Influence of haemodialysis on plasma total homocysteine concentration

Margret Arnadottir1, Anna-Lena Berg2, Jörgen Hegbrant3 and Björn Hultberg4

1Department of Medicine, National University Hospital, Reykjavik, Iceland, 2Department of Nephrology and 4Department of Clinical Chemistry, University Hospital in Lund, Lund; and 3Park Dialys, Gambro Group Renal Care, Lund, Sweden

Abstract

Background. The high prevalence of hyperhomocysteinemia in uraemic patients is of interest because of the cardiovascular risk associated with increased plasma total homocysteine (tHcy) concentration. Treatment with folic acid lowers tHcy in haemodialysis patients, however, in most patients not to normohomocysteinemic levels. With possible tHcy-lowering modifications in mind, we studied the influence of standard haemodialysis on tHcy.

Methods. In 56 folate-loaded haemodialysis patients, tHcy and parameters of dialysis adequacy were measured. In six patients, interdialytic curves of tHcy and serum creatinine concentrations were obtained and in five patients, the amount of homocysteine (Hcy) in dialysate was determined.

Results. tHcy (21.8 ± 14.4 μmol/l) correlated significantly with Kt/V (r = 0.32, P < 0.05), total Kt/V (r = 0.29, P < 0.05), nPCR (r = 0.30, P < 0.05) and serum concentrations of albumin (r = 0.28, P < 0.05) and cobalamines (r = −0.27, P < 0.05). In a multiple linear regression analysis, only serum albumin concentrations significantly predicted tHcy (r = 0.34, P < 0.05). During dialysis, tHcy decreased by 28% and remained constant for at least 8 h after treatment. The amount of Hcy recovered in dialysate was 63 μmol (12–158 μmol). There was no difference in tHcy between those who had residual renal function and those who had not.

Conclusions. The direct relationship between tHcy and Kt/V seemed to be mediated by the serum albumin concentration. The shape of the interdialytic tHcy curve suggested facilitated Hcy removal for at least 8 h after dialysis possibly due to reduced levels of inhibitory activities against relevant enzyme(s). The dialysed amount of Hcy did not seem to contribute significantly to Hcy removal. Thus, modifications of standard dialytic regimens are not likely to be effective from a tHcy-lowering point of view whereas convective procedures such as haemofiltration or haemodiafiltration might be more effective.

Key words: haemodialysis; homocysteine; renal failure

Introduction

Hyperhomocysteinemia is an independent cardiovascular risk factor according to a steadily increasing number of studies [1–6]. Although homocysteine (Hcy) probably plays a causative role in the atherothrombotic process [7,8] the mechanisms have not been clarified. Treatment with folic acid safely and effectively lowers plasma total homocysteine (tHcy) concentration [9]. As yet there are no data confirming the expected clinical benefit of such treatment.

Hcy metabolism is of particular interest in patients with end-stage renal disease (ESRD) because of their greatly increased risk of atherosclerotic complications [10] in combination with their high prevalence of hyperhomocysteinemia [11]. Indeed, hyperhomocysteinemia has been shown to be independently associated with an increase in cardiovascular risk in dialysis patients [12]. This scenario prompts many nephrologists to prescribe folic acid to their dialysis patients. In pharmacological doses, this agent lowers tHcy by 30–50% [13,14]. However, treatment with folic acid in a dose as high as 16 mg daily does not normalize tHcy in the majority of the patients [14] and there is no other agent of proven efficacy in this situation. Thus, the question arises of whether dialysis treatment can be modified for tHcy-lowering purposes. Several studies have shown that tHcy concentration decreases by 30–50% during a haemodialysis session [15–17] but the implications of these findings are not clear. In the present investigation, we studied the association between predialytic tHcy and parameters of dialysis adequacy, recorded the interdialytic course of tHcy and measured the amount of Hcy recovered in dialysate.

Patients and methods

Patients

Seventy haemodialysis patients were recruited from the dialysis departments of the National University Hospital,
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Reyjavik and the University Hospital in Lund, as well as from Park Dialys, Lund. There were 52 males and 18 females of the mean age of 65 ± 15 years. The patients received bicarbonate dialysis with low-flux dialysers 4–5 h three times a week. Folic acid 5 mg daily and pyridoxine 5 mg daily were prescribed to all patients.

