Preliminary Communication

Efficacy of methylcobalamin on lowering total homocysteine plasma concentrations in haemodialysis patients receiving high-dose folic acid supplementation

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Abstract

Background. Hyperhomocysteinaemia, which is considered to be induced by impairment of the remethylation pathway in patients with chronic renal failure (CRF), cannot be cured solely by folic acid therapy. In the present study, we investigated the additional benefit of administration of methylcobalamin, which is a co-enzyme in the remethylation pathway, on lowering total homocysteine (tHcy) plasma concentrations in haemodialysis (HD) patients receiving high-dose folic acid supplementation.

Methods. In order to assess the efficacy on lowering plasma tHcy levels (fasting concentration), 21 HD patients, were randomly assigned and provided folic acid supplementation: 15 mg/day orally (group I, n = 7); methylcobalamin 500 mg intravenously after each HD, in addition to folic acid (group II, n = 7); or vitamin B6 (B6), 60 mg/day orally, in addition to folic acid and methylcobalamin (group III, n = 7). All patients were treated for 3 weeks. A methionine-loading test was conducted before and after supplementation. The following measurements were also made before and after supplementation for each group: serum folic acid, B6, and vitamin B12 (B12) concentrations (including measurement of proportion of methylcobalamin fraction). Twelve HD patients receiving methylcobalamin alone served as the HD control group and seven healthy volunteers served as the normal control group for this study.

Results. In our randomized HD patients the proportions of methylcobalamin fraction (48.3 ± 7.5%) and plasma vitamin B6 concentration (2.9 ± 1.1 ng/ml) were significantly lower than in the normal controls (methylcobalamin 58.7 ± 2.2%, P < 0.01; B6 20.1 ± 10.8 ng/ml, P < 0.01), while folic acid and vitamin B12 were not significantly different from the normal controls. Mean percentage reduction in fasting tHcy was 17.3 ± 8.4% in group I, 57.4 ± 13.3% in group II, 59.9 ± 5.6% in group III, and 18.7 ± 7.5% in HD controls. The power of the test to detect a reduction of tHcy level was 99.6% in group II and 99.9% in group III when type I error level was set at 0.05. Groups II and III had normal results for the methionine-loading test after treatment. Treatment resulted in normalization of fasting tHcy levels (<12 ng/ml) in all 14 patients treated by the combined administration of methylcobalamin and supplementation of folic acid regardless of whether there was supplementation of vitamin B6.

Conclusion. The benefit of methylcobalamin administration on lowering plasma tHcy levels in HD patients was remarkable. Our study suggested that both supplementations of high-dose folic acid and methylcobalamin are required for the remethylation pathway to regain its normal activity. This method could be a therapeutic strategy to combat the risk associated with atherosclerosis and cardiovascular disease in patients with chronic renal failure.

Keywords: chronic renal failure; folic acid; haemodialysis; homocysteine; methylcobalamin; vitamin B12

Introduction

Hyperhomocysteinaemia was recently recognized as a risk factor for the development of atherosclerotic vascular diseases [1]. In patients with chronic renal disease, plasma total homocysteine (tHcy) levels are elevated, in an inverse relationship with the reduction in renal function [2]. Most reports showed that at least 80% of dialysis patients have markedly increased levels of tHcy [2].

Homocysteine is formed as an intermediate metabolic product of methionine at the junction
of two metabolic pathways: remethylation and trans-sulfuration [3]. Homocysteine can either be remethylated to methionine or be trans-sulfurated to cysteine. In remethylation, homocysteine receives a methyl group from 5-methyltetrahydrofolate or from betaine. Vitamin B_{12} is a necessary cofactor in the folate-dependant remethylation. Trans-sulfuration requires vitamin B_{6} as the cofactor. Impairment of remethylation is strongly implicated as the cause of hyperhomocysteinaemia in uraemic patients [4].

Folic acid is vital in humans for several metabolic reactions, including the remethylation pathway. However, clinical studies have shown that hyperhomocysteinaemia in uraemic patients cannot be cured solely by folic acid therapy [5]. Vitamin B_{12} (cyanocobalamin) supplementation alone and a combined supplementation of vitamin B_{12} (cyanocobalamin) with folic acid were reported to be effective in reducing homocysteine levels, but full normalization of hyperhomocysteinaemia was not achieved [6–8]. Hence, other strategies are needed to combat these risks associated with atherosclerosis and cardiovascular disease in patients with chronic renal failure (CRF).

