A Tale of Two Homocysteines—and Two Hemodialysis Units

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Pharmacologic doses of folic acid are commonly used to reduce the hyperhomocysteinemia of end-stage renal disease (ESRD). Vitamin B_{12} acts at the same metabolic locus as folic acid, but information is lacking about the specific effects of high doses of this vitamin on homocysteine levels in renal failure. We therefore compared the plasma homocysteine concentrations of maintenance hemodialysis patients in two McGill University–affiliated urban tertiary-care medical centers that differed in the use of vitamin B_{12} and folic acid therapy. Patients in the first hemodialysis unit are routinely prescribed high-dose folic acid (HI-F, 6 mg/d), whereas those in the second unit receive high-dose vitamin B_{12} in the form of a monthly 1-mg intravenous injection, along with conventional oral folic acid (HI-B_{12}, 1 mg/d). Predialysis homocysteine was 23.4 ± 6.8 μmol/L (mean ± SD) in the HI-F unit and 18.2 ± 6.1 μmol/L in the HI-B_{12} unit ($P < .002$). Postdialysis homocysteine was 14.5 ± 4.1 in the HI-F unit and 10.6 ± 3.4 μmol/L in the HI-B_{12} unit ($P = .0001$). Multiple regression analysis indicated that high-dose parenteral vitamin B_{12} was associated with a lower homocysteine concentration even after controlling for the potential confounders of sex, serum urea, serum creatinine, urea reduction ratio, and plasma cysteine. Because this was a cross-sectional observational study, we cannot exclude the possibility that unidentified factors, rather than the different vitamin therapies, account for the different homocysteine levels in the two units. Careful prospective studies of the homocysteine-lowering effect of high-dose parenteral vitamin B_{12} in ESRD should be undertaken.

MODERATE hyperhomocysteinemia is associated with an increased risk of atherosclerotic vascular disease and venous thrombosis.[1-4] This epidemiologic evidence, together with biological observations at the tissue and whole-body level,[5-7] has led to general agreement about the need for prospective clinical trials of homocysteine-lowering therapies for persons with cardiovascular disease and hyperhomocysteinemia.[8,9] Most persons with end-stage renal disease (ESRD) are hyperhomocysteinemic.[10,11] Since this may better predict their increased rate of cardiovascular disease than the standard risk factors,[12-14] such individuals could benefit from effective homocysteine-lowering therapy.

Homocysteine is formed from methionine and metabolized either by remethylation to methionine in a reaction catalyzed by the vitamin B_{12}–dependent enzyme, methionine synthase, or via the transsulfuration pathway, which converts it to cystathionine and then to cysteine. Since methyltetrahydrofolate is the methyl donor in the methionine synthase reaction and since the enzymes of the transsulfuration pathway are vitamin B_6–dependent, deficiencies of vitamin B_{12}, folate, or vitamin B_6 or inherited defects in the enzymes on either pathway result in hyperhomocysteinemia.[2,3,9] Among the nutritional factors affecting plasma homocysteine in ESRD, folate nutritional status is considered the most important by far.[15] Homocysteine-lowering regimens currently under consideration for ESRD invariably contain folic acid in amounts of 1 to 16 mg/d, often but not always in combination with oral vitamin B_6 and oral vitamin B_{12}.[10,16] Such regimens reduce homocysteine levels in ESRD patients, although not usually to normal. Vitamin B_{12} is required for the same enzymatic reaction as folic acid, but there is almost no information about the ability of high doses of this vitamin to reduce homocysteine levels in the absence of vitamin B_{12} deficiency.[10,16]

Patients with ESRD are appropriate to test for such an effect, since vitamin B_{12} tissue availability is normally limited by its rapid renal elimination. Thus, after parenteral vitamin B_{12} administration, a large fraction of the dose is promptly cleared by the normal kidney and excreted in the urine.[17] After an oral dose, a maximum of about 1.5 μg vitamin B_{12} is absorbed via intrinsic factor. A second transport system exists that does not require intrinsic factor, but its efficiency is only about 1%, with the consequence that even persons without pernicious anemia absorb only a few micrograms of the vitamin after oral doses of 500 to 1,000 μg.[18] This is sufficient to treat and prevent vitamin B_{12} deficiency,[19] but may not be enough to exert a pharmacologic effect on homocysteine metabolism comparable to that of high-dose folic acid. For these reasons, pharmacologic doses of vitamin B_{12} should be administered parenterally to patients with ESRD if the objective is to fully test for a homocysteine-lowering effect.

