Hyperhomocysteinaemia, folate and vitamin B12 in unsupplemented haemodialysis patients: effect of oral therapy with folic acid and vitamin B12

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Abstract

Background. Hyperhomocysteinaemia, a risk factor for atherosclerosis, is common in dialysis patients and particularly in those homozygous for a common polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (C677T transition). B-complex vitamin supplements have been shown to lower plasma total homocysteine (tHcy) concentrations, but the respective effectiveness of folate and oral vitamin B12 is not yet known. Our objectives were: (i) to determine the status of folate and vitamin B12 in a cohort of unsupplemented dialysis patients (ii) to assess the homocysteine-lowering effect of a folate supplement and then of a folate supplement with added vitamin B12. The responses were analysed for the C677T genotypes of MTHFR.

Methods. Plasma tHcy, folate and vitamin B12 were measured in 51 haemodialysis patients genotyped for the C677T MTHFR mutation (homozygotes, TT; heterozygotes, CT; without mutation, CC). All patients were then given daily supplements of 15 mg of folic acid for 2 months. They were given daily supplements of 1 mg of vitamin B12 in addition to the folate supplements for a further 2 months. Plasma tHcy, folate and vitamin B12 were monitored after each intervention.

Results. At baseline folate and vitamin B12 deficiencies were found in 10% and 6% of the patients. Initial plasma tHcy concentrations were high in all patients (mean 38.1 ± 15 μmol/l). CC patients tended to have a lower tHcy concentration than pooled CT and TT patients. After 2 months of folate therapy, tHcy concentration decreased significantly to 20.2 ± 7 μmol/l (P < 0.001) and no significant differences were observed between the different genotype subgroups (19.4 ± 6 for CC, 21.3 ± 8 for CT, 18.5 ± 4 for TT). A significant positive relationship was found between the reduction of tHcy and its initial value (r = 0.615, P < 0.0001). The impact of the added vitamin B12 was negligible since tHcy concentrations did not change for the patients as a whole (19.8 ± 7 μmol/l, NS) or in any subgroup (19.1 ± 5 for CC, 20.3 ± 9 for CT and 20 ± 7 μmol/l for TT).

Conclusions. (i) Folate and vitamin B12 deficiencies were observed in 10% and 6% respectively of our unsupplemented dialysis patients. (ii) After folate therapy, tHcy levels decreased significantly in all patients and were identical between the three C677T MTHFR genotype subgroups. (iii) Vitamin B12 supplements are useful in folate treated patients to prevent cobalamin deficiency and its neurological consequences but they did not lower tHcy plasma levels for the patients as a group or for any of the MTHFR subgroups.

Keywords: dialysis; folate; homocysteine; MTHFR genotypes; vitamin B12

Introduction

Hyperhomocysteinaemia is found in 85 to 100% of patients with end-stage renal disease (ESRD) and is considered an independent vascular risk factor for uremic patients [1].

Homocysteine (tHcy) is the transmethylation product of the essential sulphur-containing amino acid methionine. Homocysteine can be either remethylated to methionine or degraded to cysteine through the transsulphuration pathway. There are two different remethylation pathways. In the 5-methyltetrahydrofolate (5-MTHF) pathway, the active form of folate is the methyl donor in the methionine synthase reaction,
which is a vitamin B12 dependent enzyme. It is produced by a reaction catalyzed by 5,10-methylene-tetrahydrofolate reductase (MTHFR) and has a common, thermolabile and less active variant resulting from a cytidine to thymidine (C to T) substitution at position 677. After synthesis in the liver, 5-MTHF is distributed to tissues where it acts as a methyl donor for transferring to tHcy via methionine synthase, which regenerates methionine.

In uraemic patients, the pathophysiology of hyperhomocysteinaemia is still unclear. However, the conversion of homocysteine to methionine is substantially decreased in haemodialysis patients. This altered pathway is amplified in subgroups of patients with MTHFR mutation. The average plasma tHcy level is significantly higher in haemodialysis patients homozygous for the mutation (TT), compared with heterozygous patients (CT) or to patients without the mutation (CC) [3].

