Effects of High-Dose Folic Acid and Pyridoxine on Plasma and Erythrocyte Sulfur Amino Acids in Hemodialysis Patients

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Abstract. In this investigation, sulfur amino acids (sAA) and sulphydryls were determined in the plasma and erythrocytes (RBC) of 10 uremic patients on regular hemodialysis (HD) treatment and 10 healthy subjects, before and after supplementation with 15 mg/d of folic acid and 200 mg/d of pyridoxine for 4 wk. The basal total plasma concentrations of homocysteine (Hcy), cysteine (Cys), cysteinylglycine (Cys-Gly), γ-glutamylcysteine (γ-Glu-Cys), glutathione (GSH), and free cysteinylsulfenic acid (CSA) were significantly higher in HD patients when compared to healthy subjects, whereas methionine (Met) and taurine (Tau) concentrations were the same in the two groups. HD patients showed significantly higher RBC levels of Hcy and Cys-Gly, whereas the RBC concentrations of Met, Cys, Tau, and GSH were not different from those in the healthy subjects. The plasma concentrations of sAA and sulphydryls differed compared with RBC levels in the healthy subjects and HD patients. In both groups, supplementation with high doses of folic acid and pyridoxine reduced the plasma Hcy concentration. In addition, increased plasma concentrations of Cys-Gly and GSH were found in the HD patients and of CSA in the healthy subjects. After vitamin supplementation, the RBC concentrations of Hcy, Cys, and GSH increased and that of Tau decreased in healthy subjects. The only significant finding in RBC of HD patients was an increase in GSH levels after supplementation. This study shows several RBC and plasma sAA and sulphydryl abnormalities in HD patients, which confirms earlier findings that RBC and plasma pools play independent roles in interorgan amino acid transport and metabolism. Moreover, high-dose supplementation with folic acid and pyridoxine significantly reduced Hcy levels, but did not restore the sAA and sulphydryl abnormalities to normal levels. The increase that was observed in GSH after vitamin supplementation may have a beneficial effect in improving blood antioxidant status in uremic patients. Finally, the findings of elevated plasma Cys levels correlating to the elevated plasma Hcy levels in the presence of elevated plasma CSA levels, both before and after vitamin supplementation, led to the hypothesis that a block in decarboxylation of CSA is linked to hyperhomocysteinemia in end-stage renal failure.

Abnormalities in sulfur amino acid (sAA) metabolism are present in chronic renal failure (CRF) as part of the general alteration in amino acid metabolism in uremia. Increased plasma levels of total homocysteine (Hcy) and cysteine (Cys) in nondialyzed uremic patients and in those treated with hemodialysis (HD) or continuous ambulatory peritoneal dialysis (1–5), as well as plasma and muscle taurine (Tau) depletion in dialysis patients, have been reported previously (6–8). The causes of these alterations and their clinical implications have not yet been elucidated in end-stage renal disease (ESRD).

Hyperhomocysteinemia, a cardiovascular risk factor in the general population, is considered to play a role in the genesis of premature atherosclerosis in CRF patients, because increased levels of plasma Hcy and a higher incidence of cardiovascular disease have been demonstrated in these patients (3,4,9).

Tau is one of the most abundant amino acids in the human body and an end product of the metabolism of sAA with numerous described physiologic functions (10). Tau deficiency is associated with retinal dysfunction, and possibly impaired heart and skeletal muscle function, whereas cardiac disease and muscle fatigue are common in ESRD patients.

Glutathione (GSH) is a tripeptide containing a sulphydryl group formed from glutamate (Glu), cysteine, and glycine (Gly). GSH participates in the protection of cells against free radicals and toxic compounds of endogenous and exogenous origin (11). GSH deficiency has been reported in dialyzed and nondialyzed uremic patients (12–14). Although the clinical significance of GSH depletion in uremia is still unclear, decreased antioxidant capacity may contribute to anemia, erythrocyte fragility, or other pathologic conditions associated with CRF (15).

