INTRACELLULAR FREE AMINO ACIDS IN PATIENTS TREATED WITH REGULAR HAEMODIALYSIS (HD)

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Summary

Plasma and intracellular free amino acids were studied in seven haemodialysis patients with a protein and energy intake of >1g/kg BW/day and >145kJ/kg BW/day, respectively. Abnormalities in intracellular free amino acids known to be associated with uraemia, (low concentrations of threonine, valine and tyrosine) were not observed in the well-nourished haemodialysis patients. Other intracellular amino acid abnormalities were still present, suggesting that these are caused by uraemic factors not controlled by dialysis.

Introduction

Uraemic patients often exhibit signs of malnutrition and recent reports have shown that malnutrition also exists in dialysis patients on diets considered adequate for normal man [1–6]. It has also been found that patients on maintenance dialysis lose amino acids (AA) by the dialysis procedure [7–10], thus contributing to the protein depletion. There are numerous reports of abnormal plasma amino acid patterns in patients with renal failure [1–3, 5, 11–13]. This abnormal pattern is similar whether the patients are treated conservatively or by haemodialysis (HD) and it has been suggested that these derangements are related to inadequate nutrition.

The concentration of several free AA is considerably higher in the cells than in the extracellular fluid [14–16], and skeletal muscle is by far the largest intracellular pool. We have previously reported gross abnormalities in the free amino acid pattern of muscle in severely uraemic patients [11–13]. Some abnormalities such as low intracellular concentrations of valine and threonine were not corrected by long-term treatment with an 18g protein diet supplemented with EAA (2–3 times minimum requirements according to Rose) [12, 13] or by peritoneal dialysis [11].

This is a report of the extracellular and intracellular free amino acid patterns in apparently well-nourished uraemic patients treated with HD.
Patients and Methods

Seven patients who had been treated with HD for a minimum of one year were studied. The patients were dialysed 3—6 hours, three times per week, using parallel flow dialysers (Gambro Optima 13μ or 17μ). All patients were in good clinical condition. The dietary intake of protein and energy was calculated by our dietician to be more than 1g/kg BW/day and 145kJ/kg BW/day, respectively. Samples were taken from the quadriceps femoris muscle by percutaneous needle biopsy [17] after an overnight fast 12—20 hours after haemodialysis. Samples of plasma for free amino acid determination were obtained at the time of the biopsy. The method for determination of free amino acids in muscle and plasma has been described [16] as have also the calculations of the extracellular and intracellular water contents and the intracellular amino acid concentrations, based on the chloride method [16, 18, 19].

Plasma and intracellular free amino acid concentrations in the HD patients were compared with those in healthy subjects (n=40), non-dialysed uraemic patients, either 'untreated patients' without dietary restrictions or kept on a 40g protein diet (n=8), and patients treated with an 18g protein diet supplemented with a new AA preparation (n=8). This new formula contains the EAA in different proportions from those recommended by Rose [20].

Results

The concentrations of the branched-chain AA (Figure 1) were normal in intracellular fluid in the HD patients. The concentration of isoleucine was slightly elevated in plasma whereas the valine and leucine concentrations were normal.

FREE AMINO ACIDS IN UREMIA
Mean ± SE

Figure 1. The concentrations in plasma and intracellular water of the branched-chain amino acids
FREE AMINO ACIDS IN UREMIA
Mean ± SE

MUSCLE mmol/l ICW

Healthy subjects
Untreated uremia
18 g protein diet + EAA (New Formula)
HD-patients

PLASMA mmol/l

THREONINE
LYSINE
HISTIDINE

Figure 2. The concentrations of threonine, and histidine in plasma and intracellular water

FREE AMINO ACIDS IN UREMIA
Mean ± SE

MUSCLE mmol/l ICW

Healthy subjects
Untreated uremia
18 g protein diet + EAA (New Formula)
HD-patients

PLASMA mmol/l

TYROSINE
PHENYLALANINE

Figure 3. The concentrations of tyrosine and phenylalanine in plasma and intracellular water
FREE AMINO ACIDS IN UREMIA
Mean ± SE

MUSCLE mmol/l ICW

PLASMA mmol/l

CITRULLINE ORNITHINE ARGinine

Healthy subjects Untreated uremia

18 g protein diet + EAA (New Formula) HD-patients

Figure 4. The concentrations of citrulline, ornithine and arginine in plasma and intracellular water.