Several attempts have been made to reduce tHcy levels in ESRD patients. Folic acid (FA) is considered to be the most important vitamin by far to reduce tHcy levels. In contrast to the dramatic lowering effect of routine FA supplementation (1 mg/day) in the general population, the moderate hyperhomocysteinaemia characteristic of dialysis patients has been shown to be resistant to larger doses of FA (16 mg/day) given with vitamin B6 and vitamin B12 [4].

Genetic variants of MTHFR influence homocysteine remethylation, and therefore two studies have analysed the response to FA therapy in relation to MTHFR polymorphisms in ESRD. In the first study, patients homozygous for the C to T substitution were more likely to attain normal tHcy plasma concentrations with folate supplements than CC and CT patients [5]. In the second study, baseline tHcy levels were higher in TT patients, but tHcy plasma levels were similar for the three genotypes at the end of the protocol [6]. Neither study could assess the effects of folate therapy per se because different B vitamins were given concurrently. In our centre, B vitamin supplementation in dialysis patients was uncommon, but we have now changed our policy in view of the potential harm of hyperhomocysteinaemia. We have therefore taken the opportunity to study the incidence of folate deficiency and the efficiency of oral folate-only supplements to reduce tHcy level in relation to the different MTHFR genotypes.

In the general population, vitamin B12 lowers tHcy levels by 7% [7]. In uraemic patients, the effect of vitamin B12 has been seldom studied. In uraemic patients with cobalamin deficiency, intravenous administration of vitamin B12 (1 mg) weekly lowered tHcy by 30% [8]. Dialysis patients, however, rarely have vitamin B12 deficiency [9]. A recent study [10] suggests that vitamin B12 is a critical vitamin for the tHcy-lowering effect. In that study, FA supplementation was given over a short period (4 weeks) and at a low dose (1 mg/day) for dialysis patients. It is therefore difficult to discount the possibility that the tHcy reduction observed after adding vitamin B12 was not caused by the concomitant on-going FA therapy. The second part of our study aimed to determine whether oral vitamin B12 might further reduce plasma tHcy concentrations in haemodialysis patients already treated with a sufficient dose of FA and over a longer period of time.

**Subjects and methods**

**Patients and protocol**

In this prospective study, we excluded patients who were under treatment with anti-epileptic drugs or other folate antagonists or estrogens, as well as those patients having taken vitamin B12 or folate supplements over the past 12 months. Fifty-five patients gave written informed consent. Only 51 patients completed the study because two died and two moved to a self-care dialysis unit. Twenty-nine of them were women and the patients had a mean age of 63.8 ± 12 years (mean ± SD). The primary renal diseases were chronic glomerulonephritis (n = 5), chronic interstitial disease (n = 10), polycystic kidney disease (n = 4), hypertensive nephrosclerosis (n = 6), diabetic nephropathy (n = 12), systemic disease (n = 1), urogenital malformations or malignancies (n = 3) and shrunken kidneys of unknown aetiology (n = 10).

Patients were treated by regular bicarbonate haemodialysis three times a week with either high flux synthetic membranes (32 patients) or low flux synthetic or cellulosic membranes (19 patients). The length of haemodialysis treatment was 50.2 ± 7.8 months with a residual clearance of 0.3 ± 0.1 ml/min (mean ± SD). The dialysis dose determined by single pool kinetics (sp Kt/V) averaged 1.21 ± 0.04 Kt/V.

The initial evaluation determined tHcy and plasma folate concentrations. All patients received a daily oral folate supplement of 15 mg of FA (Speciafoldine®) for 2 months. At the end of the second month (at T2), we measured plasma vitamin B12, plasma tHcy and plasma folate concentrations. We added an oral dose of 1 mg of vitamin B12 daily for a further 2 months. Before the end of this second period (T4), blood samples were again drawn to measure plasma tHcy, folate and vitamin B12.

