Effect of High Dose Folic Acid Therapy on Hyperhomocysteinemia in Hemodialysis Patients: Results of the Vienna Multicenter Study

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Abstract. Homocysteine is associated with atherosclerosis and enhanced cardiovascular risk. In previous studies, treatment with folic acid up to 15 mg/d failed to correct hyperhomocysteinemia in the majority of end-stage renal disease patients. A dose of 30 or 60 mg of folic acid per day was compared with 15 mg/d in an attempt to normalize hyperhomocysteinemia in 150 hemodialysis patients. In a randomized, double-blind, multicenter study, 144 patients completed the 4-wk treatment period and 121 patients completed the 6-mo follow-up. Total homocysteine plasma levels were reduced by 32.1% (15 mg/d), 29.9% (30 mg/d), or 37.8% (60 mg/d) with no significant differences found between the three treatment groups. Baseline total homocysteine plasma concentration was an independent predictor of the response to folic acid therapy (P = 0.0001), whereas the 5,10-methylenetetrahydrofolate reductase polymorphisms (MTHFR 677C → T and 1298A → C) had no influence. Nevertheless, patients with the MTHFR 677TT genotype more frequently attained normal total homocysteine plasma levels than patients with the CC or CT genotype (P = 0.025). In response to 60 mg of folic acid per day, TT genotype patients had lower folate plasma levels compared to CC or CT genotype patients (P = 0.016). After completion of the 4-wk treatment period with 30 or 60 mg of folic acid per day, there was a marked rebound of total homocysteine plasma levels at the end of the follow-up in patients with the MTHFR 677TT genotype, which even exceeded baseline values in several patients (P = 0.0001). This study clearly demonstrates that doses of 30 or 60 mg of folic acid per day are not more effective than 15 mg/d in reducing hyperhomocysteinemia in regular hemodialysis patients. Patients with the MTHFR 677TT genotype are more likely to realize normal total homocysteine plasma levels. Folic acid at 30 or 60 mg/d but not 15 mg/d results in a rebound of total homocysteine plasma concentrations when treatment is stopped.

Plasma concentrations of homocysteine moieties, referred to as total homocysteine or homocyst(e)ine, are elevated in the majority of patients with end-stage renal failure (1–5). Total homocysteine plasma concentration is a risk factor for atherosclerosis as well as morbidity and mortality associated with cardiovascular disease (6–9). An increase of 1 μmol/L of total homocysteine enhances this risk by 1% in uremic patients (10). Although not proven, lowering total homocysteine plasma concentrations by folic acid and/or vitamin B₁₂ intervention is assumed to reduce the risk of atherosclerosis and cardiovascular disease in all hyperhomocysteinemic patients. Wilcken et al. (11) were the first to demonstrate that 5 mg/d of folic acid can normalize total free homocysteine plasma levels in some patients with renal failure. However, in a randomized, placebo-controlled study, Bostom et al. (12) showed that a daily dose of 15 mg of folic acid lowered elevated total homocysteine plasma concentrations to the normal range in only five of 15 patients on regular hemodialysis treatment. Therefore, we tested the hypothesis that 30 or 60 mg of folic acid per day for 4 wk may be more effective than 15 mg/d in lowering total homocysteine plasma concentrations in patients maintained on regular hemodialysis treatment. Because genetic variants of the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) can also influence homocysteine metabolism (13–15), we further examined whether the polymorphisms 677C → T and 1298A → C of MTHFR modulate the response to high dose folic acid therapy. We followed the patients for an additional 6 mo after treatment was stopped to assess the long-term results of folic acid therapy.
Materials and Methods

This multicenter study was conducted in four hemodialysis units including one university-based hospital (Division of Nephrology and Dialysis, Department of Medicine III, University Hospital of Vienna) and three university-affiliated teaching hospitals (Department of Medicine I, Donauspital; Department of Medicine III, Krankenhaus Lainz; Department of Medicine I, Kaiser Franz Josef Spital) in Vienna. The study was initiated in March 1997 and completed in April 1998.

Based on the assumption that the reference dose of folic acid (15 mg) can reduce a mean total homocysteine plasma level of 24 μmol/L by 30% (8 μmol/L), our study had a power of 80% (α level of 0.05) to detect an 11 μmol/L reduction (45%) of total homocysteine plasma levels by the test dose (30 or 60 mg of folic acid) with 50 patients in each treatment group.