The intracellular concentration of threonine was normal in the HD patients whereas the concentrations of lysine and histidine were elevated (Figure 2). In plasma the concentration of histidine was found to be high.

In the HD patients, the intracellular concentration of tyrosine was normal, but the concentration was elevated in plasma (Figure 3); the concentration of phenylalanine was high both in intracellular water and in plasma (Figure 3).

The concentrations of the three AA involved in the urea cycle, citrulline, ornithine and arginine, were high in both plasma and muscle in the HD-patients (Figure 4).

The plasma concentrations of methionine and several non-essential AA (taurine, aspartate, asparagine, glutamine, proline, glycine, alanine), were found to be high. High concentrations of aspartate, glycine and alanine were also found in the intracellular fluid.
Discussion

For the first time it has been shown that abnormalities in intracellular free AA known to be associated with uraemia can be normalised by haemodialysis and/or adequate nutrition.

We have earlier found low concentrations of valine in plasma and muscle of severely uraemic patients [11–13]. The plasma concentration of leucine was low, but the intracellular concentration of leucine was normal (in untreated patients, see Figure 1) or high (in patients treated with chronic peritoneal dialysis (PD)) resulting in a considerable increase in concentration gradient across the muscle cell membrane [11]. These abnormalities in branched-chain AA were only partly corrected after long-term dietary treatment with a protein-restricted diet supplemented with EAA (2–3 times Rose’s minimum requirements + histidine) [12, 13]. Imbalances and/or antagonism between these AA may induce toxic effects and decrease protein synthesis [21]. Long-term treatment with this dietary regimen also failed to correct the low intracellular threonine concentration, while intracellular depletion of tyrosine occurred [12, 13], suggesting that the requirements of these AA are increased in uraemia. This conclusion is supported by the observation that treatment with our new AA preparation with increased proportions of valine and threonine, and with addition of tyrosine resulted in normal intracellular concentrations of valine, threonine and tyrosine (Figures 1–3) and improvement of nitrogen balance [20].

Our finding that the HD patients have normal intracellular concentrations of EAA is strong evidence that the nutrition was adequate in relation to their requirements. Such patients do not need specific supplementation with EAA to compensate for abnormalities or imbalances in EAA metabolism, provided that they eat enough protein (>1g/kg BW/day) and are not in a hypercatabolic state. Presumably, the HD patients are less intoxicated by uraemia and have less deranged AA metabolism than non-dialysed patients with end-stage renal failure.

The high intracellular phenylalanine concentration was probably caused by an inhibition of the hydroxylation of phenylalanine to tyrosine, which has previously been described in uraemia [22, 23].

Abnormalities in the metabolism of the urea cycle intermediates have been observed in experimental uraemia [24]. Increased concentrations in plasma and in intracellular water of the AA involved in the urea cycle were found in untreated uraemic patients and also after long-term treatment with protein-restricted diets and EAA [11–13]. These abnormalities were not corrected in the HD patients or in the patients treated with the new AA preparation [20], indicating that these derangements are caused by uraemic factors which are not controlled by dialysis or diet, but are related more to the impaired kidney function per se. In conclusion; abnormalities in intracellular concentrations of free AA may be reversed by HD treatment and adequate nutrition. Thus, HD patients who are able to eat well appear not to require an additional supply of EAA or anaologues.

Acknowledgments

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References

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Open Discussion

CAMBI (Parma) Low serum levels of pyridoxine can worsen the amino acid balance in uraemic patients. Is it possible that in well nourished patients a higher level of this vitamin can explain the better results more than a difference in protein intake?

ALVESTRAND No, I don’t think that a higher intake of pyridoxine can explain the normalisation of the intracellular amino acid abnormalities. The reason for this is that abnormalities thought to be associated with malnutrition were not corrected in patients who were treated with an identical protein-restricted diet as that referred to in this paper but one that was supplemented with an essential amino acid preparation of a different composition. All our patients with uraemia, even those referred to as untreated in this paper, are treated with a multivitamin preparation containing large amounts of pyridoxine. Thus we don’t think that deficiency of this vitamin is of any importance for the amino acid abnormalities in the non-dialysed patients.