**Laboratory analyses**

Blood was analysed from fasting patients who were dialysed in the morning session. For patients attending dialysis in the afternoon or evening, a light snack 2 h before the session was allowed since Hultberg et al. [11] have demonstrated that levels of tHcy do not change after a meal in patients with advanced renal failure. None of the haemodialysis patients were allowed to move to another session during the protocol.

tHcy was measured by an immunoenzymological method [12]. Blood samples were collected on ice and separated within 1 h of collection by centrifugation. Reduction of disulphide-bonds by dithiothreitol allowed measurement of tHcy (free and protein-bound) with a polarization fluorescence technique (IMX; Abbott Laboratories, Oslo, Norway). We defined hyperhomocysteinaemia as a tHcy concentration greater than the 95th percentile of our control population with normal renal function (13.9 μmol/l).

We used an automated chemiluminescence system (ACS:180, Chiron Diagnostics Corporation, East Walpole, MA, USA) to determine folate and vitamin B12 concentrations in plasma. The normal range for plasma folate
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concentrations was 10–45 nmol/l, and for vitamin B12 180–812 pmol/l.

Restriction fragment length polymorphism analysis was used to identify the 677 C to T transition in the MTHFR gene according to Frosst et al. [2]. DNA was extracted from blood samples by standard methods. The C677T polymorphic site was determined by PCR amplification followed by restriction-enzyme digestion of the products and analysed on 2% agarose gels [13].

Statistical methods

Data are presented as mean ± SD. Because of the positive skewed distribution of tHcy, folate and vitamin B12 plasma concentrations, the Mann–Whitney U-test was applied to study differences between groups and the Wilcoxon rank test for the paired differences within groups. Relationships between variables were tested by the Spearman rank correlation test and by linear regression after transforming the data to natural logarithms (ln) in order to obtain a nearly normal distribution. The χ² test was used for estimating the occurrence of categorical variables. A P value of <0.05 was considered to reflect statistical differences.

Results

Table 1 shows the genotypic distribution of the (C677T) MTHFR mutation and the main characteristics of patients in the different genotype groups. No statistical differences were observed for age, length of dialysis treatment or sex ratio. The wild type, CC, was found in 37% of patients. Forty-nine per cent of patients were CT and 14% TT for the mutation.

Table 2 shows no significant difference for basal plasma tHcy between the three different genotype groups. However, CC patients tended to have lower basal values of plasma tHcy compared with pooled CT and TT patients (P = 0.06). A deficiency of folate (<10 nmol/l) was observed in five patients at baseline (one patient in group CC, one in group TT, and three in group CT). After folate therapy, all patients had higher folate concentrations at T2 and T4 than at T0. A deficiency in vitamin B12 (<180 pmol/l) was found in three patients at baseline levels. At T4, after vitamin B12 supplements, its concentration increased in all but nine patients.

Table 2 also shows the effect on tHcy plasma levels of the 2 months of treatment with FA alone, and of the subsequent 2 month treatment with FA plus vitamin B12. After FA therapy, a significant reduction in tHcy levels was observed in the three genotype groups so that plasma tHcy concentrations were similar between the different groups at T2 and T4. The percentage reduction of plasma tHcy in TT patients was significantly greater than the reduction observed in CC patients after 2 months of folate supplements. Vitamin B12 did not reduce further plasma tHcy levels for any of the genotype groups or for all patients together. Normalization of plasma tHcy concentration (<13.9 µmol/l) was obtained in six patients at T2 (four CC and two CT) and in eight patients at T4 (three CC, four CT and one TT).

The 42 patients who showed an increment of plasma cobalamin between T2 and T4 had no further reduction of plasma tHcy (Table 3). In nine patients whose plasma cobalamin concentration did not increase after oral vitamin B12 therapy (mean plasma cobalamin 415 pmol/l at T2 and 348 pmol/l at T4), baseline tHcy level was reduced from 47.5 ± 16 to 24 ± 11 µmol/l after FA alone and to 25.01 ± 13 µmol/l after the addition

Table 1. Main characteristics of patients with different MTHFR genotypes

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Sex ratio (male : female)</th>
<th>Age (mean ± SD)</th>
<th>Dialysis treatment (months, mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>19</td>
<td>8 : 11</td>
<td>65 ± 11</td>
<td>47 ± 58</td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>12 : 13</td>
<td>63 ± 14</td>
<td>46 ± 48</td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>2 : 5</td>
<td>63 ± 13</td>
<td>68 ± 93</td>
</tr>
<tr>
<td>All</td>
<td>51</td>
<td>22 : 29</td>
<td>64 ± 12</td>
<td>49 ± 58</td>
</tr>
</tbody>
</table>

Abbreviations are: CC, wild type; CT, heterozygotes; TT, homozygous mutant alleles.

