Homocysteine in the plasma of renal transplant recipients: effects of cofactors for methionine metabolism

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Summary

1. Homocysteine which is formed during the metabolism of methionine is readily oxidized and is measured by the amino acid analyser as cysteine–homocysteine mixed disulphide and homocystine. We measured plasma amino acid concentrations after an overnight fast in 27 stable long-term renal transplant recipients and 25 age- and sex-matched normal subjects with particular emphasis on sulphur-containing amino acids.

2. Plasma cysteine–homocysteine mixed disulphide was increased in the patients (mean 6.0 ± sd 3.2 μmol/l; normal 3.1 ± 0.9 μmol/l, P < 0.001) and homocystine was detectable in low concentration (<1.0 μmol/l) in 24; the elevation in cysteine–homocysteine was related to serum creatinine (r = 0.60, P < 0.002). Cystine was also increased (91.6 ± 29.3 μmol/l; normal subjects 64.0 ± 16.7 μmol/l, P < 0.001), but methionine concentrations were normal.

3. When pyridoxine, folic acid and vitamin B12, cofactors for homocysteine metabolism, were administered sequentially to 11 arbitrarily selected transplant recipients cysteine–homocysteine decreased from 7.3 ± 2.1 to 4.3 ± 0.8 μmol/l (P < 0.001) and homocystine became undetectable. The response coincided with the giving of folic acid and occurred without alteration in serum creatinine and with normal serum folate and vitamin B12 concentrations.

4. In eight patients in whom pretreatment erythrocyte folate was measured, folic acid therapy reduced cysteine–homocysteine from 9.0 ± 3.1 to 5.4 ± 1.6 μmol/l over a 4 week period (P < 0.001), the largest response being in the one patient with subnormal erythrocyte folate; values were in the low–normal or normal range in the other seven.

5. We conclude that plasma homocysteine is increased in renal transplant recipients when serum creatinine is only moderately elevated and that the homocysteine concentrations are decreased by treatment with folic acid, suggesting that both reduced homocysteine excretion and relative shortages of folic acid are responsible.

Key words: amino acids, cysteine–homocysteine mixed disulphide, cystine, folic acid, homocysteine, homocystinuria, pyridoxine, renal failure, vascular disease.

Introduction

Cysteine–homocysteine mixed disulphide and homocystine are oxidation products of homocysteine and cysteine formed in the methionine degradation pathway. The detection in the plasma of homocysteine and of the mixed disulphide, which are produced presumably during the processing of blood samples, indicates the presence of homocysteinaemia [1, 2]. We have shown that cysteine–homocysteine mixed disulphide is always detectable in low concentration in the plasma of man [3, 4]. The concentrations are increased in patients with chronic renal insufficiency [5], including those managed by regular haemodialysis [6]. Homocysteine is also detectable in the plasma when renal function is impaired [5, 6], but is not found in normal subjects [3, 4]. Restoration of renal function by transplantation is accompanied by a dramatic improvement in
blood chemistry. In this study we have explored the effects of transplantation on the abnormal sulphur-containing amino acid metabolism documented in chronic azotaemia and compared the changes in plasma sulphur-containing amino acid concentrations with those in other amino acids. We have also investigated the effects on plasma amino acid concentrations of administering co-factors for methionine metabolism.

Patients and methods

Patients

We studied 27 renal transplant recipients, 13 men and 14 women aged from 21 to 51 years (mean 41.2 ± SD 8.9 years). They had received their kidneys from 1 to 14 years before the time of study. In none had there been a recent episode of rejection and all had stable renal function as assessed by serial serum creatinine estimations. All were receiving immunosuppressive drugs, azathioprine, cyclophosphamide or chlorambucil, together with corticosteroids. All were ambulant and measurements were made at the time of outpatient visits. They were eating a normal diet, none was receiving vitamin therapy at the commencement of the study and none was obese. The disease states leading to chronic renal failure and transplantation in the patients were as follows: chronic glomerulonephritis (12), analgesic nephropathy (eight), chronic pyelonephritis (four) and congenital renal malformation (three).

