Controlled Comparison of L-5-Methyltetrahydrofolate Versus Folic Acid for the Treatment of Hyperhomocysteinemia in Hemodialysis Patients

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Background—The hyperhomocysteinemia regularly found in hemodialysis patients is largely refractory to combined oral B-vitamin supplementation featuring supraphysiological doses of folic acid. We evaluated whether a high-dose L-5-methyltetrahydrofolate–based regimen provided improved total homocysteine (tHcy)–lowering efficacy in chronic hemodialysis patients.

Methods and Results—We block-randomized 50 chronic, stable hemodialysis patients on the basis of their screening predialysis tHcy levels, sex, and dialysis center into 2 groups of 25 subjects treated for 12 weeks with oral folic acid at 15 mg/d (FA group) or an equimolar amount (17 mg/d) of oral L-5-methyltetrahydrofolate (MTHF group). All 50 subjects also received 50 mg/d of oral vitamin B6 and 1.0 mg/d of oral vitamin B12. The mean percent reductions (±95% CIs) in predialysis tHcy were not significantly different: MTHF, 17.0% (12.0% to 22.0%); FA, 14.8% (9.6% to 20.1%); P=0.444 by matched ANCOVA adjusted for pretreatment tHcy. Final on-treatment values (mean with 95% CI) were MTHF, 20.0 μmol/L (18.8 to 21.2 μmol/L); FA, 19.5 μmol/L (18.3 to 20.7 μmol/L). Moreover, neither treatment resulted in “normalization” of tHcy levels (ie, final on-treatment values <12 μmol/L) among a significantly different or clinically meaningful number of patients: MTHF, 2 of 25 (8%); FA, 0 of 25 (0%); Fisher’s exact test of between-groups difference, P=0.490.

Conclusions—Relative to high-dose folic acid, high-dose oral L-5-methyltetrahydrofolate–based supplementation does not afford improved tHcy-lowering efficacy in hemodialysis patients. The preponderance of hemodialysis patients (ie, >90%) exhibit mild hyperhomocysteinemia refractory to treatment with either regimen. This treatment refractoriness is not related to defects in folate absorption or circulating plasma and tissue distribution. (Circulation. 2000;101:2829-2832.)

Key Words: homocysteine ■ kidney ■ trials

Hyperhomocysteinemia, ie, elevated levels of plasma total homocysteine (tHcy), a putatively atherothrombotic sulfur amino acid,1,2 is observed in ≥85% of patients with end-stage renal disease (ESRD) undergoing maintenance peritoneal dialysis or hemodialysis.2 The mild hyperhomocysteinemia characteristic of dialysis patients has proved quite refractory to pharmacological doses of folic acid–based B-vitamin supplementation.1,3 For example, we reported earlier that a final on-treatment plasma tHcy <12 μmol/L could be achieved in only 1 of 15 dialysis patients (6.7%) despite 2 months of supplementation with a total of 16 mg/d folic acid, 110 mg/d vitamin B6, and 1 mg/d vitamin B12.3

Recently, findings from 2 open-label, uncontrolled investigations4,5 in which persons undergoing maintenance hemodialysis were supplemented orally4 or parenterally5 with high doses of either D,L-5-methyltetrahydrofolate4 or D,L-5-formyltetrahydrofolate4 suggested that these reduced folates might provide improved tHcy-lowering efficacy in patients with ESRD. In contrast, an additional uncontrolled study of parenteral D,L-5-formyltetrahydrofolate failed to confirm these findings.6 None of these preliminary investigations4–6 involved a controlled, direct comparison of the tHcy-lowering efficacy of a reduced folate versus folic (pteroylglutamic) acid among patients with ESRD. Accordingly, we conducted a block-randomized, controlled comparison of oral treatment with either high-dose L-5-methyltetrahydrofolate or folic acid, combined with oral vitamin B6 and vitamin B12, on predialysis plasma tHcy levels in 50 (ie, 2 matched groups of 25) maintenance hemodialysis patients.