The normal metabolism of sAA (Figure 1) requires the coenzyme function of vitamin B6, B12, and folate. Pyridoxal-5’-phosphate (PLP), the active form of vitamin B6, is the cofactor for two enzymes in the trans-sulfuration pathway.
Moreover, the biosynthesis of Tau from cysteinesulfonic acid (CSA) requires PLP as a cofactor for cysteinesulfonic acid decarboxylase. Folic acid enhances the remethylation of Hcy to methionine (Met), and supplementation with folic acid lowers plasma Hcy levels (16). A strong inverse correlation between the concentration of plasma folate and plasma Hcy has been found in both healthy subjects and ESRD patients (4,17).

Erythrocytes (RBC) contain a large proportion of free amino acids in whole blood, and the intraerythrocyte pool of free amino acids is actively involved in the interorgan transport of amino acids (18,19). The determination of RBC amino acid concentrations provides important additional information to that obtained from plasma amino acids in clinical conditions such as uremia (8). Most sAA studies in CRF patients have been carried out by determining plasma concentrations, whereas relatively little attention has been paid to sAA levels in RBC. Knowledge of the sAA distribution between plasma and RBC might yield information for a better understanding of sAA transport across the RBC membrane and also of the amino acid metabolism in RBC in healthy adults and CRF patients.

The present investigation was carried out to evaluate the distribution of RBC sAA and sulfhydryls and their relation to plasma levels and to study the effect of supplementation with high doses of folic acid and pyridoxine on RBC and plasma sAA and sulfhydryl concentrations in patients on HD treatment and in healthy subjects.

Materials and Methods

Patients

Ten HD patients (six men, four women) with a mean age of 66 yr (range, 44 to 82 yr) and 10 healthy subjects (five men, five women) with a mean age of 45 yr (range, 32 to 65 yr) were investigated. Body mass index (BMI; mean ± SD), calculated with the formula BMI = body weight in kg/height in m², was within normal range for the HD patients (22 ± 3) and healthy subjects (22 ± 2). Biochemical characteristics of the HD patients and healthy subjects are given in Table 1. The HD patients were dialyzed three times weekly, using hollow-fiber dialyzers, glucose-containing dialysate with bicarbonate as the buffer, blood flow between 300 and 350 ml/min, and a dialysate flow of 500 ml/min. The protein nitrogen appearance and the dialysis index of the HD patients, expressed as Kt/V urea, were calculated based on urea kinetic modeling (20). All patients had a protein nitrogen appearance >1.0 g protein/kg body wt per d, and the mean Kt/V urea was...
1.64 ± 0.25. Routine medication included daily supplementation with water-soluble vitamins (including t-pyridoxine chloride, 10 mg daily), sodium bicarbonate, phosphate binders, and diuretics. No patient was on folic acid or B12 supplementation during the 3 mo before the study. The nature, purpose, and potential risks of the study were carefully explained to all participants before they agreed to participate. The study protocol was approved by the local ethics committee of the Karolinska Institute at Huddinge University Hospital.

Study Procedures

Venous blood was obtained from the HD patients on the morning of a dialysis day as well as from healthy subjects after an overnight fast. Thereafter, all participants were put on a regimen of oral folic acid (15 mg) and pyridoxine hydrochloride (200 mg) daily for 1 mo. During the study period, patients and healthy subjects were encouraged to maintain their usual diet, no changes were made in the dialysis prescription, and HD patients were kept on their usual medication. At the end of the study period, blood samples were again obtained under the same conditions as before the vitamin supplementation.

Analytical Methods

Blood samples were collected in cooled ethylenediaminetetra-acetic acid (EDTA) tubes and centrifuged immediately at 4000 rpm for 10 min in a refrigerated centrifuge. Plasma was quickly separated from RBC and divided into two parts. One part (1.0 ml) was deproteinized with 100 μl of 30% (vol/wt) sulfosalicylic acid. After centrifugation, the clear supernatant was frozen at −70°C until it was analyzed for free amino acids, as described previously (21). The other part of the plasma was frozen at −70°C pending analyses of sulfhydryls, Hcy, Cys, cysteinylglycine (Cys-Gly), γ-glutamylcysteine (γ-Glu-Cys), and GSH, as well as PLP and folate.

