Original Paper

NEPHRON

Nephron 2002;91:58–63

Accepted: July 10, 2001

Randomized Trial of Methylcobalamin and Folate Effects on Homocysteine in Hemodialysis Patients

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Key Words

Chronic renal failure · Folate · Hemodialysis · Homocysteine · Hyperhomocysteinemia · Methylcobalamin

Abstract

Background: There are no data available on the effects of intravenous (i.v.) methylcobalamin (Me-Cbl), the coenzymatically active form of vitamin B_{12} that acts as a cofactor for methionine synthase in the conversion of total homocysteine (tHcy) to methionine, with or without oral folic acid (FA) supplementation, on fasting tHcy levels in hemodialysis (HD) patients. ***Methods:*** We performed a prospective randomized trial in which 62 chronic HD patients without previous vitamin supplementation were divided into four groups. Group A received Me-Cbl 500 μg twice/week plus FA 10 mg/day; group B received FA 10 mg/day alone; group C received no vitamin supplementation, and group D was on Me-Cbl 500 μg twice/week alone. Fasting tHcy, vitamin B_{12}, serum (s) FA and erythrocytic (e) FA were measured predialysis before and after 4 months of therapy. ***Results:*** Final tHcy levels were significantly lower in group A (10.2 ± 3.1 μmol/l) compared to groups C (27.3 ± 9.7 μmol/l, $p < 0.001$) and group D (24.3 ± 11.8 μmol/l, $p < 0.001$) and similar to group B (11.2 ± 1.9 μmol/l, p = n.s.). Mean tHcy levels showed a significant decrease in group A from 22.5 ± 15.6 to 10.2 ± 3.1 μmol/l (p = 0.003) and in group B from 19.9 ± 4.0 to 11.2 ± 1.9 μmol/l (p = 0.012), while no significant changes were observed in groups C (25.9 ± 9.3 vs. 27.3 ± 9.7 μmol/l, p = n.s.) and D (26.6 ± 14.3 vs. 24.3 ± 11.8 μmol/l, p = n.s.). ***Conclusion:*** Oral FA (10 mg/day) supplementation appears to be an effective approach to normalize plasma tHcy in chronic HD patients; the addition of i.v. Me-Cbl (500 μg twice/week) to this regimen showed no benefit. Separately, FA corrected hyperhomocysteinemia (HtHcy), while Me-Cbl showed no change.

Introduction

Cardiovascular disease is the major cause of death in end-stage renal disease patients, due in part to a higher prevalence of established arteriosclerotic risk factors [1, 2]. Recently, controlled evidence has shown that HtHcy occurs more commonly than other cardiovascular risk factors in patients on chronic HD [3]. According to some reports, HtHcy, defined as fasting tHcy levels > 15 μmol/l [4, 5] and classified as moderate (15–30 μmol/l), intermediate (30–100 μmol/l), and severe (>100 μmol/l) [6], occurs in 83% of the dialysis population [7, 8], and appears to contribute independently to their excess incidence of cardiovascular outcomes [9–11]. Moreover, tHcy disrupts

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0028–2766/02/0911–0058$18.50/0

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Table 1. Characteristics of 62 hemodialysis patients divided into 4 groups

Group	Age years	Males	Time on HD months	KTV	Initial albumin, g/l	Initial Hct %
Group A (n = 17)	59.70 ± 18.1 (35–80)	10	28.3 ± 21.4 (7–75)	1.2 ± 0.3	3.6 ± 0.2	32.3 ± 3.6
Group B (n = 16)	62.2 ± 10.6 (38–75)	10	13.6 ± 9.2 (7–30)	1.2 ± 0.3	3.8 ± 0.2	32.3 ± 3.2
Group C (n = 13)	50.7 ± 18.6 (19–70)	7	20.9 ± 15.2 (6–45)	1.2 ± 0.2	3.7 ± 0.1	32.6 ± 2.8
Group D (n = 16)	58.1 ± 21.2 (27–86)	8	12 ± 9.7 (5–28)	1.2 ± 0.3	3.4 ± 0.5	29 ± 6.9

HD = Hemodialysis; Hct = hematocrit.
Note: All differences between groups are nonsignificant.

several endothelium-related anticoagulant functions resulting in enhanced thrombogenicity and contributing to the occlusion of arterio-venous fistulas and grafts in HD patients [12–14]. More recently, a prospective study revealed that each 1 μmol/l increase in the tHcy level was associated with a 4% increase in the risk of access thrombosis [15].