We reported previously a decreased proportion of methylcobalamin fraction in the total serum vitamin B_{12} concentration in patients with CRF [9]. Methylcobalamin is the co-enzymatic form of the vitamin B_{12} analogues, which is required in the remethylation pathway. Therefore, we were encouraged to investigate the potential involvement of methylcobalamin in hyperhomocysteinaemia in patients with CRF. In this study, we specifically investigated the additional benefit of administration of methylcobalamin on lowering the tHcy plasma levels in haemodialysis (HD) patients with supplementation of folic acid. We also implemented a methionine-loading test in order to assess a whole body homocysteine handling.

Subjects and methods

Participants were CRF patients who started HD therapy at the Kidney Center in Nagoya City University Hospital (n = 21). Exclusion criteria were as follows: (i) presence of anaemia, haematocrit <25%, (ii) known history of diabetes mellitus, (iii) patients with homocystinuria, (iv) patients with liver dysfunction, (v) smokers, (vi) serious systemic disease, and (vii) specific indication for or contraindication to a study drug or study procedure. (Any additional vitamin other than vitamin D3 was not given during the study period.) The majority of the participants were on regular therapy with recombinant human erythropoietin and iron. Enrolment of the study participants was from October 1999 until May 2000 and from January 2001 until April 2001. Participants were randomly assigned to receive supplementation of 15 mg/day of folic acid orally (group I, n = 7); 500 μg of methylcobalamin (Methycobal, Eisai Co., Ltd, Tokyo) intravenously after each HD plus 15 mg/day of folic acid orally (group II, n = 7); or, 60 mg/day of vitamin B_{6} plus 15 mg/day of folic acid orally and 50 g methylcobalamin intravenously after each HD (group III, n = 7). All patients were treated for 3 weeks.

Twelve HD patients served as volunteers (HD control group) to receive methylcobalamin treatment without supplementation of folic acid. Exclusion criteria were the same as those for our randomized study. These patients were given 500 mg methylcobalamin intravenously after HD for 3 weeks.

All patients were dialysed three times a week for a total of 12 h weekly, using bicarbonate-based dialysate and polysulfone dialysers. We maintained K_{e} / V above 1.2 throughout the study period. Seven healthy volunteers (four men, three women) also served as the normal control group. Both the HD control group and the normal control group were forbidden to take any kind of vitamin supplement. The study protocol was approved by the institutional review board of Nagoya City University Medical School, and written informed consent was obtained from each patient and volunteer.

Measurement of total serum concentrations of folic acid, vitamin B_{6}, vitamin B_{12}, and proportion of methylcobalamin fraction of serum total vitamin B_{12}.

Serum folic acid, vitamin B_{6}, and vitamin B_{12} concentrations were measured before and after supplementation in all the subjects. Blood was sampled in the early morning at fasting condition; in the HD patients, it was drawn on a day when HD was scheduled. The proportion of methylcobalamin fraction of serum total vitamin B_{12} concentrations was measured before and after supplementation in the patients who participated in our randomized study. Determination of serum vitamin B_{12} concentration was performed by a high-performance liquid chromatography equipped with a fluorescence detector with normal range of 4.0–19.0 ng/ml. Serum concentrations of vitamin B_{12} were measured by competitive assay, while those of folic acid were by competitive immunoassay using the automated chemiluminescence systems. The normal ranges for serum concentration were 2.4–9.8 ng/ml for folic acid and 233–914 pg/ml for vitamin B_{12}.

To obtain the methylcobalamin fraction, venous blood was drawn into foil-wrapped syringes before HD, and serum was separated in a dark room under red photographic light to avoid the photolysis of vitamin B_{12} analogues including cyanocobalamin, hydroxycobalamin, deoxyadenosylcobalamin, and methycobalamin. The methylcobalamin fraction, separated using high-performance liquid chromatography, was determined by bioautographic analysis of the chromatogram using Lactobacillus leichmannii (ATCC10586) as the test organism [10,11].

Measurement of plasma tHcy concentration at fasting and methionine-loading test

The measurement of plasma tHcy concentration was conducted by a rapid, isocratic high-performance liquid chromatography assay [12]. The normal range for plasma tHcy level at fasting was 3.0–14.0 nmol/ml.

