Abstract

Objective: The authors found considerably lower plasma total homocysteine (tHcy) concentrations in patients with end-stage renal disease (ESRD) on maintenance hemodialysis, who routinely received high-dose parenteral vitamin B\textsubscript{12} than in comparable patients receiving much higher doses of folic acid but only replacement-dose oral vitamin B\textsubscript{12}. They therefore sought prospective evidence that high-dose parenterally administered vitamin B\textsubscript{12} may partially ameliorate renal failure-associated hyperhomocysteinemia.

Design: Open phase 2 clinical trial.

Setting: Outpatient hemodialysis unit.

Patients: Fourteen clinically stable patients on maintenance hemodialysis with normal baseline serum vitamin B\textsubscript{12} concentrations.

Intervention: Three parenteral injections of 1 mg vitamin B\textsubscript{12} given at 4-week intervals.

Outcome measures: Plasma tHcy and serum vitamin B\textsubscript{12} concentrations were measured before, during and 7 months after the termination of vitamin B\textsubscript{12} therapy.

Results: The mean (and standard error) baseline plasma tHcy was 26.5 (1.8) $\mu$mol/L. The plasma tHcy value fell successively after each vitamin injection to reach a value of 23.6 (1.6) $\mu$mol/L 1 month after the final injection ($p < 0.05$), while the serum vitamin B\textsubscript{12} concentration increased from 471 (42) pmol/L to 890 (74) pmol/L ($p < 0.05$). Seven months after the final injection, the serum B\textsubscript{12} concentration had fallen and tHcy had risen to near their original values.

Conclusions: Three monthly vitamin B\textsubscript{12} injections modestly but distinctly reduced tHcy concentrations in hemodialysis patients whose prior vitamin B\textsubscript{12} status was normal. Randomized placebo-controlled clinical trials of longer duration and using larger or more frequent parenteral doses are indicated to determine whether administration of this safe and inexpensive vitamin can improve hyperhomocysteinemia in ESRD.

Résumé

Objectif : Les auteurs ont trouvé des concentrations d’homocystéine totale (tHcy) plasmatique beaucoup moins élevées chez des patients atteints d’insuffisance rénale chronique au stade ultime (IRSU) en hémodialyse de maintenance qui recevaient régulièrement de fortes doses de vitamine B\textsubscript{12} par voie parentérale que chez des patients comparables recevant des doses beaucoup plus élevées d’acide folique, mais seulement des doses de
remplacement de vitamine B₁₂ par voie orale. Ils ont donc cherché à réunir des données prospectives indiquant que de fortes doses de vitamine B₁₂ administrée par voie parentérale pourraient réduire en partie l’hyperhomocystéinémie associée à l’insuffisance rénale.

**Conception** : Étude clinique ouverte de phase 2.

**Contexte** : Service d’hémodialyse externe.

**Patients** : Quatorze patients stables sur le plan clinique, en hémodialyse de maintenance, dont les concentrations de vitamine B₁₂ sérique de référence étaient normales.

**Intervention** : Trois injections parentérales de 1 mg de vitamine B₁₂ administrées à intervalles de quatre semaines.

**Mesures de résultats** : On a mesuré les concentrations de tHcy plasmatique et de vitamine B₁₂ sérique avant la thérapie à la vitamine B₁₂, pendant le traitement et sept mois après la fin du traitement.

**Résultats** : La tHcy plasmatique de référence moyenne (et l’erreur type) était de 26,5 (1,8) µmol/L. La valeur de la tHcy plasmatique a diminué successivement après chaque injection de vitamine pour atteindre 23,6 (1,6) µmol/L un mois après l’injection finale ($p < 0,05$), tandis que les concentrations de vitamine B₁₂ sérique sont passées de 471 (42) pmol/L à 890 (74) pmol/L ($p < 0,05$). Sept mois après l’injection finale, la concentration de vitamine B₁₂ sérique était tombée et la tHcy avait augmenté pour atteindre presque les valeurs de départ.