Folate, vitamin B_6 and vitamin B_{12} are the main cofactors for tHcy metabolism. Vitamin B_{12} and folate play a critical role in the remethylation of homocysteine to methionine [16, 17]. In addition, in chronic renal failure patients a significant reduction in membrane protein methyl esterification exists, and structural cellular damages accumulate, rendering proteins inadequately repaired [18]. Me-Cbl, the methylated form of cobalamin, is the cofactor for the enzyme methionine synthase that converts homocysteine to methionine [19]. In the Me-Cbl-dependent [16] reaction mediated by methionine synthase, homocysteine acquires a methyl group from methyltetrahydrofolate to form methionine. A considerable proportion of methionine is then used to form S-adenosylmethionine, a universal donor of methyl groups [1, 20].

Therefore, it seems appropriate to diminish tHcy levels particularly in HD patients. Several studies in HD patients have shown that FA supplementation at doses of 10 mg/day appear to lower fasting plasma tHcy levels by 30–50% [21–23]. In our center, because many HD patients were not taking vitamins, we have randomly selected a group which was started on i.v. Me-Cbl to avoid noncompliance and putative absorption differences among patients and assess its direct effects on tHcy alone or coupled with oral FA, overcoming the uremic-altered FA-mediated methylation processes.

Subjects and Methods

We undertook a prospective, randomized trial in which predialysis fasting plasma concentrations of tHcy, vitamin B_{12}, sFA and eFA, were determined in 62 chronic HD patients before and after 4 months of therapy with i.v. Me-Cbl and/or FA supplementation or without treatment. In this prospective study, patients were randomly divided in a blinded fashion by a random-number generator, stratified only for centre, into four groups: group A (n = 17) received 500 μg of Me-Cbl (Methycobal®; Esai, Bunkyo-ku, Tokyo, Japan) postdialysis twice a week plus FA 10 mg/day; group B (n = 16) was on 10 mg of daily FA supplementation; group C (n = 13) received no vitamin therapy and group D (n = 16) received 500 μg of Me-Cbl twice weekly postdialysis. We included patients between 19 and 86 years of age, requiring HD for at least 5 months. Patients were excluded if previous vitamin supplementation was prescribed. All patients concluded the study. All patients received epoetin 4,000 U subcutaneously twice weekly and 100 mg of i.v. iron saccharate postdialysis when necessary to maintain transferrin saturation between 25 and 50%. All groups were not different according to age, to gender, to time on HD, to KT/V (table 1), to primary renal disease, to diet, and to baseline plasma levels of tHcy, vitamin B_{12}, sFA, eFA, albumin and hematocrits (tables 2, 3). Thrice weekly high-flux HD was performed using polysulphone (F80®, Fresenius AG, Bad Homburg, Germany) or triacetate cellulose (FB 210 U®, Nipro, Japan) reused membranes with a mean QB of 380 ± 20 ml/min, QD of 500 ml/min and a mean HD session of 3.5 ± 0.5 h. Serum was processed by fluorescence polarization immunoassay for tHcy (normal: 10 ± 5 μmol/l), radioimmunoassay for vitamin B_{12} complex including all forms of cobalamins, i.e. Me-Cbl, cyano-cobalamin, hydroxycobalamin and adenosyl-cobalamin (normal: 200–900 pg/ml), and for FA determinations (sFA normal: >10 ng/ml; eFA normal: 200–700 ng/ml).

Results are expressed as the mean ± SD. Kruskall-Wallis test (one-way ANOVA by ranks) was used to assess differences between groups. Wilcoxon signed ranks test was used to evaluate intragroup differences. $p < 0.05$ as considered to be significant.