Plasma amino acids were estimated as described previously in blood drawn after an overnight fast [31 and serum creatinine concentrations were also determined. Results obtained for the neutral and acidic amino acids (basic amino acids were not measured in this study) were compared with those measured in 25 normal subjects matched for age and sex with the renal recipients. The controls had routine haematological and biochemical examinations, including serum creatinine, and normal values were obtained in all.

Vitamin therapy

After assessing the initial results we selected arbitrarily seven renal recipients who had elevated plasma cysteine–homocysteine concentrations and in these measured plasma amino acids before and after vitamin therapy. Vitamin B₁₂ (1000 μg) was injected intramuscularly and folic acid (5 mg) and pyridoxine (100 mg) were given orally daily for 2 weeks. Diet logs kept during the treatment period revealed no change in protein or caloric intake.

Since this regimen reduced plasma cysteine–homocysteine concentrations (see below), an attempt was made to determine which agent was responsible by administering each cofactor sequentially. Eleven patients were given first pyridoxine (100 mg) daily for 2 weeks. For the next 2 weeks folic acid (5 mg daily) was added to the pyridoxine. Then an injection of vitamin B₁₂ (1000 μg) was given and pyridoxine and folic acid were continued for a further 2 weeks. Blood samples were drawn for amino acid and creatinine estimations, before starting this regimen, and repeated at 2 week intervals just before the addition of each cofactor. There was a further sample taken at the end of the 6 weeks of treatment and a final sample 10 weeks after cessation of vitamin therapy. Serum folate and vitamin B₁₂ concentrations were measured before and after treatment by radioassay with a commercially available Diagnostic Products Dual-count kit. This kit uses purified intrinsic factor as the vitamin B₁₂ binder and β-lactoglobulin as the folate binder.

Because there had been no measurement of erythrocyte folate in the vitamin studies and because there was some doubt about the relative contributions of folic acid and vitamin B₁₂ to the changes in cysteine–homocysteine observed (see below), we undertook further studies in eight of these 11 patients. After an interval of 6–9 months off all vitamin therapy a control blood sample was taken for plasma amino acid and serum creatinine estimations, and then each patient was given folic acid (5 mg) daily for 4 weeks with repeat blood samples after 2 and 4 weeks of treatment. Then a vitamin B₁₂ injection (1000 μg) was given and a further blood sample drawn 2 weeks later, the folic acid being continued meanwhile. Vitamin B₁₂ and serum and erythrocyte folate were measured in the pretreatment blood sample, the last estimation in whole blood diluted 1:40 in 1% (w/v) ascorbic acid solution and then estimated as for serum folate.

Student’s t-test was used to assess the significance of the differences between the grouped data. The test was unpaired for the overall amino acid results (Table 1) and paired for the rest of the data.

Results

Amino acids

The plasma concentrations of the sulphur-containing and other neutral and acidic amino
TABLE 1. *Mean plasma neutral and acidic amino acid concentrations after an overnight fast in transplant recipients in relation to serum creatinine and in normal subjects*

Results are means ± SD. P values relate to comparisons between concentrations in the normal subjects and those in all transplant recipients as well as those with normal and elevated creatinine: * P < 0.05; ** P < 0.01; *** P < 0.001.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Plasma concentration (μmol/l)</th>
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<tbody>
<tr>
<td></td>
<td>Normal subjects (n = 25)</td>
</tr>
<tr>
<td>Taurine</td>
<td>84.8 ± 35.3</td>
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<tr>
<td>Aspartate</td>
<td>10.9 ± 6.9</td>
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<tr>
<td>Threonine</td>
<td>129.9 ± 35.1</td>
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<tr>
<td>Serine</td>
<td>121.4 ± 25.3</td>
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<tr>
<td>Glycine</td>
<td>146.4 ± 37.8</td>
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<tr>
<td>Alanine</td>
<td>232.1 ± 61.4</td>
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<tr>
<td>α-Amino-N-butyric acid</td>
<td>22.3 ± 9.2</td>
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<tr>
<td>Valine</td>
<td>225.8 ± 51.5</td>
</tr>
<tr>
<td>Cystine</td>
<td>64.0 ± 16.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>25.9 ± 6.6</td>
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<tr>
<td>Isoleucine</td>
<td>66.7 ± 20.3</td>
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<tr>
<td>Leucine</td>
<td>124.8 ± 34.7</td>
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<tr>
<td>Cysteine–homocysteine</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>58.0 ± 13.2</td>
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<tr>
<td>Phenylalanine</td>
<td>56.1 ± 12.2</td>
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Homocysteine in renal transplant recipients