**Conclusions** : Trois injections mensuelles de vitamine B₁₂ ont réduit de façon modeste mais quantifiable les concentrations de tHcy chez les patients en hémodialyse qui présentaient auparavant un statut normal quant à la vitamine B₁₂. Des études cliniques randomisées contrôlées par placebo de plus longue durée et au cours desquelles on utiliserait des doses parentérales plus importantes et plus fréquentes sont indiquées pour déterminer si l’administration de cette vitamine sûre et peu coûteuse peut améliorer l’hyperhomocystéinémie dans les cas d’IRSU.

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**Introduction**

Most people with end-stage renal disease (ESRD) have increased plasma total homocysteine (tHcy) concentrations.¹² There is much interest in identifying therapies that effectively reduce hyperhomocysteinemia, since it may better predict the risk of cardiovascular disease in these patients than standard risk factors³⁻⁵ and has been associated with an increased risk of access device thrombosis.⁶ Homocysteine is metabolized either through the transsulfuration pathway, in which it is converted to cystathionine and thence to cysteine, or by remethylation to methionine in a reaction catalyzed by the vitamin B₁₂-dependent enzyme, methionine synthase. Methyltetrahydrofolate is the methyl donor in the methionine-synthase reaction, and the enzymes of the transsulfuration pathway are vitamin B₆-dependent. Consequently, deficiencies of vitamin B₁₂, folate or vitamin B₆, or inherited defects in the enzymes on either pathway, will lead to hyperhomocysteinemia.⁷⁻⁹ A role for riboflavin (vitamin B₂) in homocysteine remethylation has also been suggested.¹⁰ Of all the vitamins, folic acid is considered to be the most important by far,¹¹ and regimens currently used to lower homocysteine concentrations in ESRD contain folic acid in supraphysiologic amounts.¹² In 1996, Bostom and associates showed that a 3-vitamin combination consisting of 16 mg of folic acid, 100 mg of vitamin B₆ and 1 mg vitamin B₁₂ per day lowered tHcy concentrations in ESRD patients by 30% more than standard folic acid supplementation of 1 mg/d.¹³ Whether this was due entirely to the very high dose of folic acid used or the combined effect of all 3 vitamins is not known. In particular, no study has shown whether large doses of vitamin B₁₂ independently reduce homocysteine levels in ESRD patients whose prior vitamin B₁₂ status is normal.¹¹² We were therefore interested to observe that patients treated in a Montreal hemodialysis unit where high-dose vitamin B₁₂ is routinely administered parenterally had considerably lower plasma tHcy concentrations than comparable patients in a nearby unit where high-dose folic acid, but only replacement-dose orally administered vitamin B₁₂ was prescribed.¹⁴ This suggested that high-dose vitamin B₁₂ could indeed have an independent homocysteine-lowering effect in ESRD.

Patients with ESRD are uniquely suitable to test for a pharmacologic effect of high-dose vitamin B₁₂ since, generally, large administered doses are promptly excreted in the urine.¹⁵ We also conjectured that parenteral administration could be important since oral bioavailability of this vitamin is low. After oral administration of vitamin B₁₂, a maximum of about 1.5 µg is absorbed by way of intrinsic factor. A second transport system exists that does not require intrinsic factor, but its efficiency is only about 1%. Thus, even patients without pernicious anemia absorb
only a few micrograms of the vitamin after oral doses of 500 to 1000 µg.16 This is sufficient to treat and prevent vitamin B₁₂ deficiency but could be insufficient to achieve a pharmacologic effect on homocysteine metabolism comparable to that of “megadose” folic acid as used by Bostom and colleagues.13,18

In this report we describe the results of an open phase 2 clinical trial whose aim was to learn whether high-dose parenteral vitamin B₁₂ can indeed reduce plasma tHcy concentrations in vitamin B₁₂-adequate ESRD patients when added to the large doses of folic acid they were already receiving.

Patients and methods

With approval from the Research Ethics Committee of the Sir Mortimer B. Davis-Jewish General Hospital, we approached 14 clinically stable ESRD patients (9 men, 5 women) on maintenance hemodialysis therapy for permission to measure their circulating tHcy, folic acid, vitamin B₁₂, and methylmalonic acid (MMA) concentrations before, during and for several months after a short course of monthly vitamin B₁₂ injections. Serum creatinine, urea, albumin and hemoglobin values, and the mean erythrocyte volume were recorded. All the subjects consumed a daily vitamin tablet providing 5 mg folic acid and, in every case but one, a multiple vitamin tablet providing 1 mg folic acid and either 6 or 10 µg of vitamin B₁₂, as has been the standard practice in this unit for many years.