Packed RBC were taken from the bottom of the tube with a pipette, after careful removal of white blood cells and platelets, and divided into two parts. The first part (0.5 g), used for the analysis of free amino acids, was rapidly hemolysed by adding 0.5 ml of 1% (vol/wt) saponin (Sigma, St. Louis, MO), vortexed, and kept on ice for 5 min. After the addition of 150 μl of 50% (vol/wt) sulfosalicylic acid for deproteinization, the samples were mixed and allowed to stand for 15 min at 4°C, then centrifuged at 13000 rpm for 20 min. The supernatant was frozen at −70°C until analyzed. The second part of the RBC was stored at −20°C until analysis of sulfhydryls and PLP. Whole blood (0.1 ml) was mixed with 2.0 ml of 1% (vol/wt) fresh ascorbic acid solution and frozen at −70°C until determination of folate.

The plasma and RBC sulfhydryls were determined with HPLC according to the method of Ubbink et al. (22), with only a minor modification in that the HPLC analysis time was extended from 6 min to 10 min leaving a reasonable time for GSH and γ-Glu-Cys to elute. In short, the RBC, after thawing, were processed as with plasma without washing, to prevent leakage from the intracellular space. Plasma (0.3 ml) or 0.3 g of RBC were mixed with 30 μl of 10% (vol/vol) tri-n-butylphosphine in dimethylformamide, and allowed to stand for 30 min at 4°C, to reduce thiols and to decouple protein-bound thiols. The proteins were precipitated with 0.3 ml of 10% TCA containing 1 mM EDTA. Plasma samples were centrifuged for 20 min at 4000 rpm, but RBC samples were centrifuged at 10,000 rpm for 15 min. After centrifugation, 100 ml of filtered supernatant was mixed with 20 μl of 1.55 M NaOH, 250 μl of 0.125 M borate buffer, pH 9.5, containing 4 mM EDTA. The mixture was derivatized with 100 μl of ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate dissolved in 0.125 M borate buffer (1 mg/ml) (vol/wt). The mixture was then incubated at 60°C for 1 h to accomplish derivatization of the plasma and RBC thiols. The thiols standard was processed similarly.

Free amino acid concentrations were determined by HPLC, after automated precolumn derivatization with orthophthalaldehyde/3-mercaptopropionic acid, as described previously (21). Concentrations of PLP in plasma and RBC were determined with HPLC according to Kimura et al. (23). Folate and vitamin B12 concentrations were determined with the Dualcount SPNB (solid phase no boi) radioassay kit from Diagnostic Product Corp. (Los Angeles, CA).

The plasma and RBC concentrations of Hcy, Cys, Cys-Gly, γ-Glu-Cys, and GSH were measured as a total, i.e., the sum of reduced, oxidized, mixed disulfide, and protein-bound species. For concentration of amino acids in plasma, micromolar refers to 1 L of plasma, whereas for those of amino acids in RBC micromolar refers to 1 L of intracellular water in the RBC. The RBC water content was assumed to be 66% of RBC weight in all samples as described by Fligel-Link et al. (24).

Statistical Analyses

Data are given as mean ± SD. The data from the HD patients and healthy subjects in this study, before as well as after vitamin supplementation, were compared with the unpaired t test. The paired t test was used for within-group comparisons, and the findings were con-
firmed by Wilcoxon matched-paired signed rank test. Spearman rank correlation analysis was used to assess the relationship between Hcy and various variables. The basal and postsupplementation Hcy values were correlated, respectively, to the basal and postsupplementation values of the other variables. \( P < 0.05 \) was considered significant.

**Results**

**Baseline Values in Patients versus Healthy Subjects**

Table 2 shows that the patients' plasma Met and Tau concentrations did not differ from those in the control subjects. However, four of the 10 patients had plasma Tau levels below the normal range, and there was also one outlier with a value far higher than the normal range (Figure 2). On the other hand, plasma Hcy, Cys, Cys-Gly, \( \gamma \)-Glu-Cys, CSA, and GSH concentrations were significantly elevated in the patients compared with healthy subjects. Among the related non-sAA, serine (Ser), Gly, and Glu, the plasma Ser concentration was far higher than the normal range (Figure 2). On the other hand, four of the 10 patients had plasma Tau levels below the normal range, and there was also one outlier with a value far higher than the normal range (Figure 2).