Acids measured in all transplant recipients and in controls matched for age and sex are shown in Table 1. Of the sulphur-containing amino acids, cysteine–homocysteine mixed disulphide and cystine are increased in the renal patients (P < 0.001 for each). There were no differences, however, in the concentrations of the precursor, methionine. Homocysteine was not detected in any normal subject but could be identified in low concentration (<1 μmol/l) in 24 transplant recipients. Of the other amino acids, alanine concentrations were also increased in the renal patients (P < 0.001) and those of serine (P < 0.01), valine and leucine were reduced (P < 0.05 for each).

When the transplant recipients were grouped into those with normal (n = 11) and those with increased serum creatinine (n = 16), as shown in Table 1, the only significant amino acid differences were in the cystine and cysteine–homocysteine mixed disulphide, both being higher in the patients with elevated creatinine (P < 0.01 and < 0.001 respectively). In Fig. 1 the plasma cysteine–homocysteine concentration in each patient is plotted against the serum creatinine measured in the same blood sample. There is a clear positive relationship between the two (r = 0.60; P < 0.002).

Vitamin studies

Fig. 2 shows the cysteine–homocysteine concentrations before and after 2 weeks treatment with vitamin B₁₂, pyridoxine and folic acid. Treatment decreased cysteine–homocysteine concentrations in all seven patients (mean reduction 35.4%, P < 0.05) and abolished the small homocysteine peaks from the chromatograms. Pre- and post-treatment serum creatinine (0.20 ± 0.10 and 0.20 ± 0.08 mmol/l), methionine (22.8 ± 5.7 and 23.7 ± 4.9 μmol/l) and cystine (105.0 ± 30.3 and 106.3 ± 31.6 μmol/l) were not different.

The serial plasma cysteine–homocysteine and serum creatinine concentrations in the 11 patients...
given the cofactors sequentially are shown in Table 2. There was no change after pyridoxine, but a significant decrease in cysteine-homocysteine after the addition of folic acid and a further small decrease after vitamin B₁₂. When concentrations were remeasured 10 weeks after cessation of vitamin therapy the mean concentration had increased to a level not different from those before treatment and after pyridoxine. After folic acid the chromatograms no longer showed the small peak due to homocystine visible before treatment. These peaks reappeared 10 weeks after cessation of vitamin therapy. Methionine and cystine concentrations were unaltered throughout. There was a change in serum creatinine, a rise, in only one patient. This occurred during the 10 weeks between the completion of treatment and the taking of the post-treatment blood sample. The increase from 0.47 to 0.72 mmol/l was associated with a rise in cysteine-homocysteine from 4.1 to 7.7 μmol/l. This patient was excluded from the post-treatment analysis (Table 2). Pretreatment serum folate and vitamin B₁₂ concentrations were within the normal range in all patients. Mean concentrations (±SD) were 6.8 ± 2.6 ng/ml and 330 ± 104 pg/ml respectively.

The results obtained in the eight patients given folic acid and vitamin B₁₂ again after an interval of 6–9 months to assess the duration of the folic acid effect and to relate it to pretreatment erythrocyte folate levels are shown in Table 3. After 2 weeks of folic acid, plasma cysteine-homocysteine was lower in all eight patients; after another 2 weeks of folic acid there was a further decline in values from six of the eight patients. The addition then of 1000 μg of vitamin B₁₂ had

![Fig. 2. Plasma cysteine-homocysteine mixed disulphide concentrations after an overnight fast in individual patients before (pre) and after (post) 2 weeks of pyridoxine (100 mg/day), folic acid (5 mg/day) and vitamin B₁₂ (1000 μg, intramuscularly). Concentrations were reduced after treatment in all seven patients and means ± SD (vertical bars) are shown. Mean pre- and post-treatment serum creatinine concentrations were 0.20 ± 0.09 and 0.20 ± 0.08 mmol/l respectively.](image)

