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Cobalamin dose regimen for maximum homocysteine reduction in end-stage renal disease

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Abstract

Plasma total homocysteine (tHcy) concentrations are markedly increased in end-stage renal disease and only partially corrected by folic acid supplementation. We and others have reported that cobalamin, administered parenterally, reduces plasma tHcy substantially below the lowest concentrations attainable with folic acid. We have now carried out a randomized controlled clinical trial to compare the plasma Hcy-lowering effect of 3 intravenous cyanocobalamin dose regimens in maintenance hemodialysis patients: 1 mg postdialysis every 28, 14, and 7 days in addition to routine oral vitamin B supplementation. All patients in the hemodialysis unit where the study was carried out routinely received 1 mg intravenous cyanocobalamin every month, so participants who were randomized to receive the vitamin every 28 days simply continued with their existing treatment program. Serum cobalamin and plasma tHcy concentrations in the control group did not change over the course of the study. As measured after 8 weeks of therapy, intravenous cyanocobalamin every 14 days increased serum cobalamin approximately 2.5-fold and reduced plasma tHcy by 11.5% (P = .035) below the concentration previously attained with monthly administration, whereas treatment every 7 days increased serum cobalamin concentrations approximately 5-fold and reduced plasma tHcy by 11.0% (P = .013). These results show that intravenous cyanocobalamin at 7- or 14-day intervals reduces plasma tHcy concentrations of hemodialysis patients below the levels brought about by prior long-term administration every 4 weeks and confirms that plasma tHcy lowering with parenteral cobalamin is a true pharmacological effect and not merely correction of a latent deficiency state.

1. Introduction

End-stage renal disease (ESRD) is associated with markedly increased plasma total homocysteine (tHcy) concentrations [1,2] which predict the high prevalence of cardiovascular disease in this patient population [3-7]. Folic acid supplementation reduces plasma tHcy concentrations by approximately 30% in ESRD [8-15], but doses greater than 1 mg per day, or different forms of the vitamin, appear to be no more effective than 1 mg per day of ordinary folic acid [13,14,16-18], and it remains to be shown that the addition of high-dose pyridoxine to folic acid is more effective than folic acid alone [19-22]. As the majority of ESRD patients remain hyperhomocysteinemic despite folic acid and pyridoxine supplementation [15,16,23-25], efforts to identify more effective Hcy-lowering treatments continue [26-28].

After observing substantially lower plasma tHcy concentrations in a hemodialysis unit where intravenous cyanocobalamin is routinely administered than in a nearby unit where this is not the practice [29], we carried out a prospective, randomized, controlled trial to test the Hcy-lowering effect of adding 1 mg/wk parenteral hydroxocobalamin to the existing regimen of high-dose oral folic acid and other B vitamins. This treatment reduced plasma tHcy concentrations 32% below the level in the control group [30]. Koyama et al [22] reported that 3 weeks of intravenous methylcobalamin (0.5 mg after each dialysis) reduced plasma tHcy approximately 50% below the level achieved with folic acid alone or folic acid combined with high-dose pyridoxine.

The dramatic effects of parenteral cobalamin on plasma tHcy concentrations differ from those of oral administration, which reduces plasma tHcy concentrations of hemodialysis patients very modestly [15] or not at all [27,31]. Owing to its limited bioavailability [32], high-dose oral cyanocobal-

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amin raises serum cobalamin concentrations of ESRD patients 3-fold at most [15,23,33], whereas parenteral hydroxocobalamin or methylcobalamin administered at 3- to 7-day intervals increases serum cobalamin concentrations 50-fold to more than 100-fold [22,30,34,35].

It appears therefore that cobalamin therapy can potently improve hyperhomocysteinemia in ESRD, but the serum (or tissue) cobalamin concentrations necessary to accomplish this are only achieved with parenteral administration. What parenteral dose of cobalamin is necessary for maximum plasma tHcy lowering? To answer this question, we carried out a prospective, randomized, controlled clinical trial comparing the plasma Hcy-lowering effect of intravenous cyanocobalamin administered at 7-, 14-, or 28-day intervals to maintenance hemodialysis patients.