There were no significant differences for these parameters between groups.

Table 2. Changes in plasma tHcy, folate and vitamin B12 concentrations, and percentage reduction of tHcy after treatment with folate only and folate with added vitamin B12

<table>
<thead>
<tr>
<th>MTHFR alleles</th>
<th>n</th>
<th>Baseline (T0)</th>
<th>2 months (T2)</th>
<th>4 months (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>19</td>
<td>34.9 ± 13</td>
<td>19.4 ± 6</td>
<td>19.1 ± 5</td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>42.9 ± 18</td>
<td>21.3 ± 8</td>
<td>20.3 ± 9</td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>42 ± 8</td>
<td>18.5 ± 4</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>All</td>
<td>51</td>
<td>39.8 ± 15</td>
<td>20.2 ± 7</td>
<td>19.8 ± 7</td>
</tr>
<tr>
<td>Folate plasma levels (nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19</td>
<td>14.6 ± 3</td>
<td>50 ± 6</td>
<td>50 ± 61</td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>17.2 ± 10</td>
<td>50 ± 6</td>
<td>50 ± 61</td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>12.5 ± 1</td>
<td>50 ± 6</td>
<td>50 ± 61</td>
</tr>
<tr>
<td>All</td>
<td>51</td>
<td>15.6 ± 8</td>
<td>50 ± 6</td>
<td>50 ± 61</td>
</tr>
<tr>
<td>Vitamin B12 plasma levels (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19</td>
<td>477 ± 400</td>
<td>726 ± 399</td>
<td>674 ± 369</td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>423 ± 288</td>
<td>601 ± 352</td>
<td>795 ± 330</td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>342 ± 139</td>
<td>795 ± 330</td>
<td>674 ± 369</td>
</tr>
<tr>
<td>All</td>
<td>51</td>
<td>432 ± 319</td>
<td>726 ± 399</td>
<td>674 ± 369</td>
</tr>
<tr>
<td>Reduction of tHcy (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19</td>
<td>42 ± 15</td>
<td>42 ± 12</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>45 ± 19</td>
<td>48 ± 20</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>55 ± 9</td>
<td>50 ± 24</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>51</td>
<td>45 ± 17</td>
<td>46 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Baseline values (T0) of plasma homocysteine (tHcy), folate and vitamin B12 concentrations and values after 2 months of folate supplements (T2) and 2 months later with added vitamin B12 (T4) are expressed as (mean ± SD). In addition the percentage reductions of tHcy observed after folate (T2 compared to T0) and after vitamin B12 in addition to folate (T4 compared to T0) are given.

Genotypes are as follows: CC, wild type; CT, heterozygous; TT, homozygous.

TT compared with CC, aP < 0.05; T0 vs T2, bbbP < 0.001, bP < 0.05; T0 compared with T4, ccccP < 0.001, cP < 0.05; T2 compared with T4, ddddP < 0.001, dP < 0.01, dP < 0.05.
of vitamin B12. These plasma tHcy values were not significantly different from the corresponding values from the 42 patients who showed an increase in plasma vitamin B12 levels.

A statistically significant positive relationship was observed between the percentage reduction of tHcy and the baseline tHcy plasma value both at T2 (\( r = 0.514, P < 0.0001 \)) and at T4 (\( r = 0.598, P < 0.0001 \)). After logarithmic transformation, the relationship was still significant at T2 (\( r = 0.500, P < 0.001 \)) (Figure 1A) and at T4 (\( r = 0.485, P < 0.001 \)) (Figure 1B). Since the baseline tHcy concentration is used to calculate the percentage of reduction of tHcy, we performed an Oldham’s transformation \([14]\) to avoid a mathematical artefact. A positive relationship was again observed at T2 (\( r = 0.739, P < 0.0001 \)) and at T4 (\( r = 0.733, P < 0.0001 \)). These correlations remained true after logarithmic transformation (T2, \( r = 0.671, P < 0.0001 \); T4, \( r = 0.642, P < 0.0001 \)).