<table>
<thead>
<tr>
<th>TABLE 2. Effect on mean plasma cysteine-homocysteine concentration of treatment with cofactors for methionine metabolism, each added sequentially at intervals of 2 weeks, in 11 renal transplant recipients</th>
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</thead>
<tbody>
<tr>
<td>Results are means ± SD. P values relate to comparisons with the pretreatment cysteine-homocysteine concentration:</td>
</tr>
<tr>
<td>*P &lt; 0.01; **P &lt; 0.001.</td>
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<tr>
<td>Before treatment (n = 11)</td>
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<tr>
<td>Cysteine-homocysteine (μmol/l)</td>
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<tr>
<td>Serum creatinine (mmol/l)</td>
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<tr>
<th>TABLE 3. Effect on mean plasma cysteine-homocysteine concentration of treatment with folic acid (5 mg/day) for 6 weeks, with an injection of vitamin B₁₂ (1000 μg) after 4 weeks in eight renal transplant recipients</th>
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</thead>
<tbody>
<tr>
<td>Results are means ± SD. P values relate to comparisons with the pretreatment cysteine-homocysteine concentration:</td>
</tr>
<tr>
<td>*P &lt; 0.002.</td>
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<tr>
<td>Before treatment (n = 8)</td>
</tr>
<tr>
<td>Cysteine-homocysteine (μmol/l)</td>
</tr>
<tr>
<td>Serum creatinine (mmol/l)</td>
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</table>
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Discussion

This study shows that patients with transplanted kidneys and stable but reduced renal function have increased plasma concentrations of the sulphur-containing amino acids cysteine and homocysteine, measured as cystine, cysteine–homocysteine mixed disulphide and homocysteine. Elevated levels of homocysteine depend both on renal clearance, as plasma concentrations are positively correlated with serum creatinine, and on the metabolism of homocysteine; treatment with folic acid, a cofactor for the remethylation of homocysteine to methionine, decreases plasma homocysteine concentrations in the absence of any change in serum creatinine and, presumably, renal clearance, and in the absence of demonstrable folate deficiency.

Of the non-sulphur-containing amino acids measured, the reductions we found in serine, valine and leucine are in accordance with the results of other studies in chronic renal insufficiency [7, 8], including our own [5]. The increase in alanine concentration, however, is likely to be a glucocorticoid effect. Wise et al. [9] showed that hyperalaninaemia occurs with acute glucocorticoid excess and it is known that alanine is an important glucocorticoid substrate [10, 11]. In our study the hyperalaninaemia was of the same magnitude in patients with normal and increased serum creatinine concentrations (Table 1), suggesting that it was independent of renal function and related to glucocorticoid administration. Also glucocorticoid excess has been shown not to alter the plasma concentrations of any amino acid other than alanine [9]. This is relevant to the interpretation of the changes in plasma amino acid concentrations found in the present study.

The possibility that the amino acid changes could result in part from liver damage, perhaps produced by azathioprine, seems unlikely because not only is azathioprine-induced liver damage rare with the doses currently used [12], but there was no clinical or biochemical evidence of liver dysfunction in the post-transplant patients as assessed by serum bilirubin and liver enzyme estimations. Furthermore the plasma concentrations of α-aminobutyric acid were within the normal range in all patients. Increased plasma concentrations of this amino acid have been shown to reflect non-specific liver injury [13]. However, immunosuppressive therapy could have contributed through mechanisms other than by producing liver damage.

The increase in plasma cystine could have been due in part to drug therapy because it occurred even in the patients with normal creatinine (Table 1). However, the increase was unrelated to the use of any particular immunosuppressive drug with prednisone and we are not aware of any published report of prednisone affecting plasma cystine. Azathioprine reacts with thiol groups of cysteine and glutathione producing a slow release of 6-mercaptopurine [14] and possibly inter-relations between cysteine, glutathione and azathioprine and subsequent metabolism may result in elevated plasma cystine. There does not seem an obvious mechanism for an effect by the non-sulphur-containing compounds cyclophosphamide or chlorambucil, which were associated with elevations of the same order. However, diminished excretion is likely to be the principal mechanism. Elevated plasma cystine is related to the severity of renal failure in patients not receiving immunosuppressive or corticosteroid therapy [5] and accumulates rapidly between treatments in patients managed with chronic haemodialysis [6].