The study protocol was approved by the review boards of each of the four hospitals according to the Declaration of Helsinki and the Austrian Law on Gene Technology. Two hundred and two regular hemodialysis patients were eligible for the study, and all 150 patients who enrolled in the study gave written informed consent. Randomization to treatment was performed according to a blocked code generated by a computer at the Institute of Medical Statistics at the University of Vienna. Capsules containing 15, 30, or 60 mg of folic acid were manufactured at the pharmacy of the University Hospital of Vienna under supervision of a pharmacist. Patients were consecutively assigned to treatment at the four dialysis units. Both study physicians and patients were blinded to the treatment code. The patients were instructed to bring back the capsule container for compliance control at the end of the study. Patient inclusion criteria were: a minimum age of 19 yr, maintenance on regular hemodialysis treatment for at least 4 wk, and normal or elevated total homocysteine plasma concentration. Exclusion criteria were: hemoglobin of ≤8 g/dl, the presence of megaloblastic anemia due to vitamin B12 deficiency without concomitant vitamin B12 therapy, and current treatment with anticonvulsive agents. The majority of the patients were on regular therapy with recombinant human erythropoietin and iron.

Study Protocol

Patients were randomly assigned to take 15, 30, or 60 mg of folic acid per day for 4 wk (treatment period), and were followed for 6 mo after stopping folic acid therapy (follow-up period) in an attempt to document the decline of folate plasma levels and any increase in total homocysteine plasma concentrations over time. Based on the study of Bostom et al. (12), which showed that 8 wk of folic acid therapy was not more effective than 4 wk, a 4-wk treatment period was chosen. Predialysis blood samples for analysis of total homocysteine, folate, and vitamin B12 plasma levels, as well as for analysis of MTHFR 677C → T and 1298A → C genotypes, were collected at baseline. Total homocysteine, folate, and vitamin B12 plasma levels were measured weekly during the 4-wk treatment period, and every 4 wk thereafter until completion of the 24-wk follow-up period. Potential side effects of treatment were assessed by interview during each hemodialysis session.

Biochemical Assays

Before the dialysis session, blood anticoagulated with citrate was drawn, immediately placed on ice, and centrifuged within 30 min at 2000 × g at 4°C (20 min). Plasma aliquots and 500 μl of citrated blood for DNA isolation were snap-frozen and stored at −70°C.

Using the method originally described by Araki and Sako (16) as a basis for this study, total homocysteine (= free and protein-bound homocysteine-derived moieties in either sulfhydryl or disulfide form) plasma concentrations were determined by automated HPLC with reversed-phase separation and fluorescence detection. Hyperhomocysteinemia was defined as total homocysteine levels >15 μmol/L. Intraassay variability was 1.4 to 1.7% and interassay variability was 1.5 to 1.9%.

Folate (5-methyltetrahydrofolate) and vitamin B12 plasma levels were measured with a radioassay to allow for simultaneous determination of both vitamin concentrations in a single reaction tube (SimuTRAC – SNB; Becton Dickinson, Toronto, Ontario, Canada). Folate deficiency was defined as a plasma concentration of <3.4 nmol/L. Vitamin B12 deficiency was defined as a plasma concentration of <118 pmol/L.

Identification of the 677C → T transition and the 1298A → C transversion in the MTHFR gene was performed according to Frosst et al. (13) and Weisberg et al. (15), using restriction fragment length polymorphism analysis. According to the coding sequence of MTHFR (GenBank accession no. U09806), the correct positions of the two polymorphisms 677C → T and 1298A → C are 668C → T and 1298A → C, respectively. However, we refer to the primarily published nucleotide positions. The identification of the 677T allele is possible by restriction enzyme cleavage of a 198-bp PCR product using Hinfl (13). The presence of the mutation creates a Hinfl recognition sequence that leads to cleavage of the 198-bp product into fragments of 175 and 23 bp. Therefore, heterozygous subjects show three fragments (198, 175, and 23 bp, respectively), whereas individuals homozygous for the mutant T allele display only the two fragments of 175 and 23 bp. The identification of the 1298A → C polymorphism was performed according to the protocol of Weisberg et al. (15), because it allows for the discrimination of the 1298A → C polymorphism from a silent 1317T → C transition in the same exon, which is not the case using the restriction enzyme MboII (14). The PCR primers published by Weisberg et al. (15) generate a 138-bp PCR fragment that is cleaved by the restriction enzyme Fnu4HI into 119- and 19-bp fragments in the presence of the mutation.

Statistical Analyses

Descriptive statistics included mean values ± SD for continuous data and percentages for categorical data. Mean values with 95% confidence intervals (CI) are also expressed for the key end points of total homocysteine and folate plasma levels.