2. Materials and methods

The study was carried out in the hemodialysis unit of the Royal Victoria Hospital, Montreal, with the approval of the hospital's research ethics committee. All English- or French-speaking patients on the 3 times per week maintenance hemodialysis morning and afternoon treatment schedule were approached to participate if they were clinically stable, had a blood hemoglobin concentration of at least 100 g/L, and a serum albumin concentration of at least 30 g/L. Participants were randomly assigned to 1 of 3 regimens: 1 mg intravenous cyanocobalamin postdialysis every 28, 14, or 7 days for a period of 8 weeks. As all patients routinely receive 1 mg intravenous cyanocobalamin postdialysis every month, the participants who were randomized to receive the vitamin every 4 weeks simply continued with their existing treatment program. The study was designed to interfere as little as possible with the busy clinical activity on the unit. Blood draws were scheduled to coincide with the routine drawing of the monthly biochemical profile. Serum cobalamin, serum folic acid, and plasma tHcy concentrations were measured at baseline and after 4 and 8 weeks. All patients on the unit are routinely prescribed a vitamin B supplement containing folic acid and, in some cases, additional folic acid. The study participants were advised to continue their current prescription.

Blood for plasma tHcy was collected in potassium ethylenediaminetetraacetic acid–lined tubes and kept on ice until the plasma was separated and stored at −30°C. The analysis was by high-performance liquid chromatography with fluorescence detection as described earlier [30]. Serum cobalamin and folate were analyzed after appropriate dilution by automated chemiluminescent immunoassay (Immulite, Diagnostic Products, Corp, Los Angeles, Calif) as in our earlier study [30]. The normal ranges for this assay are 180 to 660 pmol/L for cobalamin and 6 to 40 nmol/L for folate.

All variables were normally distributed (Kolmogorov-Smirnov test), so changes over time were compared using 2-way repeated-measures analysis of variance. The source of significant differences was identified using the Student-Newman-Keuls post hoc multiple comparison test. Statistical significance is based on a 2-tailed $P < .05$. Statistical analysis was done using SigmaStat version 2.0 (San Rafael, Calif). The results are expressed as mean ± SEM.

3. Results

Of the 94 patients assessed, 29 did not meet the inclusion criteria, and 4 eligible patients declined to participate, leaving 61 participants who were randomly assigned to the 3 treatment groups. All the participants tolerated intravenous cyanocobalamin without side effects. One participant in the control group (cyanocobalamin every 28 days) was found to have severe hyperhomocysteinemia (125 μmol/L) despite elevated serum folate and cobalamin concentrations. He was referred for metabolic testing, and his plasma tHcy concentration fell to close to normal (18 μmol/L) in response to high-dose pyridoxine therapy. This patient's results were excluded from the data analysis. A blood sample was inadvertently omitted for 1 patient in this group at the 4-week time point; his other data were valid and hence included in the analysis. One patient in the group receiving cyanocobalamin every 14 days withdrew after the baseline sample. A second patient in this group died of small intestinal infarction before the 8-week sample, and the 8-week blood sample was inadvertently omitted for another one. The data for the 4-week time point were valid for these 2 participants and hence were included in the analysis. One patient in the group receiving cobalamin every 7 days withdrew from the study after the 4-week time point, and a second was transferred to another center. The data for the 4-week time point were valid for these 2 participants and were included in the analysis.