Procedure

Blood sampling was performed before a mid-week dialysis in all patients. The blood was collected for routine analysis of the concentrations of blood folate, serum cobalamin and serum albumin. A sample, drawn in an EDTA-containing test tube, was placed directly on ice and centrifuged within half an hour. The plasma was stored at −20°C for analysis of tHcy in one series.

All urine between the end of the mid-week dialysis session and the start of the subsequent session was collected. Serum urea and creatinine concentrations were determined in samples drawn at the beginning and end of the collection period and the mean concentrations were then calculated. Residual urea and creatinine clearances were calculated according to the formula:

\[
\text{Residual clearance} = \frac{\text{urine volume (ml) \times \text{urea concentration}}}{\text{time (min) \times \text{mean serum concentration}}}
\]

Serum concentration was analysed in samples drawn before and after the mid-week dialysis and before the subsequent dialysis. Single-pool \(K_t/V\) was then estimated according to Daugirdas’ second-generation formula [18]:

\[
K_t/V = \frac{-\ln(R - 0.008 \times R) + (4 - 3.5 \times R) \times \text{UF/W}}{V}
\]

Total \(K_t/V\) was calculated with the formula [19]:

\[
\text{Total } K_t/V = K_t/V + 5.5 \times \text{residual urea clearance}/V.
\]

Normalized protein catabolic rate (nPCR) was calculated from the predialytic serum concentration and \(K_t/V\) [20].

In six patients, samples for analysis of tHcy, as well as serum concentrations of folate, cobalamin and creatinine, were collected before a mid-week dialysis, directly after and at 0.5, 2, 8, 20, and 44 h after the end of the dialysis session. The patients recruited for this procedure were characterized by small interdialytic fluid accumulation (<2 l). In five patients, samples for analysis of tHcy were collected before and after a mid-week dialysis. During this dialysis session, samples of dialysate were collected every h for <30 min for analysis of tHcy concentration. Taking the profile of dialysate tHcy concentration, flow of dialysate and treatment time into consideration the total amount of tHcy recovered in the dialysate was calculated.

Analyses

tHcy was measured by high performance liquid chromato- graphy (HPLC) after the reduction of disulphide-bonds by dithiothreitol [21]. For analysis of Hcy in dialysate, the method was modified as follows: 400 μl dialysate, 30 μl 0.1 M dithiothreitol and 70 μl 0.3 M Trizma® buffer (pH 8.5) were mixed and incubated for 15 min at 37°C. Thereafter, 100 μl 10% sulphosalicylic acid was added and the mixture was kept at 4°C for 30 min and then centrifuged at 4000 \(\times\) g, 4°C, for 10 min. The supernatant (80 μl) was analysed. The detection limit for Hcy in dialysate was 0.05 μmol/l (defined as a signal-to-noise ratio > 5). To test the influence of matrix differences in dialysate and the calibration sample, Hcy was added to dialysate. The recoveries were between 96 and 102%. Interassay precision was 8% at 1.0 μmol/l.

Folate concentrations, in blood, as well as in serum, and serum cobalamin concentrations were analysed by radioassays using purified intrinsic factor and purified folate binding protein (vitamin B₁₂ folate dual RIA kit, Amersham International, Amersham, UK). The reference interval for blood folate concentrations was 125–500 nmol/l and for cobalamin concentrations was 110–650 pmol/l.

Serum urea and creatinine concentrations were measured by enzymatic methods and serum albumin concentrations by using the Bromocresol Green method.

Statistical methods

Data are presented as means ± SD except in the small subgroups where they are presented as medians (ranges). Nonparametrical statistical methods were used because of the skewed distribution of tHcy. The Mann–Whitney U-test was applied to study differences between groups and the Wilcoxon’s rank test for the paired case for differences within groups. Relationships between variables were tested by the Spearman rank correlation test. Multiple linear regression analysis was applied to test the independency of the relationships between tHcy and the variables of dialysis adequacy.

Before inclusion into the multiple regression analysis, tHcy was log transformed. A P-value of less than 0.05 was considered to reflect statistical significance.