Vitamin B₁₂ (1 mg of cyanocobalamin, 1 mg/mL) was administered by subcutaneous injection at the end of the dialysis session. Serum creatinine, urea, albumin and hemoglobin values, and the mean erythrocyte volume were recorded. All the subjects consumed a daily vitamin tablet providing 5 mg folic acid and, in every case but one, a multiple vitamin tablet providing 1 mg folic acid and either 6 or 10 µg of vitamin B₁₂, as has been the standard practice in this unit for many years.

On the test days, a predialysis blood sample was collected from the arteriovenous fistula into a 4-mL tube containing 7.2 mg of ethylenediaminetetraacetic acid (EDTA) (Sarstedt, Montreal). The tube was promptly chilled in crushed ice, brought to the laboratory and centrifuged within 10 minutes at 3000 rpm at 4 °C. The resulting plasma was stored at −30 °C until analyzed for tHcy and total cysteine concentrations by high-pressure liquid chromatography (HPLC) with fluorescence detection using the method of Araki and Sako as modified for isocratic separation by Feussner and associates.20 All disulfides were converted to their reduced sulfhydryl form by adding 20 µL of 10% tris-(2-carboxyethyl)-phosphine hydrochloride (Pierce, Rockford, Ill.) to 200 µL of plasma, followed by incubation at room temperature for 30 minutes, as described by Gilfix and associates.21 Plasma proteins were precipitated by adding 200 µL of ice-cold 10% trichloroacetic acid containing 1 mmol of EDTA (Fisher Chemicals, Montreal). To 100 µL of the resulting plasma supernatant was added 200 µL of 0.1 M potassium tetraborate (Sigma Aldrich Canada, Oakville, Ont.) containing 4 mmol of Na₂EDTA. The samples were converted to fluorescent derivatives by the addition of 100 µL of a solution of ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F; Sigma Aldrich Canada; 1 mg of SBD-F per 1 mL of 0.1 M potassium tetraborate) and incubated for 60 minutes at 60 °C in a water bath then stored at −30 °C. Samples were filtered through a 0.45-µm syringe-driven filters units (Millex-HV, Millipore, Montreal) before injection onto the HPLC column.

The separation was performed on a Waters NovaPak C18 reverse phase column with a NovaPak C18 precolumn insert (Waters Canada, Mississauga, Ont.). The system was equipped with a Waters fluorescence detector (Model 420) and a Waters autosampler (Model 717 Plus). The samples were separated in isocratic mode with a mobile phase consisting of 0.06 M sodium acetate buffer, pH 4.0, containing 1% methanol. The flow rate was 0.6 mL/min, the pump pressure approximately 1100 psi, and the injection volume 20 µL. Fluorescence was measured with the excitation and emission filters set at 385 and 515 nm, respectively.

Serum MMA was measured by gas chromatography mass spectrometry as described by Montgomery and Mamer. Serum vitamin B₁₂ and folic acid were measured with use of the BioRad Quantaphase II radioassay (Bio-Rad Diagnostics, Hercules, Calif.)
with the samples counted on a 1270 Rachgamma II gamma counter (LKB Wallac, Stockholm, Sweden) in the hospital’s biochemistry laboratory. Serum, creatinine, urea and albumin levels were measured on the Hitachi model 917 multiple-channel analyzer (Hitachi, Tokyo) located in the same laboratory.

Repeated measures analysis of variance (ANOVA) was used to determine the significance of changes in the circulating concentrations of tHcy, cysteine, vitamin B₁₂ and MMA (SigmaStat version 1.0, Jandel Corp., San Rafael, Calif.). When the ANOVA indicated significance, the Newman–Keuls test was used to determine the source of difference. All results are presented as the mean (and standard error of the mean).