For age, time on treatment, and the baseline values of total homocysteine and folate plasma levels, a one-way ANOVA of the grouping variable doses was performed to exclude potential differences between the three different dose groups at baseline. Differences in gender and mode of dialysis treatment were analyzed by χ² test. Differences between the four study centers were also assessed by ANOVA.

The effect of the 30- or 60-mg folic acid dose per day on total homocysteine plasma concentrations compared to the standard dose of 15 mg/d was analyzed using data from all 144 patients who completed the 4-wk treatment period. Comparisons over time were performed by an ANOVA with repeated measurements for 4 wk (time point 0, and weeks 1, 2, 3, and 4). This analysis included the grouping variable MTHFR 677C → T genotype (CC, CT, TT), the regression variable doses (15, 30, or 60 mg of folic acid per day), the interaction “doses × MTHFR 677C → T,” and the values of the baseline total homocysteine plasma concentrations as covariates (using the individual deviations from the median of the total sample). A similar analysis was performed for the folate plasma concentrations and for the grouping variable MTHFR 1298A → C (AA, AC, CC).

For the follow-up period, an ANOVA with repeated measurements
was performed for the time points of weeks 4, 8, 12, 16, 20, 24, and 28 with the grouping variable \( \text{MTHFR} \, 677C \rightarrow T \) (CC, CT, TT), the covariable doses (15, 30, or 60 mg/d), and the interaction term “doses \( \times \text{MTHFR} \, 677C \rightarrow T \).” This analysis was performed for total homocysteine and folate plasma levels for all 121 patients who completed the follow-up period.

Comparison of the number of patients who had hyperhomocysteinemia at baseline and who completed the 4-wk study period (\( n = 120 \)) with normal total homocysteine plasma concentrations (<15 \( \mu \text{mol/L} \) versus patients presenting with persistent hyperhomocysteinemia at time point week 4, according to the \( \text{MTHFR} \, 677C \rightarrow T \) genotype (TT genotype versus pooled CC and CT genotypes), was performed by Mantel-Haenszel \( \chi^2 \) test.

Comparison of the proportion of patients who had folate plasma levels of <100 nmol/L at 4 wk after stopping folic acid therapy who were either treated by low-flux dialysis or high-flux dialysis and hemodiafiltration was performed by \( \chi^2 \) test for each treatment group. All calculations were performed using the statistical software package SAS (SAS Institute, Inc., Cary, NC).

**Results**

**Participation Data and Dropouts**

Of the 150 randomized participants, 148 underwent baseline testing (two patients refused to participate after the randomization procedure), 144 completed the treatment period, and 121 completed the entire study including the 6-mo follow-up period (Figure 1). The four patients who did not complete the treatment period either received a kidney graft or died from myocardial infarction, a stroke, or gastrointestinal bleeding. Of the remaining 23 patients who did not complete the follow-up period, seven received a kidney graft, 14 died, and two were moved to another center and lost to follow-up. All three deaths during the intervention period were not related to treatment with the study medication as judged by the physicians in charge of the patients’ care. Few adverse events were reported during the treatment period. One patient developed a general, short-lasting exanthema during the treatment period that was judged to be unrelated to the study medication by an independent dermatologist. Another patient complained about nebulous gastrointestinal symptoms in conjunction with the intake of the study medication but agreed to continue the study and completed it without further symptoms.

**Demographic and Clinical Characteristics**

Selected demographic and clinical parameters of the 148 patients who underwent baseline testing are shown in Table 1. Patient characteristics and dialysis modalities according to the four participating centers are given in Table 2. ANOVA revealed that there was no difference in baseline values of total homocysteine and folate plasma levels between the three treatment groups (Tables 3 and 4). There was also no difference in age, gender, and time on treatment and number of patients treated either by low-flux dialysis, high-flux dialysis, or hemodiafiltration between the three treatment groups.

![Figure 1. Flow of participants. R, randomization; TX, kidney transplantation.](image-url)
Effect of High Dose Folic Acid Therapy on Total Homocysteine Plasma Concentrations

Total homocysteine plasma concentrations declined rapidly within 1 wk of high dose folic acid therapy and then remained stable in the majority of patients throughout the rest of the treatment period (Table 3). Total homocysteine plasma concentrations of the 144 patients according to the three folic acid dose groups are displayed in Figure 2.