Table 3 shows that after 4 wk of vitamin supplementation, there were significant increases in RBC Hcy, Cys, and GSH concentrations, whereas Tau, Ser, Gly, and Glu concentrations were significantly reduced. No significant changes in RBC Met and Cys-Gly were observed.

**Effect of Vitamin Supplementation in Healthy Subjects**

The vitamin supplementation resulted in a significant reduction of plasma Hcy and an increase in CSA and Gly concentrations. However, the plasma Met, Tau, Cys, Cys-Gly, \( \gamma \)-Glu-Cys, GSH, Ser, and Glu levels did not differ from the basal value (Table 2).

The changes in the plasma concentrations of the HD patients, after 4 wk of folic acid and vitamin B6 supplementation, are presented in Table 2. Plasma levels of Met, CSA, Cys, \( \gamma \)-Glu-Cys, and Ser showed no significant changes. The mean plasma Tau concentrations of the patients did not change significantly after treatment, but Tau levels of four patients who had basal values below the normal range were normalized after treatment (from 29, 31, 33, 35 to 42, 52, 55, and 45 \( \mu \)mol/L, respectively). On the other hand, plasma Hcy concentrations decreased significantly by 28%, but were not normal-

**Table 2. Plasma-free amino acid, sulfhydryl, and vitamin concentrations in hemodialysis patients and healthy subjects before and after vitamin supplementation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Subjects</th>
<th>HD Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (n = 10)</td>
<td>After (n = 10)</td>
</tr>
<tr>
<td>Methionine</td>
<td>29 ± 4</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>11 ± 2</td>
<td>8 ± 2 ( b )</td>
</tr>
<tr>
<td>Cysteine</td>
<td>263 ± 36</td>
<td>277 ± 67</td>
</tr>
<tr>
<td>Taurine</td>
<td>48 ± 5</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Cysteinesulfonic acid</td>
<td>1.1 ± 0.38</td>
<td>1.8 ± 0.6 ( e )</td>
</tr>
<tr>
<td>Cysteinylglycine</td>
<td>29 ± 6</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>( \gamma )-glutamylcysteine</td>
<td>3.8 ± 1.1</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>Glutathione</td>
<td>2.9 ± 1.1</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td>Serine</td>
<td>110 ± 10</td>
<td>103 ± 9</td>
</tr>
<tr>
<td>Glycine</td>
<td>222 ± 45</td>
<td>262 ± 34 ( b )</td>
</tr>
<tr>
<td>Glutamate</td>
<td>44 ± 13</td>
<td>42 ± 18</td>
</tr>
<tr>
<td>PLP (nmol/L)</td>
<td>53 ± 42</td>
<td>477 ± 140 ( d )</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>19 ± 12</td>
<td>91 ± 19 ( d )</td>
</tr>
<tr>
<td>B12 (pmol/L)</td>
<td>377 ± 125</td>
<td>381 ± 159</td>
</tr>
<tr>
<td>Hcy/Met ratio</td>
<td>0.36 ± 0.05</td>
<td>0.28 ± 0.07 ( e )</td>
</tr>
<tr>
<td>Hcy/Cys ratio</td>
<td>0.040 ± 0.008</td>
<td>0.028 ± 0.003 ( d )</td>
</tr>
</tbody>
</table>

\( a \) Values are given as mean ± SD. The amino acids and sulfhydryl concentrations are in \( \mu \)mol/L. HD, hemodialysis; PLP, pyridoxal-5'-phosphate; Hcy, homocysteine; Met, methionine; Cys, cysteine.

\( b \) \( P < 0.01 \), compared with basal values in each group.

\( c \) \( P < 0.001 \), comparing basal values in patients with control subjects.

\( d \) \( P < 0.001 \), compared with basal values in each group.

\( e \) \( P < 0.05 \), compared with basal values in each group.

\( f \) \( P < 0.05 \), comparing basal values in patients with control subjects.
ized in any patient. In addition, Glu levels also decreased, whereas Cys-Gly, GSH, and Gly concentrations increased significantly.

The only significant change observed in the HD patients’ RBC (Table 3), after supplementation, was the increase in GSH, whereas Hcy and Cys remained at the same level. The RBC Tau level decreased, but this change did not reach significance.