At a marginal level, a negative relationship was found between the percentage reduction of tHcy at T2 and the baseline concentration of plasma folate (\( r = -0.266, P = 0.05 \); after logarithmic transformation \( r = 0.297, P < 0.05 \)). No such relationship was found between the percentage reduction of tHcy and the initial plasma vitamin B12 concentration.

**Discussion**

This study shows that 15 mg/day of FA lowers total tHcy plasma concentration in haemodialysed patients, irrespective of the MTHFR 677 genotype. After 2 or 4 months of folate therapy, tHcy plasma levels in the three groups with different MTHFR 677 C to T polymorphisms were similar, although baseline plasma tHcy values were higher in heterozygote or homozygote patients for the mutation. This confirms the findings of two previous studies, which showed that this mutation does not impair the response to high doses of FA \([5,6]\).

The difference in the initial concentrations of tHcy related to MTHFR polymorphisms might be clinically relevant to haemodialysis patients. In a cross-sectional study of haemodialysis patients, the prevalence of the TT genotype was lower for older patients and for a longer duration of dialysis \([15]\). This suggests that the risk of death increased with the length of dialysis in ESRD patients homozygous for the mutation. The reduction of tHcy levels could be critical in the TT and CT subgroups of uraemic patients whose baseline tHcy levels are higher than normal. A prospective study with folate therapy is needed to test this hypothesis.

Plasma tHcy basal values in our patients were higher than the values generally reported for dialysed patients \([3–5]\), which is explained by the lack of previous multivitamin supplementation. Without vitamin supplementation, baseline tHcy concentrations have previously been found to be in the same range as our values and sometimes higher \([6]\). FA-fortified cereal grain flour is rarely used in France and therefore, unlike many other countries, dialysis patients along with the general population are not usually supplemented with B vitamins. A true folate deficiency (<10 nmol/l) was observed in 10% of our dialysis