One hundred and twenty of the 144 patients who completed folic acid therapy presented with hyperhomocysteinemia at baseline. Demographics and clinical characteristics of 148 hemodialysis patients who were enrolled in the study are shown in Table 1. The majority of patients were male (90/58), with a mean age of 56.0 ± 14.6 yr. The duration of dialysis treatment ranged from 1.3 to 6.0 yr, with a mean of 2.1 ± 1.6 yr. Baseline total homocysteine (tHcy) plasma concentration was 25.0 ± 11.3 μmol/L, with 123 patients having a concentration above 15 μmol/L. Baseline folate plasma concentration was 26.4 ± 31.8 nmol/L.

Table 1. Demographics and clinical characteristics of 148 hemodialysis patients who were enrolled in the studya

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Center</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>No. of patients</td>
<td>47</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50.5 ± 14.0b</td>
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<tr>
<td>Male/female</td>
<td>31/16</td>
</tr>
<tr>
<td>Duration of dialysis treatment (yr)</td>
<td>2.6 ± 2.7</td>
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<tr>
<td>Mode of treatment (LF/HF/HDF)</td>
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<td>Baseline tHcy plasma level (μmol/L)</td>
<td>25.3 ± 9.0</td>
</tr>
<tr>
<td>Baseline folate plasma levels (nmol/L)</td>
<td>30.3 ± 35.9</td>
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</tbody>
</table>

a Results are given as mean ± SD. tHcy, total homocysteine; MTHFR, 5,10-methylenetetrahydrofolate reductase.

b P < 0.05 versus all other centers.

c P < 0.05 versus all other centers.

Table 2. Patient characteristics according to study centera

<table>
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<th>Center</th>
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<tr>
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<tr>
<td>Daily Folic Acid Dose</td>
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</tr>
<tr>
<td></td>
<td>15 mg</td>
</tr>
<tr>
<td></td>
<td>tHcy (μmol/L)</td>
</tr>
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<td>16.3 ± 7.6</td>
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<td>2</td>
<td>16.9 ± 7.5</td>
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<tr>
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<td>16.5 ± 7.2</td>
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<td>8</td>
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<td>12</td>
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<td>17.5 ± 7.3</td>
</tr>
<tr>
<td>20</td>
<td>18.4 ± 7.2</td>
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<tr>
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<td>18.6 ± 8.5</td>
</tr>
<tr>
<td>28</td>
<td>21.6 ± 11.2</td>
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</table>

a Results are given as mean ± SD. n = number of patients. tHcy, total homocysteine; CI, confidence interval.
b Baseline ANOVA, NS.
baseline and had their total homocysteine plasma concentration reduced by an average of 32.8% (range, –82.9 to +26.4%) at the end of the treatment period. At week 4, 38 of these 120 patients (31.7%) had their total homocysteine plasma levels normalized. Nine patients (7.5%) had higher total homocysteine plasma concentrations compared to baseline. Four of these patients had folate plasma levels <1000 nmol/ml and were presumably noncompliant.

In contrast, 12 of 24 patients (50%) with normal total homocysteine plasma levels at baseline had even higher plasma levels at week 4. Two of these 12 presented with moderate hyperhomocysteinemia at this time point (mean reduction of total homocysteine plasma levels in all 24 patients by 2.2%; range, –36.0 to +43.2%). One of these patients had low folate plasma levels, indicating noncompliance with folic acid therapy.

Standard dose of 15 mg of folic acid per day resulted in a reduction of mean total homocysteine plasma concentration by 32.8% (range, –82.8 to +26.4%). However, there was no significant improvement in treatment efficacy in patients treated with 30 mg (–29.9%; range, –69.6 to +43.2%) or 60 mg of folic acid (–37.8%; range, –76.4 to +39.6%) per day (P = 0.1433 and P = 0.2880 for both MTHFR polymorphism models). The MTHFR 677C → T and 1298A → C polymorphisms had no influence on treatment efficacy in this analysis (P = 0.980 and P = 0.2240, respectively). There also was no significant effect based on the interaction “folic acid doses × MTHFR 677C → T genotype” or “folic acid doses × MTHFR 1298A → C genotype” (P = 0.123 and P = 0.922, respectively). A highly significant independent predictor of the effect of folic acid therapy on total homocysteine plasma concentrations was the total homocysteine plasma level before treatment in both MTHFR genotype models (P = 0.0001 and P = 0.0001, respectively).