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Methods

The institutional review board at Rhode Island Hospital, Providence, RI, approved the study protocol, and all participants provided written informed consent. Participants were 50 chronic (ie, hemodialysis [duration $\geq$ 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 $\geq$ 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 folic acid, 10.0 mg vitamin $B_{6}$, and 0.012 mg vitamin $B_{12}$. This baseline supplementation regimen was continued throughout the 12-week investigation. Study participants were matched on the basis of sex, dialysis center, and their screening (initial) nonfasting, prehemodialysis tHcy levels according to the following algorithm: tHcy 12 to 15 $\mu$mol/L ($n=2$), matched within $\pm 1$ $\mu$mol/L; tHcy >15 to 25 $\mu$mol/L ($n=38$), matched within $\pm 2$ $\mu$mol/L; tHcy >25 to 35 $\mu$mol/L ($n=4$), matched within $\pm 3$ $\mu$mol/L; tHcy >35 to 45 $\mu$mol/L ($n=4$), matched within $\pm 4$ $\mu$mol/L; and tHcy >45 $\mu$mol/L ($n=2$), matched within $\pm 5$ $\mu$mol/L. They were then randomly assigned in blocks to 1 of 2 treatment regimens: the folic acid (FA) group: folic acid 15.0 mg/d, vitamin $B_{6}$ 50.0 mg/d, vitamin $B_{12}$ 1.0 mg/d (n = 25) or the 5-methyltetrahydrofolate (MTHF) group: L-5-methyltetrahydrofolate (Eprova) 17.0 mg/d (ie, equimolar to 15.0 mg/d folic acid), vitamin $B_{6}$ 50.0 mg/d, vitamin $B_{12}$ 1.0 mg/d (n = 25). 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 week 12 of treatment, as described elsewhere. Plasma tHcy levels were determined by high-performance liquid chromatography (HPLC) with fluorescence detection, plasma total folate levels were measured by a microbiological (Lactobacillus casei) assay, plasma pyridoxal 5'-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay, and plasma vitamin $B_{12}$ levels were ascertained by radioassay. The distribution of erythrocyte and plasma folates was determined by affinity/HPLC, with electrochemical (coulometric) detection. This method separates and identifies folates on the basis of their pteridine ring structure and number of glutamate residues. Serum creatinine and albumin were measured by standard automated clinical chemistry laboratory techniques. To eliminate interassay variability, all analytes were batch-assayed from aliquots (which had been cryopreserved at $-70^\circ C$) obtained during each of the 4 study visits.

Using tHcy data obtained from all 50 participants at the initial pretreatment screening, with 25 subjects block-randomized to each of the 2 groups, we estimated that there was 85% power at a 2-tailed value of $\alpha=0.05$ to detect a 5.5-$\mu$mol/L difference in the pretreatment to posttreatment change in tHcy comparing the MTHF and FA treatment groups.

All laboratory analyte values reported are based on averages of 2 pretreatment and posttreatment values. Descriptive statistics included arithmetic or geometric means (with 95% CIs or complete ranges) and frequencies (percentages). Baseline continuous variables included arithmetic or geometric means (with 95% CIs or complete ranges) and frequencies (percentages). Baseline continuous variables were compared by paired t tests, and categorical variables were compared by $\chi^{2}$ analysis. Continuous variables were assessed with both untransformed and (natural log) transformed values. Treatment effects on percentage changes in tHcy levels were presented as (average posttreatment level−average pretreatment level)/average pretreatment level $\times 100$ and were compared by general linear modeling with ANCOVA. To assess the relative independent effects of the 2 treatments, the ANCOVA accounted for the pretreatment matching and adjusted for the pretreatment levels of tHcy. Overall compliance with the study capsules was confirmed by assessing the mean increase (percentage change) in plasma PLP and vitamin $B_{12}$ levels among all 50 participants by paired t tests. Reported probability values were based on 2-tailed calculations. All statistical analyses were performed with SYSTAT software (version 7.0.1, SPSS).