The measurement of plasma tHcy concentration at fasting and the methionine-loading test were conducted before and after supplementation in all the patients who participated in our randomized study and in 12 HD controls. All of the patients and control subjects received 0.05 g of methionine per kg of body weight after fasting for 12 h. The oral methionine challenge (100 mg/kg) is useful for diagnosis of cystathionine-beta-synthase deficiency.
or MTHFR reductase deficiency [13]. Because HD patients have shown an exaggerated increase in plasma tHcy level after the methionine loading [14], we considered that a half dose of methionine (50 mg/kg) loading was sufficient to assess the metabolic pathway of homocysteine in HD patients as reported by Hirose et al. [15]. In HD patients, methionine was loaded on a day when HD was not performed. As methionine has a slightly unpleasant smell, we administered it orally with a sugar-based non-protein containing flavour. The plasma tHcy concentrations were measured prior to and 2 and 4 h after methionine administration.

**Statistical analysis**

All numeric data, including the primary end points of plasma tHcy concentration were expressed as the mean ± SD, and the level P < 0.05 was considered to be statistically significant. Mean values with 95% confidence intervals (CI) are also expressed for the primary end points of post-treatment plasma tHcy levels. Secondary end points were serum concentrations of vitamins: folic acid, vitamin B₆, vitamin B₁₂, and the proportion of methylcobalamin fraction of serum total vitamin B₁₂.

For the baseline values of plasma tHcy concentrations at fasting, serum concentrations of folate, vitamin B₁₂ and vitamin B₆, age, HD duration, sex, haematocrit, serum urea nitrogen, serum creatinine, β₂-microglobulin, and albumin concentrations, a one-way ANOVA of the grouping variable was performed to exclude potential differences between HD patients groups including the three randomized HD groups and HD controls. The proportion of methylcobalamin fraction in total serum vitamin B₁₂ concentration was analysed by a one-way ANOVA between the randomized HD groups. Differences in gender were analysed by χ²-test. Treatment effects on percentage changes in fasting plasma tHcy levels were presented as [(average pretreatment level–average post-treatment level)/average pretreatment level] × 100. The difference in the change in fasting plasma tHcy levels between the treatment groups was evaluated by a two-way repeated-measures ANOVA (type I error level of statistical analysis was set at α = 0.05).

Effects of vitamin supplementation on serum vitamin concentrations in each group were analysed by the Student’s t-test. The results from the methionine-loading test were analysed by a two-way repeated-measures ANOVA to assess the effect of each vitamin supplementation regimen (folic acid alone, folic acid with methylcobalamin, folic acid with methylcobalamin and vitamin B₆, methylcobalamin alone) on the metabolic pathway of homocysteine. This analysis included the grouping variable, time course variable (prior, 2 and 4 h after methionine loading), and the interaction ‘supplementation effect × time course’ as co-variables.

Baseline values of plasma tHcy concentrations at fasting, serum concentrations of folic acid, serum concentrations of vitamin B₆, vitamin B₁₂, and the proportion of methylcobalamin fraction of serum total vitamin B₁₂ concentration in each randomized HD group were compared with those values of the normal control group by the Student’s t-test. Post-treatment values of fasting plasma tHcy in each HD group were compared with fasting plasma tHcy values of the normal control group by the Student’s t-test.

**Results**

All of the 21 randomized participants and 12 HD volunteers underwent baseline testing. No adverse events were reported during the treatment period. There were no clinical abnormalities following the methionine loading in any of the study participants. Additional details on participant recruitment and retention are provided in Figure 1.

**Demographic and clinical characteristics**

The demographic and clinical characteristics of each group are shown in Table 1. ANOVA revealed that there were no significant differences in baseline values of plasma tHcy levels and serum concentrations of folic acid, vitamin B₆, vitamin B₁₂, and the proportion of methylcobalamin fraction in total serum vitamin B₁₂ concentration among the three randomized HD groups. There were also no significant differences with respect to age, HD duration, sex, haematocrit, serum urea nitrogen, serum creatinine, β₂-microglobulin, and albumin concentrations between the three randomized HD groups.