### Table 4. Folate plasma concentrations before (0), during 4 wk of daily 15, 30, or 60 mg of folic acid therapy (1 to 4), and during 24 wk after stopping folic acid treatment (8 to 28)a

<table>
<thead>
<tr>
<th>Week</th>
<th>15 mg</th>
<th></th>
<th>30 mg</th>
<th></th>
<th>60 mg</th>
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<tr>
<td></td>
<td>Folate (nmol/L)</td>
<td>95% CI</td>
<td>n</td>
<td>Folate (nmol/L)</td>
<td>95% CI</td>
<td>n</td>
</tr>
<tr>
<td>0b</td>
<td>32.5 ± 45.6</td>
<td>19.5 to 45.5</td>
<td>50</td>
<td>26.1 ± 26.3</td>
<td>18.6 to 33.6</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>2261 ± 1740</td>
<td>1761 to 2761</td>
<td>49</td>
<td>4758 ± 3088</td>
<td>3871 to 5645</td>
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<td>2024 to 2990</td>
<td>49</td>
<td>4742 ± 2811</td>
<td>3934 to 5550</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>2896 ± 2287</td>
<td>2239 to 3553</td>
<td>49</td>
<td>6181 ± 4285</td>
<td>4950 to 7412</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>1899 ± 1490</td>
<td>1471 to 2327</td>
<td>49</td>
<td>4696 ± 3431</td>
<td>3699 to 5692</td>
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<tr>
<td>5</td>
<td>79.4 ± 39.7</td>
<td>67.7 to 91.1</td>
<td>47</td>
<td>117.7 ± 95.3</td>
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<tr>
<td>6</td>
<td>43.6 ± 25.7</td>
<td>35.9 to 51.2</td>
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<td>45.7 ± 29.4</td>
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<tr>
<td>7</td>
<td>31.8 ± 18.8</td>
<td>26.1 to 37.5</td>
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<td>8</td>
<td>33.2 ± 50.0</td>
<td>17.7 to 48.8</td>
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<td>49.7 ± 160.8</td>
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<td>45</td>
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<tr>
<td>9</td>
<td>28.7 ± 24.7</td>
<td>21.0 to 36.4</td>
<td>42</td>
<td>21.2 ± 10.6</td>
<td>18.0 to 24.5</td>
<td>44</td>
</tr>
<tr>
<td>10</td>
<td>26.2 ± 13.8</td>
<td>21.8 to 30.6</td>
<td>41</td>
<td>24.8 ± 20.6</td>
<td>18.5 to 31.3</td>
<td>42</td>
</tr>
</tbody>
</table>

a Results are given as mean ± SD. n = number of patients. CI, confidence interval.  

b Baseline ANOVA, NS.

### Effect of High Dose Folic Acid Therapy on Folate Plasma Concentrations

Folate plasma concentrations increased rapidly with folic acid therapy and remained high throughout the treatment period. The folate plasma levels of all three patient groups are displayed in Table 4. The time course of folate plasma levels in the 144 patients who completed the intervention period are shown in Figure 2. There was a significant difference in folate plasma levels in the three different dose groups (P = 0.0001). Neither MTHFR 677C → T nor MTHFR 1298A → C demonstrated an independent influence on folate plasma levels. Baseline folate plasma levels did not influence the response to folic acid therapy as measured by folate plasma levels in both MTHFR models (P = 0.1378 and P = 0.1929, respectively). Conversely, there was a significant interaction between “doses × MTHFR 677C → T” (P = 0.0163): We observed a significant smaller increase of folate plasma levels in response to 60 mg of folic acid per day in patients who were homozygous for the MTHFR 677C → T transition (TT genotype) compared with MTHFR 677CT or MTHFR 677CC (Figure 3). This interaction was not significant for the other MTHFR polymorphism (“doses × MTHFR 1298A → C”, P = 0.1002).

### Total Homocysteine Plasma Levels during the Follow-Up Period

The follow-up values of total homocysteine plasma levels of all patients are given in Table 3. Figure 4 shows the time course of the total homocysteine plasma levels of the 121 patients who completed the follow-up period. There was a significant increase in total homocysteine plasma levels between week 12 and week 16 compared with total homocysteine plasma levels after the 4-wk treatment period in all groups (P = 0.0002). The difference in increase of total homocysteine plasma levels between the three folic acid dose groups was not
significant (P = 0.0767). However, there was a significant interaction of “time × doses” (P = 0.0001) and “time × doses × MTHFR 677C → T” (P = 0.0001), indicating a dose-dependent rebound of total homocysteine plasma levels over time in the various MTHFR 677C → T genotypes. This effect is illustrated by the more pronounced increase of total homocysteine plasma levels in patients who had received 30 or 60 mg of folic acid per day during the treatment period and who were homozygous for the MTHFR 677TT allele (TT genotype). This result of stopping high dose folic acid therapy is outlined in Figure 5.

