Hydroxocobalamin Reduces Hyperhomocysteinemia in End-Stage Renal Disease

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Renal failure causes hyperhomocysteinemia, an important risk factor for cardiovascular disease and venous access thrombosis in end-stage renal disease (ESRD). Folic acid is necessary for homocysteine (Hcy) metabolism, and therapy with 1 mg/d or more of folic acid reduces plasma total Hcy (tHcy) concentrations in ESRD, although seldom to normal. In contrast to folic acid, the Hcy-lowering effect of vitamin B_{12} has not been well studied in ESRD. We performed a prospective randomized controlled clinical trial involving 24 maintenance hemodialysis patients with normal or supranormal serum folate and vitamin B_{12} concentrations who received either standard therapy, which included 5 to 6 mg folic acid, 5 to 10 mg pyridoxine, and 6 to 10 μg oral vitamin B_{12} per day, or standard therapy plus 1 mg hydroxocobalamin administered subcutaneously once per week after dialysis. Plasma tHcy and serum methylmalonic acid (MMA) concentrations were measured before and after 8 and 16 weeks of continuous treatment. Hydroxocobalamin reduced plasma tHcy by an average of 32% ($P < .005$) and serum MMA by an average of 19% ($P < .001$). The Hcy-lowering effect of hydroxocobalamin was independent of baseline serum vitamin B_{12}, folic acid, and MMA concentrations. Patients with higher baseline plasma tHcy concentrations had the greatest response ($r = 0.80$; $P < .002$). These results show that parenteral hydroxocobalamin reduces plasma tHcy dramatically in vitamin B_{12}-replete hemodialysis patients. Persons with considerable persisting hyperhomocysteinemia despite high-dose folic acid therapy are likely to respond to the addition of hydroxocobalamin, irrespective of their serum vitamin B_{12} concentrations.

MODERATE hyperhomocysteinemia is an important, biologically plausible risk factor for atherosclerotic disease and venous thrombosis.[1-4] Hard endpoint clinical trials of therapies that reduce plasma homocysteine (Hcy)[5] concentrations are underway, and their results are awaited with interest.[6] Effective Hcy-lowering therapy, once identified, would be especially useful for persons with end-stage renal disease (ESRD), whose their plasma total Hcy (tHcy)[5] concentrations are typically 2 to 3 times higher than normal and associated with a high risk of cardiovascular disease[7-10] and venous access thrombosis.[11]

Hcy is metabolized either via the transsulfuration pathway, in which it is converted to cystathionine and then to cysteine, or by remethylation to methionine in a reaction catalyzed by the methylcobalamin-dependent enzyme, methionine synthase. Methyltetrahydrofolate is the methyl donor in the methionine synthase reaction, and the enzymes of the transsulfuration pathway are pyridoxal phosphate–dependent. Consequently, inherited defects in the enzymes of either pathway or deficiencies of folic acid, vitamin B_{12}, or vitamin B_6 cause hyperhomocysteinemia.[1,12,13] Folic acid is widely perceived as the most important of these vitamins,[7,14] and almost all Hcy-lowering regimens provide folic acid, often in very large doses.[15,16] By comparison, the Hcy-lowering effect of vitamin B_{12} has not been well studied in ESRD, except in deficiency states.[16,17]

When considering the pharmacologic use of vitamin B_{12} it is important to recognize its limited oral bioavailability. A maximum of approximately 1.5 μg/d is absorbed via intrinsic factor, and while a second, intrinsic factor–independent transport system exists, its capacity is limited: only about 1% of a large oral vitamin B_{12} dose is absorbed via this system. Consequently, normal persons absorb only a few micrograms of vitamin B_{12} following oral doses of 500 to 1,000 μg.[18] Large daily oral doses of vitamin B_{12} can prevent or treat pernicious anemia,[19] but they may not be sufficient to affect Hcy metabolism in a manner comparable to the pharmacologic doses of folic acid used to treat hyperhomocysteinemia in ESRD. Persons with reduced renal function are well suited to test whether pharmacologic parenteral vitamin B_{12} affects Hcy metabolism, since when renal function is a normal, large parenteral doses are rapidly cleared into the urine.[20]

We performed a randomized controlled trial to test the Hcy-lowering effect of parenteral hydroxocobalamin in folate- and vitamin B_{12}–replete hemodialysis patients.