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**Table 3. Changes in plasma tHcy, vitamin B12 and folate concentrations, and percentage reduction of tHcy after T2 and T4 in patients who showed a plasma vitamin B12 increment between T2 and T4**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (T0)</th>
<th>2 months (T2)</th>
<th>4 months (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total homocysteine plasma levels (( \mu \text{mol/l} ))</td>
<td>38.1 ± 15 (^{aa})</td>
<td>19.4 ± 5 (^{b})</td>
<td>18.7 ± 5 (^{b}bb)</td>
</tr>
<tr>
<td>Folate plasma levels (( \mu \text{mol/l} ))</td>
<td>16.3 ± 8 (^{a})</td>
<td>&gt; 50 (^{aa})</td>
<td>&gt; 50 (^{bb})</td>
</tr>
<tr>
<td>Vitamin B12 plasma levels (( \mu \text{mol/l} ))</td>
<td>435 ± 348 (^{c})</td>
<td>744 ± 367 (^{e}e)</td>
<td></td>
</tr>
<tr>
<td>Reductions of tHcy (%)</td>
<td>44.7 ± 17</td>
<td>46 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Values are given for 42 patients selected on the basis of plasma vitamin B12 increment between T2 and T4. T0 compared with T2, \( ^{aa}P < 0.001 \); T0 compared with T4, \( ^{bb}P < 0.001 \); T2 compared with T4, \( ^{e}P < 0.001 \).
controlled comparisons of oral treatment with either FA given to haemodialysis patients, normal-
plasma levels after treatment is a better measure of the level. The percentage of patients with normal tHcy
efficiency of various forms of folate therapy cannot be
with FA (20–50%). These figures are higher than the usual values observed the average pre-treatment tHcy levels were observed.
However, a large reduction in tHcy after folate supplements does not infer a normalization of plasma tHcy levels. In the Vienna multicenter study [5], signifi-
cantly more patients in the TT group normalized their tHcy plasma level than those in the other groups (52.4% in the TT group vs 27.3% of the CT and CC groups pooled, $P = 0.025$). Our study did not show that trend towards a greater percentage of normalization of tHcy in the TT group, since normal-
ization of tHcy was observed in 21% of CC, 8% of CT and 0% of TT patients at T2, and in 16% of CC, 12% of CT and 14% of TT patients at T4. Even if the MTHFR genotype influences the response to FA supplementation, patients with pre-treatment tHcy concentrations close to the cut-off value for the normal range probably normalize tHcy levels most easily. The Austrian group [16] showed, with logistic regression analysis, that patients with low baseline tHcy plasma concentrations were more likely to normalize their tHcy plasma levels but patients with greater baseline levels showed better relative responses.
Two open-label investigations reported a dramatic efficiency on the reduction of tHcy levels with reduced folates [17,18]. Respective reductions of 73 and 67% in the average pre-treatment tHcy levels were observed. These figures are higher than the usual values observed with FA (20–50%) [4–6]. However, because pre-treatment tHcy plasma level is a powerful predictor of the response to folate supplements, the respective efficiency of various forms of folate therapy cannot be based only on the percentage reduction of tHcy plasma level. The percentage of patients with normal tHcy plasma levels after treatment is a better measure of the efficiency of different forms of folate supplements. With FA given to haemodialysis patients, normal-
ization of pre-dialysis plasma tHcy concentration (tHcy < 15 μmol/l) ranges from 0–33% [4–6]. Two controlled comparisons of oral treatment with either 15 mg/day of FA or an equimolar amount of reduced folates have been carried out [19,20]. After 12 weeks of treatment, only about 8% in each group reached a normalized tHcy concentration (< 12 μmol/l). Hauser et al. [16] compared i.v. folinic acid to i.v. FA in a randomized, controlled, double-blind trial, and obtained a normalization of tHcy in 30.3% of the patients treated with FA and in 18.2% of the patients with folinic acid. This difference was not significant. The results of these three comparative studies show that reduced folates are no more effective than FA for lowering plasma tHcy levels in haemodialysis patients. In order to analyse the results of tHcy plasma normalization, the cut-off value defining the upper limit of the normal range is important. With a strict definition of normalization of plasma tHcy concentration (< 12 instead of < 13.9 μmol/l), in our haemodialysis patients, only four out of 51 (8%) had achieved normal plasma tHcy levels at T2 and only six (12%) at T4. These figures are similar to those observed in two recent controlled studies comparing FA to folinic acid [19,20]. The resistance of the dialysis population to folate supplements seems to be independent of the route and form of administration, namely given either in its reduced form (folinic acid) or FA.
Our data show that oral vitamin B12 has no additional homocysteine-lowering effects over folate supplements in ESRD patients with a normal cobalamin concentration. Four previous trials have examined the role of vitamin B12 therapy in patients with ESRD.
One randomized placebo-controlled 8-week study carried out in haemodialysis patients compared a combination of FA (15 mg/day) and vitamin B12 (1 mg/day) with FA (1 mg/day) and vitamin B12 (12 μg/day) [4]. Plasma tHcy concentrations were 26% lower in patients who received the high dose B-vitamin treatment. However, this double inter-
vention does not allow discrimination between the separate tHcy-lowering effects of each vitamin tested.
One small uncontrolled study showed that vitamin B12 alone induces a tHcy-lowering effect in 14 patients with a low serum cobalamin concentration (< 180 pmol/l). After intravenous injections of 1 mg per week of vitamin B12 for 1 month, tHcy concentrations decreased by 35% [8]. In our study, tHcy concentrations for the three patients with low vitamin B12 did not change after oral vitamin B12 therapy (from 20.17 to 20.33 μmol/l), with serum cobalamin increasing from 166 to 403 pmol/l. Because vitamin B12 is highly protein bound, most reports in the liter-
ature show normal or high plasma levels for vitamin B12 in unsupplemented dialysis patients. In this popu-
lated, the prevalence of true vitamin B12 deficiency is only 6% [9].
A recent study enrolled haemodialysis patients with vitamin B12 concentrations in the normal range and showed that tHcy concentrations decreased after three parenteral injections of 1 mg vitamin B12 given at a 4-week interval. The tHcy plasma values in 14 patients...
consuming daily vitamin tablets providing 5 mg of FA fell successively after each injection to reach a plateau of 23.6 μmol/l 1 month after the final injection. From a baseline plasma tHcy concentration of 26.5 μmol/l, there was therefore a 13% tHcy-lowering effect, which could be clinically significant [21].

