Pathophysiology and Treatment of Hyperhomocysteinemia in End-Stage Renal Disease Patients

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The pathophysiology of hyperhomocysteinemia in end-stage renal disease (ESRD) patients includes impaired remethylation of homocysteine (Hcy) to methionine, inhibition of extrarenal Hcy metabolism by uremic solutes, a block in decarboxylation of cysteinesulfinic acid, impaired [adenosylmethionine]/[adenosylhomocysteine] ratio, and a probable impairment of renal Hcy metabolism and excretion.

Treatment of hyperhomocysteinemia in ESRD patients includes administration of folic acid (1 – 15 mg per day). No additional effects have been observed with higher folic acid doses, folinic acid, or 5-methyltetrahydrofolate. Oral supplementation with vitamin B_6 and vitamin B_{12} has no effect, but some studies reported a decrease of plasma Hcy with high intravenous vitamin doses. Effective reduction of plasma total Hcy (tHcy) in patients treated with super-flux hemodialyzers suggests the removal of uremic toxins with inhibitory activities against enzymes involved in the extrarenal Hcy metabolism.

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Key words

Homocysteine, renal failure, folic acid

Introduction

Moderate hyperhomocysteinemia (15 – 30 μmol/L) occurs in most patients with chronic renal failure [1]. Hyperhomocysteinemia is an adverse cardiovascular risk factor in end-stage renal disease patients [2–4]. Plasma total homocysteine (tHcy) consists of approximately 70% protein-bound homocysteine (Hcy), bound via disulfide bonds mainly to plasma albumin [5], and about 25% – 30% free mixed disulfides, mostly homocysteine–cysteine [6,7], in the circulation. Approximately 2% of circulating tHcy is in the reduced form (rHcy). Recently, Hoffer *at al.* [8] developed a sensitive method for measuring plasma rHcy concentrations. They found an average plasma tHcy concentration of 8.47 ± 0.58 μmol/L in normal adults whose rHcy concentration was 0.24 ± 0.03 μmol/L. The pre-dialysis tHcy concentration in end-stage renal disease patients was 21.5 ± 1.1 μmol/L. Their pre-dialysis rHcy level was 0.72 ± 0.04 μmol/L. Hemodialysis therapy significantly lowered both tHcy and rHcy concentrations [8].

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Homocysteine metabolism

Fig. 1 shows a diagram of Hcy metabolism. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the folate cycle. In the cell, 5-methyltetrahydrofolate (5-CH_3-H_4-folate) serves as a methyl donor and as a source of tetrahydrofolate (H_4folate), synthesized by methionine synthase reductase (MSR) and methionine synthase (MS). One of the reactions requiring 5,10-methylenetetrahydrofolate and 5-methyltetrahydrofolate is the synthesis of methionine from homocysteine, a remethylation pathway of the homocysteine metabolism. The second remethylation pathway involves betaine–homocysteine methyltransferase (BHMT) to form dimethylglycine from betaine.

Homocysteine in the cell is derived from methionine. This reaction involves many transmethylation reactions (R: methyl group acceptor), producing *S*-adenosylhomocysteine (AdoHcy) from *S*-adenosylmethionine (AdoMet). Cystathionine β-synthase (CBS) catalyzes the trans-sulfuration of homocysteine to cystathionine and cysteine.

Several factors contribute to the hyperhomocysteinemia of end-stage renal disease patients. Because urinary excretion of homocysteine is negligible in healthy subjects [5], a lack of elimination due to impaired renal function is improbable. On the other hand, even in renal failure patients, the kidney may contribute to homocysteine metabolism. Impaired non-renal disposal owing to inhibition of crucial enzymes in the methionine–homocysteine metabolism by the uremic

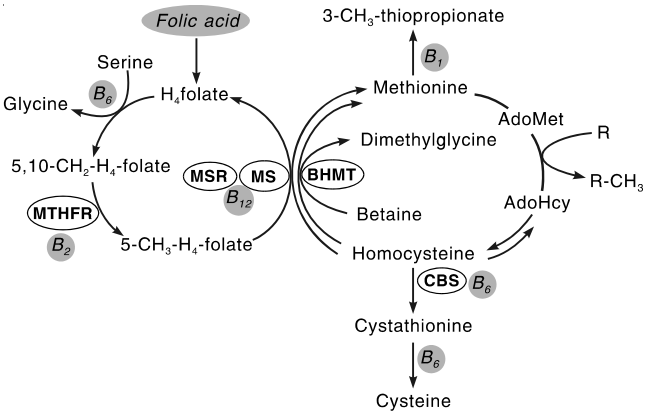

FIGURE 1 Diagram of homocysteine metabolism.

milieu has been suggested [9]. High-dose folic acid, however, reduces plasma tHcy concentration by improving tissue Hcy remethylation [10].