MATERIALS AND METHODS

All patients of the Jewish General Hospital in Montreal receiving regular daytime hemodialysis treatment for at least 3 months were considered for participation in this study, which involved random allocation to standard treatment, consisting of 5 mg folic acid plus a multiple B vitamin containing 0 to 1 mg folic acid, 5 to 10 mg pyridoxine, and 6 to 10 μg vitamin B_{12} per day (DiaVite, R&D Laboratories, Marina del Rey, CA; or Beminal C Fortis, Wyeth-Ayerst Canada, St-Laurent, Quebec, Canada) or standard treatment plus 1 mg hydroxocobalamin (Schein Pharmaceuticals, Florham Park, NJ) administered subcutaneously once per week after dialysis. Predialysis blood samples were obtained just prior to starting hydroxocobalamin and after 8 and 16 weeks of continuous treatment. Participants were instructed not to change their usual oral vitamin regimen. The study protocol was approved by the hospital's research ethics committee, and all participants gave written informed consent.

Blood for plasma tHcy was collected in potassium ethylenediamine tetraacetic acid-lined tubes and kept on ice until the plasma was

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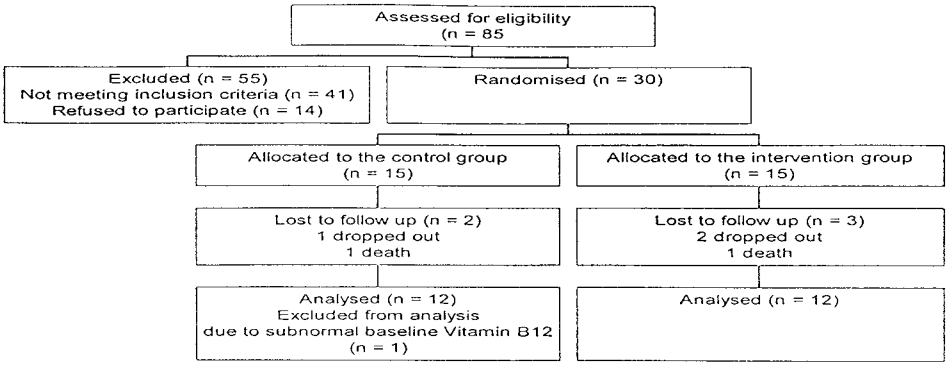

Fig 1. Trial profile.

separated and stored at -30 °C. The analysis was by high-performance liquid chromatography with fluorescence detection according to the method of Araki and Sako[21] as modified by Gilfix et al[22] and Feussner et al,[23] and described previously in detail.[24] Serum vitamin B_{12} and folate were analyzed after appropriate dilution by automated chemiluminescent immunoassay (Immulite, Diagnostic Products Corp, Los Angeles, CA). The normal ranges for this assay are 120 to 600 pmol/L for vitamin B_{12} and 6 to 40 nmol/L for folate. Serum methylmalonic acid (MMA) was measured by gas chromatography–mass spectrometry using a modification of the method of Montgomery and Mamer.[25] The enzyme methylmalonyl-coenzyme A mutase requires vitamin B_{12} for activity. Serum MMA concentrations are therefore increased in vitamin B_{12} deficiency, but also in ESRD.[17,26,27]

Since all variables were normally distributed (Kolmogorov-Smirnov test), Student's *t* test was used to compare the treatment and standard therapy groups at baseline. Changes over time were compared using 2-way repeated-measures analysis of variance (ANOVA). The source of significant differences was identified using Student Newman-Keuls post-hoc multiple comparison test. Statistical significance is based on a 2-tailed *P* value less than .05. Statistical analysis was done using SigmaStat version 2.0 (SPSS, Inc, Chicago, IL). Results are expressed as the mean ± SEM.