In light of this, the contrasting vitamin regimens used in 2 McGill University–affiliated hemodialysis units presented a unique opportunity. These units are in tertiary-care teaching hospitals located 6 km apart in the Montreal urban area, serve similar patients, and are similar in their criteria for selecting patients for dialysis. In the first unit, the majority of approximately 55 patients on maintenance hemodialysis take 5 mg/d oral folic acid in addition to the 1 mg folic acid already provided in their daily multiple-vitamin tablet (HI-F unit). In the second unit, which treats about 125 patients, maintenance dialysis patients routinely receive a monthly intravenous injection of 1 mg cyanocobalamin at the end of dialysis (HI-B_{12} unit). This is

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based on a report that high serum vitamin B_{12} levels are associated with better nerve conduction velocity in ESRD,[20] rather than evidence of vitamin B_{12} deficiency, which is generally thought to be uncommon in this population.[21-23] Most patients in the HI-B_{12} unit also receive a daily oral multiple-vitamin preparation providing 1 mg folic acid. We wondered whether the high-dose parenteral vitamin B_{12} used in the HI-B_{12} unit is associated with a higher or lower plasma homocysteine concentration versus the HI-F unit.

SUBJECTS AND METHODS

After obtaining approval from the institutional review boards of the 2 hospitals, we approached ESRD patients on stable maintenance hemodialysis in the 2 units for permission to measure their plasma homocysteine and cysteine concentrations just prior to 1 standard dialysis treatment, at a time that coincided with the normal monthly blood sample (which included serum urea, creatinine, and albumin). We also measured postdialysis homocysteine, cysteine, urea, and creatinine levels. Serum folic acid and vitamin B_{12} and erythrocyte folic acid levels were measured in most of the participants. Patients with serious anemia or advanced debilitating disease that precluded unnecessary blood sampling and patients with hypoalbuminemia (serum albumin <35 g/L) were excluded from the study. The patients at the 2 hospitals received regular hemodialysis using polysulfone or polyacrylonitrile hollow-fiber, 1.6- to 2.1-m^2 surface area dialyzers.

Plasma total homocysteine and cysteine levels were measured by reverse-phase high-performance liquid chromatography with fluorometric detection as described by Feussner et al.[24] The plasma was obtained in chilled test tubes containing EDTA, mixed, and immediately placed into a bucket of crushed ice. The plasma was separated by centrifugation and frozen at −30°C. At the time of analysis, thawed mixed plasma was treated with tri-*n*-butylphosphine to convert all sulfhydryl molecules to their reduced form, and then reacted with the sulfhydryl-reactive compound 7-fluorobenzofurazane-4-sulfonic acid to convert them to stable fluorescent derivatives. Derivatized homocysteine and cysteine were separated on a 30-cm C-18 Waters (Montreal, Canada). Novapak column using solvents and conditions similar to those described by Fortin and Genest.[25]

Serum urea, creatinine, and albumin concentrations of HI-F unit patients were measured on the Hitachi (Tokyo, Japan) model 917 multiple-channel analyzer in the core biochemistry laboratory of that hospital, and the same parameters of HI-B_{12} unit patients were measured on the DAX 96 (Bayer, Toronto, Canada) in the biochemistry laboratory of the other hospital. These analyzers reportedly produce closely similar results.[26] When the difference in homocysteine levels between the 2 units became apparent, we decided to correct for any systematic differences in the results by simultaneously reanalyzing stored frozen sera from all HI-F unit patients on the DAX 96 in the HI-B_{12} hospital. The resulting linear regression equations were used to correct the original Hitachi 917 results in the HI-F hospital to the values that would have been obtained if analyzed on the DAX 96 originally. Fasting serum vitamin B_{12} and folic acid and erythrocyte folic acid levels were measured in patients in both hospitals on an ACS 180 Chemiluminescence Analyzer (Bayer) located in the HI-B_{12} hospital.

