic acid-based supplementation does not afford improved tHcy-lowering efficacy in hemodialysis patients. The preponderance of hemodialysis patients (that is, >90%) exhibits mild hyperhomocysteinemia refractory to treatment with either regimen.

Key words: homocysteine, end-stage renal disease, vitamin B, maintenance hemodialysis.

METHODS

The institutional review board at Rhode Island Hospital (Providence, RI, USA) approved the study protocol,
and all participants provided written informed consent. Participants were 48 chronic (that is, hemodialysis duration ≥6 months), stable hemodialysis patients free of malignancy, end-stage congestive heart failure, active liver or thyroid disease, uncontrolled diabetes, and clinical malnutrition, whose serum albumin was ≥3.0 mg/dL.

As per the standard of care for hemodialysis centers in Rhode Island, all patients were prescribed a daily multivitamin that contained 1.0 mg FA, 10.0 mg vitamin B₆, and 0.012 mg vitamin B₁₂. This baseline supplementation regimen was continued throughout the 12-week investigation. Study participants were matched in specific pairs on the basis of sex, dialysis center, and their screening (initial) nonfasting, prehemodialysis tHcy levels (that is, within ≦2 μmol/L; mean between pair difference = 0.7 μmol/L). They were then randomly assigned in paired blocks to one of two treatment regimens: FA group-FA 15.0 mg/day, vitamin B₆ 50.0 mg/day, vitamin B₁₂ 1.0 mg/day (N = 24); 5-folic acid (FNA) group-[5,10-methylenetetrahydrofolate] (Eprova, Zurich, Switzerland) 20.0 mg/day (that is, equimolar to 15.0 mg/day FA), vitamin B₆ 50.0 mg/day, vitamin B₁₂ 1.0 mg/day (N = 24). Treatment assignments were made blinded to all other aspects of the study. Laboratory analyses, data entry, and data analyses were performed by code so that treatment assignments remained concealed. Compliance with treatment was assessed by pill counts and determination of the change in plasma vitamin status.

Nonfasting, prehemodialysis blood samples were collected twice before treatment and twice during the 12th week of treatment, as described elsewhere [3]. Plasma tHcy levels were determined by high-performance liquid chromatography (HPLC) with fluorescence detection [5]. Plasma total folate levels were measured by a microbiological (Lactobacillus casei) assay [6]. Plasma pyridoxal 5’-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay [7], and plasma vitamin B₁₂ levels were ascertained by radioassay. To eliminate interassay variability, all analytes were batch assayed from aliquots (which had been cryopreserved at −70°C) obtained during each of the four study visits.

Using tHcy data obtained from all 48 participants at the initial pretreatment screening, with 24 subjects block randomized to each of the two groups, we estimated, a priori, that there was 86% power at a two-tailed α of 0.05 to detect a 4.5 μmol/L (that is, a clinically relevant) difference in the pretreatment to post-treatment change in tHcy comparing the 5-folic acid and FA treatment groups [8].

All laboratory analyte values reported are based on averages of two pretreatment and post-treatment values. Descriptive statistics included arithmetic means (with 95% CIs), and frequencies (percentages). Baseline continuous variables were compared by paired t tests, and categorical variables were compared by chi square analysis. Continuous variables were assessed using both untransformed and (natural log) transformed values. Treatment effects on percentage changes in tHcy levels were presented as [(average pretreatment level − average post-treatment level) ÷ average pretreatment level] × 100, and were compared by paired t tests. Overall compliance with the study capsules was confirmed by assessing the mean increase (percentage change) in plasma PLP and vitamin B₁₂ levels among all 48 participants, using paired t tests. Reported P values were based on two-tailed calculations. All statistical analyses were performed by using SYSTAT software (version 9.0; SPSS, Chicago, IL, USA).

RESULTS

As depicted in Table 1, block randomization was successful with respect to the key baseline covariates. All 48 patients completed the entire study protocol. Average compliance by pill count was 90.0% (91.1% in the FNA group and 88.5% in the FA group), a finding confirmed by marked, significant (by paired t tests) increases in the mean plasma levels of both PLP (49.9%, P = 0.001) and vitamin B₁₂ (152.4%, P < 0.001) in all 48 subjects. Following treatment, both groups evidenced similar, marked elevations in mean plasma total folate [FNA (N = 24) = +371.7 ng/mL; FA (N = 24) = +512.6 ng/mL; P = 0.119 by paired t test].