Whereas the relationship between cysteine–homocysteine and serum creatinine (Fig. 1) suggests that reduced excretion is an important mechanism contributing to the elevated plasma cysteine–homocysteine, a similar relationship was also present in our previous study over a wider range of serum creatinine in patients not requiring transplantation or haemodialysis [5], diminished excretion cannot provide the whole explanation in view of the reduction obtained after treatment with cofactors for homocysteine metabolism. In the 11 patients given pyridoxine, folic acid and vitamin B₁₂ sequentially (Table 2) significant decreases in plasma cysteine–homocysteine mixed disulphide and abolition from the
within the normal range in these patients. But pretreatment serum folate concentrations were all occurred only after folic acid was given. Pre-treatment serum folate concentrations were all within the normal range in these patients. But serum folate may not represent metabolically active cellular concentrations, whereas erythrocyte folate does reflect this \[^{[15]}\]; it is unfortunate that we were unable to measure pretreatment erythrocyte folate in these patients. Nevertheless, their response to folic acid is consistent with there being a relative shortage of this cofactor, which was sufficient to affect homocysteine metabolism. This was confirmed in the subsequent study designed to assess specifically the folic acid effect and identify any possible associated vitamin B\(_{12}\) contribution (Table 3). The response coincided with the giving of folic acid and there was no effect with vitamin B\(_{12}\).

Folic acid acts with vitamin B\(_{12}\) as a cofactor in the remethylation of homocysteine to methionine and folate deficiency may occur in severely ureaemic patients \[^{[7]}\], whereas vitamin B\(_{12}\) deficiency is not a concomitant of chronic azotaemia \[^{[7, 16]}\]. The change which coincided with the giving of vitamin B\(_{12}\) in the first sequential study was due to a continuing folic acid effect, the full response being seen after 4 weeks of treatment. That the decrease in plasma cysteine–homocysteine was related to treatment is evidenced by the increase to pretreatment concentrations within 10 weeks of cessation of therapy in the absence of any clinical change or alteration in serum creatinine or diet (Table 2).

In the eight patients in whom there were erythrocyte folate measurements (Table 3) there was unequivocal deficiency in only one (who had the largest response), but there were low normal readings in a further three (<540 nmol/l). Yet all responded to folic acid with a decline in cysteine–homocysteine. Long-term immunosuppression produces complex metabolic changes and perhaps relative deficiencies develop when the serum creatinine is only moderately elevated; under these circumstances excess folate may increase remethylation of homocysteine to methionine. However, it may well be that chronic renal failure patients not receiving immunosuppressive therapy will also respond to folic acid. This remains to be tested.

Pyridoxine deficiency has been reported in severely ureaemic patients \[^{[7, 17]}\]. The giving of pyridoxine alone, however, produced no significant change in the mean cysteine–homocysteine concentration. Pyridoxine is a cofactor for cystathionine synthase, which mediates the condensation of homocysteine with serine to produce cystathionine \[^{[18]}\], and in pyridoxine-deficient patients could reduce plasma homocysteine concentrations and therefore those of the mixed disulphide. In one patient there was a large decrease in cysteine–homocysteine after pyridoxine, without any change in the serum creatinine, and it is possible that this patient was pyridoxine-deficient.

Finally, we have no information about any possible clinical relevance of the biochemical changes documented in the present study. Very high plasma homocysteine concentrations, some 10–20 times those found here, are associated with vascular disease in children with homocystinuria and vascular disease is the usual cause of death \[^{[18]}\]. It has been reported that chronic high-dose homocysteine infusions produce atherosclerosis in a primate model \[^{[19]}\]. From our earlier observations elevated plasma cysteine–homocysteine concentrations are likely to be present for many years in patients with slowly deteriorating renal function: cysteine–homocysteine concentrations in excess of 7.0 \(\mu\)mol/l occur when the creatinine is above 0.5 mmol/l. There is a high incidence of occlusive vascular disease after transplantation \[^{[20–23]}\], and many factors must be involved. The present study documents elevated plasma homocysteine concentrations in these patients when the serum creatinine is only moderately increased; the homocysteine concentrations are decreased by treatment with folic acid and this may be beneficial.

Acknowledgments

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References

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