ANOVA also revealed that there were no significant differences in baseline values of fasting tHcy plasma levels, serum concentration of folic acid, vitamin B₆, and vitamin B₁₂, age, sex, haematocrit, serum concentration of urea nitrogen, creatinine, β₂-microglobulin, and albumin among the randomized HD patients and HD controls. The mean fasting plasma tHcy concentration (nmol/ml) in the HD patient groups overall (n = 33) was 22.3 ± 6.9, being significantly higher than in the normal control group (8.3 ± 1.9, n = 7, P < 0.01). Proportions of methylcobalamin fraction in the randomized HD patients (n = 21) (48.1 ± 6.4%) and serum vitamin B₆ concentration in the HD patient groups overall (n = 33) (2.9 ± 0.9 ng/ml) were significantly lower than in the normal control group (methylcobalamin 59.4 ± 2.1%, P < 0.01; vitamin B₆ 21.9 ± 11/6 ng/ml, P < 0.01), while folic acid and vitamin B₁₂ in the HD patient groups overall (n = 33) were not significantly different from the normal controls.

**Effects of vitamin supplementation on serum vitamin concentrations in each group**

Table 2 shows effects of vitamin supplementation on serum vitamin concentrations (folic acid, vitamin B₆, and vitamin B₁₂) in each group. Vitamin supplementation effectively increased serum concentrations of folic acid and vitamin B₁₂ in each group of patients with supplements. Vitamin B₆ increased in group III only. Supplementation of methylcobalamin resulted in the remarkable increase of serum total vitamin B₁₂ concentration, while the proportion of methylcobalamin fraction was not significantly changed in any group.
Efficacy of vitamin supplementation on reducing plasma tHcy levels and findings of methionine-loading test (Tables 3–5)

Mean percentage reduction (per cent reduction) in plasma tHcy level were 17.3 ± 8.4% in group I, 57.4 ± 13.3% in group II, 59.9 ± 5.6% in group III, and 18.7 ± 7.5% in HD controls (Table 3). The reductions of plasma tHcy levels in groups II and III are both significantly remarkable (P < 0.01). The power of the test to detect a reduction of plasma tHcy levels is 99.6% in group II and 99.9% in group III when type I error level of statistical analysis was set at α = 0.05. Post-treatment plasma tHcy levels (± 95% CI) were 15.8 ± 2.3 ng/ml (13.6–18.0) in group I, 8.3 ± 1.4 ng/ml (6.5–10.0) in group II, 8.2 ± 1.9 ng/ml (7.0–9.6) in group III, and 21.0 ± 8.1 ng/ml (15.8–26.2) in HD control group. Group I and the HD control group showed significantly higher post-treatment fasting plasma tHcy levels compared with baseline.
Table 2. Effects of vitamin supplementation on serum vitamin concentrations in each group

<table>
<thead>
<tr>
<th>Vitamin B6 (ng/ml)</th>
<th>After supplementation (baseline)</th>
<th>P-values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 15.9 ± 2.5</td>
<td>9.3 ± 3.2 (8.7 ± 2.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Group II 15.0 ± 2.0</td>
<td>3.4 ± 1.6 (3.3 ± 1.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Group III 15.6 ± 2.6</td>
<td>26.2 ± 14.6 (2.9 ± 1.0)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin B12 (ng/ml)</th>
<th>After supplementation (baseline)</th>
<th>P-values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 3.3 ± 0.6 (2.7 ± 0.5)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Group II 3.4 ± 1.6 (3.3 ± 1.0)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Group III 26.2 ± 14.6 (2.9 ± 1.0)</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>HD control 3.2 ± 1.2 (2.9 ± 1.1)</td>
<td>ns</td>
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</tbody>
</table>

*Compared with baseline by t-test. %m-B12, proportion of methylcobalamin fraction.

Table 3. Treatment effect on reducing tHcy level in HD patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean reduction of tHcy by treatment (ng/ml)</th>
<th>Per cent (%) reduction in fasting tHcy by treatment</th>
<th>Power*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.4 ± 1.8</td>
<td>17.3 ± 8.4</td>
<td>98.3%</td>
</tr>
<tr>
<td>Group II</td>
<td>12.6 ± 6.0</td>
<td>57.5 ± 3.3</td>
<td>99.6%</td>
</tr>
<tr>
<td>Group III</td>
<td>13.1 ± 5.5</td>
<td>59.9 ± 5.6</td>
<td>99.9%</td>
</tr>
<tr>
<td>HD control (n=12)</td>
<td>4.5 ± 1.8</td>
<td>18.7 ± 7.5</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

*The power of the test to detect a reduction of tHcy level when type 1 error level of statistical analysis was set at α = 0.05.

