No change in impaired endothelial function after long-term folic acid therapy of hyperhomocysteinaemia in haemodialysis patients

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Abstract

Background. Hyperhomocysteinaemia is frequent in chronic haemodialysis patients. Because of its potential role in athero-and thrombogenesis, the effects of long-term homocysteine-lowering treatment on endothelial function are of interest.

Methods. We conducted a randomized, controlled trial in 35 haemodialysis patients. In phase 1, patients were treated with 5 mg folic acid or 5 mg folic acid and 4 g betaine per day for 12 weeks, and in phase 2 with 1 or 5 mg folic acid daily for 40 weeks. In phase 3, all patients received 15 mg folic acid daily for four weeks. Endothelial function was assessed before and after 52 weeks of treatment by determination of flow-mediated vasodilatation of the brachial artery, and by measuring plasma levels of endothelium-derived proteins.

Results. Non-fasting predialysis plasma total homocysteine was markedly elevated at baseline (46.9 ± 6.3 μmol/l) and decreased rapidly after initiation of therapy. Significant differences in plasma homocysteine between the groups were found neither during phase 1 nor phase 2. Plasma total homocysteine had normalized in only two out of 30 patients at the end of phase 2. Increasing the daily folic acid dose to 15 mg did not further reduce plasma total homocysteine. Endothelial function parameters did not improve.

Conclusions. We concluded that betaine is not effective in conjunction with folic acid in the treatment of hyperhomocysteinaemia in haemodialysis patients. Normalization of plasma total homocysteine is seldom achieved with 1, 5 or 15 mg folic acid daily, which may explain why long-term homocysteine-lowering treatment with 1 or 5 mg folic acid does not ameliorate endothelial function.

Key words: Hyperhomocysteinaemia; endothelial function; haemodialysis; folic acid; betaine

Introduction

Life expectancy of dialysis patients is only 20–30% of that of the general population, i.e. 5.5–8.5 years in patients aged 40–50 years [1]. The risk of fatal ischaemic heart disease in end-stage renal disease (ESRD) is increased 16- to 19-fold [2]. Atherogenesis in ESRD cannot be fully explained by traditional risk factors such as hypertension, hypercholesterolaemia, smoking and diabetes mellitus.

Hyperhomocysteinaemia is a recently established independent risk factor for cardiovascular disease not only in the general population [3–5], but also in ESRD patients [6], in whom hyperhomocysteinaemia is extremely common [6–8]. Animal and in vitro studies have demonstrated that homocysteine can induce dysfunction of the vascular endothelium [9,10], which plays a key role in the development of atherosclerosis and thrombosis [11]. Endothelial function is impaired in chronic haemodialysis patients, as shown by a decreased endothelium-dependent vasodilatation [12], and by increased plasma levels of endothelium-derived proteins [13,14].

Previous studies on homocysteine-lowering treatment have been short-term (≤4 months) and have not investigated cardiovascular outcomes [8,15,16].

In view of these data, we investigated: (i) to what extent long-term (1 year) treatment with folic acid can normalize plasma homocysteine levels in haemodialysis patients; and (ii) second, whether such long-term treatment is associated with amelioration of endothelial dysfunction. A plasma homocysteine concentration of ≤15 μmol/l was considered to be normal, as prospective studies have found a definite increase in myocardial infarction and stroke above this level [5,17].

Subjects and methods

Subjects

The protocol had been approved by the local ethics committee and all 35 participants gave their informed consent.
Hyperhomocysteinaemia in haemodialysis patients

Patients on maintenance haemodialysis for at least 3 months were recruited from the Department of Nephrology of the University Hospital Vrije Universiteit and Dietael, both in Amsterdam. The renal diagnoses were hypertensive nephrosclerosis (n = 5), immunoglobulin (Ig)A-nephropathy (n = 5), chronic pyelonephritis (n = 5), polycystic disease (n = 4), renal failure of unknown origin (n = 4), focal glomerular sclerosis (n = 3), cholesterol embolism, Goodpasture’s syndrome, reflux nephropathy, interstitial nephritis, analgesic nephropathy, postpartum bilateral cortical necrosis, systemic lupus erythematosus, Henoch Schönlein purpura and diabetic nephropathy (all n = 1). Patients were dialysed 2–3 times a week for 4–5 h. All used regular dialysis medication, i.e. erythropoetin, phosphate binders, supplements of vitamin C and D, and multivitamin B tablets containing 2 mg of vitamin B6, but no folic acid or vitamin B12. Antihypertensives were used by 15 patients: β-blockers in 11, ACE-inhibitors in nine and calcium-antagonists in four. Five patients had a history of cardiovascular disease [aortobifemoral prosthesis in nine and calcium-antagonists in four]. Table 1 shows baseline characteristics. Plasma homocysteine was elevated in all patients.