Hcy/Met and Hcy/Cys Ratios

The plasma and RBC Hcy/Met ratios were significantly higher in the HD patients than in the healthy subjects (Tables 2 and 3). After vitamin supplementation, the RBC ratio increased significantly and the plasma ratio tended to decrease, without reaching significance in healthy subjects ($P = 0.06$). Vitamin supplementation had no effect on the plasma and RBC Hcy/Met ratios in the HD patients.

The plasma Hcy/Cys ratio (Table 2) was significantly higher in the HD patients than in the healthy subjects, whereas the RBC ratio (Table 3) was not significantly different between the two groups. Vitamin supplementation led to a significant reduction in the plasma Hcy/Cys ratio in both groups. The RBC Hcy/Cys ratio decreased significantly in the healthy subjects, but not in the HD patients, after vitamin supplementation.

Homocysteine Correlations

Basal plasma Hcy was positively correlated to RBC Hcy ($r = 0.82$, $P < 0.05$) and plasma Cys (Figure 4), whereas RBC Hcy was positively correlated to plasma Cys ($r = 0.73$, $P < 0.05$) in HD patients. Plasma and RBC Hcy were inversely correlated, respectively, to plasma folate (Figure 3) and RBC folate ($r = -0.83$, $P < 0.05$ and $r = -0.97$, $P < 0.01$, respectively) in HD patients. There was a negative correlation

Table 3. Erythrocyte-free amino acid, sulfhydryl, and vitamin concentrations in hemodialysis patients and healthy subjects before and after vitamin supplementation

<table>
<thead>
<tr>
<th>Variable</th>
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<tr>
<td></td>
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<td>After (n = 10)</td>
</tr>
<tr>
<td>Methionine</td>
<td>27 ± 4</td>
<td>26 ± 4</td>
<td>27 ± 5</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>3.9 ± 0.6</td>
<td>6.8 ± 1.0$^b$</td>
<td>5.6 ± 1.9$^c$</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>Cysteine</td>
<td>8 ± 4</td>
<td>25 ± 8$^b$</td>
<td>13 ± 7</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Taurine</td>
<td>142 ± 64</td>
<td>89 ± 28$^d$</td>
<td>153 ± 64</td>
<td>113 ± 68</td>
</tr>
<tr>
<td>Cysteinylglycine</td>
<td>12 ± 2</td>
<td>12 ± 3</td>
<td>15 ± 4$^e$</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Glutathione</td>
<td>2853 ± 493</td>
<td>3029 ± 579$^d$</td>
<td>2812 ± 512</td>
<td>3068 ± 459$^d$</td>
</tr>
<tr>
<td>Serine</td>
<td>229 ± 29</td>
<td>166 ± 28$^b$</td>
<td>179 ± 43$^e$</td>
<td>169 ± 48</td>
</tr>
<tr>
<td>Glycine</td>
<td>502 ± 63</td>
<td>392 ± 86$^d$</td>
<td>517 ± 118</td>
<td>547 ± 128</td>
</tr>
<tr>
<td>Glutamate</td>
<td>355 ± 114</td>
<td>248 ± 79$^f$</td>
<td>474 ± 141$^e$</td>
<td>461 ± 158</td>
</tr>
<tr>
<td>PLP (nmol/L)</td>
<td>152 ± 49</td>
<td>788 ± 280$^b$</td>
<td>301 ± 89$^g$</td>
<td>1395 ± 477$^b$</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>888 ± 388</td>
<td>2333 ± 725$^b$</td>
<td>1324 ± 408$^e$</td>
<td>5707 ± 1296$^b$</td>
</tr>
<tr>
<td>Hcy/Met ratio</td>
<td>0.15 ± 0.04</td>
<td>0.27 ± 0.06$^b$</td>
<td>0.21 ± 0.06$^g$</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>Hcy/Cys ratio</td>
<td>0.56 ± 0.23</td>
<td>0.29 ± 0.09$^f$</td>
<td>0.56 ± 0.40</td>
<td>0.50 ± 0.14</td>
</tr>
</tbody>
</table>

$^a$ Values are given as mean ± SD. The amino acids and sulfhydryl concentrations are in μmol/L intracellular water. Abbreviations as in Table 2.

$^b$ $P < 0.001$, compared with basal values in each group.