RESULTS

There were 56 patients on the hospital's morning and afternoon hemodialysis schedule. Twelve patients were not considered for participation due to intercurrent illness, anemia (blood hemoglobin < 100 g/L), or hypoalbuminemia (serum albumin < 35 g/L). Of the remaining 44 patients, 14 declined to participate, leaving 30 volunteers, 15 of whom were randomized to continue standard therapy and 15 to standard therapy plus weekly hydroxocobalamin injections. All the participants were known to have normal or supranormal serum vitamin B_{12} and folate concentrations before entering the study. Three persons dropped out before the 8 week blood test because of lack of interest (2 in the treatment group, 1 in the standard therapy group). One person in each group died, during study weeks 14 and 16; their 8-week results, although not included in the analysis, would strengthen the study's conclusions. One standard therapy patient who completed the study was subsequently found to have been vitamin B_{12}-deficient (average serum vitamin B_{12}, 127 pmol/L; average plasma tHcy, 116 μmol/L), even though her serum vitamin B_{12} concentration a month earlier was well within the normal range (209 pmol/L). As this patient was atypical, her results were not included in the analysis (see Fig 1). The causes of renal failure in the 24 eligible patients who completed the study were diabetic nephropathy (n = 8), hypertensive glomerulosclerosis (4), focal segmental glomerulosclerosis (n = 4), renal artery stenosis (n = 2), glomerulonephritis (n = 1), multiple myeloma (n = 1), obstructive uropathy (n = 1), polycystic kidneys (n = 1), sarcoidosis (n = 1), and unknown (n = 1). Randomization resulted in treatment and standard therapy groups that were similar with respect to sex, age, dry body weight, blood hemoglobin, mean corpuscular volume, serum albumin, serum urea, serum creatinine, serum vitamin B_{12}, serum folate, serum MMA, and plasma tHcy (Table 1).

The average serum vitamin B_{12} concentration increased more than 60-fold during hydroxocobalamin therapy ($P < .001$), which reduced plasma tHcy by an average of 32% ($P < .005$) and serum MMA by an average of 19% ($P < .001$) from their baseline concentrations by the 8 week time point, remaining equivalently low thereafter (Table 2). Plasma tHcy was reduced in all 12 treated subjects, and by at least 25% in 7 of them. There was no change in plasma tHcy, serum vitamin B_{12}, or MMA in the standard therapy group, and serum folate remained constantly highly elevated (>14 times the upper normal limit) in both groups throughout the study.

Hydroxocobalamin reduced plasma tHcy most in those patients with the highest baseline plasma tHcy concentrations

Table 1. Baseline Patient Characteristics

Characteristic	Hydroxocobalamin (n = 12)	Standard Therapy (n = 12)	*P* Value
Age (yr)	64 ± 4	71 ± 3	.17
Sex (% male)	58	67	.69
Dry weight (kg)	66 ± 3	65 ± 3	.79
Blood hemoglobin (g/L)	118 ± 5	119 ± 3	.76
Mean cell volume (fL)	95 ± 2	92 ± 1	.20
Serum albumin (g/L)	38 ± 1	40 ± 1	.23
Serum urea (mmol/L)	28 ± 2	28 ± 2	.89
Serum creatinine (μmol/L)	934 ± 58	965 ± 77	.75
Serum vitamin B_{12} (pmol/L)	625 ± 90	505 ± 43	.24
Serum folate (nmol/L)	567 ± 96	627 ± 115	.69
Serum MMA (μmol/L)	0.84 ± 0.07	0.86 ± 0.07	.83
Plasma tHcy (μmol/L)	25.9 ± 1.9	25.8 ± 1.9	.98

NOTE. Values are expressed as means ± SEM.

Table 2. Effect of Hydroxocobalamin and Standard Therapy on Plasma Homocysteine, Serum Methylmalonic Acid, and Serum B-Vitamin levels

	Hydroxocobalamin			Standard Therapy		
	0 Weeks	8 Weeks	16 Weeks	0 Weeks	8 Weeks	16 Weeks
Plasma tHcy (μmol/L)	25.9 ± 1.9[a]	17.7 ± 1.1[b]	18.4 ± 1.2[b]	25.8 ± 1.9[a]	28.4 ± 1.4[a]	26.5 ± 2.0[a]
Serum vitamin B_{12} (pmol/L)	625 ± 90[a]	30000 ± 3100[b]	40400 ± 2900[c]	505 ± 43[a]	472 ± 50[a]	442 ± 43[a]
Serum folate (nmol/L)	567 ± 96[a]	534 ± 105[a]	538 ± 113[a]	627 ± 115[a]	754 ± 130[a]	723 ± 147[a]
Serum MMA (μmol/L)	0.84 ± 0.07[a]	0.68 ± 0.04[b]	0.68 ± 0.04[b]	0.86 ± 0.07[a]	0.86 ± 0.06[a]	0.86 ± 0.06[a]

NOTE. Values are expressed as mean ± SEM. Differing superscripts in any line indicate statistically significant differences ($P < .005$).

