Original Article

Hyperhomocysteinaemia therapy in haemodialysis patients: folinic versus folic acid in combination with vitamin B6 and B12

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Abstract

Background. In a recent uncontrolled retrospective report we suggested that the long-term supplementation of high-dose, i.v. folinic acid combined with high-dose i.v. pyridoxine was highly effective in correcting plasma total homocysteine (tHcy) concentrations in haemodialysis patients. To confirm these findings, we conducted a randomized, controlled trial aimed at evaluating whether i.v. or oral folinic acid provided improved tHcy-lowering efficacy in haemodialysis patients compared with oral folic acid.

Methods. In a 6-month prospective, randomized, controlled trial, 60 chronic haemodialysis patients, matched for age, gender, dialysis duration, and average screening pre-treatment-fasting tHcy levels, were given either 50 mg/week of i.v. calcium folinate (group 1), 50 mg/week of oral calcium folinate (group 2), or 45 mg/week oral folic acid (group 3). All 60 patients also received 750 mg/week of i.v. vitamin B6 and 3 mg/week of oral vitamin B12.

Results. Fasting tHcy decreased significantly and to a similar extent in the three groups after 2 months of treatment and remained stable at 4 and 6 months (16.6 ± 3.5, 18.3 ± 4, and 19.1 ± 3.1, in groups 1, 2, and 3, respectively, P = NS). Mean percentage reduction at 6 months was also similar in the three treatment groups (46, 43, and 42% in groups 1, 2, and 3, respectively, P = NS).

Conclusions. These findings show that the tHcy-lowering effects of high-dose i.v. folinic acid, oral folinic acid, or oral folic acid were comparable, suggesting that the hyperhomocysteinaemia observed in haemodialysis patients is not due to abnormal folate metabolism. Furthermore, they are compatible with the view that other abnormalities are also involved in the impaired clearance of homocysteine in uraemic patients.

Keywords: dialysis; folic acid; folinic acid; homocyst(e)ine; vitamin B6; vitamin B12

Introduction

The moderate elevation of fasting total homocysteine (tHcy) concentrations in plasma, which is observed in at least 85% of haemodialysis patients, can be lowered by appropriate cofactor supplementation. However, administration of folic acid at pharmacological doses, either alone or combined with vitamin B6 and B12, is only partially effective in reducing plasma tHcy; in fact, few dialysis patients normalize their tHcy concentration entirely [1]. On the other hand, oral treatment with the reduced form of folate, i.e. methyltetrahydrofolate (MTHF), has been found to lead to a more marked decrease (70%) in plasma total Hcy levels in haemodialysis patients [2]. In a retrospective study, we found that a once weekly i.v. administration of 50 mg calcium folinate (racemic mixture of L- and D-isomers of formyltetrahydrofolate), which is the immediate precursor of MTHF, when given together with i.v. pyridoxine, was very effective; the treatment normalized plasma tHcy concentrations in 78% of haemodialysis patients [3]. Three possible hypotheses have been advanced to explain this efficacy. Firstly, reduced forms of folate are more efficient than folic acid itself in normalizing plasma tHcy concentrations, probably due to the abnormal metabolism of folic acid in uraemia [4–5]. Secondly, the i.v. use of folinic acid may have improved tHcy-lowering potency in such patients more than oral treatment by overcoming the inhibition of cellular folate transport, which has been reported in uraemic patients [6]. Thirdly, the addition of i.v. pyridoxine to i.v. folinic acid supplementation could have contributed to the efficacy of our strategy, since vitamin B6 does not only play a key role as a coenzyme in the trans-sulphuration pathway, but also...
as a coenzyme for serine hydroxymethyltransferase (SMHT) [7]. SMHT is responsible for the conversion of tetrahydrofolate (THF) to 5,10-methylene-THF [7]. A portion of the 5,10-methylene-THF is transformed by 5,10-methylene-THF reductase to 5-MTHF, which serves as a methyl donor for homocysteine and thereby causes its remethylation to methionine [7]. Vitamin B6 deficiency in rats has been shown to substantially affect both methyl group production for homocysteine remethylation and the flux through whole-body trans-sulphuration [8].

However, our previous study had an important limitation, namely the lack of a controlled and prospective study design, which precluded a definite conclusion as to whether folinic acid had a better tHcy-lowering efficacy than folic acid. Recently, two controlled trials examined the relative efficacy of high-dose oral reduced forms of folate (i.e. L-5 MTHF or L-folinic acid) compared with high-dose oral folic acid [9,10]. In both studies, mean percentage reductions in pre-dialysis tHcy were close to 20%, which were not significantly different from those observed with folic acid [9,10]. In another, randomized, controlled trial, Hauser and colleagues [11] demonstrated that a 4-week course of i.v. folinic acid three times weekly was not superior to i.v. folic acid three times weekly in reducing tHcy plasma levels in haemodialysis patients (34% vs 32% respectively). Taken together, these data suggest that short-term (<3 months) supplementation with reduced forms of folate does not afford improved tHcy-lowering efficacy in haemodialysis patients. However, these authors did not explore the effect of long-term supplementation with high-dose i.v. reduced forms of folate or the possible beneficial effects in association with high-dose i.v. pyridoxine, as suggested by our previous retrospective study.