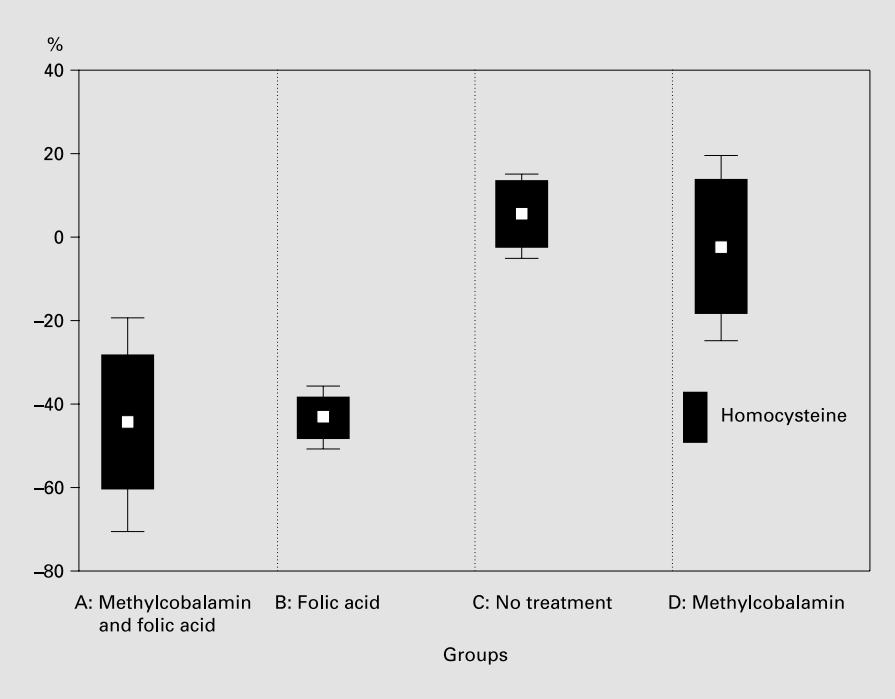

Fig. 1. Percent variations of homocysteine in all groups.

Table 2. Initial and final values and percent variations of homocysteine in all groups

Group	Initial Hcy µmol/l	Final Hcy µmol/l	% Hcy variation
Group A	22.5 ± 15.6	10.2 ± 3.1[a]	–44
Group B	19.9 ± 4.0	11.2 ± 1.9[b]	–43
Group C	25.9 ± 9.3	27.3 ± 9.7	5
Group D	26.6 ± 14.3	24.3 ± 11.8	–2.4

Hcy = Homocysteine; Hct = hematocrit.
[a] p = 0.003; [b] p = 0.012.

Results

The effects of Me-Cbl and FA on tHcy are shown in figure 1 and changes in vitamin levels are summarized in table 3.

Effects of Me-Cbl on tHcy

In group D, final tHcy levels (24.3 ± 11.8 µmol/l) were significantly higher than final levels in groups A (10.2 ± 3.1 µmol/l, $p < 0.001$) and B (11.2 ± 1.9 µmol/l, $p < 0.001$); no statistical difference with respect to final levels in group C (27.3 ± 9.7 µmol/l) and with respect to initial values in group D (26.6 ± 14.3 µmol/l).

Effects of FA on tHcy

Final tHcy levels in group B (11.2 ± 1.9 µmol/l) were similar to final levels of group A (10.2 ± 3.1 µmol/l) and significantly lower than final levels of groups C (27.3 ± 9.7 µmol/l, $p < 0.001$) and D (24.3 ± 11.8 µmol/l, $p < 0.001$). Final tHcy levels decreased significantly when compared to initial values (11.2 ± 1.9 vs. 19.9 ± 4.0 µmol/l, p = 0.012); percent reduction 43%.

Combined Me-Cbl and FA Effects on tHcy

In group A, tHcy final levels were similar to group B (10.2 ± 3.1 vs. 11.2 ± 1.9 µmol/l, p = n.s.) and showed a significant decrease when compared to final levels of groups C (27.3 ± 9.7 µmol/l, $p < 0.001$) and D (24.3 ± 11.8 µmol/l, $p < 0.001$). Final group A tHcy concentrations were significantly lower than initial values (10.2 ± 3.1 vs. 22.5 ± 15.5 µmol/l, p = 0.003); percent decrease 44% (fig. 1).

Finally, it was observed that by the first month of treatment (data not shown), 15 patients from group A (90%) but only 11 from group B (70%) had normalized their tHcy levels. In the second month, all patients from both groups had normal tHcy plasma levels.

