Original Article

Treatment with different doses of folic acid in haemodialysis patients: effects on folate distribution and aminothiol concentrations

Margret Arnadottir1, Vilmundur Gudnason2 and Björn Hultberg3

1Department of Medicine, National University Hospital, Reykjavik, 2Icelandic Heart Association Heart Preventive Clinic, Reykjavik, Iceland and 3Department of Clinical Chemistry, Lund University Hospital, Lund, Sweden

Abstract

Background. Hyperhomocysteinaemia is highly prevalent among haemodialysis patients and may contribute to their increased cardiovascular risk. Treatment with pharmacological doses of folic acid lowers the plasma homocysteine concentration in these patients. The purpose of the present study was to expand the knowledge about such treatment by testing the effects of stepwise increases in the dose of folic acid on the concentrations of plasma and red blood cell folate as well as the total plasma concentrations of homocysteine (tHcy), cysteine (tCys), and glutathione (tGSH) in patients on chronic hemodialysis.

Methods. Fourteen stable haemodialysis patients completed the study which consisted of four consecutive periods, each of 6 weeks duration: (i) no treatment with folic acid (control period); (ii) 5 mg of folic acid three times per week (15 mg/week); (iii) 5 mg of folic acid daily (35 mg/week); (iv) 10 mg of folic acid daily (70 mg/week).

Results. Neither plasma or red cell folate nor plasma aminothiol concentrations changed significantly during the control period. The mean red cell folate concentration doubled during the administration of folic acid at the dose of 15 mg/week but at higher doses the further rise was only marginal. The mean folate concentration in plasma increased steeply especially at the higher doses of folic acid. During treatment with 15 mg/week of folic acid, tHcy fell by a mean of 36%, tGSH increased by a mean of 34%, but tCys was unaffected. Increases in the dose of folic acid did not augment these responses.

Conclusions. The maximal effect on tHcy seemed to be obtained already at the lowest given dose of folic acid (15 mg/week). At that dose, the red blood cells approached folate saturation, which may reflect the situation in other cells that participate in homocysteine metabolism and explain why further increases in the dose of folic acid are not effective from a tHcy-lowering point of view.

Keywords: cysteine; folate; glutathione; haemodialysis; homocysteine; renal failure

Introduction

Hyperhomocysteinaemia has been recognized as an independent atherothrombosis risk factor [1,2] potentially explaining as much as 10% of the coronary artery disease in the general population [3]. The mechanisms behind this have not been fully elucidated but seem to involve endothelial dysfunction and increased thrombogenicity [4,5]. The plasma concentration of total homocysteine (tHcy) can be lowered by treatment with water-soluble vitamins (folic acid, vitamin B12 and pyridoxine (vitamin B6)), whose precursors act as substrates or enzyme cofactors in the homocysteine removal processes [6]. In the general population, only treatment with folic acid has consistently decreased fasting tHcy even in replete subjects [7]. Thus, folic acid has become the agent of choice for tHcy-lowering treatment, although as yet there is no evidence that therapy influences cardiovascular morbidity or mortality [8].

For unknown reasons, renal insufficiency is associated with hyperhomocysteinaemia [9]. In haemodialysis patients, mean tHcy is roughly threefold that of the general population. The development of tHcy-lowering strategies for these patients is of particular interest due to their greatly increased mortality in atherosclerotic complications [10]. In addition, in this patient category folic acid has proved effective, lowering tHcy in dialysis patients by 30–50% [11–15]. However, the optimal dose has not been established. The largely unknown pattern of folate distribution associated with different doses of folic acid may be relevant in this context.

The aminothiol cysteine is derived from homocysteine catabolism and, in turn, the synthesis of glutathione depends on the availability of cysteine. In dialysis patients, the total plasma concentration of cysteine (tCys) is increased [16,17] whereas that of glutathione (tGSH) has been reported to be similar.
to that of control subjects [18]. Little is known about the effect of treatment with folic acid on the plasma concentrations of these aminothiols in renal failure.

The present study included a group of stable haemodialysis patients who were characterized with regard to the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene. The main purpose of the study was to document the effects of stepwise increases in the dose of folic acid on the folate concentrations in plasma and red blood cells as well the plasma total concentrations of the aminothiols homocysteine, cysteine, and glutathione.