Folate Plasma Levels during the Follow-Up Period

Table 4 shows folate plasma levels at baseline, during 4 wk of treatment, and during the follow-up period for all patients. The time course of folate plasma levels of the 121 patients who completed the 6-mo follow-up period is illustrated in Figure 4. There was a rapid decline in folate plasma levels at week 8 (4 wk after stopping high dose folic acid therapy), and by the end of the study folate plasma levels nearly reached baseline values (P = 0.0001). The folate plasma level at week 4 significantly influenced folate plasma levels in the follow-up period (the higher the folate plasma level at week 4, the less the folate plasma levels declined during follow-up; P = 0.0018) and a significant interaction “time × folate plasma level at week 4” (P = 0.0373), but no effect of the daily folic acid dose on the posttreatment behavior of folate plasma levels was found (P = 0.3119).

At week 8 the proportion of patients who reached plasma levels of <100 nmol/L was equal in the low-flux dialysis or high-flux dialysis/hemodiafiltration group in all three folic acid dose groups (Table 5).

MTHFR 677C → T and Normalization of Total Homocysteine Plasma Levels

Of 144 patients who completed the 4-wk treatment period, 120 patients had total homocysteine plasma concentrations >15 μmol/L at baseline. These patients were subdivided according to their MTHFR 677C → T alleles. Only 27 of 99 (27.3%) pooled MTHFR 677CC and CT genotype patients had their total homocysteine plasma concentrations normalized at the end of 4 wk of treatment. In contrast, 11 of 21 MTHFR 677TT genotype patients (52.4%) with hyperhomocysteinemia at baseline had normal total homocysteine plasma concentrations at the end of the treatment period (P = 0.025).

Vitamin B₁₂ Plasma Levels

Five of 148 patients had subnormal vitamin B₁₂ plasma levels at baseline (97 to 105 pmol/L), four of 144 patients had subnormal vitamin B₁₂ plasma levels at the end of the treatment period (91 to 115 pmol/L), and four of 121 patients had low vitamin B₁₂ plasma levels at the end of the follow-up (104 to 117 pmol/L). Because 42 patients at one center received routine intravenous vitamin therapy including thiamin, pyridoxine, and cobalamin, which resulted in supranormal vitamin B₁₂ plasma levels, we have not included these data in the analysis. However, there was no significant change in mean vitamin B₁₂ plasma levels during folic acid therapy or folate.
low-up in patients without intravenous vitamin supplementation (data not shown).

**Compliance**

Capsule containers were returned at the end of the intervention period for counting by 136 of the 144 hemodialysis patients (94.4%) who completed the intervention period. Eight patients had lost the containers after the intervention period. As estimated from counting capsules, average compliance was 96%. Twenty of the returned containers held one to three, 12 contained four to seven, and five contained more than seven capsules (eight to 15 capsules). Folate plasma levels were measured in all patients during the intervention and during the follow-up. Mean folate plasma levels (from week 1 to week 4) remained <100 nmol/L in three patients, <500 nmol/L in four patients, and <1000 nmol/L in four patients.

**Discussion**

The present study demonstrates that 30 or 60 mg of folic acid per day is not superior in lowering total homocysteine
plasma concentrations in hemodialysis patients than 15 mg of folic acid per day. The major predictor of the response to folic acid therapy is the baseline total homocysteine plasma concentration: The higher the plasma level, the better the response to folic acid therapy. In patients with normal total homocysteine plasma levels, high dose folic acid therapy produces almost no effect. Patients who are homozygous for the MTHFR 677T allele (TT genotype) have a better chance of normalizing their total homocysteine plasma concentration compared to patients with a CC or CT genotype (52.4% vs. 27.3%). Patients with the MTHFR 677TT genotype who received 60 mg of folic acid per day had lower folate plasma levels compared to CC and CT patients during the treatment period. Between 8 and 12 wk after cessation of folic acid therapy, total homocysteine plasma levels increased again. In contrast to patients who were treated with 15 mg of folic acid per day, there was a significant effect of high dose folic acid therapy (30 or 60 mg/d) on total homocysteine plasma levels in patients homozygous for the MTHFR 677T allele during the follow-up period (Figure 5). Folic acid at 30 or 60 mg/d resulted in a remarkable rebound of total homocysteine plasma levels when treatment was stopped in patients with the MTHFR 677TT genotype, exceeding baseline values in several patients.