We have presented results evaluating the between groups change in tHcy levels based on the untransformed continuous variable data only, as use of the transformed data did not alter the findings. There was no significant between-group difference in tHcy-lowering treatment responsiveness (Table 2). Mean percentage (%) reductions (±95% CIs) in predialysis tHcy were as follows: FNA group, 22.1% (11.8 to 31.4%); FA group, 20.7% (11.7 to 30.5%); P = 0.950. Final on-treatment tHcy values (mean with 95% CI) were as follows: FNA group, 15.9 μmol/L (14.0 to 18.0); FA group, 16.9 μmol/L (14.8

### Table 1. Baseline characteristics by treatment group

<table>
<thead>
<tr>
<th></th>
<th>L-folic acid</th>
<th>Folic acid</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td>Sex, N (% women)</td>
<td>10 (41.7%)</td>
<td>10 (41.7%)</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 (15)</td>
<td>64 (12)</td>
<td>0.383</td>
</tr>
<tr>
<td>tHcy (μmol/L)</td>
<td>20.4 (19.0–21.8)</td>
<td>21.3 (19.9–22.7)</td>
<td>0.239</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>36.6 (28.0–45.2)</td>
<td>30.2 (22.7–37.7)</td>
<td>0.258</td>
</tr>
<tr>
<td>PLP (nmol/L)</td>
<td>119.3 (77.4–161.2)</td>
<td>82.6 (55.7–109.5)</td>
<td>0.200</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>736 (563–910)</td>
<td>631 (554–709)</td>
<td>0.270</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>8.6 (6.9–10.3)</td>
<td>8.4 (7.2–9.6)</td>
<td>0.807</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.8 (3.7–4.0)</td>
<td>3.7 (3.5–3.8)</td>
<td>0.549</td>
</tr>
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*Based on paired t test or Fisher’s exact test

*Mean (standard deviation); [complete range]

*Mean (95% confidence interval)
to 18.8). Finally, we performed a post hoc, unpaired analysis among all patients with pretreatment tHcy levels ≥14 μmol/L (that is, 46 of the total N = 48). These data revealed that neither treatment resulted in “normalization” of tHcy levels (that is, final on-treatment values <12 μmol/L) among a significantly different or clinically meaningful number of patients [FNA = 2 out of 22 (9.1%); FA = 2 out of 24 (8.3%); Fisher’s exact test of between groups difference, P = 1.000].

**DISCUSSION**

Our investigation represents the initial controlled comparison of oral high-dose l-folinic acid [l-5-formyltetrahydrofolate] versus equimolar FA-based supplementation for the treatment of mild hyperhomocysteinemia among chronic, stable hemodialysis patients. Relative to FA-based supplementation, we have demonstrated that comparable supraphysiological-dose l-folinic acid-based supplementation does not provide significantly greater reductions in fasting tHcy levels, assessed as either changes in mean levels, or the proportion of individuals with mild pretreatment hyperhomocysteinemia whose tHcy levels were normalized by treatment. The preponderance of hemodialysis patients (that is, >90%) who were in our study exhibited a mild hyperhomocysteinemia refractory to normalization with either regimen.

Touam et al, in an open-label, uncontrolled study of 37 hemodialysis patients that mean pretreatment tHcy levels of 37.3 μmol/L were lowered to a mean of 12.3 μmol/L post-treatment by administering 50 mg of intravenous d,l-folinic acid once per week after dialysis [4]. In contrast, another uncontrolled study reported by Bayes et al revealed that postdialysis treatment with 10 mg intravenous d,l-5-formyltetrahydrofolate (folic acid) three times per week (that is, a total of 30 mg/week) lowered mean tHcy levels to 21 μmol/L post-treatment from a pretreatment mean of 38 μmol/L [abstract; *J Am Soc Nephrol* 10:274A, 1999]. Using a controlled design, we could not confirm the earlier findings by Touam et al [4]. There are probably three main reasons for these discordant results. First, inflated effect size estimates are characteristic of the uncontrolled, quasi-experimental design employed by Touam et al [4], due to a host of threats to internal validity [9]. Second, several of the subjects in the study by Touam et al also received high-dose (that is, 1 mg/day) oral vitamin B12, and mean levels of vitamin B12 for the entire study group actually doubled over the duration of the investigation [4]. Third, the standard daily multivitamin regimen prescribed to essentially all U.S. hemodialysis patients, including those we studied who complied with the regimen, eliminates potential cases of folate deficiency and perhaps vitamin B12 deficiency as well. In contrast, the hemodialysis patients studied by Touam et al were selected on the basis of either not receiving, or being noncompliant with, oral FA-based B vitamin supplementation [4]. Finally, it should be noted that the preliminary uncontrolled data reported by Perna et al in 1997 indicated that two months of oral supplementation with 5-methyltetrahydrofolate at 15 mg per day in 10 hemodialysis patients whose pretreatment tHcy levels were between approximately 13 and 72 μmol/L caused a mean reduction in their predialysis tHcy levels of approximately 61% (that is, from a pretreatment mean of ~38 μmol/L to ~15 μmol/L post-treatment) [10]. However, we recently reported the results from a randomized controlled comparison of the tHcy-lowering efficacy of equimolar amounts of L-5-methyltetrahydrofolate (MTHF; 17 mg/day) versus FA (15 mg/day)-based B vitamin supplementation in 50 (that is, two matched groups of 25) hemodialysis patients [11]. After 12 weeks of treatment, the mean percentage reductions in predialysis tHcy were not significantly different (MTHF, 17.0%; FA, 14.8%; P = 0.444) [11].