**Subjects and methods**

**Patients**

Twenty haemodialysis patients were recruited at the dialysis department of the National University Hospital in Reykjavik. Three patients died of cardiovascular causes during the control or low-dose study periods (see below) and three patients were excluded due to lack of cooperation. Thus, 14 patients completed the study. The patients were treated with polysulphone filters (F6, F7, F8) for 4–5 h three times per week. Age, sex, time on dialysis, Kt/V, PCR, and the serum concentrations of albumin as well as cobalamin are shown in Table 1. At the start of the study, two patients had red cell folate concentrations below the reference range, all the patients had serum vitamin B12 concentrations within the reference range, and only had received daily supplementation with a multivitamin preparation for at least 3 months. This supplementation, containing 15 mg of pyridoxine but no folic acid or vitamin B12 was continued throughout the study.

**Procedure**

The study consisted of four consecutive periods, each of 6 weeks duration:

- Weeks 1–6: no treatment with folic acid was given (control period).
- Weeks 7–12: folic acid was given at the dose of 5 mg three times per week directly after the haemodialysis sessions (15 mg/week).
- Weeks 13–18: folic acid was given at the dose of 5 mg daily (35 mg/week).
- Weeks 19–24: folic acid was given at the dose of 10 mg daily (70 mg/week).

Blood samples were collected five times during the study, i.e. at study start and at the end of each period, for analysis of folate concentrations in plasma and red blood cells as well as the concentrations of tHcy, tCys and tGSH in plasma. Samples of whole blood were collected in EDTA tubes for genotype analysis regarding the C677T polymorphism of the MTHFR gene.

The samples were taken after a light breakfast immediately before a midweek haemodialysis session. The samples intended for analysis of aminothiols were placed directly on ice, centrifuged within an hour, and the plasma stored at −20 °C for analysis in the same series.

**Laboratory investigations**

Folate concentrations were analysed by a radioassay, using purified folate-binding protein (vitamin B12/folate dual kit, Amersham International, Amersham, UK).

Plasma was treated with dithiothreitol in order to cleave the disulphide bonds. Thereafter, the concentrations of tHcy, tCys and tGSH were measured by high-performance liquid chromatography [19]. The intra-assay variation of the method for homocysteine analysis was 1.5% and the interassay variation was 2.5%.

The C677T polymorphism was determined as previously described using polymerase chain reaction and digestion with HinfI restriction endonuclease [20].

**Statistical analyses**

Data are presented as mean ± standard deviations except data regarding the C677T/MTHFR polymorphism that are presented as medians (ranges). Friedman’s test was applied to test the variance between the study periods and, in the case of a significant difference, the data were directly compared by the Wilcoxon test for paired data. Correlation analyses were performed with Spearman’s rank correlation test. A P value < 0.05 was considered to reflect statistical significance.

**Results**

With increasing doses of folic acid the folate concentrations rose markedly, indicating good patient compliance (Figure 1). During treatment with the lowest dose of folic acid, a steep rise in the mean red blood cell folate concentration was observed, followed by much smaller further increases at the higher doses. In contrast, the mean plasma folate concentration rose steeply at the higher doses of folic acid.

The effects of treatment with folic acid on plasma tHcy, tCys, and tGSH are shown in Figure 2 and Table 2. According to Friedman’s test, the variance between the study periods was significantly different regarding tHcy (P = 0.0001) and tGSH (P = 0.0001). None of the aminothiols changed significantly during the control period. tHcy fell significantly by 36% during treatment with 15 mg/week of folic acid but did not decline further as the dose of folic acid was increased, i.e. there was no statistical difference between tHcy at the end of different treatment protocols. Before treatment no patient was normohomocysteinaemic (tHcy < 16.0 μmol/l) but after treatment two patients were. Plasma tGSH increased significantly by 34% during treatment but in this case also no additional changes

| Table 1. Demographic variables and measures of dialytic as well as nutritional adequacy in 14 haemodialysis patients |
|-------------|---|
| Age (years) | 59 ± 16 |
| Sex (male/female) | 8/6 |
| Time on dialysis (months) | 23 ± 23 |
| Kt/V | 1.46 ± 0.28 |
| PCR (g/kg/day) | 0.99 ± 0.28 |
| Serum albumin (g/l) | 38 ± 4 |
| Serum vitamin B12 (pmol/l) | 539 ± 332 |

Data, except for sex, are given as means ± standard variations.
Fig. 1. The upper curve (rectangles) shows the folate concentrations in the red blood cells of 14 haemodialysis patients before and after treatment with different doses of folic acid. The lower curve (circles) shows the corresponding folate concentrations in plasma. Data are given as means ± standard variations.

were observed at higher doses of folic acid. Plasma tCys was not affected by treatment.