$^c$ $P < 0.05$, comparing basal values in patients with control subjects.

$^d$ $P < 0.05$, compared with basal values in each group.

$^e$ $P < 0.01$, comparing basal values in patients with control subjects.

$^f$ $P < 0.01$, compared with basal values in each group.

$^g$ $P < 0.001$, comparing basal values in patients with control subjects.
between basal plasma Hcy and plasma folate levels in healthy subjects (Figure 3).

After vitamin supplementation, the plasma Hcy was positively correlated with RBC Hcy \((r = 0.63, P < 0.05)\) in HD patients and with plasma Cys in the HD and healthy subjects (Figure 4). All HD patients and healthy subjects had high levels of blood folate due to vitamin supplementation, which probably explains the lack of correlation between Hcy and folate in the two groups, but despite that, a negative correlation between RBC Hcy and RBC folate \((r = 0.77, P < 0.05)\) was observed in healthy subjects.

Discussion

To our knowledge, this is the first report demonstrating the effect of folic acid and pyridoxine supplementation on both plasma and RBC sAA and sulfhydryl levels in uremic patients undergoing HD treatment and in healthy subjects. The study shows that the concentrations of most of the plasma and RBC sAA and sulfhydryls are higher in the HD patients than in the healthy subjects, except for Met and Tau.

Our results show that in both the HD patients and healthy subjects, the concentrations of sAA and sulfhydryls in RBC differ significantly from those in plasma, except for Met. Specifically, GSH and Tau were higher in RBC, whereas the concentrations of the other sulfhydryls were higher in plasma. These findings are in agreement with studies showing that RBC and plasma play independent and frequently opposing roles in amino acid interorgan transport in several mammalian species (18,19). CSA was detected in plasma but not in RBC, similar to the previous findings in muscle (25). \(\gamma\)-Glu-Cys could not be determined in RBC samples due to interference of its chromatographic peak in the present analysis method.

Homocysteine

In agreement with our previous studies (5,26), hyperhomocysteinemia, accompanied by hypercysteinemia, was noted in the HD patients. Moreover, in the HD patients the levels of RBC Hcy were significantly increased and correlated to the plasma levels. In the patients, we also observed that the increases in plasma and RBC Hcy were negatively and significantly correlated with basal plasma and RBC folate levels (Figure 3). Furthermore, the high level of the plasma Cys in our patients was significantly correlated to the basal plasma Hcy values (Figure 4), as reported earlier by us (26), and the correlation remained after vitamin supplementation. Hong et al. (27) recently confirmed that plasma Hcy and Cys were correlated in ESRD patients and they also found that hyperhomocysteinemic patients with normal renal function did not have elevated plasma cysteine concentrations. CSA is also elevated in ESRD patients in the presence of low Tau levels in plasma and muscle, which we interpreted to be due to an inhibition of cysteinesulfinic acid decarboxylase caused by uremia (21). These results suggest that hyperhomocysteinemia in renal failure patients may develop by mechanisms other than in nonuremic individuals. On the basis of our observations of strongly elevated Hcy levels in RBC and plasma, along with elevated levels of cysteine and CSA, we hypothesize that hyperhomocysteinemia in ESRD patients may, at least in part, be caused by a block in decarboxylation of CSA, leading to accumulation of the precursor metabolites.

The levels of folate, vitamin B6, and vitamin B12 are regarded as important factors influencing the metabolism of sAA. Patients with uremia are at risk for deficiency of B6 (28,29) but our patients were routinely given daily supplements of 10 mg of pyridoxine. Accordingly, their plasma and RBC PLP baseline concentrations were higher than normal, reflecting the supplemental intake. None of the patients received vitamin B12 or folic acid supplementation before the study, and their mean folate and B12 levels were the same, or even higher, as those in the healthy subjects. Therefore, it is unlikely that folic acid, PLP, and vitamin B12 depletion contributed to the hyperhomocysteinemia observed in our patients. The
mechanism of hyperhomocysteinemia in chronic renal failure has not yet been elucidated (30). Renal excretion is not considered to be a major cause of hyperhomocysteinemia in kidney failure, since urinary Hcy clearance is less than 1% of creatinine clearance (31,32) and the fractional clearance of Hcy increases as renal function worsens (32). Decreased catabolism of Hcy in the proximal tubular cells may contribute to hyperhomocysteinemia (33). Moreover, the extrarenal Hcy metabolism may decrease due to inhibition by retained metabolites (34). Disturbed methylation reaction due to accumulation of S-adenosylhomocysteine in CRF has also been suggested to affect Hcy metabolism (35–37). Increased sulfate levels in CRF may reduce folate conjugase activity (38), which could be an additional factor contributing to hyperhomocysteinemia.