The details of the trial profile are shown in Fig. 1. Randomization resulted in 3 comparison groups with similar physical and biochemical parameters, as shown in Table 1. The study participants used a variety of vitamin B supplements, and their serum folate concentrations were above the

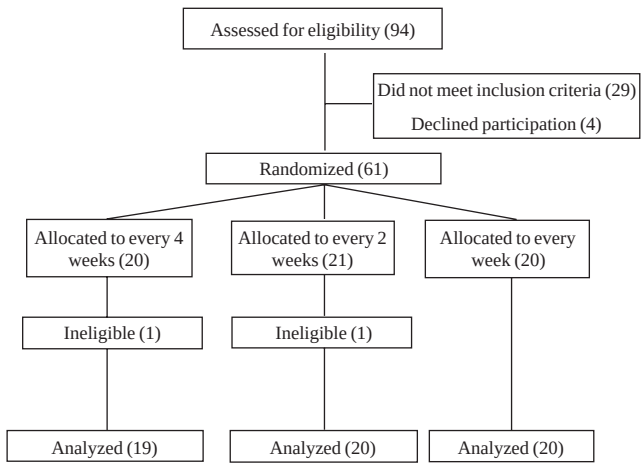

Fig. 1. Trial profile.

Table 1
Baseline characteristics of patients receiving intravenous cyanocobalamin

Characteristic	Every 28 days (n = 19)	Every 14 days (n = 20)	Every 7 days (n = 20)
Age (y)	63 ± 5	65 ± 3	68 ± 3
Height (cm)	166 ± 2	162 ± 3	168 ± 2
Body mass index (kg/m^2)	28.6 ± 2.0	28.2 ± 1.5	30.1 ± 1.4
Serum creatinine (μmol/L)	745 ± 62	779 ± 53	689 ± 51
Serum urea (mmol/L)	20.5 ± 1.5	20.0 ± 1.0	21.2 ± 1.4
Serum albumin (g/L)	32.5 ± 1.0	34.5 ± 0.6	33.4 ± 1.0
Blood hemoglobin (g/L)	121 ± 4	134 ± 3	129 ± 3
Serum glucose (mmol/L)	7.3 ± 0.7	6.8 ± 0.8	7.8 ± 0.0

Values are expressed as the means ± SEM.

upper limit of the assay in the great majority of cases. Average oral folic acid, pyridoxine, and cyanocobalamin intakes in the control group (intravenous cyanocobalamin every 28 days) were 2.4 ± 0.5 mg/d, 3.6 ± 0.5 mg/d, and 6.1 ± 0.05 μg/d, respectively. The corresponding intakes for the group receiving intravenous cyanocobalamin every 14 days were 2.5 ± 0.5 mg/d, 4.2 ± 0.4 mg/d, and 8.2 ± 0.9 μg/d, respectively, and for the group receiving cyanocobalamin every 7 days were 2.8 ± 0.5 mg/d, 3.4 ± 0.4 mg/d, and 8. 3 ± 0.8 μg/d, respectively; none of these intakes differ significantly among the groups either clinically or statistically.

The aim of the study was to determine the Hcy-lowering effect of parenteral cyanocobalamin in a setting of adequate folic acid status. For this reason, serum folic acid concentrations greater than the upper limit of the assay (45.2 nmol/L) were not further quantified, and in tabulating the results, all values above 45.2 nmol/L were conservatively entered as 45.2. Baseline average serum folate concentrations were 34.8 ± 3.4 nmol/L for the control group, 39.1 ± 2.9 nmol/L for the group receiving cobalamin every 14 days, and 39.3 ± 2.5 nmol/L for the group receiving cobalamin every 7 days (not significant) and did not change significantly over the course of the clinical trial. A subnormal serum folate concentration was observed in only 1 patient (in the group receiving weekly cyanocobalamin) at the 8-week time point; the corresponding plasma tHcy concentration was 13.4 μmol/L. Table 2 shows the serum cobalamin concentrations by treatment group. Cyanocobalamin administered every 14 days approximately doubled serum cobalamin concentrations by the 4-week time point, whereas administration every 7 days approximately quadrupled them, with modest further increases in both groups by the 8-week time point.