Van Tellingen *et al.* [11] prospectively assessed tHcy levels in patients undergoing regular hemodialysis with either high-flux polysulfone dialyzers (F60: Fresenius Medical Care, Bad Homburg, Germany) or super-flux dialyzers [polysulfone (F500S: Fresenius) and cellulosic triacetate (Tricea 150 G: Baxter Healthcare, Osaka, Japan)]. Total Hcy levels remained stable during high-flux dialysis therapy. However, tHcy decreased significantly to 21.5 ± 8.5 μmol/L (week 12) from 29.6 ± 9.9 μmol/L (week 1) during hemodialysis with F500S, and to 15.3 ± 3.7 μmol/L (week 12) from 24.4 ± 8.7 μmol/L (week 1) during hemodialysis with Tricea 150 G. Because the molecular weight of free homocysteine is less than 268 Da (and not responsible for the observed reduction during super-flux dialysis), the authors concluded that the most likely explanation seems to be the removal of uremic toxins with inhibitory activities against enzymes involved in the extrarenal homocysteine metabolism [11]. This mechanism has also been suggested by other authors [12,13] as an alternative explanation for the elevated tHcy levels in end-stage renal disease patients. However, the reduction of plasma tHcy in patients treated with super-flux hemodialyzers may be the result of albumin loss, given that Hcy is 70% protein-bound. It is noteworthy that the reduction in tHcy during hemodialysis is not markedly different between high-flux and low-flux devices [14].

An elevation in plasma homocysteine concentration leads to an increased intracellular level of its precursor, adenosylhomocysteine [15], a potent inhibitor of all adenosylmethionine-dependent transmethylation reactions [16,17]. Consequently, the [adenosylmethionine]/[adenosylhomocysteine] ratio has been used as a key metabolic parameter to evaluate the degree of such inhibition [15,18].

Treatment of hyperhomocysteinemia with folates

Perna *et al.* [19] studied the metabolic effects of oral methyltetrahydrofolate, the active form of folic acid, on regular hemodialysis patients. Two months of therapy led to a significant reduction of plasma tHcy and to a significant amelioration of the ratio between adenosylmethionine and adenosylhomocysteine. The data, however, could not be confirmed in another study. Bostom *et al.* [20] treated two groups of 25 hemodialysis patients for 12 weeks with oral folic acid (15 mg daily) or an equimolar amount (17 mg daily) of oral L-5-methyltetrahydrofolate. All 50 subjects also received oral vitamin B_6 (50 mg daily) and vitamin B_{12} (1 mg daily). The mean percentage reductions were comparable between the two groups (14.8% vs 17.0%). Table I summarizes these studies and other homocysteine-lowering trials [21–46].

Suliman *et al.* [35] investigated the effects of supplementation with high doses of folic acid (15 mg daily) and pyridoxine (200 mg daily) on sulfur amino acid metabolism in red blood cells and plasma of regular hemodialysis patients and healthy subjects. This therapy reduced the plasma tHcy concentration in both groups. In addition, plasma concentrations of cysteinylglycine, glutathione, and free cysteinesulfinic acid were significantly higher in hemodialysis patients as compared with healthy subjects. The authors suggested that a block in decarboxylation of free cysteinesulfinic acid is linked to hyperhomocysteinemia in ESRD patients [35].

The hyperhomocysteinemia of patients with chronic renal insufficiency can usually be normalized with folic acid doses of 2 – 5 mg daily [47–49]. In contrast, end-stage renal disease patients are, to varying degrees, refractory to available treatments, including folates, vitamin B_6, and vitamin B_{12}, even at high doses. It has been shown that, in nearly all hemodialysis patients, folic acid doses as high as 60 mg daily fail to normalize plasma tHcy levels [37]. In a study by Tremblay *et al.* [40], the tHcy lowering effect of 10 mg of folic acid thrice weekly intravenously (IV) was not superior to 1 mg of folic acid orally per day.

Touam *et al.* [36] determined plasma tHcy concentrations before and during IV supplementation of folinic acid (50 mg once weekly), together with IV supplementation of pyridoxine (250 mg three times weekly). On folinic acid treatment, mean plasma tHcy levels decreased significantly to 12.3 ± 5.4 μmol/L from 37.3 ± 5.8 μmol/L at baseline. At the end of follow-up, 29 of the 37 patients (78%) investigated had normal plasma tHcy levels. No adverse effects attributable to folinic acid treatment were observed.