Therefore, we conducted a 6-month, block-randomized, controlled comparison of the effects of i.v. calcium folinate, oral calcium folinate and oral folic acid, each combined with high-dose i.v. vitamin B6 and oral vitamin B12, on plasma tHcy levels in chronic haemodialysis patients.

Subjects and methods

We conducted a multicentre study in two haemodialysis units in France, including one university-based hospital (Hôpital Saint Jacques, Besançon) and one university-affiliated hospital (Hôpital André Ballanche, Montbéliard). The study was initiated in May 2000 and completed in October 2000. The study protocol was approved by the review board of Franche-Comté according to the Declaration of Helsinki and the French law on bioethics, and all participants provided written informed consent.

We assumed that i.v. folinic acid would reduce pre-treatment plasma tHcy concentrations by 67%, as found in our retrospective study [3]. Based on the hypothesis that oral folic acid or folinic acid can reduce tHcy by 30%, our study had a power of 80% at a two-tailed \( \alpha \) of 0.05 to detect a 37% difference in the tHcy-lowering effect between these therapeutic modalities, and adjusted for potential lost patients (+10%), with 20 patients in each treatment group.

Study population

A total of 60 chronic haemodialysis patients, having given written consent, participated in this prospective, randomized, open study (Figure 1). The randomization procedure was centralized in the office of the Nephrology Division of the Saint Jacques Hospital, Besançon. Sixty files, each with a fixed number (1–3), were randomly distributed in 60 opaque envelopes to define the groups. The selection was made by an independent person at the time of randomization. Inclusion criteria were an age of between 18 and 75 years, haemodialysis for at least 3 months and a weekly dialysis time of 12 h or more. Exclusion criteria were: hypersensitivity to vitamin B; acute illness at the time of inclusion; and systemic disease. Study participants either did not use vitamin supplements or had abstained from taking supplements containing folic acid, vitamin B6, or vitamin B12 for at least 6 weeks before the start of the study.

Study protocol

Study participants were matched for age, gender, dialysis duration, and average pre-treatment fasting tHcy levels measured one month before and at the start of the study (<25 \( \mu \)mol/l, between 25 and 35 \( \mu \)mol/l, or >35 \( \mu \)mol/l, respectively). They were then randomly assigned to one of three regimens, namely i.v. folinic acid (Calcium folinate\( ^{8} \), Dakota Pharm, Créteil, France) 50 mg/week (group 1; \( n=20 \)), oral folinic acid (Calcium folinate\( ^{9} \)) 50 mg/week (group 2; \( n=20 \)), or oral folic acid (Specia-Rhône-Poulenc-Rorer, Montrouge, Paris) 15 mg/3 weeks (group 3; \( n=20 \)). All patients received i.v. vitamin B6 (Specia-Rhône-Poulenc-Rorer), 250 mg/3 weeks and oral vitamin B12 (Roche, Neuilly sur Seine, France), 1 mg/3 weeks over the entire study period. The dose of folic acid (i.e. 50 mg/week) was similar to those used in our retrospective study [3]. The dose of folic acid (i.e. equivalent to \( \approx 2.5 \) equimolar to 50 mg/week of folinic acid) was selected based on general practice in France (5 mg/day). To avoid the problem of poor compliance, treatments were given after each dialysis session as three pills of 5 mg each for practical reasons, bearing in mind the lack of an increase in the efficacy of higher amounts of folic acid supplementation, namely \( \approx 5 \) mg/day [12]. Compliance with treatment was assessed by dialysis nurses who gave the i.v. or oral medication at the end of the dialysis sessions. Fasting plasma tHcy levels were measured pre-dialysis twice before treatment, and at 2, 4 and 6 months after the start of the study.

Laboratory methods

Total plasma Hcy was measured as follows. Blood samples were drawn prior to the dialysis session. They were centrifuged within 15 min, and the plasma was kept frozen at \(-20^\circ\)C. The tHcy concentration, and the sum of the soluble acids (reduced Hcy, homocysteine, disulphide, and homocysteine-cysteine mixed disulphide) and protein-bound moieties were measured by high-performance liquid chromatography. This assay involved the following steps: reduction of the sample with tri-n-butylphosphine, precipitation of proteins, alkalinization of the supernatant with
sodium borate, derivitization with 7-fluoro-2-oxa-1,3-diazole-4 sulphonate, followed by 8-aminonaphthale-1,3,6-trisulphonic acid, and HPLC separation with fluorescence detection. The normal values of plasma Hcy concentration ranged from 7 to 15 μmol/l. The precision of the assay corresponded to a coefficient of variation <3%.