Study design

The four main options for treatment of hyperhomocysteinemia are folic acid, vitamin B12, vitamin B6 and betaine. We wished to select a regimen with an optimal chance of achieving normalization of plasma homocysteine levels. We, therefore, conducted this randomized and open study in three phases. In phase 1, patients were randomized to receive 5 mg folic acid once daily without (FA group) or with betaine 2 g twice daily (FA + B group).

As betaine had no short-term effect on plasma total homocysteine (see Results), it was not included in Phase 2, which consisted of 40 weeks’ treatment in which we compared two folic acid doses [5 mg (FA5 group) versus 1 mg folic acid (FA1 group)]. The patients were re-randomized per stratum of phase 1. The FA1 patients formed the control group as a daily folic acid dose of 1 mg is commonly used in dialysis centres.

In view of recent findings that further reduction in plasma total homocysteine can be achieved by high doses of B vitamins [16], we decided to add a third phase consisting of a 4 week treatment with 15 mg folic acid once daily in all remaining patients. The total treatment period therefore was 56 weeks. Total homocysteine levels were determined at baseline and at 1, 2, 4, 6, 12, 18, 28, 38, 52 and 56 weeks, folate levels at baseline and at 1, 2, 4, 12, 18, 28, 38 and 52 weeks, and endothelial function at baseline and at 52 weeks.

Measurement of plasma homocysteine

Non-fasting predialysis blood samples were drawn and immediately centrifuged. Plasma was stored at −20°C. Plasma total (free plus protein bound) homocysteine (tHcy) was measured by HPLC with fluorescence detection [20] (reference value: ≤15 μmol/l). Intra- and interassay coefficients of variation are 2.1 and 5.1% respectively [21].

Endothelial function

A gold standard for endothelial cell function is not available. We, therefore, determined both the endothelium-dependent, flow-mediated vasodilatation in the brachial artery [22,23], which closely correlates with endothelial function of the coronary arteries [24], and plasma levels of endothelium-derived proteins, high levels of which are associated with endothelial injury [25,26] and an adverse cardiovascular prognosis [27–30].

Measurement of endothelium-dependent vasodilatation was performed once day prior to a regular dialysis session and after having refrained from smoking and from use of caffeine-containing beverages for at least 10 h. We used a novel wall-motion detector system (Wall Track System, Neurodata, Bilthoven, The Netherlands), which consists of an ultrasound imager (Ultramark IV, ATL, Bothell, USA) connected to a data acquisition and processing unit. In M-mode, the arterial diameter can be obtained with an accuracy of 0.1–0.2 mm [31]. The shunt arm was never used. Baseline end diastolic brachial artery diameter and the peak systolic velocity (PSV) were measured after 15 min of supine rest. Reactive hyperaemia was induced by release of a blood pressure tourniquet that had been inflated on the forearm for 4 min at a pressure of 100 mm Hg above the systolic blood pressure. The brachial artery diameter and the PSV were again measured between 45 and 60 s after release of the cuff. After a 15 min rest, a second baseline measurement was performed. The final measurement was performed 5 min after administration of 0.4 mg of glyceryl trinitrate sublingually. The change in PSV, which was used as the quantitative estimate of the reactive hyperaemia, was expressed as a percentage of the baseline PSV.