The correlations between plasma tHcy and folate concentrations in plasma and red blood cells did not reach statistical significance at any time point. Before treatment the coefficient of correlation between plasma tHcy and red blood cell folate was −0.30 but after the different treatment protocols it ranged between −0.02 and 0.02. In addition, the treatment-induced changes in plasma tHcy did not correlate with post-treatment folate concentrations or changes in folate concentrations. The same applied to the other aminothiols except that before treatment tGSH correlated inversely with red blood cell folate concentrations ($r = -0.70$, $P < 0.05$).

Plasma tHcy before and after treatment correlated directly ($r = 0.65$, $P < 0.05$). There was a significant inverse correlation between tHcy before treatment and the relative treatment-induced changes in tHcy ($r = -0.58$, $P < 0.05$). Thus, higher pre-treatment tHcy concentrations were associated with greater treatment-induced reductions. Before treatment, plasma tHcy tended to correlate directly with plasma tCys ($r = 0.52$, $P = 0.06$) and plasma tGSH ($r = 0.53$, $P = 0.06$). After treatment, the correlation between tHcy and tCys grew stronger ($r = 0.82$, $P < 0.01$) whereas the tendency toward correlation between tHcy and tGSH disappeared.

Two patients were homozygous with respect to the C677T mutation in the MTHFR gene, four patients were heterozygous, and eight patients manifested the wild-type alleles. The respective median tHcy concentrations (µmol/l) of these groups before treatment were 40.8 (35.5–46.1), 30.3 (24.4–61.6) and 29.0 (23.8–57.8), and the respective median changes (%) in tHcy were −38 ((−28)–(−48)), −31 ((4)–(−58)), and −37 ((−20)–(−62)).

Discussion

In haemodialysis patients, plasma tHcy declined by 36% during treatment with folic acid at the dose of 15 mg/week. This treatment also induced an increase in plasma tGSH of 34% but did not affect plasma tCys. Increasing the dose of folic acid did neither augment the changes observed in the aminothiols nor markedly elevate the folate concentration in red blood cells.
Table 2. Plasma concentrations of tHcy, tCys, and tGSH (μmol/l) before treatment and after treatment with folic acid at the doses of 15 mg/week, 35 mg/week, and 70 mg/week

<table>
<thead>
<tr>
<th>Treatment with folic acid</th>
<th>tHcy</th>
<th>tCys</th>
<th>tGSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment 1</td>
<td>36.5±10.6</td>
<td>398±93</td>
<td>4.6±1.3</td>
</tr>
<tr>
<td>No treatment 2</td>
<td>37.5±12.9</td>
<td>401±74</td>
<td>5.4±1.8</td>
</tr>
<tr>
<td>15 mg/week</td>
<td>22.3±5.0**</td>
<td>415±64</td>
<td>6.9±1.5**</td>
</tr>
<tr>
<td>35 mg/week</td>
<td>23.4±6.5**</td>
<td>416±105</td>
<td>6.5±1.8**</td>
</tr>
<tr>
<td>70 mg/week</td>
<td>22.3±5.3**</td>
<td>415±81</td>
<td>7.9±2.2**</td>
</tr>
</tbody>
</table>

**P<0.01 as compared with ‘no treatment 2’. Data are given as means± standard deviations.

Homocysteine is an intermediate in the metabolism of the essential amino acid methionine. It is removed either by irreversible breakdown to smaller sulphate compounds via the trans-sulphuration pathway where pyridoxal-5-phosphate acts as an enzyme cofactor or by remethylation back to methionine. The main remethylation pathway is catalysed by the enzyme methionine synthase, which requires vitamin B₁₂ as a cofactor and methylenetetrahydrofolate as a substrate. This constitutes the theoretical base for using the B-vitamins for tHcy-lowering purposes. In the general population, the administration of folic acid lowers tHcy in most individuals whereas treatment with vitamin B₁₂ or pyridoxine is of less clinical significance [6,7]. The same pattern of vitamin response has been found in patients with renal insufficiency, who generally manifest hyperhomocysteinaemia of unknown mechanism; several studies found no independent tHcy-lowering effect of treatment with vitamin B₁₂ or pyridoxine [11,13,21] while treatment with folic acid at different doses lowered tHcy by 30–50% [11–15].