Cobalamin and folate (DPC, La Garenne Colombes, France) in plasma were determined by radioassay using purified intrinsic factor and purified folate-binding protein. Normal values for cobalamin and folate were 250 pg/ml and 10±7 ng/ml, respectively. Plasma vitamin B6 levels were measured by high-performance liquid chromatography (Biochemistry Laboratory, CHU St Jacques, Besançon, France). Normal values ranged from 52 to 145 nmol/l. The precision of the assay corresponded to a coefficient of variation <5%.

Statistical analyses

Descriptive statistics included mean values ± SD for continuous data and percentages for categorical data. For age, time on dialysis, dialysis session duration, Kt/V and the baseline values of tHcy, folate, cobalamin and vitamin B6 plasma levels, a one-way ANOVA was performed to exclude potential differences between the three groups at baseline. Comparisons over time were performed by an ANOVA with repeated measurements for 6 months (baseline, 2, 4 and 6 months). The analyses were performed on an intention to treat basis (including all randomized patients). Comparisons of the proportion of the number of patients in each group who had hyperhomocysteaemia at baseline and who completed the study with normal tHcy (15.1 μmol/l) was performed by χ^2 tests. The Spearman/Pearson ranks test was used to evaluate the relationship between numerical variables. P values <0.05 were considered to be significant.

Results

Of the 60 randomized patients, 53 completed the study (Figure 1). Seven patients withdrew from the study (two, one and four patients from groups 1, 2 and 3, respectively). Two patients withdrew from the study because of abdominal pain related to the vitamin B therapy (one patient from group 1 and one from group 3), four patients underwent renal transplantation during the protocol period (one, one and two patients from groups 1, 2 and 3, respectively), and one patient...
from group 3 died (myocardial infarction). No other clinically relevant side effects related to the treatment (e.g. neurotoxicity) were observed. The demographic and clinical parameters of the patients are shown in Table 1. At baseline, the three groups did not differ in age, duration of dialysis sessions, total length of time on dialysis treatment, and plasma tHcy and vitamin B concentrations (Table 1). Baseline tHcy levels were above normal values in all the patients (32.7 ± 8.6 μmol/l; range, 21–60). Mean baseline folate concentration was in the low normal range (4.2 ± 1 ng/ml) whereas both cobalamin (385 ± 107 pg/ml) and vitamin B6 (134 ± 41 nmol/l) concentrations were in the normal range. Baseline tHcy levels were negatively related to baseline folate concentrations (r = −0.47; P < 0.001). There was no relationship between either baseline tHcy and baseline cobalamin, or baseline tHcy and baseline vitamin B6.

The tHcy levels significantly decreased in all groups. The decrease was similar in the three groups after 2 months of treatment and remained stable at 4 and 6 months (Table 2). Mean percentage reduction was also similar in the three treatment groups (Table 2). Normalization of tHcy concentration (<15.1 μmol/l) at 2 months was achieved in 40%, 35%, and 20% of patients of groups 1, 2 and 3, respectively; at 4 months in 52%, 40% and 26% of patients; and at 6 months in 50%, 26% and 6% of patients, respectively. This latter result for group 3 is mainly due to the withdrawal of two patients whose tHcy levels were normalized by the treatment at 2 months. The decrease in tHcy level at 6 months was positively related to baseline tHcy (r = 0.55; P < 0.0001), to plasma vitamin B6 concentrations on treatment (6 months) (r = 0.34; P < 0.02), and to vitamin B12 concentrations on treatment (6 months) (r = 0.32; P < 0.02), but not to plasma folate concentrations.

Discussion

Our data show that the administration of i.v. folinic acid to chronic haemodialysis patients is equally effective in correcting tHcy concentrations as oral folinic acid or oral folic acid, when associated with pyridoxine and vitamin B12 supplements. This comparable efficacy is true when considering reductions of absolute mean levels of pre-dialysis tHcy concentrations, as well as percentage changes under treatment. There were fewer patients whose tHcy levels were normalized by oral folinic acid or oral folic acid groups than by i.v. folinic acid. However, the trial was not powered to identify potential differences in the proportion of patients whose tHcy levels were normalized by each treatment modality.