Table 2
Serum cobalamin concentrations

Treatment group	Baseline	Week 4	Week 8
Every 28 d	1054 ± 71	1435 ± 127	1343 ± 160
Every 14 d	1259 ± 108	2328 ± 168	3040 ± 503
		P = .028[a]	P = .002[a]
			P = .004[b]
Every 7 d	1011 ± 87	4206 ± 494	4805 ± 1009
		P < .001[a]	P < .001[a]
		P < .001[b]	P < .001[b]
		P < .001[c]	P = .005[c]

Values are expressed in picomoles per liter as the means ± SEM. Reference range is 180 to 660 pmol/L.
[a] Significantly different from baseline value.
[b] Significantly different from every 28 days' treatment.
[c] significantly different from every 14 days' treatment.

Table 3
Plasma tHcy concentrations

Treatment group	Baseline	Week 4	Week 8
Every 28 d	19.0 ± 1.3	19.8 ± 1.6	19.5 ± 1.6
Every 14 d	20.8 ± 1.5	19.1 ± 1.5	18.4 ± 1.7
		P = .046	P = .035
Every 7 d	20.0 ± 1.0	17.6 ± 1.0	17.8 ± 1.2
		P = .006	P = .013

Values are expressed in micromoles per liter as the means ± SEM. Normal plasma tHcy concentration is less than 12 μmol/L. P values are for differences from the baseline plasma tHcy concentration; there were no significant differences among the different treatment groups.

Plasma tHcy concentrations are shown in Table 3. At baseline, the plasma tHcy concentration in the 3 groups was approximately 20 μmol/L, in agreement with our earlier observations in this hemodialysis unit [29]. These concentrations are approximately 25% less than the average concentration in a nearby hemodialysis unit where high-dose folic acid, but no parenteral cobalamin, is routinely prescribed [29,30]. At the 8-week time point, plasma tHcy concentrations were reduced in both active treatment groups by approximately 11% below the level previously accomplished by monthly cyanocobalamin administration (P < .05).

4. Discussion

We have shown that cyanocobalamin administered intravenously at 7- or 14-day intervals reduces plasma tHcy concentrations of hemodialysis patients below the already-low concentrations brought about by prior long-term administration every month. This confirms that the Hcy-lowering effect of parenteral cobalamin we [30] and others [22] recently reported is a pharmacological effect and not merely correction of a latent deficiency state. Deficiency was impossible because the participants in the present trial all had supraphysiological serum cobalamin concentrations upon entry, owing to the monthly intravenous cyanocobalamin routinely administered in this unit.

These results are consistent with our previous observations, which indicate that, as compared with patients naive to parenteral cobalamin, monthly parenteral therapy reduces plasma tHcy by approximately 25% [29], whereas weekly parenteral therapy reduces plasma tHcy by approximately 32% [30]. The present results indicate that switching from monthly to weekly therapy reduces plasma tHcy by an additional 11%, almost precisely the incremental lowering

that would have been predicted from the earlier results. Although intravenous cyanocobalamin every 7 or 14 days is superior to monthly therapy, there was insufficient statistical power to determine whether therapy every 14 days is equivalent or inferior to therapy every 7 days. A small pilot study found that in cobalamin-naive patients, 1 mg of parenteral hydroxocobalamin at 2-week intervals resulted in only a 23% reduction in plasma tHcy and hence was possibly inferior to weekly therapy [36].

Others have reported that while a regimen of 10 mg/d folic acid plus intravenous methylcobalamin 0.5 mg twice weekly reduced plasma tHcy concentrations of hemodialysis patients by 44%, patients in a comparison group receiving 10 mg folic acid only experienced a similar reduction, implying that intravenous methylcobalamin confers no additional benefit to the Hcy-lowering caused by high-dose folic acid alone [37]. However, it was also reported that the average serum cobalamin concentration in the folic acid–only group increased to 4700 pmol/L, more than 7 times the upper normal value, during the course of the clinical trial. One must therefore suspect that some patients in the folic acid–only group in that study were inadvertently administered methylcobalamin. This could well have reduced average plasma tHcy concentrations for the group as a whole.