Statistical Analysis

Since the patients in the 2 hemodialysis units differed in a number of ways, multiple linear regression analysis (including standard tests for the appropriateness of the regression models developed) was used to adjust for confounding factors that might affect plasma homocysteine. Data are reported as the mean, since all variables were found to be normally distributed (Kolmogorov-Smirnov test). Because 2 important but highly (negatively) correlated attributes of the 2 units were the high-dose oral folic acid therapy used almost exclusively in the HI-F unit and the high-dose parenteral vitamin B_{12} therapy used exclusively in the HI-B_{12} unit, we examined multicolinearity between vitamin B_{12} therapy and supplemental folic acid intake (variance inflation factor and lability of coefficients) in a multiple regression model that included both site (HI-F unit *v* HI-B_{12} unit) and supplemental folic acid as independent variables. This was possible because, unlike vitamin B_{12} therapy, which was perfectly segregated into the HI-B_{12} unit, supplemental folic acid intake varied somewhat in both units from 0 to 6 mg/d. In addition to examining for the effects of usual potential confounders, we tested the novel hypothesis that plasma cysteine concentrations might act as a confounder, for the following reason. Cysteine levels are increased in ESRD.[27-29] The explanation for this is not known,[30] but if (as appears true for hyperhomocysteinemia[31]) it is largely due to reduced renal cysteine catabolism, then plasma cysteine might usefully predict the residual metabolic renal mass, a valid potential confounder of plasma homocysteine. All analyses were performed using SAS Statistical Software (SAS Institute, Cary, NC) or Microsoft Excel.

RESULTS

In the HI-F unit, 26 patients participated (46%), and in the HI-B_{12} unit, 67 participated (54%). Participants in the 2 units were generally similar in age, sex, prevalence of diabetes, and blood hemoglobin concentration. There was no apparent bias in their selection. They differed by a clinically unimportant but statistically significant amount for predialysis serum albumin and urea, and they differed significantly in the mean urea reduction ratio and hemodialysis-associated weight reduction (Table 1).

Serum folic acid and vitamin B_{12} levels (the latter measured 1 month after the previous injection) were measured in 18 patients in the HI-F unit and 61 patients in the HI-B_{12} unit. Serum folic acid was very high and did not differ between the units (HI-F unit *v* HI-B_{12} unit, 42 ± 9 *v* 40 ± 11 nmol/L, mean ± SD), but erythrocyte folic acid was significantly higher for the HI-F unit versus the HI-B_{12} unit (2,540 ± 790 *v* 1,800 ± 530 nmol/L, $P < .001$). Serum vitamin B_{12} in the HI-B_{12} unit was more than twice the level in the HI-F unit (1,270 ± 740 *v* 520 ± 220 pmol/L, $P < .0001$). Despite the lack of supplemental vitamin B_{12} in the HI-F unit, the vitamin B_{12} levels were well within the normal range.

Predialysis homocysteine was significantly lower in the

Table 1. Subject Characteristics

Characteristic	HI-F Unit	HI-B_{12} Unit	*P*
No. of patients	26	67	
Age (yr)	65 ± 13	61 ± 17	.3
Men (%)	62	57	
Diabetics (%)	27	25	
Hemoglobin (g/L)	112 ± 16	119 ± 17	.06
Predialysis parameters			
Weight (kg)	71 ± 20	70 ± 16	.9
Albumin (g/L)	41 ± 2	39 ± 3	<.0001
Urea (mmol/L)	28 ± 8	24 ± 7	.026
Creatinine (μmol/L)	757 ± 271	719 ± 208	.52
Postdialysis parameters			
Weight reduction (kg)	2.3 ± 1.2	2.9 ± 1.2	.02
Urea (mmol/L)	9 ± 4	10 ± 3	.27
Urea reduction ratio	0.67 ± 0.10	0.57 ± 0.1	<.0001
Creatinine (μmol/L)	306 ± 169	310 ± 107	.91

NOTE. Values are the mean ± SD.