Results

As depicted in Table 1, block randomization was successful with respect to the key baseline covariables. In a subset of 10 patients for whom erythrocyte folate distribution was determined at baseline, 9 had 100% 5-methyltetrahydrofolate. One subject had evidence of formylated folates, consistent with the percent prevalence (ie, $=10\%$) of homozygosity for the C677T transition in the gene encoding methylenetetrahydrofolate reductase among both general and ESRD populations. Mean ($\pm 95\%$ CI) glutamate chain length of erythrocyte folates was 5.4 (5.2 to 5.6), comparable to previously reported normative control values. Baseline plasma folate distribution analyses from a separate subgroup of 10 randomly selected patients (ie, 5 MTHF versus FA group matched pairs) revealed that 5-methyltetrahydrofolate was the predominant fraction in both groups (MTHF, 76.2%; FA, 86.3%), and all folates were present as monoglutamate. All 50 patients completed the entire study protocol. Average compliance by pill count was 89.8% (91.2% in the MTHF group; 88.4% in the FA group), a finding confirmed by marked, significant ($P<0.001$ by paired t tests) increases in the mean plasma levels of both PLP (+69.0%) and vitamin $B_{12}$ (+40.5%). After treatment, both groups evidenced similar, marked elevations in plasma total folate: mean increase ($\pm 95\%$ CI): MTHF, +369.3 (266.5 to 472.1) ng/mL; FA, +436.5 (336.0 to 537.0) ng/mL; $P=0.379$ by ANCOVA adjusted for pretreatment plasma total folate. Consistent with an earlier report by Schmitz et al in healthy, nonuremic volunteers, ingestion of the large oral dose of folic (pteroylglutamic) acid did result in a sizable increase in this moiety in the FA group only. However, the posttreatment plasma folate distribution data from the random subgroup analyzed also confirmed that the enormous increase in plasma total folate resulted in similar, markedly elevated final on-treatment (geometric mean) levels of 5-methyltetrahydrofolate monoglutamate among both groups: MTHF, 277.2 ng/mL; FA, 230.6). Finally, as observed in the pretreatment analyses, all final on-treatment plasma folates were monoglutamated.
We present ANCOVA results evaluating the between-groups change in tHcy levels based on the untransformed continuous variable data only, because use of the transformed data did not alter the findings. ANCOVA (see Table 2) accounting for the pretreatment matching and adjusted for pretreatment levels of fasting tHcy did not reveal significant group differences in tHcy-lowering treatment responsiveness. Mean percent reductions (±95% CIs) in predialysis tHcy were MTHF group, 17.0% (12.0% to 22.0%); FA group, 14.8% (9.6% to 20.1%); for the final treatment mean tHcy levels were MTHF group, 20.0 μmol/L (18.8 to 21.2 μmol/L); FA group, 19.5 μmol/L (18.3 to 20.7 μmol/L). Finally, all patients had pretreatment tHcy levels ≥14 μmol/L, and neither treatment resulted in normalization of tHcy levels (ie, final on-treatment values <12 μmol/L) among a significantly different or clinically meaningful number of patients: MTHF, 2 of 25 (8%); FA, 0 of 25 (0%); Fisher's exact test of between-groups difference, P = 0.490.

**Discussion**

Our study represents the initial controlled comparison of oral high-dose l,5-methyltetrahydrofolate versus equimolar folic acid–based supplementation for the treatment of hyperhomocysteinemia among chronic, stable hemodialysis patients. We have demonstrated that at comparable supraphysiological doses, l,5-methyltetrahydrofolate–based supplementation does not afford significantly greater reductions in fasting tHcy levels relative to folic acid–based supplementation, gauged as either changes in mean levels or the proportion of individuals with mild pretreatment hyperhomocysteinemia whose tHcy levels were normalized by treatment. Indeed, the preponderance of hemodialysis patients (ie, >90%) we studied exhibited a mild hyperhomocysteinemia refractory to normalization with either regimen.