Limited experimental observations and human data have fostered suggestions that there may be decreased intestinal absorption, as well as general transmembrane transport of reduced folates, in uremia [12–14]. Live et al have further speculated that uremia could result in defective folate glutamation [15]. In a recent review, it was proposed that reduced folate administration could circumvent these speculative “defects” in folate metabolism and more effectively lower tHcy levels in ESRD compared with FA [16]. Two independent studies by our group, initially involving L-5-MTHF [11] and now L-folinic acid, have provided carefully controlled, definitive evidence that reduced folates afford no improved tHcy-lowering efficacy relative to FA among hemodialysis patients. Moreover, elegant folate distribution analyses performed in a subsample of hemodialysis patients from our study of MTHF [11] did not support any of the prior speculations regarding defective folate metabolism in ESRD [12–16]. Specifically, we observed both normal baseline levels of plasma 5-methyltetrahydrofolate and significant increases in total plasma folate, predominantly as plasma 5-methyltetrahydrofolate, after oral treatment with either FA, or L-5-methyltetrahydrofolate [11]. In addition, although other tissues were not sampled, normal baseline erythrocyte folate distributions

<table>
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<tr>
<th>Group</th>
<th>Decrease in tHcy %</th>
<th>Final tHcy level μmol/L</th>
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<tbody>
<tr>
<td>FNA (N = 24)</td>
<td>22.1† (11.8–31.4)</td>
<td>15.9† (14.0–18.0)</td>
</tr>
<tr>
<td>FA (N = 24)</td>
<td>20.7† (11.7–30.5)</td>
<td>16.9† (14.8–18.8)</td>
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</table>

Definitions are: FNA, 20.0 mg l-folinic acid, 50.0 mg vitamin B1, and 1.0 mg vitamin B12; FA, 15.0 mg folic acid, 50.0 mg vitamin B1, and 1.0 mg vitamin B12; tHcy, total homocysteine.

Values are mean (with 95% CI). *P = 0.950 for between groups difference by paired t test
with respect to both methyltetrahydrofolate predominance and glutamate chain length were observed [11]. Moreover, 5-methyltetrahydrofolate monoglutamate was the predominant folate form observed in plasma among all subjects sampled at baseline and post-treatment [11]. These findings also help explain the lack of difference in tHcy-lowering efficacy between orally and parenterally administered FA in the controlled comparison study reported by Tremblay et al [17]. Indeed, based on areaunder-the-curve analyses, there is no evidence that parenteral administration of FA or reduced folates results in greater circulating 5-MTHF levels relative to equimolar administration of oral FA, or reduced folates, such as folinic acid [18].

The folate refractory hyperhomocysteinemia in dialysis-dependent ESRD may reflect an inability to compensate for losses of normal renal homocysteine uptake and metabolism [19, 20], as well as the influence of unidentified factors causing extrarenal impairment in homocysteine metabolism [21, 22]. Data from our earlier study of L-5-MTHF [11], as well as the present study, strongly suggest that defects in folate absorption, or circulating plasma and tissue distribution, do not contribute to this persistent hyperhomocysteinemia.

In summary, relative to high dose FA, high-dose oral L-folic acid-based B vitamin supplementation does not afford improved tHcy-lowering efficacy in hemodialysis patients. The preponderance of hemodialysis patients (that is, >90%) exhibit mild hyperhomocysteinemia refractory to treatment with either regimen.

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REFERENCES