Table 1. Baseline characteristics of the study group. Values shown are means (SEM) with range between brackets.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (range)</th>
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</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>19/16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.3 (2.6)</td>
</tr>
<tr>
<td>Smoker (yes/no)</td>
<td>9/26</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 (0.6)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>146.8 (4.2)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>82.7 (2.4)</td>
</tr>
<tr>
<td>Time on dialysis (years)</td>
<td>6.8 (1.0)</td>
</tr>
<tr>
<td>Predialysis plasma total homo­cysteine (μmol/l)</td>
<td>46.9 (6.3)</td>
</tr>
<tr>
<td>Serum folate (nmol/l)</td>
<td>16.1 (0.9)</td>
</tr>
<tr>
<td>Serum vitamin B6 (nmol/l)</td>
<td>83.4 (10.5)</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l)</td>
<td>360.8 (25.6)</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>5.4 (0.3)</td>
</tr>
</tbody>
</table>
Biopool, Umeå, Sweden; normal range 1.84–9.80 ng/ml). PAI-1 (Innotest PAI-1, Innogenetics, Zwijndrecht, Belgium; normal range 6.0–96.0 ng/ml), and ES (R&D Systems, Minneapolis, USA; normal range 29.1–63.4 ng/ml). Plasma immunoreactive ET was measured by radioimmunoassay (Nichols Institute, formerly ITS, Wijchen, The Netherlands; normal range 1.6–4.9 pg/ml) after extraction on 'Sep-Pak C18' cartridges (Waters, Milford, MA, USA). Recovery rate for this assay is 92.4%. Sensitivity of the assay is 1 pg/ml; cross-reactivity with endothelin-2 amounts to 52%, with endothelin-3 to 96% and with 'big' endothelin to 7%. All samples were assayed in one run in order to avoid inter-assay variation. Intra-assay coefficients of variation were <5%.

At baseline, the study power (at $\alpha=0.05$) to detect a normalization of endothelium-dependent vasodilatation and vWF in the total study group was calculated to be 0.80 and 0.98 respectively.

Other measurements

Serum folate (normal >3.4 nmol/l) and vitamin B12 (normal: 120–716 pmol/l) levels were determined by radioassay (ICN Pharmaceuticals, Costa Mesa, USA). Serum pyridoxal phosphate was measured by fluorescence HPLC (normal: 15–100 nmol/l), and serum cholesterol was determined enzymatically by the CHOD-PAP method (Boehringer, Mannheim, Germany).

Statistical analysis

Values are given as mean ± SEM. The statistical analysis was performed on an intention-to-treat basis. Fisher’s exact test was used for the primary endpoint, i.e. the proportion of patients with $t\text{Hcy} \leq 15 \text{ mmol/l}$. Data were tested for skewness and log-transformed when appropriate. Wilcoxon’s test was used if skewness remained. Otherwise, Student’s $t$-tests were used. Correlations were calculated with Pearson’s or Spearman’s test as appropriate. Analysis of variance (ANOVA) for repeated measurements was used for comparison between the FA1 and FA5 groups during phase 2. Analysis of covariance (ANCOVA) was used for the within subjects relation between tHcy and folic acid levels. A two-tailed $P<0.05$ was accepted as the level of significance.

Results

Of the 35 patients, 34 (97%), 30 (86%) and 23 (66%) completed 12, 52 and 56 weeks of treatment. Patients were lost due to kidney transplantation ($n=7$), non-compliance ($n=2$) and switch to peritoneal dialysis, switch to another dialysis centre and cessation of dialysis (all $n=1$). Baseline measurements of endothelium-dependent vasodilatation and plasma markers were not determined in five and three patients respectively (due to altered vascular anatomy of both arms in three, and due to non-compliance in two). Folic acid and betaine were well tolerated by all patients and no side-effects were reported or noticed.

None of the patients had deficiencies of folate or vitamin B12 (Table 1); one had a subnormal serum vitamin B6 level and received vitamin B6 supplements (25 mg daily). The FA group ($n=18$) and the FA+B group ($n=17$) had similar initial tHcy levels: 47.6±9.2 and 46.3±8.9 μmol/l ($P=0.71$). Folate and tHcy were negatively correlated ($r=-0.52; P<0.01$). Plasma tHcy was not correlated significantly with either vitamin B6 ($r=-0.27; P=0.13$) or with B12 ($r=-0.20; P=0.24$). Endothelial function parameters were not correlated significantly with either plasma tHcy or time on dialysis.

Phase 1

Plasma tHcy decreased rapidly after initiation of therapy (Figure 1). At week 12, only one (6%) patient in the FA group and two (13%) in the FA+B group had reached a plasma tHcy level ≤15 μmol/l ($P=0.59$). Plasma tHcy at week 12 was 20.4±1.3 and 23.6±2.4 μmol/l in the FA and FA+B group ($P=0.35$). Serum folate levels showed a steep and similar increase in both groups (Figure 1). Betaine thus had no additional effect on plasma tHcy and was discontinued (see Study design).