Results

The mean age of the patients was 73 (3) years and they had been receiving maintenance dialysis therapy for 47 (8) months. They were clinically stable and their average weight was 68 (4) kg. Predialysis urea (29 [2] mmol/L) and creatinine (880 [79] µmol/L) concentrations were typical of this patient population. The mean hemoglobin was 115 (2.3) g/L, mean corpuscular volume 94 (2) fL, and serum albumin 35 (1) g/L. Serum folic acid concentrations were uniformly greater than 45 nmol/L (normal range from 5.0 to 36.3 nmol/L) in keeping with the pharmacologic folic acid therapy all the patients received. The baseline plasma vitamin B₁₂ concentration was 471 (42) pmol/L, ranging from 158 to 677 pmol/L (normal range, 80 to 600 pmol/L). The only patient not using an oral vitamin B₁₂ supplement had the lowest serum B₁₂ level (158 pmol/L), still well within the normal range. His plasma tHcy of 30.1 µmol/L was within the 95% confidence interval for the group. Exclusion of his response does not affect our conclusions.

The results of the vitamin injection therapy are shown in Table 1 and illustrated graphically in Fig. 1. The 3 monthly vitamin injections increased average serum vitamin B₁₂ concentrations by approximately 90% (p < 0.0001). Three months after the final injection, B₁₂ concentrations had decreased, although not yet completely to normal. There was a successive reduction in plasma tHcy after each vitamin injection, with a return to the original value 7 months after the final injection. Plasma total cysteine did not change significantly. Baseline serum MMA was markedly increased (1.13 [0.13] µmol/L, range from 0.64 to 2.31 µmol/L; reference range from 0.05 to 0.26 µmol/L). Despite the suspicion of a downward trend, the reduction in average serum MMA during vitamin B₁₂ therapy was not statistically significant.

Discussion

The important result of this study is that a brief course of parenteral vitamin B₁₂ therapy modestly, but distinctly, reduced plasma tHcy in patients with ESRD whose baseline serum vitamin B₁₂ concentrations were within the normal range. Vitamin B₁₂ must, therefore, have reduced their tHcy concentrations through a pharmacologic mechanism, presumably one

<table>
<thead>
<tr>
<th>Measurement time</th>
<th>Homocysteine, µmol/L</th>
<th>Cysteine, µmol/L</th>
<th>Vitamin B₁₂, pmol/L</th>
<th>Methylmalonic acid, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1st injection</td>
<td>26.5 (1.8)</td>
<td>448 (26)</td>
<td>471 (42)</td>
<td>1.13 (0.13)</td>
</tr>
<tr>
<td>4 wk after 1st injection</td>
<td>24.8 (1.6)</td>
<td>487 (30)</td>
<td>(p = 0.07)*</td>
<td>0.99 (0.07)</td>
</tr>
<tr>
<td>4 wk after 2nd injection</td>
<td>24.0 (2.0)</td>
<td>491 (35)</td>
<td>(p = 0.035)</td>
<td>0.95 (0.05)</td>
</tr>
<tr>
<td>4 wk after 3rd injection</td>
<td>23.6 (1.6)</td>
<td>484 (25)</td>
<td>(p = 0.002)</td>
<td>0.94 (0.05)</td>
</tr>
<tr>
<td>16 wk after 3rd injection</td>
<td>23.1 (1.8)</td>
<td>464 (24)</td>
<td>(p = 0.017)</td>
<td>632 (58)</td>
</tr>
<tr>
<td>28 wk after 3rd injection</td>
<td>27.0 (2.3)</td>
<td>488 (25)</td>
<td>(p = 0.749)</td>
<td>602 (197)</td>
</tr>
</tbody>
</table>

*p values are versus initial value.
analogous to that of the “megadose” folic acid used to lower tHcy concentrations in non-folate-deficient ESRD patients\textsuperscript{13,18} and renal transplant recipients.\textsuperscript{23} The participants in this study continued to take the 5 mg of folic acid orally and a multiple vitamin containing 1 mg of folic acid and either 6 or 10 µg vitamin B\textsubscript{12} that is standard in this unit. We do not know whether the vitamin B\textsubscript{12} effect requires concurrent, high-dose oral folic acid or whether 16 mg/d of folic acid without supplemental vitamin B\textsubscript{12} would have been as effective or more effective without high-dose vitamin B\textsubscript{12}. It is also possible that oral dosing with large amounts of vitamin B\textsubscript{12} can independently lower plasma tHcy in ESRD patients, despite our conjecture that the parenteral route ought to be superior.