HI-B_{12} unit (18.2 ± 6.1 μmol/L) versus the HI-F unit (23.4 ± 6.8 μmol/L, $P < .002$). Postdialysis homocysteine was also significantly lower in the HI-B_{12} unit (10.6 ± 3.4 *v* 14.5 ± 4.1 μmol/L in the HI-F unit, $P = .0001$; Table 2). Despite the different urea reduction ratios in the 2 units, the homocysteine reduction ratios were closely similar, 0.38 (HI-F) and 0.41 (HI-B_{12}), equivalent to homocysteine reductions reported elsewhere.[10]

Multiple regression analysis was used to control for factors other than the vitamin B_{12} therapy in the HI-B_{12} unit patients that could affect plasma homocysteine and hence act as confounders. Of 26 study subjects in the HI-F unit, 23 consumed 6 mg supplemental folic acid per day, and 1 each consumed 5, 1, and 0 mg. One patient in the HI-B_{12} unit consumed 5 mg supplemental folic acid per day, 59 consumed 1 mg, and 7 consumed 0 mg. To probe the problem of multicolinearity between folic acid intake and vitamin B_{12} therapy (see the Methods), we first examined a multiple regression model that included both site (HI-F unit *v* HI-B_{12} unit) and folic acid therapy as independent variables.

According to this model, vitamin B_{12} therapy and increasing folic acid intake were both associated with a reduction in plasma homocysteine. However, the presence of multicolinearity rendered the coefficients for the variables unreliable. To confirm this, we explored a model that contained folic acid intake but not site (thus ignoring any effect of vitamin B_{12} therapy). This model indicated a biologically implausible positive association between increasing folic acid intake and increasing plasma homocysteine. This was confirmatory evidence that the strong association between site and folic acid intake overwhelmed any independent effect of folic acid itself. We therefore chose as a valid model one that included site but excluded folic acid intake as an independent variable. Using this model, the variables that yielded the best results for the regression analysis were sex, predialysis body weight, plasma cysteine concentration, and urea reduction ratio. The final model (R^2 adjusted = .52) was predialysis homocysteine (μmol/L) = 7.8 + 5.5 [site] + 1.2 [sex] − 0.054 [body weight (kg)] + 0.044 [cysteine (μmol/L)] − 4.2 [urea reduction ratio], where site = 1 for the HI-F unit and 0 for the HI-B_{12} unit, and male sex = 1 and female sex = 0. The site, cysteine, and urea reduction ratio were all highly significant variables in this model ($P < .005$). There was a nonsignificant trend ($P = .08$) for body weight, and sex was not significant ($P = .24$). Other variables that were omitted based on standard regression modeling strategies included age, serum albumin, and serum creatinine. This analysis demonstrated that the site at which patients were treated remained an important factor accounting for the lower plasma homocysteine in the HI-B_{12} unit even after controlling for suspected confounding factors. The only site-specific factor that could be identified to explain the lower homocysteine levels found in the HI-B_{12} unit was the parenteral vitamin B_{12} therapy used therein.

Table 2. Plasma Homocysteine and Cysteine (μmol/L)

Parameter	HI-F Unit	HI-B_{12} Unit	*P*
Predialysis			
Homocysteine	23.4 ± 6.8	18.2 ± 6.1	.0017
Cysteine	397 ± 93	382 ± 96	.51
Postdialysis			
Homocysteine	14.5 ± 4.1	10.6 ± 3.4	.0001
Cysteine	198 ± 58	174 ± 46	.16
Reduction ratio			
Homocysteine	0.38 ± 0.09	0.41 ± 0.09	.16
Cysteine	0.50 ± 0.09	0.54 ± 0.08	.09

NOTE. Values are the mean ± SD. Normal homocysteine concentration, 8.4 ± 2.2 μmol/L (n = 34); normal cysteine concentration, 231 ± 28 μmol/L (n = 34).

DISCUSSION

We measured plasma homocysteine concentrations in patients at 2 apparently similar hemodialysis units to explore the possibility that the high-dose parenteral vitamin B_{12} therapy used in one of them specifically decreases plasma homocysteine concentrations in ESRD. Both predialysis and postdialysis plasma homocysteine concentrations were substantially lower in this unit, despite a far lower mean folic acid intake versus the HI-B_{12} unit. No factor other than these injections was identified that could explain this difference.

Cigarette smoking is associated with an increase of plasma homocysteine of about 1 μmol/L.[32] We did not record the smoking history of the participants in this study, but fewer than 1 in 5 of the hemodialysis patients in either unit use tobacco; nor is there any reason to anticipate a difference in the smoking habits of the study participants in the 2 units, who were closely similar in sex ratio, age, and place of residence. It is therefore highly unlikely that cigarette smoking was a significant confounder.