Phase 2

Re-randomization allocated 17 patients each to the FA1 and the FA5 groups. Plasma tHcy was similar: 22.3±2.1 versus 21.5±1.7 μmol/l ($P=0.72$), as were body mass index, systolic and diastolic blood pressure, age, gender, smoking status, and time on dialysis (in all cases $P>0.35$). Only serum total cholesterol tended to be higher in the FA5 group: 6.0±0.5 vs 4.9±0.3 mmol/l in the FA1 group ($P=0.08$). No further significant changes in tHcy level occurred in either group (Figure 2). There was also no significant difference in tHcy level between the two groups at any point ($P=0.39$ by ANOVA). At week 52, none of the patients in the FA1 group and two (13%) in the FA5 group had a plasma tHcy ≤15 μmol/l ($P=0.48$).
Hyperhomocysteinaemia in haemodialysis patients

Baseline plasma tHcy levels were 27.2 ± 2.6 and 23.7 ± 1.8 μmol/l [difference (95% confidence interval): 3.4 (-3.0 to 9.8) μmol/l; P = 0.49]. The levels of tHcy and folate during Phases 1 and 2 in the whole group were negatively correlated within subjects (r = −0.25; P < 0.001 by ANCOVA). Reactive hyperaemia, assessed by the percentage increase in PSV, was not different before and after treatment (Table 2).

The differences in endothelium-dependent and -independent vasodilatation before and after 52 weeks of treatment were neither significant in the total group, nor in the FA1 and FA5 groups separately (Table 2). When patients with previous cardiovascular disease and smokers were excluded from analysis, a weak trend for improvement of endothelium-dependent vasodilatation was found in the total group: 3.3 ± 1.4 vs 5.6 ± 1.1% after treatment (n = 17; P = 0.18). Baseline levels of vWF, TM, tPA, PAI-1, ES and ET were not different between the groups and did not correlate with endothelium-dependent vasodilatation. After 52 weeks of treatment, levels of vWF, TM, PAI-1, tPA and ET had not changed in the total group, whereas ES had increased (Table 3). Exclusion of patients with previous cardiovascular disease and smokers yielded similar results. Plasma levels after treatment were not significantly different between the FA1 and FA5 group for vWF (P = 0.54), TM (P = 0.53), tPA (P = 0.13), ES (P = 0.64), and ET (P = 0.68). Plasma PAI-1 was higher in the FA5 group compared with the FA1 group (P = 0.04) after treatment, but the increment was not significantly different: 1.0 ± 4.4 (FA1 group) versus 9.6 ± 5.7 ng/ml (FA5 group); (P = 0.24). No significant associations were found between the decrease in tHcy (measured as level before phase 1 minus the mean of all tHcy levels during treatment) and the change in endothelium-dependent vasodilatation or in plasma levels of markers of endothelial function.

Table 2. Brachial artery measurements of the patients before and after 52 weeks of folic acid therapy (paired observations only)

<table>
<thead>
<tr>
<th>Brachial artery</th>
<th>Before treatment</th>
<th>After 2 weeks</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline diameter (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 26)</td>
<td>3.8 (0.1)</td>
<td>3.8 (0.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>FA1 (n = 14)</td>
<td>3.8 (0.2)</td>
<td>3.8 (0.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>FA5 (n = 12)</td>
<td>3.8 (0.2)</td>
<td>3.7 (0.2)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Endothelium-dependent vasodilatation (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 26)</td>
<td>3.5 (1.2)</td>
<td>4.7 (1.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>FA1 (n = 14)</td>
<td>2.0 (1.2)a</td>
<td>5.3 (1.4)b</td>
<td>0.052</td>
</tr>
<tr>
<td>FA5 (n = 12)</td>
<td>5.2 (2.1)</td>
<td>3.9 (1.8)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Endothelium-independent vasodilatation (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 25)</td>
<td>11.3 (1.6)</td>
<td>8.4 (1.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>FA1 (n = 13)</td>
<td>13.7 (2.4)c</td>
<td>9.7 (2.2)d</td>
<td>0.07</td>
</tr>
<tr>
<td>FA5 (n = 12)</td>
<td>8.6 (1.9)</td>
<td>7.0 (1.4)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Reactive hyperaemia (% increase in PSV)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (n = 26)</td>
<td>197 (6)</td>
<td>191 (5)</td>
<td>0.32</td>
</tr>
<tr>
<td>FA1 (n = 14)</td>
<td>190 (9)</td>
<td>188 (8)</td>
<td>0.87</td>
</tr>
<tr>
<td>FA5 (n = 12)</td>
<td>205 (9)</td>
<td>194 (7)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*a P = 0.17; b P = 0.33; c P = 0.15 and d P = 0.30 compared with FA5.
FA1 = group receiving 1 mg folic acid per day; FA5 = group receiving 5 mg folic acid per day; PSV = peak systolic velocity.