Our results confirm recent reports demonstrating that short-term supplementation of chronic haemodialysis patients with oral [9,10] or i.v. [11] reduced forms of folate does not lower tHcy levels more than supplementation with oral [9,10] or i.v. [11] folic acid. They extend these observations by showing that even long-term administration of reduced forms of folate did not further improve tHcy-lowering efficacy in haemodialysis patients. These data suggest that our initial hypothesis regarding abnormal folate metabolism in these patients was incorrect [1,3]. They are in line with data published by Bostom et al. [9] who studied normal folate metabolism, as assessed by normal baseline levels of plasma 5-MTHF, and found significant increases in total plasma folate (predominantly plasma 5-MTHF) after oral treatment with either folic acid or L-5-MTHF. Thus, the reason for the reduction in plasma homocysteine clearance observed in haemodialysis patients remains unidentified. It is possible that the reduction is also due to multiple abnormalities of the remethylation pathway that are not related to folate [13]. For example, recent data suggest that the reduction of betaine-homocysteine methyl transferase activity, which is probably due to an accumulation of dimethylglycine in the plasma of uraemic patients, may be an important contributor to hyperhomocysteinaemia [14]. Since vitamin B12 plays a role in the remethylation pathway [13], it is possible that part of the Hcy-lowering effect can be ascribed to the simultaneous vitamin B12 treatment. In a recent study, Manns et al. [15] demonstrated that oral vitamin B12 (1 mg/day) supplements given to haemodialysis patients who were not vitamin B12-deficient by usual

Table 1. Baseline demographic, clinical and biological characteristics in the three groups of patients

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 20)</th>
<th>Group 2 (n = 20)</th>
<th>Group 3 (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 10</td>
<td>53 ± 14</td>
<td>56 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Males/females</td>
<td>12/8</td>
<td>13/7</td>
<td>13/7</td>
<td>NS</td>
</tr>
<tr>
<td>Time since first dialysis (months)</td>
<td>28 ± 17</td>
<td>30 ± 16</td>
<td>27 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>Weekly dialysis session duration (h)</td>
<td>12.4 ± 0.6</td>
<td>12.4 ± 0.6</td>
<td>12.5 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>High flux membrane (%)</td>
<td>95</td>
<td>90</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>35 ± 3</td>
<td>34 ± 4</td>
<td>36 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma tHcy (μmol/l)</td>
<td>31.6 ± 8.8</td>
<td>32.7 ± 8.6</td>
<td>33 ± 8.9</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma folate (ng/ml)</td>
<td>4.3 ± 1</td>
<td>4.2 ± 1.4</td>
<td>4.2 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma cobalamin (pg/ml)</td>
<td>372 ±110</td>
<td>382 ±108</td>
<td>401 ±110</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma vitamin B6 (nmol/l)</td>
<td>124 ± 42</td>
<td>137 ± 52</td>
<td>133 ± 50</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group 1, i.v. folinic acid; group 2, oral folinic acid; group 3, oral folic acid; tHcy, total homocyst(e)ine.
TREATMENT OF HYPERHOMOCYSTEINAEMIA IN HEMODIALYSIS PATIENTS

Table 2. Variations in plasma tHcy, folate, cobalamin and vitamin B6 concentrations during the study period in the three groups

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4 months</td>
<td>6 months</td>
</tr>
<tr>
<td>(n=20)</td>
<td>(n=20)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>Plasma tHcy (nmol/l)</td>
<td>31.6±8.8*</td>
<td>17.0±4.0</td>
</tr>
<tr>
<td>Plasma folate (ng/ml)</td>
<td>4.3±1.0*</td>
<td>22.6±1.8</td>
</tr>
<tr>
<td>Plasma cobalamin (pg/ml)</td>
<td>372±10*</td>
<td>792±21</td>
</tr>
<tr>
<td>Plasma vitamin B6 (nmol/l)</td>
<td>124±12*</td>
<td>193±62</td>
</tr>
</tbody>
</table>

Plasma tHcy, folate, cobalamin and vitamin B6 concentrations during the study period in the three groups.

Group 1: i.v. folinic acid, group 2: oral folinic acid; group 3: oral folic acid; tHcy, total homocyst(e)ine; *P<0.0001 baseline vs. 2, 4 and 6 months, **P<0.05 group 1 vs. groups 2 and 3.

In summary, the results of our present study show that long-term supplementation with either folic acid or folinic acid, combined with high-dose i.v. vitamin B6 and oral vitamin B12, have comparable efficacy in reducing tHcy levels in haemodialysis patients. These findings suggest that the hyperhomocysteinaemia observed in haemodialysis patients is not due to abnormal folate metabolism. They also suggest that the reduction of plasma homocysteine clearance may be due to multiple abnormalities of the remethylation pathway that may not be related to folate alone.

References

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