($r = 0.80$; $P < .002$). The magnitude of the effect was unrelated to baseline serum folate, vitamin B_{12}, and MMA concentrations, nor was it related to the magnitude of the increase in serum vitamin B_{12} during hydroxocobalamin therapy. Univariate regressions of the change in plasma tHcy at 16 weeks versus plasma tHcy at baseline, and versus initial serum vitamin B_{12}, are shown in Fig 2.

DISCUSSION

This prospective, randomized clinical trial tested the independent Hcy-lowering effect of vitamin B_{12} in ESRD. It shows that parenteral hydroxocobalamin can reduce plasma tHcy concentrations of maintenance hemodialysis patients to levels substantially below the best previously attained with high-dose folic acid therapy that was sufficiently intense to increase serum folate to more than 14 times the upper normal limit. Hydroxocobalamin treatment was effective in patients whose prior vitamin B_{12} concentrations were normal or supranormal. The maximum effect was obtained within 8 weeks of treatment.

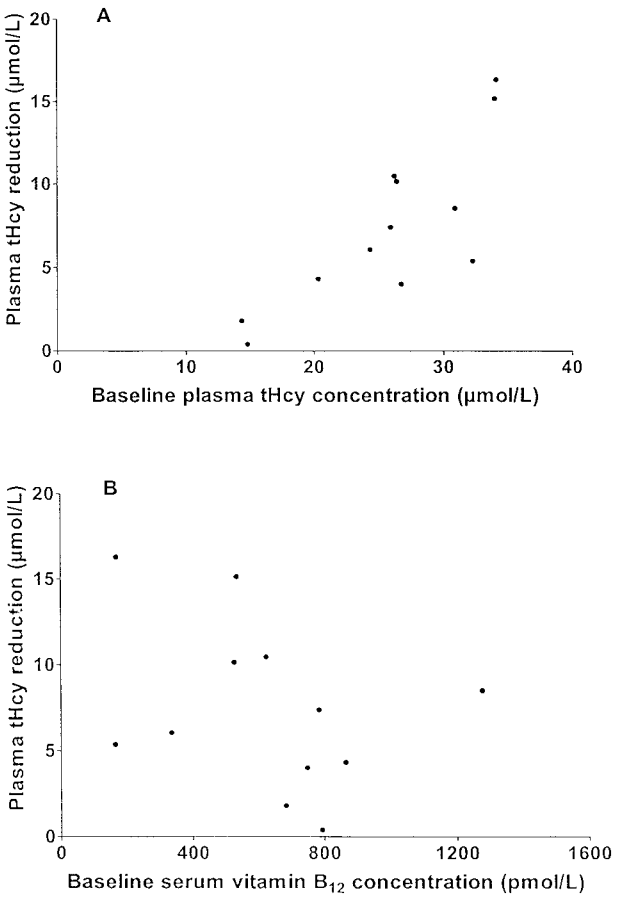

Fig 2. (A) Linear correlation between reduction in plasma tHcy and baseline plasma tHcy concentration ($r = 0.80$; $P < .002$). (B) Linear correlation between the reduction in plasma tHcy and baseline serum vitamin B_{12} concentration ($r = -0.33$; $P = .29$).

Except for renal failure itself,[28] folate nutritional status is the most consistent predictor of plasma tHcy in ESRD,[7,14,29] so it is not surprising that efforts to identify more effective Hcy-lowering strategies in ESRD have focused on different doses and forms of this vitamin.[30-34] By contrast, serum vitamin B_{12} concentrations above the deficiency level predict plasma tHcy less consistently in ESRD,[7,14-16] and vitamin B_{12} deficiency is not especially common.[7,35] It is presumably for these reasons the specific Hcy-lowering potential of vitamin B_{12} has not been well examined in ESRD.