Instead, the possibility that high-dose vitamin B_{12} therapy can reduce homocysteine levels is biologically plausible. In a recent clinical trial designed to test this, combination therapy with oral vitamin B_{12} (1 mg/d), folic acid (15 mg/d), and pyridoxine (100 mg/d) was found to effectively reduce homocysteine levels in ESRD patients.[33] In a study in persons without renal disease, vitamin B_{12} (1 mg), folic acid (1 mg), and vitamin B_6 (5 mg) were administered parenterally for 3 weeks to elderly subjects residing at home or in hospital.[34] The conversion of methylmalonyl-coenzyme A(CoA) to succinyl-CoA requires the vitamin B_{12}–dependent enzyme methylmalonyl-CoA mutase, and as is common in elderly persons[35] (as well as persons with renal failure[36]), the serum methylmalonic acid levels were increased despite normal serum vitamin B_{12} levels. The combined therapy reduced serum methylmalonic acid by more than 50% and plasma homocysteine from 12 μmol/L to 8 μmol/L. However, the specific contribution of high-dose vitamin B_{12} to the homocysteine-lowering effect observed cannot be determined from these studies, since it was combined with folic acid and vitamin B_6 in both of them.

In fact, there is almost no information specifically addressing the possibility that high-dose vitamin B_{12} given alone can decrease plasma homocysteine in persons lacking clinically apparent vitamin B_{12} deficiency. Araki et al[37] reported that parenteral vitamin B_{12} (1 mg daily for 3 weeks) decreased plasma homocysteine in 10 diabetic subjects from 15 to 10 μmol/L. On the other hand, Wilcken et al[38] reported no reduction in the serum homocysteine-cysteine mixed-disulfide concentration in 8 renal transplant patients with mild renal dysfunction (serum creatinine 180 μmol/L) tested 2 weeks after a single 1-mg intramuscular injection of vitamin B_{12}.

The causes of hyperhomocysteinemia in ESRD have been investigated in some detail.[10,11,16] According to recent comprehensive reviews, there is no evidence that the cause of renal failure, length of time on dialysis, hemodialysis membrane type, dialysis adequacy (urea reduction ratio), or residual renal function have any significant specific effect on plasma homocysteine in maintenance hemodialysis patients.[10,16] In our study, the urea reduction differed between the units and was predictive of plasma homocysteine, but this was independent of the site and hence did not account for the difference in plasma homocysteine between sites, nor did the homocysteine reduction ratio differ between the units.

In addition to the nutritional status with regard to folic acid, vitamin B_6, and vitamin B_{12}, a number of factors, including sex, serum albumin, and serum creatinine, have been associated with plasma homocysteine in persons with or without renal failure.[10,39] In the present study, neither serum albumin nor creatinine were predictive of plasma homocysteine, but, as recently described by Suliman et al,[40] we found plasma cysteine to be highly predictive (univariate $R^2 = .38$, $P < .001$). Cysteine concentrations are increased in ESRD,[27-29] and a direct relationship between plasma cysteine and homocysteine has previously been observed in both normal persons and those with renal disease.[41-44] There is evidence that the hyperhomocysteinemia of ESRD is largely the result of diminished renal metabolic mass[31]; perhaps diminished renal metabolism also limits cysteine catabolism. Indeed, the strong correlation we observed between plasma concentrations of the 2 amino acids supports this possibility. However, unlike homocysteine, cysteine concentrations were similar in the 2 hemodialysis units.

In conclusion, we observed an important difference in predialysis and postdialysis plasma homocysteine concentrations in 2 similar hemodialysis units, with no apparent explanation other than the high-dose parenteral vitamin B_{12} administered in 1 of the units. Since this was an observational study, we cannot exclude the possibility that an unidentified confounder accounted for the different homocysteine levels in these 2 units. However, the hypothesis that pharmacologic parenteral vitamin B_{12} can reduce homocysteine levels in ESRD is biologically plausible and, if correct, of considerable clinical importance. Parenteral vitamin B_{12} is safe, inexpensive, convenient, and probably far more potent than oral administration in ESRD. Thus, the more than 2-fold elevation in serum vitamin B_{12} that was present fully 4 weeks after a 1-mg injection in patients in the HI-B_{12} unit was equivalent to the serum levels reported with a regimen of daily oral megadose vitamin B_{12} therapy.[33] The possibility that parenteral vitamin B_{12} therapy can improve the hyperhomocysteinemia of ESRD merits careful prospective testing.

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