Phase 3

A total of 30 patients participated; 23 completed 4 weeks of treatment with 15 mg folic acid per day. A significant decrease in plasma tHcy was neither found in the total group (from 25.4 ± 1.8 to 24.0 ± 1.9 μmol/l; P = 0.16), nor in the FA1 group (from 28.3 ± 3.2 to 27.4 ± 2.8 μmol/l; P = 0.14), or in the FA5 group (from 23.2 ± 2.0 to 21.3 ± 2.4 μmol/l; P = 0.50).

Table 3. Plasma levels of endothelium-derived regulatory proteins before and after 52 weeks of folic acid (FA) therapy (paired observations only)

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After 2 weeks</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand factor (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 29)</td>
<td>242.3 (18.2)</td>
<td>248.6 (20.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>227.4 (26.5)</td>
<td>269.3 (35.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>258.3 (25.1)</td>
<td>226.3 (18.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Thrombomodulin (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 29)</td>
<td>367.4 (18.4)</td>
<td>368.5 (20.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>394.6 (28.2)</td>
<td>381.1 (31.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>338.3 (21.7)</td>
<td>354.9 (26.6)</td>
<td>0.61</td>
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<tr>
<td>E-selectin (ng/ml)</td>
<td></td>
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</tr>
<tr>
<td>All pts (n = 29)</td>
<td>50.3 (4.6)</td>
<td>57.6 (5.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>47.2 (5.3)</td>
<td>54.1 (5.9)</td>
<td>0.21</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>53.7 (7.7)</td>
<td>61.4 (9.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 29)</td>
<td>25.3 (4.4)</td>
<td>30.4 (5.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>21.1 (3.9)</td>
<td>22.1 (5.0)</td>
<td>0.89</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>29.7 (8.1)</td>
<td>39.3 (8.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 29)</td>
<td>29.3 (1.7)</td>
<td>35.3 (2.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>26.4 (2.3)</td>
<td>32.4 (2.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>33.7 (3.2)</td>
<td>40.3 (3.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>ET (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All pts (n = 29)</td>
<td>5.6 (0.2)</td>
<td>5.5 (0.2)</td>
<td>0.82</td>
</tr>
<tr>
<td>FA1 (n = 15)</td>
<td>5.4 (0.3)</td>
<td>5.4 (0.3)</td>
<td>0.92</td>
</tr>
<tr>
<td>FA5 (n = 14)</td>
<td>5.7 (0.3)</td>
<td>5.6 (0.2)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

FA1 = group receiving 1 mg folic acid per day; FA5 = group receiving 5 mg folic acid per day; PAI-1 = plasminogen activator-inhibitor-I; tPA = tissue-type plasminogen activator; ET = endothelin.
Discussion

This is the first study of the long-term effects of folic acid supplementation on plasma homocysteine levels and the impaired endothelial function in chronic haemodialysis patients. The results indicate that 5 mg of folic acid is not sufficient to normalize plasma homocysteine levels and that folic acid treatment with 1 or 5 mg has no effect on the endothelial dysfunction, which accompanies ESRD and chronic haemodialysis [12]. In addition, we found, in the first part of our study, that adding betaine to folic acid supplementation does not further lower plasma homocysteine levels, suggesting that, in chronic haemodialysis patients, the betaine-dependent remethylation of homocysteine cannot be stimulated further by exogenous betaine.

Since betaine did not add to the homocysteine-lowering effect of 5 mg folic acid daily in phase 1, it was discontinued in phase 2. We considered the inclusion of a placebo group in phase 2 less relevant as it is common practice nowadays to supplement dialysis patients with 1 mg folic acid per day. A cross-over design was discarded since folic acid has a long-lasting carry-over effect [8].