Table 3. Initial and final levels of vitamin B_{12}, serum folic acid and erythrocytic folic acid in all groups

Group	Initial vitamin B_{12}, pg/ml	Final vitamin B_{12}, pg/ml	Initial sFA ng/ml	Final sFA ng/ml	Initial eFA ng/ml	Final eFA ng/ml
Group A	2,352 ± 1,453	23,553 ± 11,334[a]	5.7 ± 2.7	407 ± 422[a]	743 ± 847	5,401 ± 1,926[a]
Group B	2,489 ± 2,423	6,372 ± 5,378	6.6 ± 2.4	267 ± 182[b]	485 ± 122	3,259 ± 1,600[b]
Group C	2,152 ± 1,100	2,205 ± 1,206	7 ± 2.3	6.9 ± 2.2	334 ± 120	316 ± 102
Group D	1,691 ± 1,360	17,422 ± 4,819[c]	8.6 ± 3.3	9.7 ± 5.5	778 ± 488	700 ± 439

sFA = Serum folic acid; eFA = erythrocytic folic acid.
[a] p = 0.003; [b] p = 0.012; [c] p = 0.003.

Changes in Vitamin Levels

With respect to vitamin levels (table 3), all patients had initial vitamin B_{12} values within normal limits and initial low sFA blood levels, despite eFA were normal. When Me-Cbl was prescribed, final levels in groups A and D were significantly higher than the initial ones (23,553 ± 11,334 vs. 2,352 ± 1,453 pmol/ml, p = 0.003 in group A; 17,422 ± 4,819 vs. 1,691 ± 1,360 pmol/ml, p = 0.003 in group D). Interestingly, despite baseline normal vitamin B_{12} and eFA blood levels, when considerable high concentrations of Me-Cbl, sFA and eFA were achieved after vitamin supplementation, tHcy decreased, while tHcy actually showed a modest nonsignificant increase in group C. When final values of vitamins were compared between groups, Me-Cbl final levels were significantly higher in group A (p = 0.001) and sFA final levels were significantly higher in groups A and B than in the rest (p = 0.001). Noteworthy, eFA levels were significantly higher when Me-Cbl and FA were given together (5,401 ± 1,926 ng/ml, p = 0.001); in group B eFA final concentrations were also different (3,258 ± 1,600 ng/ml, p = 0.001) from those in groups C (316 ± 101 ng/ml) and D (699 ± 439 ng/ml). No adverse effects could be ascribed to Me-Cbl or FA therapy during the study.

Discussion

Our data show that oral administration of 10 mg FA is safe and highly effective in normalizing tHcy blood levels in chronic HD patients, and i.v. Me-Cbl alone did not significantly reduce tHcy levels; when prescribed with oral FA supplementation, Me-Cbl achieved a similar decrease in tHcy levels than the one obtained with FA alone.

Our results emphasize the importance of FA in the regulation of plasma tHcy levels in chronic HD patients. A daily oral intake of 5–10 mg FA decreases tHcy levels by 20–30%, provided vitamin B_{12} levels are within normal limits. A primary function of FA is to participate as a methyl donor for the conversion of homocysteine to methionine. The methyl group is first transferred from methyl-tetrahydrofolate to cobalamin to form Me-Cbl, which then donates the methyl group onto homocysteine to generate methionine [16]. Due to the methylation abnormalities present in chronic renal failure, Me-Cbl concentration may be diminished, and the activity of methionine synthase could be abnormal. This enzyme impairment could result in abnormal DNA synthesis and methylation abnormalities, thereby altering erythropoiesis and protein synthesis, which would then translate into anemia and hypoalbuminemia, respectively. It is also important to emphasize that FA distribution in blood is mainly divided into two compartments: eFA, which represents the circulating deposits of FA and was within normal limits in all patients in this study (table 3); and, sFA, the metabolically available or physiologically active compartment, which was low in all groups (table 3), regardless of the time the patients were on HD. After oral FA loading, sFA dramatically increased in all groups and tHcy levels normalized (tables 2, 3). Thus, sFA and not eFA must be monitored in chronic HD patients to assess its ability to lower tHcy levels.

With respect to the sole administration of Me-Cbl, albeit vitamin B_{12} is important as a cofactor in Hcy metabolism, we were unable to demonstrate its capability to significantly diminish tHcy levels when it was prescribed either alone or in combination with FA, despite the remarkable high levels of vitamin B_{12} achieved. This may be due to the fact that both cofactors must be present for a correct performance of methionine synthase [16]. Moreover, all HD patients in this study had normal baseline vitamin B_{12} concentrations. In one report, the preva-