Treatment with other agents of theoretical interest such as betaine [22] or serine [23] did not significantly lower tHcy in dialysis patients. In spite of folic acid obviously being the cornerstone of tHcy-lowering treatment in haemodialysis patients, few studies have compared the tHcy-lowering effect or documented the folate distribution in response to different doses of folic acid. There is as yet no consensus about the optimal dosage of folic acid in this setting, while the literature gives the general impression that very high doses are needed for maximal tHcy-lowering effect. The maximal tHcy-lowering response to folic acid seemed to be obtained already at the dose of 15 mg/week administered directly after the haemodialysis sessions (Figure 2 and Table 2). The individual curves in Figure 2 in no case gave the impression of a substantial or consequent increase in response to the successively increased dose of folic acid. It is of interest to note that during treatment with 15 mg/week of folic acid, the mean red blood cell folate concentration doubled, whereas subsequent manifold increases in the dose of folic acid only resulted in marginal further increases in the red blood cell folate concentration. Thus, the red blood cells seemed to approach folate saturation already at the lowest given dose of folic acid. These results probably reflect the situation in other cells than red blood cells, a notion supported by the steep increases in plasma folate concentrations at the higher doses. Moreover, it has been shown in rats that increased administration of folic acid results in proportional elevations in the folate concentrations in red blood cells and liver cells [24]. It is hardly conceivable that an increase in the dose of folic acid can further stimulate the remethylation of tHcy without even gaining intracellular access. Therefore, the present results indicate that there is no rationale for administering very high doses of folic acid to haemodialysis patients. The results of the study by Dierkes et al. (no statistical difference in the tHcy-lowering effect between folic acid 7.5 mg/week and 15 mg/week [15]), Bostom et al. (26–30% increase in the tHcy-lowering effect when the dose of folic acid was increased from 1 mg daily to 16 mg daily [12]) in combination with the present results suggest that the optimal dose of folic acid in haemodialysis patients may be in the range of 1–2 mg daily.

Even during treatment with 16 mg of folic acid, only a minority of haemodialysis patients were reported to be normohomocysteinaemic [12]. Accordingly, none of our patients was normohomocysteinaemic before treatment but after treatment, two patients were. One patient did not respond at all. This patient was heterozygotic for the C677T mutation and, as judged by his folate concentrations, he was compliant with the treatment. Thus, in most cases of haemodialysis patients, treatment with folic acid substantially lowers tHcy but not to normohomocysteinaemic levels. This probably reflects a unique influence exerted by the uraemic environment on homocysteine metabolism. However, those who are in the greatest need for treatment, i.e. those who manifest the highest tHcy concentrations, respond most markedly. This is illustrated in Figure 2 by the treatment-induced narrowing of the tHcy range. Possibly, treatment with methyltetrafolate, the active form of folate, will prove to be superior. A promising treatment trial has been performed with methyltetrahydrofolate, but in that study no comparison was made with folic acid [25].

In those haemodialysis patients who lie beneath the median in folate concentration, homozygosity for the thermodabile variant of the enzyme MTHFR (C677T mutation) has been shown to predispose to increased tHcy [26]. In the present study there was no difference in the relative response to folic acid between the two patients who were homozygous for the above-mentioned mutation and those eight patients who did not carry this mutation at all. However, the groups were too small for statistical analysis.

Catabolism of homocysteine takes place in the trans-sulphuration pathway. In the first steps, catalysed by cystathionine-β-synthase, cysteine is formed. Cysteine, in turn, is necessary for glutathione synthesis. In the present study, treatment with folic acid lowered tHcy, left tCys unaffected and raised tGSH. These findings seem to be paradoxical. The folate-induced increase in the remethylation would be expected to reduce the load on the trans-sulphuration pathway, which should
result in a decrease in tCys and tGSH unless intracellular homocysteine is still in considerable excess. It can be speculated whether treatment with folic acid also facilitates the trans-sulphuration process, a hypothesis that reconciles all our aminothiol results. This would also be in accordance with the findings of van Gulden et al. [27], who observed a reduction in both tHcy and the increment in tHcy after methionine loading during treatment with folic acid. Alternatively folate may in some way influence the metabolism of glutathione. It must be taken into consideration that the analysis of glutathione in stored plasma may to some extent be disturbed by endogenous gamma-glutamyl transferase activity [28] and therefore, the present results need to be confirmed on fresh samples.

In summary, in the present study, the maximal tHcy-lowering effect seemed to be obtained already at the lowest given dose of folic acid (15 mg/week). At that dose the red blood cells approached folate saturation, possibly explaining why further increases in the dose of folic acid are not effective from a tHcy-lowering point of view.

References


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