Wilcken et al. (11) investigated the effect of 5 mg of folic acid per day on total free homocysteine (total non-protein-bound homocysteine) plasma concentrations in 21 predialysis patients. Only 10% of these patients presented with normal homocysteine plasma levels at the end of the study. A higher pretreatment homocysteine plasma level resulted in a better response to folic acid therapy. These findings are in line with the results of the present study, clearly demonstrating that the pretreatment total homocysteine plasma level is the most powerful predictor of the response to folic acid therapy.

Two separate studies of hemodialysis patients showed that 5 mg of folic acid per day lowered mean total homocysteine plasma concentrations by 32.9% from 32.6 ± 10.0 to 21.9 ± 6.9 (17) or 52.5% from 50.5 ± 14.3 to 24.0 ± 1.8 μmol/L (18), respectively. Dierkes et al. (19) treated 70 hemodialysis patients with 2.5 or 5 mg of folic acid three times weekly. The percentage of patients with normal predialysis total homocysteine plasma concentrations increased from 0 to 16%. Total homocysteine plasma levels after dialysis were normal in 50% of the patients before folate treatment. After folic acid therapy, 74% of the patients had normal postdialysis total homocysteine levels (19).

In the first placebo-controlled study of hyperhomocysteinemic end-stage renal failure patients, Bostom et al. (12) showed that multivitamin therapy resulted in a 25.8% reduction of total homocysteine plasma levels from 29.5 to 21.9 μmol/L within 8 wk. Only five of 15 patients treated with 15 mg of folic acid, 100 mg of vitamin B6, and 1 mg of vitamin B12 per day in addition to chronic low dose vitamin supplementation exhibited normalized total homocysteine plasma levels after 8 wk of treatment. There was no difference in total homocysteine plasma levels at week 4 compared to week 8 of folic acid and vitamin therapy (12).

Two other studies focused on the effect of up to 15 mg of folic acid per day on endothelial function in renal failure patients (20,21). In both studies, only a minority of patients had normal total homocysteine plasma levels after treatment. No beneficial effect of folic acid therapy on functional and blood-derived markers of blood vessel function was observed.

Therefore, the question arose as to whether higher doses of folic acid would be more effective. Our study clearly demonstrates that 30 or 60 mg of folic acid per day provides no additional benefit in lowering total homocysteine plasma levels compared to daily doses of 15 mg of folic acid in regular hemodialysis patients.

The effect of genetic variants of MTHFR on the response to folic acid therapy was also analyzed. The enzyme 5,10-methyltetrahydrofolate reductase provides 5-methyltetrahydro-
folic acid, which serves as methyl-donor in the methylation reactions building methionine from homocysteine. Previous studies have shown that a polymorphism in the MTHFR gene (MTHFR 677C → T) aggravates hyperhomocysteinemia in patients on renal replacement therapy (22–24). Another polymorphism in this gene (MTHFR 1298A → C) also has recently been shown to influence total homocysteine plasma concentrations and to be a risk factor for neural tube defects (14, 15). In the present study, no independent influence of these MTHFR polymorphisms on the response to folic acid treatment could be discerned. Nevertheless, patients homozygous for the C to T transition at nucleotide position 677 were more likely to normalize their total homocysteine plasma concentrations than the pooled CC and CT genotype patients. This is in line with the observation of Malinow et al. (25), who reported that the MTHFR 677C → T transition has some influence on the response to folic acid therapy in subjects without renal failure. Not unexpectedly, TT genotype patients treated with 60 mg of folic acid per day had significantly lower folate plasma levels during the treatment period than patients with the CC or CT genotype. This effect in TT genotype patients is due to a decreased intracellular formation of 5-methyltetrahydrofolate, the principal form of folate in the plasma (26).

Another finding in our study that is of considerable importance is the rebound of total homocysteine plasma concentrations above baseline plasma levels when treatment was stopped in several patients with the TT genotype who received 30 or 60 mg of folic acid per day. One possible explanation for this phenomenon might be the presence of excess formylated tetrahydrofolates, which were recently shown to accumulate in red blood cells of healthy individuals with the MTHFR 677TT genotype (27). Based on these results, Bagley and Sellhub (27) speculated that the formation of folate species other than 5-methyltetrahydrofolate may be partly responsible for the elevation of total homocysteine plasma concentrations in subjects with the MTHFR 677TT genotype due to effects on methylation reactions.

There may be also some influence of the dialysis method on folate levels, thus also influencing total homocysteine plasma concentrations. However, the proportion of patients whose plasma folate levels returned to <100 nmol/L at week 8 of this study (that is 4 wk after stopping high dose folic acid therapy) was not different in patients on low-flux dialysis compared with patients maintained on high-flux dialysis or hemodiafiltration within each folic acid dose group.