lence of suboptimal levels of vitamin B_{12} in apparently healthy men with HtHcy was approximately 57% [24]. This difference could obviously be because patient characteristics are different but also because total body stores of vitamin B_{12} are considerable (approximately 2–5 mg, one-half of which is in the liver). This suggests that from 3 to 5 years must elapse until cobalamin deficiency exists [25] (time on HD of our patient population ranged between 5 months and 6.3 years, average 18.7 months), unless an alteration in vitamin B_{12} absorption or prolonged inadequate intake are present [25]. It is probable that in the uncommon situation of vitamin B_{12} deficiency, FA alone may not be capable of lowering tHcy concentrations either. In addition, the method by which vitamin B_{12} blood levels are quantitated is a radioimmunoassay that measures all cobalamins indistinctly, and as methylation abnormalities are present in chronic renal failure [18], Me-Cbl plasma levels could be certainly low, while the total measured amount of cobalamin is normal. Normally, human plasma contains fractions of about 0.6 Me-Cbl, 0.3 adenosyl-cobalamin, and less than 0.1 of hydroxy- and cyano-cobalamin [26]; hydroxy-cobalamin, another cobalamin, is also methylated to form Me-Cbl in human tissue [16].

In relation to this apparent FA-dependent response to lowering tHcy levels observed in this study, an intriguing possibility to be analyzed is that tHcy may not be a cause of cardiovascular disease or of endothelial dysfunction but otherwise a marker of another risk factor involved in tHcy metabolism, as low sFA levels or a decreased physiological bioavailability of FA due to the uremic state. Therefore, the prescription of FA would not only reduce tHcy levels (the marker of endothelial dysfunction) but more importantly, provide the FA (if low, the cause of cardiovascular disease) necessary to contribute in the correction of some of the endothelial derangements present in HtHcy.

Approximately 70–80% of circulating tHcy is bound to large proteins as albumin [4, 27], which is considered as an independent determinant of tHcy, unrelated to B vitamin status [1, 27]. However, in our study albumin and hematocrits differences were non-significant among groups (table 1) when simultaneously high vitamin B_{12} blood levels were measured, and consequently cannot explain the Me-Cbl failure to correct tHcy by itself. In other words, tHcy was corrected only when FA was prescribed, either alone (group B) or with Me-Cbl (group D).

In addition to vitamin influence, tHcy concentration also depends on nutritional intake, genetic factors, on the degree of kidney failure and cellular metabolism and to impairments in the remethylation of homocysteine to methionine [20, 28, 29]. If, for instance, the remethylation reaction is impaired due to vitamin deficiencies or to alterations in the methylation process itself, homocysteine intracellular levels will increase and after its cellular extrusion HtHcy ensues [20]. High intracellular concentrations of homocysteine result in the cellular accumulation of S-adenosyl homocysteine, an extremely toxic compound that inhibits methyltransferases [30]. Several theories suggest that homocysteine causes vascular intimal thickening, elastic lamina disruption, luminal platelet accumulation and increases the proliferation of vascular smooth muscle cells, and hallmarks of atherosclerosis [12, 28, 31].

In contrast with other studies which show that high doses of FA do not normalize Hcy in HD patients [21, 22, 32], our findings demonstrate that with 10 mg FA a day Hcy levels normalize. In patients with chronic renal failure the proposed dose of FA varies between 1 and 15 mg/day, while vitamin B_{12} dose oscilates between 400 μg to 1 mg/day [1, 6, 33, 34]. As in other studies, in which Me-Cbl was used to evaluate its effects on diabetic or uremic neuropathy, such high levels were not related to adverse reactions [35, 36]. In addition, some patients have referred improvement of chronic paresthesias. As to the time it takes to normalize tHcy plasma levels, it can range from two weeks up to seven months [37–39], depending on the vitamins used, on the doses prescribed and on the population under consideration.

Conclusion

We believe 10 mg FA can safely be given to chronic HD patients to lower tHcy levels, regardless of normal baseline eFA plasma levels (sFA may be low). In the setting of normal baseline vitamin B_{12} blood levels, the prescription of i.v. Me-Cbl showed no additional benefits to diminish tHcy levels. Our study includes a small number of patients; in the absence of data from randomized, controlled trials demonstrating a reduction in cardiovascular outcomes or in thrombotic events with successful treatment of HtHcy in the HD population, careful conclusions must be drawn from our report and the previously published uncontrolled, open-label investigations.

Acknowledgements

We thank trb pharma s.a. for providing methylcobalamin vials for all patients and Mr. Gabriel Converso for his statistical analysis.

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