Results

Blood folate

Fifty-six patients had blood folate concentrations higher than 1500 nmol/l, which in our clinical experience is compatible with a regular intake of 5 mg of folic acid daily. Fourteen patients had much lower folate concentrations (430 ± 161 nmol/l) indicating noncompliance regarding the intake of folic acid. The compliant group had significantly lower tHcy than the noncompliant one (21.8 ± 14.4 vs 25.5 ± 8.9 μmol/l, \(P < 0.05\)).

Results in folate-loaded patients

In order to eliminate the confounding effect of folate concentrations only the folate-loaded patients were included in further studies (40 males and 16 females, mean age 66 ± 15 years). Forty-two patients (75%) were hyperhomocysteinemic [as defined by tHcy higher than the 95th percentile (16 μmol/l) of a healthy control group consisting of 40 males and 20 females, aged 65 ± 13 years, with concentrations of serum creatinine, serum albumin and blood folate within the normal ranges].

Mean serum concentrations of albumin and cobalamines were 445 ± 368 pmol/l and 35 ± 6 g/l, respectively. Sixteen patients had residual renal function, i.e. creatinine clearances ranging from 0.3 to 6.3 ml/min whereas 35 patients were anuric (renal function was not known in five patients). There was no significant difference in tHcy between those who were anuric and those who had residual renal function (22.5 ± 17.6 vs 19.4 ± 6.2 μmol/l). \(K_t/V\) of treatment was 1.35 ± 0.32,
total Kt/V was 1.41 ± 0.32 and nPCR was 1.00 ± 0.19 g/kg/day.

There were significant correlations between tHcy and serum concentrations of albumin (r = 0.28, P < 0.05) and cobalamines (r = −0.27, P < 0.05), Kt/V of treatment (r = 0.32, P < 0.05), total Kt/V (r = 0.29, P < 0.05) and nPCR (r = 0.30, P < 0.05). A multiple linear regression analysis showed that these variables explained 34% of tHcy (n = 40). Serum albumin concentration was the only variable that independently predicted Hcy (r = 0.34, P < 0.05) whereas serum cobalamin concentration tended to (r = −0.28, P = 0.086). tHcy correlated neither with age nor residual renal function.

tHcy before and after dialysis

In 11 representative patients, tHcy was 24.0 ± 9.3 μmol/l before and 17.3 ± 6.3 μmol/l after a haemodialysis session. Thus, dialysis induced a significant reduction in tHcy of 28 ± 14% (P < 0.05). There was a direct correlation between the tHcy before and after dialysis (r = 0.83, P < 0.05) but not between tHcy and the reductions in tHcy induced by dialysis.

The median values of interdialytic tHcy and serum creatinine concentrations, obtained in six patients, are shown in Table 1. After dialysis, the tHcy curve was flat for at least 8 h, in contrast to the creatinine production which is around 20 000 μmol/day [24]. Only about 10% of the reductions in tHcy induced by dialysis.

Amount of Hcy in dialysate

The amount of Hcy in dialysate was 63 μmol (12–158 μmol). The individual values of the amount of Hcy in dialysate, the predialytic tHcy and the dialysis-induced decrease in tHcy in five patients are given in Table 2.

Discussion

tHcy correlated directly with Kt/V, nPCR and serum albumin concentrations suggesting that the improved nutrition associated with adequate dialysis is of greater importance for tHcy than the dialysis-induced removal of Hcy. Indeed, a relatively small amount of Hcy was recovered in dialysate. However, in contrast to the immediate postdialytic rise of serum creatinine concentrations, the tHcy curve was flat for at least 8 h after dialysis possibly due to the elimination of inhibitors of enzymes catalysing Hcy removal.