This investigation was triggered by the results of an observational study.[36] All the patients in a McGill University–affiliated hemodialysis unit are routinely administered 1 mg cyanocobalamin intravenously each month as a measure to preserve nerve conduction in uremia[37]; they also receive 1 mg of oral folic acid per day. These patients had plasma tHcy concentrations 22% lower than otherwise similar patients in a nearby hemodialysis unit where a much higher folic acid dose (6 mg/d) and a standard multiple vitamin containing approximately 5 μg of vitamin B_{12} are routinely provided. We conjectured that high-dose vitamin B_{12} is more effective at lowering plasma tHcy than even very-high-dose folic acid, as long as at least 1 mg/d of folic acid is provided.[36] The present results support this view.

Hydroxocobalamin, rather than the more widely available cyanocobalamin, was used for 2 reasons. First, hydroxocobalamin has been used safely in massive doses for more than 25 years as a treatment for cyanide poisoning.[38-40] Second, we speculated that it might be more efficacious. Infants with the genetic disease, methylmalonic acidemia with homocystinuria, are routinely administered massive parenteral doses of vitamin B_{12} (1 mg/d intramuscularly) to bypass a presumed defect in the conversion of cobalamin to active cofactor status for methylmalonyl-coenzyme A mutase and methionine synthase.[41] Hydroxocobalamin is superior to cyanocobalamin for treating this condition.[41] The precise etiology of ESRD-related hyperhomocysteinemia is controversial, but a defect in the remethylation of Hcy to methionine has been implicated.[42] It is conceivable that uremia induces a milder form of the same defect as in

methylmalonic acidemia with homocystinuria, and indeed, serum MMA concentrations were reduced by 19% ($P < .001$) in the hydroxocobalamin treatment group. An additional possibility is that uremia impairs reactivation of methionine synthase by methionine synthase reductase,[43,44] and that very high tissue concentrations of hydroxocobalamin can overcome or compensate for this block.

The average serum vitamin B_{12} concentration rose to 30,000 pmol/L (0.03 μmol/L) within 8 weeks of weekly 1 mg hydroxocobalamin injections. There were no adverse effects in any patient, as was also reported in one previous study in which hemodialysis patients were given 0.5 mg intravenous methylcobalamin 3 times weekly to treat peripheral neuropathy,[45] and another in which 106 patients received 2.5 mg vitamin B_{12} intravenously after every dialysis for 3 years.[46] Nor is there evidence that high concentrations of vitamin B_{12} are toxic in tissue or cell culture systems.[39,47] Notwithstanding the lack of toxicity of high-dose hydroxocobalamin, it is possible that the vitamin dose used in this study was greater than necessary for maximum plasma tHcy lowering. It remains to be determined in future studies whether an interdose interval of 2 or even 4 weeks (as in our observational study[36]) can be as effective as the weekly interval used here.

This study could be criticized for a number of drawbacks. First, while the trial was prospective and randomized, it was not double-blind. Blinding is desirable in clinical trials, but it is generally accepted that open randomized controlled clinical trials can provide valid results when blinding is not feasible (as in this study) and the outcome measure is objective.[48-50] A second drawback is the relatively small number of participants, 12 in each group. However, the results of the preliminary, open study[24] that preceded this one indicated that these subject numbers would be more than adequate to detect a treatment effect even smaller than actually occurred. The possibility that study participants were not compliant with their folic acid therapy is extremely unlikely, since baseline serum folate concentrations were more than 14 times the upper normal limit for both groups, and remained unchanged for the duration of the study. Moreover, baseline plasma tHcy concentrations were comparable to those in other studies of similar patients receiving high-dose folic acid (1 mg or more per day) without high-dose vitamin B_{12}.[29,51-54] Thus, while the number of study participants was small, and the design of the 16-week, randomized, prospective clinical trial was not double-blind, neither of these drawbacks justifies rejecting its striking findings. Rather, they should stimulate efforts to replicate these important findings in other centers.

