SHORT COMMUNICATION

Accumulation of sulphur-containing amino acids including cysteine–homocysteine in patients on maintenance haemodialysis

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Summary

1. Plasma sulphur-containing amino acids were measured in 19 patients with renal failure on chronic haemodialysis and in 22 normal subjects, to determine the rate of accumulation of these amino acids in chronic azotaemia.

2. Cysteine–homocysteine mixed disulphide was significantly increased in patients before dialysis and homocysteine was detected in low concentration in 10 patients. Cystine and taurine were also increased. Changes in other neutral and acidic amino acids were similar to those reported in chronic renal insufficiency.

3. In 3–4 h of dialysis serum creatinine was decreased by a mean of 55%, cysteine–homocysteine by 41% and cystine by 58.5% (P < 0.001 for each). Methionine concentrations were normal throughout.

4. We conclude that sulphur-containing amino acids, except methionine, accumulate in chronic renal failure as rapidly as creatinine.

Key words: amino acids, β-aminoisobutyric acid, cysteine–homocysteine mixed disulphide, haemodialysis, homocysteine, homocystinuria, methionine metabolism, renal failure, vascular disease.

Introduction

We have shown that cysteine–homocysteine mixed disulphide is always present in the plasma of normal man (Gupta & Wilcken, 1978) and that plasma concentrations of the principal sulphur-containing amino acids, except methionine but including cysteine–homocysteine, are increased in chronic renal failure (Wilcken & Gupta, 1979). In this study we have assessed the effect of regular haemodialysis on plasma concentrations of these sulphur amino acids.

Patients and methods

We studied 19 patients of mean age (+/− SD) 42 + 14 years (14 women and five men) on chronic haemodialysis and compared the findings with those obtained in 22 normal volunteer subjects of similar age (43 ± 5 years) and sex distribution. The chronic renal failure was due to: analgesic nephropathy (six patients), glomerulonephritis (four), congenital renal malformation (four), bilateral renal calculi (two), Goodpasture's syndrome (one), diabetic nephropathy (one) and hypertensive nephropathy (one). The patients had been on regular haemodialysis from 2 to 74 months every second day for 3–4 h. Protein intake was 40 g daily in six patients, 50 g in ten and 60 g in three.

Venous blood samples were drawn immediately before and immediately after haemodialysis. The patients were in the post-absorptive state and did not eat during dialysis. The plasma was immediately separated, deproteinized with sulphosalicylic acid and stored at −30°C until analysed. Neutral and acidic amino acids were measured by ion-exchange chromatography with a JEOL amino acid analyser (JLC-6AH) and lithium buffers as described previously (Gupta & Wilcken, 1978).
The values obtained for neutral and acidic amino acids measured in fasting normal subjects and renal patients before and after haemodialysis

Mean values ± SD are shown. β-Aminoisobutyric acid was detected in all pre-dialysis samples in a mean concentration of 11.9 ± 10.4 μmol/l (n = 16), excluding three patients with very high values (see the text). Homocystine was also detected in concentrations between 0.5 and 0.8 μmol/l (n = 10). Neither was identified in the normal subjects. P₁ refers to comparisons between concentrations in the pre-dialysis samples and the normal subjects and P₂ to those between pre- and post-dialysis concentrations.

### Table 1. Plasma concentrations of basic and neutral amino acids measured in fasting normal subjects and renal patients before and after haemodialysis

<table>
<thead>
<tr>
<th>Plasma concentration (μmol/l)</th>
<th>Normal subjects (n = 22)</th>
<th>Renal patients: pre-dialysis (n = 19)</th>
<th>Renal patients: post-dialysis (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>83.9 ± 37.0</td>
<td>135.1 ± 70.6</td>
<td>103.2 ± 54.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>134.3 ± 33.7</td>
<td>126.5 ± 27.0</td>
<td>95.4 ± 27.2</td>
</tr>
<tr>
<td>Serine</td>
<td>122.7 ± 26.0</td>
<td>82.2 ± 21.4</td>
<td>66.3 ± 17.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>149.5 ± 39.1</td>
<td>11.4 ± 6.2</td>
<td>12.1 ± 6.3</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>22.9 ± 8.7</td>
<td>117.9 ± 42.2</td>
<td>148.1 ± 29.3</td>
</tr>
<tr>
<td>Valine</td>
<td>235.4 ± 50.4</td>
<td>189.6 ± 65.7</td>
<td>83.3 ± 27.5</td>
</tr>
<tr>
<td>Cystine</td>
<td>63.5 ± 15.7</td>
<td>62.4 ± 16.3</td>
<td>62.4 ± 16.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>25.9 ± 6.4</td>
<td>82.2 ± 30.2</td>
<td>52.7 ± 14.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>69.0 ± 20.3</td>
<td>66.4 ± 47.5</td>
<td>64.8 ± 13.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>62.4 ± 16.3</td>
<td>16.9 ± 6.9</td>
<td>15.7 ± 189.6</td>
</tr>
<tr>
<td>Cysteine–homocysteine</td>
<td>125.1 ± 32.0</td>
<td>22.8 ± 7.1</td>
<td>14.6 ± 52.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.3 ± 3.7</td>
<td>6.3 ± 3.7</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>134.3 ± 33.7</td>
<td>39.7 ± 23.9</td>
<td>32.1 ± 17.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>56.2 ± 12.7</td>
<td>58.5 ± 21.6</td>
<td>52.7 ± 14.6</td>
</tr>
</tbody>
</table>

The normal subjects were studied after an overnight fast. They all had normal renal function and haematological and biochemical profiles, which included measurements of serum creatinine, urate and blood urea by standard methods. In the renal patients, pre- and post-dialysis body weight, serum creatinine, haemoglobin and packed cell volume were also recorded. The significance of differences in the grouped data was assessed by using Student’s t-test.