Until recently, high-dose cobalamin was thought to have slight, if any, Hcy-lowering effect in ESRD [14,38-40]. It is now clear that people with normal renal function rapidly eliminate unbound cobalamin in their urine [41], whereas people with ESRD may accumulate high serum and tissue cobalamin concentrations when the vitamin is administered parenterally [22,30,34,35]. This accumulation either partially overcomes uremia-related cobalamin resistance [42,43] or, as observed in cultured cells, increases methionine synthase activity [44] which, in turn, increases Hcy metabolism [45].

The question of cobalamin toxicity should be addressed. In our previous controlled trial, we used hydroxocobalamin rather than cyanocobalamin, partly because hydroxocobalamin has been used safely in massive doses for more than 25 years as a treatment of cyanide poisoning [46-48] and partly because the mechanism by which cobalamin reduces plasma tHcy concentrations in cobalamin-sufficient ESRD patients is uncertain, and hydroxocobalamin is superior to cyanocobalamin for treating certain inborn errors of cobalamin metabolism [49]. There is no evidence that high concentrations of cobalamin are toxic in tissue or cell culture systems [47,50]. Cyanocobalamin is converted in the tissues to hydroxocobalamin and methylcobalamin, and the only difference between the molecules is the presence or absence of cyanide. Each milligram of cyanocobalamin contains 19 μg of cyanide, so weekly administration of cyanocobalamin could release a theoretical maximum of 3 μg/d, whereas the normal dietary intake of cyanide from natural cyanide-containing compounds is in the milligram range. Cyanocobalamin was chosen for use in this study because it is readily available and inexpensive in Canada, and our patient population, which has been receiving it on a monthly basis for many years, provided a natural control group to answer our experimental question.

It is important to appreciate that parenteral cobalamin will reduce plasma tHcy only if folic acid status is normal [34,37]. Furthermore, low-frequency treatment such as 1 mg or less every month [51,52] may be incorrectly interpreted as ineffective unless continued for the many months that may be needed to accumulate sufficiently high cobalamin tissue stores.

We previously observed that once-weekly parenteral hydroxocobalamin increased serum cobalamin concentrations 48- to 65-fold [30]; Koyama et al [22] reported a 70-fold increase in serum cobalamin after administering 0.5 mg methylcobalamin intravenously 3 times per week. However, in the present study, 8 weeks of intravenous cyanocobalamin increased the average serum cobalamin concentration only 5-fold. Notwithstanding this substantially lower concentration, the plasma Hcy lowering achieved appears to have been equivalent to what we previously observed with hydroxocobalamin.

How could this come about? Unlike some commercial cobalamin assays, the assay we used detects very high serum concentrations accurately as long as the appropriate diluent is used. Because the form of cobalamin administered to patients is reflected in their serum [53], we tested the possibility that our assay is less sensitive to cyanocobalamin than hydroxocobalamin at very high concentrations by adding known authentic standards of the 2 molecules to control sera. There was appropriate and equal recovery of cyanocobalamin and hydroxocobalamin.

Hydroxocobalamin is known to be better retained in the body, to produce much higher circulating cobalamin concentrations, and to have a longer half-life than cyanocobalamin when studied in people with normal renal function; this is attributed to its extensive nonspecific plasma protein binding once specific cobalamin binding sites are saturated [54,55]. We speculate that when injected after dialysis, hydroxocobalamin's greater nonspecific protein binding partially protects it from clearance during subsequent dialysis treatments. Large doses of cobalamin in any form penetrate and accumulate within many tissues [54,56]. Thus, hydroxocobalamin and cyanocobalamin could accumulate to a similar extent in the liver and other tissues, reducing plasma tHcy with comparable potency, despite cyanocobalamin's much lower serum concentration. The present results nevertheless raise the possibility that hydroxocobalamin is superior to cyanocobalamin for plasma tHcy reduction in ESRD. A controlled study is currently in progress to test this.

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