Other investigators could not confirm these data, however. In a study by Yango *et al.* [45], two groups of 24 hemodialysis patients were treated for 12 weeks with oral folic acid (15 mg daily) or an equimolar amount (20 mg daily) of oral L-folinic acid. All patients also received oral vitamin B_6 (50 mg daily) and vitamin B_{12} (1 mg daily). The mean percentage reductions in pre-dialysis tHcy were comparable between hemodialysis patients on L-folinic acid [22.1% (range: 11.8% – 31.4%)] and patients on folic acid [20.7% (11.7% – 30.5%)]. The investigators concluded that, relative to high-dose folic acid, high-dose oral L-folinic acid–based supplementation does not improve tHcy-lowering efficacy in hemodialysis patients [45].

In a study by Hauser *et al.* [43], 66 hemodialysis patients were allocated to two groups of 33 patients each, one group receiving 15 mg of folic and the other, an equimolar amount (16.1 mg) of folinic acid. The folic and folinic acid was given IV at the end of each hemodialysis session, three times per week for 4 weeks. The tHcy levels in all participating subjects decreased to an average of 19.4 ± 7.9 μmol/L at week 4 from an average of 31.4 ± 24.9 μmol/L at baseline. Normalization of tHcy plasma levels after 4 weeks of treatment was achieved in 16 patients (24.2%). No significant difference was seen in the efficacy of the two substances to lower elevated tHcy levels in hemodialysis patients.

Betaine supplementation may lower tHcy plasma levels in healthy individuals, but the tHcy-lowering effect seems

TABLE I Therapy of hyperhomocysteinemia in dialysis patients.

Author	*Study type*	*Substance and dosis*	*Main effect on tHcy*
Wilcken *et al.*, 1981 [21][a]	US	5 mg FA/PO/d + 100 mg B_6/PO/d + 1 mg B_{12}/IM	35% reduction of cysteine–homocysteine
Wilcken *et al.*, 1988 [22][a]	US	5 mg FA/PO/d	49% reduction of total fHcy
Arnadottir *et al.*, 1993 [23]	US	300 mg B_6/PO/d, then 5 mg FA/PO/d	B_6 no effect, 30% reduction by FA
Bostom *et al.*, 1995 [24]	US	6 g betaine/PO/d	No effect
Bostom *et al.*, 1995 [25]	US	3–4 g serine/PO/d	No effect
Bostom *et al.*, 1996 [26]	US	1200 mg N-acetylcysteine pre-HD	16% reduction
Bostom *et al.*, 1996 [27]	RCT	15 mg FA/PO/d + 100 mg B_6/PO/d + 1 mg B_{12}/PO/d vs. low dose vitamins	30% reduction by high dose vitamins
Perna *et al.*, 1997 [19]	US	15 mg MTHF/PO/d	72% reduction
van Guldener *et al.*, 1998 [28]	RCT	5 mg FA/PO/d vs 5 mg FA + 4 g betaine/PO/d	No additional effect of betaine
	RCT	then 5 mg FA/PO/d vs 1 mg FA/PO/d	5 mg FA not better than 1 mg FA
	US	then + 15 mg FA/PO/d	No additional effect
van Guldener *et al.*, 1998 [29]	RCT	5 mg FA/PO/d vs 5 mg FA + 4 g betaine/PO/d	No additional effect of betaine
	RCT	then 5 mg FA vs 1 mg FA/PO/d	5 mg FA not better than 1 mg FA
van Guldener *et al.*, 1999 [30]	RCT	5 mg FA/PO/d vs 4 g betaine/PO/d	Betaine not better than FA (F + PML)
	RCT	then 1 mg FA/PO/d vs 5 mg FA/PO/d	5 mg FA not better than 1 mg FA (F + PML)
Dierkes *et al.*, 1999 [31]	RCT	2.5 mg FA/PO/HD vs 5 mg FA/PO/HD	5 mg FA not better than 2.5 mg FA
Dierkes *et al.*, 1999 [32]	US	1 mg B_{12}/IV/w	35% reduction (B_{12}-deficient patients)
Spence *et al.*, 1999 [33]	US	1 mg FA/PO/d vs 5 mg FA/PO/d	5 mg FA not better than 1 mg FA
Kunz *et al.*, 1999 [34]	RCT	10 mg FA/PO/d vs placebo	36.6% reduction
Suliman *et al.*, 1999 [35]	US	200 mg B_6/PO/d + 15 mg FA/PO/d	Decrease of fHcy
Touam *et al.*, 1999 [36]	US	50 mg FTHF/IV/w + 250 mg B_6/IV/HD	78% normalization (retrospective study)
Sunder–Plassmann *et al.*, 2000 [37]	RCT	15 mg vs 30 mg vs 60 mg FA/PO/d	30 mg and 60 mg FA not better than 15 mg FA
Bostom *et al.*, 2000 [38]	RCT	15 mg FA/PO/d vs 17 mg MTHF/PO/d	MTHF not better than FA
Arnadottir *et al.*, 2000 [39]	US	15 mg, then 35 mg, then 70 mg FA/PO/w	35 or 70 mg FA not better than 15 mg FA
Tremblay *et al.*, 2000 [40]	RCT	1 mg FA vs 1 mg FA/PO/d + 10 mg FA/IV/HD	10 mg FA IV no additional effect
Thambyrajah *et al.*, 2000 [41][b]	RCT	5 mg FA/PO/d vs placebo	25% reduction vs placebo
Manns *et al.*, 2001 [42]	US	DiaVite™,[c], addition of 1 mg B_{12}/PO/d	16.7% reduction
	RCT	Addition of placebo vs 5 mg vs 20 mg FA/PO/d	5 mg and 20 mg FA not better than 1 mg FA
	US	addition of 8 g serine/PO/d	No effect
Hauser *et al.*, 2001 [43]	RCT	15 mg FA/IV/HD vs 16.1 mg FTHF/IV/HD	FTHF not better than FA
Suliman *et al.*, 2001 [44]	US	200 mg B_6 + 15 mg FA/PO/d	Decrease of PML tHcy
Yango *et al.*, 2001 [45]	RCT	15 mg FA/PO/d vs 20 mg FTHF/PO/d	FTHF not better than FA
Dierkes *et al.*, 2001 [46]	RCT	1.6 mg FA + 12 µg B_{12} + 20 mg B_6/PO/HD vs 0.32 mg FA + 20 mg B_6/PO/HD vs placebo	1.6 mg FA + 12 µg B_{12} vs placebo better than 0.32 mg FA vs placebo