Table 4. Post-treatment tHcy level and n/total (%) subjects with after tHcy levels <12 ng/ml

<table>
<thead>
<tr>
<th>Group</th>
<th>After treatment fasting tHcy level (ng/ml) (95% CI)</th>
<th>n/total (%) subjects with after treatment tHcy levels &lt;12 ng/ml</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>15.8 ± 2.3 (13.6–18.0)</td>
<td>0/7 (0%)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>8.3 ± 4.6 (6.5–10.0)</td>
<td>7/7 (100%)</td>
<td>ns</td>
</tr>
<tr>
<td>Group III</td>
<td>8.2 ± 9.0 (7.0–9.6)</td>
<td>7/7 (100%)</td>
<td>ns</td>
</tr>
<tr>
<td>HD control</td>
<td>21.0 ± 8.1 (15.8–26.2)</td>
<td>1/12 (12%)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

*Compared with baseline of normal control (8.3 ± 9 ng/ml) by t-test. CI, confidence interval.

Discussion

From this study we should make special note of the fact that, in HD patients, the hyperhomocysteinaemia, which has proven quite refractory to pharmacological doses of folic acid supplementation [16,17], is cured by co-administration of methylcobalamin and high-dose folic acid supplementation. This method of therapy for hyperhomocysteinaemia could combat the risk associated with atherosclerosis and cardiovascular disease in patients with CRF.

Folate is vital in humans for several metabolic reactions involved in the formation and transfer of one-carbon units, such as formyl, methylene, or methyl (–CH3). In the remethylation pathways a methyl group is transferred from 5-methyl-tetrahydrofolate, a folate-related derivative, to produce methionine. In our study, the fasting plasma tHcy concentration was reduced 17 ± 8.4% by supplementation with high-dose folic acid. Our results were similar to those of Bostom’s study and support the Vienna Multicenter Study, which clearly demonstrated that hyperhomocysteinaemia in end-stage renal disease patients cannot be cured solely by folic acid supplementation [18].

Supplementation of L-5-methyltetrahydrofolate did not prove to be any more beneficial than folic acid in treating hyperhomocysteinaemia [5].

Administration of methylcobalamin, which is co-enzyme in the methionine remethylation pathway, was anticipated to be another strategy to cure hyperhomocysteinaemia. Our study has indicated that administering methylcobalamin alone to HD patients was not sufficient to normalize hyperhomocysteinaemia in CRF. That result supported our consideration that methylcobalamin would require a sufficient amount of intracellular of L-5-methyltetrahydrofolate in remethylation. Vital processes in folate disposition, however, also include intestinal absorption and receptor and carrier-mediated transport across cell membranes. And, it is known that the 677C→T transition of methylene tetrahydrofolate reductase is the cause of hyperhomocysteinaemia. Arnadottir et al. reported
that, at a folic acid dose of 15 mg/week, red blood cells approached folate saturation and the maximum effect on tHcy seemed to be obtained at that dose in HD patients [19]. Bostom et al. indicated that, in comparison to high-dose folic acid (15 mg/day), high-dose oral 5-methyltetrahydrofolate-based supplementation (17 mg/day) did not afford improved tHcy-lowing efficacy among HD patients [5]. Plassmann demonstrated that supplementation of 15 mg/day folic acid resulted in the maximum effect on lowering tHcy regardless of type of MTHFR genotype (677CC, 677CT, or 677TT) [18]. These studies indicated that the body cells could be saturated with 5-methyltetrahydrofolate by supplementation with 15 mg/day folic acid regardless of the type of MTHFR genotype.

Homocysteine loading is considered to be the most effective method to assess the quality of the remethylation pathway [20]. Taking into account that homocysteine is a candidate uraemic toxin that affects cardiovascular risk, we inferred that homocysteine loading may not be suitable for the patients in this study. HD patients have shown an exaggerated increase in plasma tHcy level after methionine loading, and because their trans-sulfuration pathway activity was reported as not significantly decreased [4], the methionine loading was, therefore, considered to be more appropriate to evaluate the quality of the remethylation pathway in uraemic patients. We elected to load half of this diagnostic dose of methionine (50 mg.kg), which was a sufficient loading dose to achieve our objective for this study.

Our study suggested that both supplements of high-dose folic acid and methylcobalamin are required for the remethylation pathway to regain its normal activity. Based upon these results, we deduced that deterioration of the remethylation pathway is related not only to an inhibition of folate enzymes but also to a deficiency of methylcobalamin in uraemic patients.