Preliminary, uncontrolled data reported by Perna et al indicated that 2 months of oral supplementation with 5-methyltetrahydrofolate at 15 mg/d in 14 hemodialysis patients caused a mean reduction in their predialysis tHcy levels of ~73% (ie, from a pretreatment mean of ~70 to 19 μmol/L after treatment). Even after 4 patients with pretreatment tHcy levels >100 μmol/L had been eliminated and the analysis restricted to the 10 patients whose pretreatment tHcy levels were between ~13 and 72 μmol/L (pretreatment mean of ~38 μmol/L), the posttreatment mean tHcy was ~15 μmol/L, a 61% reduction. In addition, 3 of 9 patients with pretreatment tHcy levels >20 μmol/L had final on-treatment levels maintained <12 μmol/L. Similar tHcy-lowering efficacy was reported in an open-label, uncontrolled study of 37 hemodialysis patients by Touam et al. By treating their subjects after dialysis with 50 mg of intravenous d,L-5-formyltetrahydrofolate (folic acid) once per week, these investigators reported that mean pretreatment tHcy levels of 37.3 μmol/L were lowered to a mean of 12.3 μmol/L after treatment. In contrast, another uncontrolled study reported by Bayes et al revealed that postdialysis treatment with 10 mg d,L-5-formyltetrahydrofolate (folic acid) IV 3 times per week (ie, a total of 30 mg/wk) lowered mean tHcy levels to 21 μmol/L after treatment from a pretreatment mean of 38 μmol/L. Using a controlled design, we could not confirm the earlier findings by Perna et al or Touam et al. There are probably 3 main reasons for these discordant results. First, inflated effect size estimates are characteristic of the uncontrolled, quasi-experimental design used by Perna et al and Touam et al because of a host of threats to internal validity. Second, Perna et al provide no data regarding either baseline or within-study changes in the plasma status of folate or vitamins B12 and B6 (ie, as PLP). In addition, several of the subjects in the study by Touam et al also received high-dose (ie, 1 mg/d) oral vitamin B12, and mean levels of vitamin B12 for the entire study group actually doubled over the duration of the investigation. Third, when complied with, the standard-of-care daily multivitamin regimen prescribed to essentially all US hemodialysis patients, including those we studied, eliminates potential cases of folate deficiency and perhaps B12 deficiency as well. In contrast, the hemodialysis patients studied by Perna et al were withdrawn from any supplementation with B vitamins for 2 months before receiving 2 months of oral d,L-methyltetrahydrofolate, whereas those investigated by Touam et al were similarly selected on the basis of either not receiving or being noncompliant with oral folic acid–based B-vitamin supplementation.

Experimental observations and very limited human data have fostered suggestions that there may be decreased intestinal absorption, as well as general transmembrane transport of reduced folates, in uremia. Livant et al have further speculated that uremia could result in defective folate glutationation. Moreover, a recent review proposed that reduced folate administration could circumvent these speculative "defects" in folate metabolism and more effectively lower tHcy levels in ESRD compared with folic acid. In addition to presenting carefully controlled, definitive evidence that reduced folates provide no improved tHcy-lowering efficacy relative to folic acid, our study does not support any of the previous speculations regarding defective folate metabolism in ESRD.

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 folic acid or l-5-methyltetrahydrofolate. Moreover, although other tissues
were not sampled, normal baseline erythrocyte folate distributions with respect to both methyltetrahydrofolate predominance and glutamate chain length were observed. Finally, 5-methyltetrahydrofolate monoglutamate was the predominant folate form observed in plasma among all subjects sampled at baseline and after treatment. The folate-refractory hyperhomocysteinemia in dialysis-dependent ESRD may reflect an inability to compensate for losses of normal renal homocysteine uptake and metabolism,\textsuperscript{21,22} as well as the influence of unidentified factors causing extrarenal impairment in homocysteine metabolism.\textsuperscript{23,24} Data from 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 folic acid, high-dose oral l-5-methyltetrahydrofolate–based B-vitamin supplementation does not afford improved tHcy-lowering efficacy in hemodialysis patients. The preponderance of hemodialysis patients (ie, >90%) exhibit mild hyperhomocysteinemia refractory to treatment with either regimen. The treatment refractoriness of the hemodialysis population stands in stark contrast to what we have demonstrated in the chronic stable renal transplant recipient population, ie, a mild hyperhomocysteinemia that can be consistently normalized with standard high-dose folic acid–based B-vitamin supplementation regimens. Specifically, in comparison to renal transplant recipients with equivalent baseline tHcy levels, the mild hyperhomocysteinemia of maintenance hemodialysis patients is much more refractory to tHcy-lowering B-vitamin treatment regimens featuring supraphysiologically amounts of folic acid or the reduced folate l-5-methyltetrahydrofolate.\textsuperscript{27} Accordingly, renal transplant recipients are a preferable target population for controlled clinical trials testing the hypothesis that tHcy-lowering B-vitamin intervention may reduce arteriosclerotic cardiovascular disease event rates in patients with chronic renal disease.\textsuperscript{28}

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