[a] First folic acid treatment studies in renal failure conducted with kidney graft recipients and chronic renal failure patients.
[b] One of the largest studies conducted in chronic renal failure patients.
[c] R&D Laboratories, Marina del Rey, CA, U.S.A.
tHcy = total homocysteine; US = uncontrolled study or case report; FA = folic acid; PO = orally; d = day; IM = intramuscularly; fHcy = free homocysteine; HD = hemodialysis session; RCT = randomized controlled trial; MTHF = 5-methyltetrahydrofolate; F = fasting; PML = post methionine loading; IV = intravenously; w = week; FTHF = 5-formyltetrahydrofolate (folinic acid).

smaller than that established by folic acid therapy [50]. Mutations of betaine–homocysteine methyltransferase do not influence tHcy plasma levels [51].

Hyperhomocysteinemia has been shown to be associated with impaired endothelium-dependent NO-mediated vasodilation [52–54]. This endothelial dysfunction in hyperhomocysteinemic subjects with [55] or without [56,57] clinically manifest vascular disorders is improved, in patients with normal kidney function, by the administration of folates. Supplementation with folic acid also improves endothelial dysfunction in patients with familial hypercholesterolemia and normal tHcy [58]. In contrast, folates failed to significantly improve endothelial dysfunction in patients undergoing regular hemodialysis treatment [28], in patients on peritoneal dialysis [29], and in patients with pre-dialysis renal failure [41]. High-dose IV folic acid or folinic acid also failed to improve mean arterial pressure or pulse pressure in hemodialysis patients [59].

Massy *et al.* [60] found a significant correlation between moderate hyperhomocysteinemia and plasmatic activity of glutathione peroxidase in a large cohort of uremic patients. Folinic acid supplementation has also been shown not only to decrease tHcy but also to prevent lipid peroxidation in hemodialysis patients [61]. These data suggest that moderate hyperhomocysteinemia may predispose to endothelium dysfunction through a mechanism that involves the generation of reactive oxygen species [60].

Oxidative stress may be involved in abnormalities of coagulation associated with hyperhomocysteinemia. Reactive oxygen species, generated during Hcy auto-oxidation, initiate

lipid peroxidation in cell membranes and circulating lipoproteins, resulting in platelet activation and hemostatic abnormalities [62]. Markedly elevated Hcy levels in patients with inborn errors of Hcy metabolism result in thromboembolic disease [63].

Conclusion

The pathophysiology of hyperhomocysteinemia in ESRD patients is not entirely understood. Most of these patients are refractory to the usual therapies. Hopefully, this important issue will be resolved.

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