Homocysteine condenses with serine to form cystathionine via the trans-sulfuration pathway. The kidney is the major site for metabolism of glycine to serine (39). Thus, in the failing kidney, the synthesis of serine might be impaired, resulting in decreased plasma and RBC serine concentrations, as observed in this study. However, it cannot be excluded that the high Hcy levels in uremic patients may lead to enhanced condensation with serine, thereby contributing to serine depletion.

Treatment with folic acid and pyridoxine for 1 mo significantly reduced plasma Hcy in the patients and healthy subjects, despite the normal range of vitamin concentration before treatment. Pyridoxine supplementation was not expected to change Hcy concentration in uremic patients (16,40–42), as this effect is attributed to folic acid supplementation, which has been shown to have a plasma Hcy-lowering effect at pharmacologic doses in subjects with normal renal function (43) and in uremic patients (40). The high folate dose used in our study reduced plasma Hcy by 28% in the HD patients but failed to normalize the level in any patient. The same percentage of reduction was reported by Bostom et al. (44), but in contrast to our results, plasma Hcy levels were normalized in five of their 15 patients who participated in the study. The lack of normalization of Hcy levels in our patients is in agreement with most previous studies, in which folic acid supplementation failed to normalize the Hcy level regardless of the pretreatment level and the duration of the supplementation (40–42,45). Failure of Hcy normalization may be due to impairment of cysteinesulfenic acid decarboxylase activity in uremic patients, as discussed previously (see above). Moreover, genetic determinants are also thought to play a role in hyperhomocysteinemia in uremic patients (46) and may influence the therapeutic efficiency of folic acid supplementation. The most probable mechanism by which folic acid supplementation reduces plasma Hcy concentration is that, after it is converted to 5-methyltetrahydrofolate (Figure 1), folic acid enhances the remethylation of Hcy to methionine (40,43). If folic acid led to a decrease in Hcy levels via enhancement of the remethylation, plasma and RBC methionine levels should be increased, which was not the case in either group in this study and thus is similar to the findings of Wilcken et al. (40). On the other hand, Perna et al. (36) observed an increase in methionine and S-adenosylmethionine levels in HD patients, after supplementation with the active form of folate, methyltetrahydrofolate.

Folic acid has been administered orally at doses of up to 60 mg/d for 2 yr, and 500 mg/d for 2 wk (47). Given the lack of known toxicity of an elevated folate level and its potential role in the reduction of vascular morbidity and mortality by reducing Hcy levels, folate supplementation has been suggested to be used routinely in HD patients, even in patients with moderately elevated folate levels. In previous studies (41,44,45) and in the present study, treatment with 5 to 16 mg of folic acid daily decreased Hcy by 25 to 50% in HD patients without any obvious relation between the dose given and Hcy-lowering effect. Additional studies are warranted to determine the optimal dose of folic acid needed to provide long-term sustained reduction of Hcy level in uremic patients.

The Hcy/Met and Hcy/Cys ratios reflect the catabolism of Hcy through remethylation and trans-sulfuration pathways. These ratios could be affected by alteration in the enzyme activity and/or vitamin availability, caused by endogenous or...
exogenous factors. In this study, the basal plasma Hcy/Cys and Hcy/Met ratios (Table 2) were significantly higher in the HD patients than in healthy subjects, as in our previous finding (5). The abnormality in the plasma ratio may reflect alterations that occur in Hcy catabolism during CRF. Although the basal RBC Hcy/Met ratio, like the plasma ratio, was higher in the HD patients, the RBC Hcy/Cys ratio was similar in HD patients and healthy subjects (Table 3). The reduction observed in these ratios after vitamin supplementation might indicate enhancement and improvement in the Hcy metabolism in healthy subjects and HD patients. Since the change in the plasma ratio was not accompanied, in HD patients, by a change in RBC ratio, the reduction in RBC ratio observed in the healthy subjects might be due to the increase in RBC Hcy (twofold) and RBC Cys (threelfold) levels after vitamin supplementation (Table 3).