A final objection could be that the subjects in the treatment group responded because they were vitamin B_{12}-deficient. If so, this is a condition shared by the majority of hemodialysis patients, for the baseline serum vitamin B_{12} concentrations in our subjects were comparable to, or higher than, those reported in other hemodialysis populations.[7,29,30,51-54] No subject in this study had a serum vitamin B_{12} concentration in the deficient range (see Fig 2). If the vitamin treatment merely represented correction of a deficiency state, the magnitude of the response should have correlated with baseline serum vitamin B_{12} or MMA concentrations, and it did not (Fig 2). Rather, plasma tHcy decreased in every hydroxocobalamin-treated patient, and by at least 25% in the majority of them, despite normal or elevated baseline serum vitamin B_{12} concentrations (Fig 2).

There is, in addition, independent evidence that the Hcy-lowering mediated by the high-dose parenteral vitamin B_{12} used in this study was pharmacologic, rather than nutritional. In the open clinical trial in hemodialysis patients that preceded this one, we observed that 3 monthly injections of cyanocobalamin were sufficient to double serum vitamin B_{12} concentrations, while reducing plasma tHcy by 13%.[24] In another uncontrolled trial, Manns et al[53] added 1 mg/d of oral vitamin B_{12} to the vitamin regimen of maintenance hemodialysis patients. This, too, doubled the average serum vitamin B_{12} concentration and reduced plasma tHcy by a comparable amount, 17%. Thus, both earlier studies indicate that a treatment which approximately doubles or triples serum vitamin B_{12} concentrations—more than sufficient to correct any deficiency state—will only slightly reduce plasma tHcy concentrations. The more potent, parenteral route of vitamin B_{12} administration, used in the present study, may be the only way to achieve the pharmacologic serum concentrations necessary for optimum Hcy-lowering.

While our study strongly suggests that parenteral hydroxocobalamin can substantially reduce plasma tHcy in ESRD, it does not prove the parenteral route is always necessary. In a randomized, controlled study involving 27 patients, Bostom et al[51] reported that 8 weeks of a daily oral B-vitamin combination providing 16 mg folic acid, 100 mg vitamin B_6, and 1 mg vitamin B_{12} reduced plasma tHcy concentrations of ESRD patients by 30% more than did 1 mg daily folic acid alone, a treatment effect similar to the present study. This large effect could not have been solely due to the ultra-high dose of folic acid used, since several subsequent studies have shown that 2.5, 5, and 15 mg or more of folic acid per day are no more effective than 1 mg/d for lowering plasma tHcy in ESRD.[30-33,53] It is possible, therefore, that the specific combination of ultra-high oral doses of folic acid, vitamin B_6 and vitamin B_{12} are as effective as parenteral vitamin B_{12}. More research is necessary to confirm or refute this.

However, even if future studies should demonstrate that the specific daily oral intake of ultra-high doses of vitamin B_{12}, folic acid, and vitamin B_6 can reduce plasma tHcy as effectively as a daily multivitamin containing 1 mg of folic acid and weekly 1 mg subcutaneous vitamin B_{12}, the parenteral route of vitamin B_{12} administration has important advantages for ESRD patients. These are convenience, assured compliance, low cost, and the ease of attaining a therapeutic serum concentration in every patient. In the studies cited above by Bostom et al[51] and Mann et al,[53] average serum vitamin B_{12} concentrations increased 2- to 3-fold after prolonged daily ingestion of 1 mg of the vitamin. In the present study, average serum vitamin B_{12} rose more than 60-fold after hydroxocobalamin injections of 1 mg given only once per week.

Finally, we draw attention to the broad clinical implications of the present results. Our study was essentially population-based, and the baseline serum folate and vitamin B_{12} concen-

trations of the participants were as high as or higher than reported in patients in other hemodialysis centers. The majority of persons receiving hydroxocobalamin experienced at least a 25% reduction in plasma tHcy, with the greatest lowering occurring among those whose baseline plasma tHcy concentrations were highest ($r = 0.80$; $P < .002$), without relation to baseline serum folate, vitamin B_{12}, or MMA concentrations. This suggests that any ESRD patient whose hyperhomocysteinemia remains considerable despite folic acid therapy is likely to respond well to the combination of folic acid and parenteral hydroxocobalamin, irrespective of his or her serum vitamin B_{12} concentration.

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