Folic acid in doses up to 60 mg/d was previously well tolerated from subjects without renal failure (28). Nevertheless, high dose folic acid therapy may lead to adverse events like allergic reactions or gastrointestinal symptoms. However, in our study we observed no major side effects during the treatment period, suggesting that high dose folic acid therapy can be well tolerated by renal failure patients.

A potential limitation in our study is that serum albumin and serum creatinine, which are also predictors of total homocysteine plasma levels in renal failure patients, have not been included in this analysis. Serum albumin levels may have some influence on the response to folic acid therapy since albumin may serve as a carrier protein for plasma folate. The importance of serum creatinine is difficult to evaluate, particularly in patients undergoing regular hemodialysis therapy. It can be argued that red blood cell folate is a better indicator for folate status in hemodialysis patients compared with plasma folate levels (29). Red blood cell folates were not determined in our patients, but a recent study failed to detect a change in red blood cell folates following treatment with 10 mg of folic acid per day in hemodialysis patients (29). Another point of concern is the potential influence of compound heterozygosity for the MTHFR 677TT allele and the MTHFR 1298C allele, which was not analyzed in this study. Because neither heterozygosity for the MTHFR 677C → T transition nor the MTHFR 1298A → C transversion showed an independent influence on the overall response to folic acid therapy in the present study, it is not likely that compound heterozygosity would have had an important role in this particular setting.

Therapy with folic acid also may have some effect on the response to treatment with recombinant human erythropoietin (30). However, a subgroup analysis of 21 patients from one center who had stable hemoglobin levels and a constant erythropoietin and iron dose for at least 3 mo before folic acid therapy showed no change of hemoglobin level or need for erythropoietin dose adjustment during the 3 mo after high dose folic acid therapy (data not shown).

A potential beneficial effect of folic acid supplementation in renal failure patients is the normalization of the S-adenosylmethionine/S-adenosylhomocysteine ratio. Perna et al. (31) demonstrated convincingly that treatment with 5-methyltetrahydrofolate (the active form of folate) resulted in a more pronounced increase of S-adenosylmethionine than S-adenosylhomocysteine. The result was an increase of the S-adenosylmethionine/S-adenosylhomocysteine ratio, which likely indicated some improvement of methylation reactions in uremic subjects. 5-Methyltetrahydrofolate therapy (10 mg/d) also lowered the total homocysteine plasma levels substantially by 72.2% from 67.99 ± 14.89 to 18.87 ± 2.37 μmol/L in this study (31). Nine of the 14 patients who had initial total homocysteine plasma levels >40 μmol/L. Five of 13 (38%) patients with hyperhomocysteinemia (>20 μmol/L) had their total homocysteine plasma levels normalized after treatment (31). We have not measured S-adenosylmethionine/S-adenosylhomocysteine ratios in our patients. Although Perna et al. (31) used 5-methyltetrahydrofolate in their study, this ratio also may have been improved in some of our patients on high dose folic acid therapy.

A possible cause for the limited response of elevated total homocysteine plasma concentrations to folic acid therapy in uremic subjects may be the accumulation of cysteinesulfenic acid (29). The hypothesis that a block in decarboxylation of cysteinesulfenic acid is linked to hyperhomocysteinemia in end-stage renal failure was raised after treatment with 15 mg of folic acid and 200 mg of pyridoxine per day for 4 wk, where cysteinesulfenic acid plasma levels remained unchanged in hemodialysis patients (29).

In summary, our study shows that high doses of 30 or 60 mg of folic acid per day have no more beneficial effect on total
homocysteine plasma levels in hemodialysis patients than a dose of 15 mg/d. Patients with the MTHFR 677TT genotype are more likely to respond to folic acid therapy by normalizing their total homocysteine plasma levels than MTHFR 677CC and CT genotypes. TT genotype patients treated with 60 mg of folic acid per day had lower folate plasma levels during the treatment period compared with CC and CT patients. For those patients who were homozygous for the MTHFR 677T allele, high dose folic acid therapy resulted in a total homocysteine rebound when treatment was stopped. The consequences of such a rebound may be detrimental to the health of the patients. This study clearly demonstrates that hyperhomocysteinemia in end-stage renal disease patients cannot be cured solely by folic acid therapy regardless of the dose. Therefore, other therapeutic strategies need to be developed to combat the risk associated with atherosclerosis and cardiovascular disease.

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References


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