In a recent study, Manns et al. [10] suggest that oral vitamin B12 reduces tHcy concentrations in ESRD patients, independently of folate supplements. Eighty-one haemodialysis patients received 1 mg/day of FA for 4 weeks, and 1 mg/day of vitamin B12 was added for a further 4 weeks along with the FA therapy. Screening tHcy levels (mean, 27.7 μmol/l) fell by 19.2% after FA alone. When vitamin B12 supplements were given, tHcy was reduced significantly further from 22.3 to 18.6 μmol/l (mean reduction, 16.7%). This suggests that vitamin B12, independently of FA, has an tHcy-lowering effect in haemodialysis patients whose prior vitamin B12 status is normal. This study, despite having a similar design to ours, gives conflicting results. This may be because the 16.7% decrease of tHcy concentration after oral vitamin B12 supplements was caused by several factors. In two studies, the maximum tHcy-lowering effect of oral folate therapy was observed after 4 weeks but with a daily dose of 15 mg of FA or more [5,16]. A dose of FA at least fifteen times as low is unlikely to produce a maximum reduction of tHcy concentration after 4 weeks in ESRD patients. In our study, plasma tHcy levels were measured with exactly the same assay and the upper limit of the normal range was similar (13.7 in Manns et al.’s study vs 13.9 μmol/l in our study). In our patients, after 2 months with FA alone, tHcy plasma was 20.2 μmol/l. This is similar to the mean tHcy concentration observed after 8 weeks in Manns et al.’s study (18.6 μmol/l). In both studies, a normal tHcy plasma level was achieved in similar percentages of patients, with 13.6% after vitamin B12 and FA in Manns et al.’s trial and 12% with FA alone in our study.

In addition to the classic route of absorption via gastric intrinsic factor, cobalamin can be absorbed by a second transport system that does not require intrinsic factor, but with an efficiency of only about 1%. Patients without pernicious anaemia absorb only a few micrograms of the vitamin after oral doses of 500–1000 μg and gastritis could further reduce this amount in elderly people. After vitamin B12 therapy, the lack of increase in serum cobalamin concentration in nine of our patients could have been caused by lack of compliance or poor absorption. We therefore selectively studied the 42 patients showing an increase in serum cobalamin concentration with vitamin B12 supplements. Table 3 shows no significant reduction in tHcy plasma concentration after vitamin B12 therapy in this group of patients (i.e. 19.4 μmol/l after FA alone and 18.7 μmol/l after vitamin B12 was added to FA). Oral vitamin B12 in itself does not appear to have a significant tHcy-lowering effect. As parenteral vitamin B12 seems to lower tHcy plasma level in dialysis patients [21], vitamin B12 might reduce tHcy concentration through a pharmacological mechanism, presumably analogous to that of the ‘megadose’ FA used to lower tHcy concentrations in non-folate deficient ESRD patients. With oral vitamin B12 (1 mg/day), this pharmacological action does not seem obvious in haemodialysis patients.

However, we think that oral vitamin B12 should be given to ESRD patients, despite its lack of tHcy-lowering effect, for three reasons. Cobalamin deficiency assessed by high methylmalonic acid concentrations is sometimes observed in spite of normal vitamin B12 serum concentrations. High-flux haemodialysis treatment and the use of erythropoietin could induce subnormal serum B12 levels. Folate supplements in vitamin B12 depleted patients mask haematological features of cobalamin deficiency (megaloblastosis) while its neurological consequences are not curbed and therefore its diagnosis can be grossly delayed.

To summarize, (i) in haemodialysis patients unsupplemented with B vitamins, 10 and 6% were folate or vitamin B12 deficient, respectively. (ii) Response to FA supplement is independent of the MTHFR 677 C to T genotypes since tHcy concentration decreases in all genotypes. (iii) After folate supplementation no further differences between TT and CT or CC genotypes are observed. (iv) Oral Vitamin B12 does not further reduce tHcy plasma concentration in ESRD patients already treated with folate supplements.

References

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