Although the 3 vitamin B\textsubscript{12} injections lowered average tHcy by only 13%, even this modest effect could be clinically significant. When plasma tHcy exceeds 20 µmol/L, as in ESRD, even a moderate lowering is associated with a large reduction in cardiac risk.\textsuperscript{24} Also, the time trend of the tHcy concentration after the 3 vitamin injections suggests the level might have continued to fall with a treatment of longer duration or with larger vitamin doses, or both. Thus, the mean serum vitamin B\textsubscript{12} concentration 1 month after the third injection was 890 (278) pmol/L, whereas in our recent observational study, the average vitamin B\textsubscript{12} concentration of ESRD patients receiving monthly vitamin B\textsubscript{12} parenterally for an indefinite period was considerably higher than this (1270 [740] pmol/L, \(p = 0.06\)) and their plasma tHcy concentrations 22% lower than in patients receiving high-dose folic orally acid but no vitamin B\textsubscript{12} parenterally.\textsuperscript{14} Vitamin B\textsubscript{12} given parenterally has been safely administered to ESRD patients in a dose of 0.5 mg 3 times per week for many months.\textsuperscript{25}

The cause of the hyperhomocysteinemia of ESRD is uncertain. It is plausible to suppose that reduced renal metabolic mass plays an important role,\textsuperscript{1} and this could explain the hypercysteinemia of ESRD as well.\textsuperscript{14} However, unlike in the rat kidney, which extracts considerable amounts of homocysteine,\textsuperscript{26} the renal arteriovenous plasma homocysteine gradient of normal humans was recently reported to be zero, arguing against impaired renal homocysteine catabolism as the cause of hyperhomocysteinemia in ESRD.\textsuperscript{27} The fact that supraphysiologic doses of folic acid partially lower plasma tHcy in ESRD\textsuperscript{13} suggests that this therapy partially overcomes a block in homocysteine remethylation, and some support for this concept has emerged from a recent human whole-body tracer study, which showed a defect in remethylation rather than transsulfuration in ESRD patients.\textsuperscript{28} It was with this possibility in mind that we tested the effectiveness of high-dose parenteral vitamin B\textsubscript{12} as add-on therapy to the high-dose oral folic acid the patients in our study were already using.

Baseline serum MMA concentrations were

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**Fig. 1:** Top: plasma total homocysteine concentrations in response to parenterally administered vitamin B\textsubscript{12}. Bottom: serum vitamin B\textsubscript{12} (closed circles) and methylmalonic acid (open circles) levels in response to parenterally administered vitamin B\textsubscript{12}.

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markedly increased in our patients, as has been previously reported in patients with chronic renal failure. This has been attributed to impaired renal MMA clearance rather than a biochemical vitamin B12 deficiency.29,30 Dierkes and associates31 recently showed that MMA levels can be substantially reduced by vitamin replacement therapy in ESRD patients with documented vitamin B12 deficiency. The short course of vitamin B12 therapy we used did not significantly reduce our patients’ serum MMA concentrations, but these patients were not vitamin B12 deficient, and, as is commonly observed,2,32,33 their average vitamin B12 serum concentrations were in the high-normal range. We cannot predict whether a higher dose or longer duration of parenterally administered vitamin B12, or both, would have significantly lowered their serum MMA concentrations, for the mechanism by which supraphysiologic vitamin B12 lowers tHcy could be independent of its MMA-lowering properties. It would be of considerable interest to test this in future studies of longer duration and involving larger numbers of subjects.

As this was not a controlled clinical trial, we cannot rule out the possibility that the tHcy-lowering reported here was simply time-related or due to an unidentified confounding variable. favouring the possibility that this was indeed a vitamin B12 effect are the constancy of plasma cysteine concentrations over the same period and the return of tHcy concentrations to baseline values 7 months after the final vitamin injection, in association with a fall in serum vitamin B12 to close to pre-treatment levels.

The results of this open phase 2 clinical trial support the hypothesis that high-dose parenteral vitamin B12 could prove to be an important addition to a comprehensive Hcy-lowering regimen for patients with ESRD. The treatment is convenient, safe and inexpensive, and on theoretical grounds superior to oral therapy. The present results should stimulate controlled studies of longer duration and using larger doses of parenteral vitamin B12.

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References


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