### Results

The values obtained for neutral and acidic amino acids measured in renal patients and normal subjects are shown in Table 1. With regard to the sulphur-containing amino acids, pre-dialysis concentrations of cysteine–homocysteine, cystine and taurine were increased, but that of methionine was normal. Homocystine was detected before but not after dialysis in 10 of the 19 patients. The concentrations were low, being 0.5 μmol/l in two patients and between 0.5 and 0.8 μmol/l in the remaining eight patients. Homocystine was not detected in any normal subject. Of the other amino acids, glycine was increased, serine, α-aminobutyric acid, valine, leucine and tyrosine were reduced, and threonine, isoleucine and phenylalanine concentrations were normal. β-Aminoisobutyric acid, which was not identified in any normal subject, was found in all pre-dialysis samples; concentrations were high in three patients in whom the values were 131.1, 117.6 and 65.4 μmol/l. Dialysis reduced these to 137.1, 44.5 and 37.0 μmol/l respectively.

Dialysis markedly reduced the elevated concentrations of cysteine–homocysteine and cystine (Table 1). Although haemodialysis reduced substantially the concentrations of the other elevated amino acids, it decreased those with normal or low pre-dialysis concentrations to a much lesser degree. Mean pre- and post-dialysis creatinine concentrations (± SD) for the group were 0.93 ± 0.22 and 0.42 ± 0.15 mmol/l respectively representing a reduction of 55 ± 6% compared with reductions of 41 and 58.5% respectively for cysteine–homocysteine and cystine (P < 0.001 for each). Dialysis was associated with 2.9 ± 2.6% reduction in body weight and 7.5 ± 7% increase in haemoglobin, consistent with removal of water.

### Discussion

The study shows that patients on regular, alternate-daily haemodialysis have substantially increased plasma cysteine–homocysteine concen-
Cysteine—homocysteine in haemodialysis

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Cysteine—homocysteine, which was not detected in any normal subject, was present in 10 of the 19 patients. As in our previous study of patients with less severe renal failure not requiring haemodialysis (Wilcken & Gupta, 1979), the plasma concentrations of the other principal sulphur-containing amino acids, except methionine, were also increased. Since a brief period of dialysis reduces these amino acids virtually to normal, there must be rapid accumulation in the plasma of these sulphur-containing amino acids in dialysis patients. The reasons why sulphur-containing amino acids might accumulate in azotaemia were reviewed in our earlier report (Wilcken & Gupta, 1979). In the present study greatly reduced or absent renal function and reduced sulphur excretion is presumably the principal mechanism.

Our results for the other amino acids measured (Table 1) are similar to those already reported in the literature on plasma amino acid changes in chronic renal failure (Chami, Reidenberg, Wellner, David, Rubin & Stenzel, 1978; Kopple, Jones, Fukuda & Swendsen, 1978). Of interest is the detection of substantial amounts of β-aminoisobutyric acid, in confirmation of the recent demonstration that plasma β-aminoisobutyric acid is increased in uraemia (Gejyo, Kinoshita & Ikenaka, 1976; Wilcken & Gupta, 1979), reflecting presumably increased tissue breakdown.

There is a moderate loss of amino acids during haemodialysis, approximately 0.5 g/h, which should be replenished easily from dietary sources (Aviram, Peters & Gulassy, 1971; Kopple, Swendsen, Shinaberger & Umezawa, 1973). Although important determinants of the loss during dialysis of individual amino acid solutes are molecular weight and the diffusion coefficient in water (Aviram et al., 1971), the plasma content is the main factor; the quantity removed is proportional to the pre-dialysis plasma concentration (Kopple et al., 1973). Of the sulphur-containing amino acids, in our study cystine must have constituted the principal loss. Plasma amino acids are quantitatively only a small part of the body pool of free amino acids, so that losses from the plasma during dialysis are restored, presumably, by mobilization from tissue pools (Bergström, Furst, Noree & Vinnars, 1978). Bergström et al. (1978) found increased amounts of both cystine and taurine in intracellular water of uremic patients and a large gradient between the elevated plasma concentrations and intracellular water concentrations. Therefore, removal from the plasma during dialysis is followed by re-equilibration with the total body pool within at least 48 h, since, in our study, this was the time interval between treatments.

Finally, we wished to see whether cysteine—homocysteine mixed disulphide concentrations were increased despite regular dialysis, since this could constitute a possible additional mechanism contributing to the largely unexplained premature development of vascular disease in these patients (Lowrie, Lazarus, Mocelin, Baily, Hampers, Wilson & Merrill, 1973; Linder, Charra, Sherrard & Schribner, 1974; Wilcken & Gupta, 1979). The plasma concentrations of cysteine—homocysteine mixed disulphide and homocysteine measured by the amino acid analyser reflect those of plasma homocysteine in vivo, since the latter is readily oxidized (Perry, Hansen, MacDougall & Warrington, 1967). Chronic infusions of homocysteine in baboons producing plasma concentrations of between 100 and 200 μmol/l have been reported to produce atherosclerotic lesions after 3 months (Harker, Ross, Slichter & Scott, 1976). These concentrations are about twice those we found in untreated homocystinuric children, in whom there is a high incidence of vascular disease (McKusick, 1972). The significance in relation to vascular disease of the very much lower concentrations identified in the present study can only be speculative.

Acknowledgments

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References