It has been reported that synthesis of Hcy occurs in RBC (48) and Hcy is known to easily cross the RBC membrane (49). Perna et al. (50) have reported increased RBC levels of Hcy and S-adenosylhomocysteine in HD patients. Our results confirm their finding of higher Hcy RBC levels in HD patients. Moreover, the Hcy RBC levels did not change after vitamin supplementation in the HD patients but increased significantly in the healthy subjects. The decreased plasma Hcy levels found in healthy subjects after vitamin supplementation may offset a response by increasing production of Hcy in RBC, as plasma levels after vitamin supplementation may be low for normal individuals. The same phenomenon was observed for RBC Cys in healthy subjects but not in HD patients, although vitamin treatment had no effect on plasma Cys in patients or healthy subjects. The biochemical and possible clinical relevance of these findings are still unknown, and further investigations will be needed to clarify them.

**Taurine**

Earlier studies from our laboratory have reported low muscle and plasma Tau levels and increased RBC Tau concentrations in uremic patients (5–8,21). However, normal or elevated levels of plasma taurine have also been reported previously (51–53). In the present study, the plasma and RBC Tau levels in the HD patients did not differ significantly from normal. However, Figure 2 shows that four of the 10 patients had plasma Tau levels below the normal range. Differences in plasma Tau concentrations may be due to alterations in dietary intake of Tau or its precursor sAA, or due to impairment of Tau synthesis. PLP is required as a cofactor for the synthesis of Tau, and low plasma Tau levels in chronic dialysis patients on daily pyridoxine supplementation have been found (5,21). The basal PLP levels were normal in plasma and higher in RBC in HD patients than in healthy subjects, presumably because of routine pyridoxine supplementation before the study. Hence, the low plasma Tau levels observed in four of the patients are unlikely due to PLP deficiency. However, since these values normalized after treatment, it cannot be excluded that a pharmacologic dose of pyridoxine may promote endogenous synthesis of Tau. Unexpectedly, we observed that vitamin supplementation led to a significant reduction in RBC Tau concentration in the healthy subjects and a similar tendency in HD patients, despite unchanged plasma Tau levels in both groups.

**Glutathione**

Previous studies have reported low levels of RBC and whole blood GSH in patients undergoing both HD and continuous ambulatory peritoneal dialysis (12,13). In contrast, high RBC GSH levels have been reported in nondialyzed patients with varying degrees of renal insufficiency and patients on regular HD (54). These apparently conflicting results may be due to differences in methods of analysis or to the clinical state of the patients investigated. We observed in our patients elevated plasma GSH levels compared with the levels in healthy subjects, whereas RBC GSH levels were not significantly different. Since RBC concentration represents 99% of the GSH in whole blood (55), the changes noted in plasma GSH in our patients do not reflect a real change in whole blood GSH. Recent studies (12,56) have shown that intravenous administration of GSH to HD patients increased the RBC GSH levels, which led to improvement in RBC survival, correction of hemoglobin levels, and a reduction in the dose of erythropoietin. In the present study, we found that vitamin supplementation increased RBC and plasma GSH level in HD patients and healthy subjects. Additional studies may be needed to determine whether this observed increase in GSH levels has a beneficial effect on the blood antioxidant status of uremic patients.

In summary, we report that abnormalities in sAA metabolism are present in both RBC and plasma of hemodialysis patients. The sAA and sulfhydryl concentrations in plasma and RBC differ in both patients and healthy subjects. Furthermore, vitamin supplementation resulted in a decrease in plasma Hcy and an increase in RBC and plasma GSH levels, suggesting that longitudinal follow-up studies are required to evaluate the long-term effects of vitamin supplementation on sAA metabolism. On the basis of our observations that plasma Hcy and Cys levels were both elevated and correlated in the presence of elevated plasma levels of CSA, we propose the hypothesis that a block in decarboxylation of CSA causes or contributes to hyperhomocysteinemia in ESRD.

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