Vitamin B12 has several analogues: cyanocobalamin, hydroxycobalamin, deoxyadenosylcobalamin, and methylcobalamin. Each fraction can be estimated by measuring the proportion of that fraction of the serum total vitamin B12 concentration. The prevalence of these analogues, and their metabolism, has not been elucidated clearly. Wilson et al. reported that an increase in the proportion of the cyanocobalamin fraction indicates accelerated cyanide (CN) detoxication via cyanocobalamin synthesis [21]. We reported previously that the ability to detoxify CN is impaired by reduced renal function [9]. In that study, we indicated that vitamin B12 is utilized to detoxify CN, resulting in an increase in the proportion of cyanocobalamin and a decrease in the proportion of methylcobalamin. Based on our results, we can deduce that deficiency of methylcobalamin could be induced by deterioration of renal function.

The accumulation of homocysteine in CRF patients causes the hydrolysis of S-adenosylhomocysteine (AdoHcy) to slow down, resulting in accumulation of S-adenosylhomocysteine (AdoMet) and decreased AdoMet:AdoHcy ratios [22]. The concentration of AdoHcy, and even more importantly the ratio of AdoMet:AdoHcy, exert their potent inhibitory effect on the transmethylation reaction [23]. Because methylcobalamin is synthesized through a transmethylation reaction [24], the proportion of the methylcobalamin fraction, therefore, decreases with the accumulation of homocysteine. Through these mechanisms, the hyperhomocysteinaemia and the deficiency of methylcobalamin become a vicious cycle.

Cyanocobalamin has to be changed and transmethylated into methylcobalamin to act as a co-enzyme. This methylcobalamin synthesis is inhibited by accumulation of AdoMet, hence we deduce that methylcobalamin is more potent than cyanocobalamin for reducing plasma tHcy concentrations in uraemic patients.

In our study, the proportion of methylcobalamin fraction was not changed, while serum total vitamin B12 concentration increased remarkably after i.v. methylcobalamin administration. This result indicates that administered methycobalamin supplied its methyl group and was changed into other analogues of vitamin B12. It can be inferred that a decrease in plasma tHcy concentrations would accelerate transmethylation reactions.

Table 5. Plasma tHcy levels after methionine loading test

<table>
<thead>
<tr>
<th>Vitamin supplementation</th>
<th>Before supplementation time course</th>
<th>After supplementation time course</th>
<th>P-value by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting (0 h)  2 h  4 h</td>
<td>Fasting (0 h)  2 h  4 h</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>19.2 ± 2.9  23.8 ± 3.9  28.2 ± 4.4</td>
<td>15.8 ± 2.3  20.0 ± 2.9  23.9 ± 3.6</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>20.9 ± 5.7  26.0 ± 6.3  30.4 ± 7.5</td>
<td>8.3 ± 1.4   11.6 ± 1.7  14.0 ± 2.4</td>
<td>P &lt; 0.01  P &lt; 0.01  P &lt; 0.01</td>
</tr>
<tr>
<td>Group III</td>
<td>21.3 ± 7.3  26.4 ± 8.4  31.1 ± 9.4</td>
<td>8.2 ± 1.9   11.1 ± 2.6  13.6 ± 3.1</td>
<td>P &lt; 0.01  P &lt; 0.01  P &lt; 0.01</td>
</tr>
<tr>
<td>Normal control*</td>
<td>8.6 ± 1.7   12.3 ± 2.3  14.0 ± 2.5</td>
<td>2.9 ± 2.3   23.8 ± 2.5  23.9 ± 2.6</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>HD control</td>
<td>25.6 ± 8.4  30.9 ± 9.4  36.2 ± 10.6</td>
<td>21.0 ± 8.1  25.8 ± 9.2  30.7 ± 10.5</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

*P < 0.01 vs HD groups before supplementation. Homocysteine elevations after methionine loading in groups II and III were normalized by vitamin supplementation. No significant difference among HD groups (groups I–III, HD control) before supplementation.
Statistically, the effect of methylcobalamin administration along with folic acid supplementation in lowering plasma tHcy concentrations was considered to be remarkable. However, in our study the patient number in each group was very low. Therefore, our results should be confirmed by a large study, including an adequate number of patients.

In conclusion, plasma tHcy concentrations are normalized by combined administration of methylcobalamin and supplementation of high-dose folic acid in HD patients. Our study suggests that both supplementation of high-dose folic acid and methylcobalamin are required for the remethylation pathway to regain its normal activity. This treatment for hyperhomocysteinemia could be a therapeutic strategy to combat the risk associated with atherosclerosis and cardiovascular disease in patients with CRF.

References


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