Adenosylmethionine, the principal methyl donor in mammals, is the immediate precursor of Hcy. After a methyl transfer reaction, adenosyl-Hcy is hydrolysed to Hcy and adenosine. The liver is probably the main site of this process [22]. In healthy individuals, Hcy production is around 20 000 μmol/day [23] of which roughly 1200 μmol/day is exported to the plasma [24]. Only about 10 μmol/day of Hcy are excreted unchanged in urine [15] indicating cellular reuptake and metabolism as the main plasma removal mechanism. The site of elimination of Hcy is uncertain but the necessary enzymes are found mainly in hepatocytes and renal proximal tubular cells [25,26]. Hcy is either irreversibly broken down via the transulfuration pathway or remethylated back to methionine. Many enzymes involved in these processes and the water-soluble vitamins B<sub>6</sub>, B<sub>12</sub> and folic acid are precursors of necessary cofactors or substrates for these enzymes.

tHcy correlates inversely with glomerular filtration rate [27] and in haemodialysis patients, tHcy is roughly threefold that of the general population [11]. The elimination of Hcy is slow in renal failure [28] but the exact mechanism behind the uraemic hyperhomocysteinaemia is not known. The inhibition of enzyme(s) of Hcy removal by uraemic toxins is a potential explanation [11,15].

In the present study, all the participants had a blood folate concentration threefold higher than the upper reference limit and a serum cobalamin concentration within the normal range. They were also supplemented with pyridoxine. We assumed that the study subjects were optimally or almost optimally supplemented with vitamins given that, in dialysis patients, the folate concentration seems to be the most important determinant of tHcy [29] and there is no data to support the independent effect of treatment with either vitamin B<sub>6</sub> or B<sub>12</sub>. In this group of folate-loaded patients, we found that tHcy correlated directly with Kt/V of treatment, total Kt/V and nPCR, as well as serum...
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albumin and cobalamine concentrations. However, a multiple linear regression analysis revealed that only serum albumin concentration independently predicted tHcy. These data indicate the improved nutrition associated with a larger dose of dialysis resulting in a higher serum albumin concentration which, in turn, leads to increased tHcy probably due to the extensive protein binding of Hcy. In contrast, Bostom et al. [29] found no relationship between urea reduction ratios and tHcy in haemodialysis patients with widely ranging folate concentrations.

On a weekly basis, the amount of Hcy that was recovered in dialysate was about threefold higher than the amount that healthy individuals excrete in their urine [15]. Still, the dialysed amount of Hcy only constituted a few per cent of the approximately 1200 mmoles of Hcy that are removed from the circulation of a healthy individual every day [24]. Even though the findings of studies on healthy individuals cannot be directly extrapolated to the uraemic situation, the present results indicate that the dialysed amount does not contribute substantially to the removal of Hcy. The dialysed amount also suggests that there is an insignificant increase in Hcy export from cells during dialysis (Table 2).

There were clear differences between the interdialytic curves of tHcy and serum creatinine concentrations (Table 1). The dialysis-induced relative reduction of tHcy was smaller than that of creatinine, which is in accordance with the protein binding of Hcy. Interestingly, tHcy did not rise for at least 8 h after dialysis in contrast to the serum creatinine concentration. Both substances would have been expected to show similar postdialytic behaviour since their production is linked by a methylation reaction [23] and their concentrations generally show a direct correlation [5]. Conceivably, the elimination of Hcy from plasma is facilitated after haemodialysis, e.g. such treatment may to some extent remove uraemic toxins with inhibitory activities against one or more of the enzymes of remethylation and/or the transsulphuration pathway. Under such circumstances the uptake and elimination of Hcy might keep pace with the export to the plasma until the inhibitors have accumulated again.

The present study described the relationship between tHcy and parameters of dialysis adequacy and provided an interdialytic tHcy curve as well as a measure of the Hcy lost in dialysate. The implication of the results is that an increase in tHcy will follow the improvement of the adequacy of standard dialysis. Since adequacy of dialysis is well known to be positively associated with outcome [30] it is not acceptable to reduce Kt/V for tHcy-lowering purposes. Thus, the results do not seem to open up possibilities for tHcy-lowering modifications of standard haemodialysis. However, the shape of the interdialytic tHcy curve is compatible with the hypothesis that relevant enzyme-inhibitory activities circulate in uraemic patients, and in that case, convective treatments such as haemofiltration and haemodiafiltration may be more effective tHcy-lowering treatments than standard dialysis. Awaiting such data, the most effective tHcy-lowering approach is probably to ascertain that the patients are adhering to their prescribed medication. In the present study, we actually found that 20% of the patients were not compliant regarding the intake of folic acid as judged by blood folate concentrations and supported by their significantly higher tHcy as compared with the other patients.

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Received for publication: 28.5.98
Accepted in revised form: 18.9.98