Short-term studies will fail to detect slow responders to treatment and do not establish whether responses to treatment are sustained. Our long-term study shows that the response to folic acid treatment is of rapid onset and sustained. However, only 7% of the patients reached a plasma tHcy level of \( \leq 15 \mu mol/l \) after 1 year. Furthermore, when given after a 12 week period of 5 mg folic acid daily, similar plasma homocysteine levels were sustained by 1 and 5 mg folic acid per day despite a large difference in serum folate, suggesting that 1 mg folic acid per day is sufficient to maintain a serum folate level above which no further reduction in plasma tHcy can be achieved.

Bostom et al. [16] have recently shown that, by supplementation of a multivitamin tablet containing 15 mg folic acid, 100 mg vitamin B6 and 1 mg vitamin B12, plasma tHcy could be reduced to 15 \( \mu \)mol/l or less in one third of dialysis patients who had been receiving 1 mg folic acid daily. Because treatment with vitamin B6 in dialysis patients [15], or with vitamin B12 in renal transplant patients [18], does not lower plasma homocysteine level, and because disturbances in folate metabolism have been demonstrated in haemodialysis patients [32], it is likely that folate acid was responsible for that reduction. However, we were unable to detect a similar decrement in plasma tHcy after treatment with 15 mg folic acid only. Therefore, it appears that the homocysteine-lowering effect observed by Bostom et al. somehow depended on the addition of vitamin B12 and/or B6, although a plausible pathophysiological mechanism for such an effect is lacking. A recent study by Robinson et al. [6] suggests that, in comparison with normal control subjects, higher blood levels of folate, vitamin B12 and B6 are required in dialysis patients for a comparable plasma homocysteine level. Further studies are therefore required to test the efficacy of multivitamin treatment.

We investigated whether long-term homocysteine-lowering treatment in haemodialysis patients would result in an amelioration of endothelial function but found no such effect. However, as plasma tHcy levels in the FA5 and in the control (FA1) groups remained comparable, this study cannot exclude an effect of a change in plasma tHcy level on endothelial function, for it is unknown to what extent endothelial function changes in time when plasma tHcy levels remain markedly elevated.

Two important parameters of endothelial dysfunction were clearly abnormal in our patients: flow-mediated vasodilatation of the brachial artery and plasma vWF. After completion of the study, the power (at \( \alpha = 0.05 \)) to detect normalization or a 50% reduction in the difference with the normal value were calculated to be 0.95 and 0.45 respectively, for flow-mediated vasodilatation and 0.99 and 0.90 respectively, for plasma vWF when the total group of patients was considered (thereby representing an uncontrolled design). In the controlled design (FA5 vs FA1), these values were 0.73 and 0.25 for flow-mediated vasodilatation, and 0.98 and 0.61 for vWF.

In contrast to our study, van den Berg et al. [33] observed a decrease in plasma vWF and TM in non-ESRD patients with premature vascular disease after 1 year of homocysteine-lowering treatment. In addition, cholesterol-lowering treatment for 3–6 months improves endothelium-dependent vasodilatation in forearm and coronary arteries [34,35]. Relatively short-term reductions in risk factor levels can, therefore, result in improvements of endothelial function. Thus, our study period was not unreasonably short. It is, however, possible that the reductions in tHcy levels may not have been sufficient to result in an improvement of endothelial function. Alternatively, hyperhomocysteinaemia may play only a minor role in the endothelial dysfunction of haemodialysis patients; the repeated haemodialysis procedures per se may be more injurious to the endothelium. This would be in accordance with our previous observations that being a haemodialysis patient is associated with a decreased endothelium-dependent vasodilatation, and 0.98 and 0.61 for vWF.

Whatever the explanation, if homocysteine-lowering therapy is to reduce cardiac morbidity and mortality in haemodialysis patients, it is likely that, in view of the shortened life expectancy, any beneficial effects on endothelial function of treatment should be apparent at 1 year to be clinically meaningful. Our results, however, overall did not show an improvement of...
endothelial function after 1 year, which in our patient group comprises on average ~15% of their remaining years of life. Although the findings of our study suggest that the treatment we used may not influence the cardiovascular outcome of haemodialysis patients, we realise that only large-scale intervention studies with hard clinical endpoints are necessary to establish the eventual beneficial effects of homocysteine-lowering treatment. In addition, it remains to be investigated whether homocysteine-lowering therapy is effective in reducing endothelial dysfunction when given before end-stage renal failure has developed. Finally, new treatment strategies should be developed to normalize plasma tHcy completely in chronic haemodialysis patients.

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