INFLUENCE OF ESSENTIAL AMINO ACIDS AND KETO ACIDS ON PROTEIN METABOLISM AND THE ANAEMIA OF PATIENTS ON CHRONIC INTERMITTENT HAEMODIALYSIS

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Summary

Ten patients were treated with 10 g essential amino acids per day orally; 9 patients received 9.5 g of a mixture of essential amino acids (Lys, Thr, Try, His, Tyr) and ketoanalogues of Ile, Leu, Phe, Val, Met per day and a control group of 11 patients received no supplementation. All patients were on a liberal food intake amounting to 1 g protein/kg body weight and 31 kcal/kg body weight daily. Before and three months after the beginning of supplementation the following parameters were measured: serum concentrations of albumin, transferrin, urea, creatinine, blood haemoglobin content and haematocrit, activities of the enzymes δ-aminolevulinic acid dehydrase and porphobilinogen desaminase, and globin synthesis in peripheral red blood cells. After treatment with either essential amino acids or keto acids a significant stimulation of globin synthesis occurred. None of the other parameters was altered. It is concluded that in well-nourished patients supplements of essential amino acids or keto acids are ineffective.

Introduction

It has been suggested that both the biochemical and clinical condition of uraemic patients on a low protein diet could be improved by supplements of essential amino acids1,2,3. Heidland et al4 observed a beneficial effect of infused essential amino acids on protein metabolism and on anaemia even in patients on an adequate protein and food intake.

The keto acid analogues of some essential amino acids augment the reutilisation of nitrogen-containing retention products by transamination to the corresponding essential amino acids. Therefore, it has been proposed that keto acids should be administered rather than the essential amino acids themselves5,6. We administered essential amino acids and keto acids to dialysis
patients for two reasons:

1 Despite adequate food and protein intake most of our patients had serum transferrin levels lower than normal. We wanted to know whether supplementation with essential amino acids or keto acids could improve this disturbance.

2 Our previous investigations had shown that, in uraemia, the reaction chain of haemoglobin synthesis is impaired at three levels: the synthesis of porphobilinogen, the conversion of porphobilinogen to porphyrins, and globin synthesis. Since a significant improvement of uraemic anaemia has been reported after the administration of essential amino acids or keto acids$^4,5$ we wanted to know whether one or the other impaired reaction could be stimulated by such treatment.

Methods

Thirty patients on chronic intermittent haemodialysis were randomised into three groups after a dietary protocol had established that protein and food intake did not differ significantly among the patients (1 g protein/kg x day, 31 kcal/kg x day). Ten patients received daily 10 g essential amino acids orally (EAS oral, Fresenius). Nine patients received a mixture of essential amino acids (Lys, Thr, Tyr, His, Try) and keto analogues of essential amino acids (keto analogues of Ile, Leu, Phe, Val, Met), administered orally (Ketosteril, Fresenius, 9.5 g/day). Eleven patients received no supplementation (control group). All 30 patients were seen regularly by nutritionists to ascertain that the desired food and protein intake was maintained throughout the experimental period.

All the patients were dialysed either 3 x 4 hours weekly with the EX 29 1.4 m$^2$ coil dialyser (Extracorporeal) or 3 x 6 hours using the 1.0 m$^2$ Lundia Nova parallel flow dialyser (Gambro) throughout the investigation. Between these techniques there was no difference in amino acid losses during dialysis (unpublished).

Before and three months after the beginning of the supplementation, the following parameters were measured: haemoglobin, haematocrit, serum albumin, urea, creatinine and transferrin, using routine methods$^7$.

Estimation of the enzymes δ-aminolevulinic acid dehydrase and porphobilinogen desaminase were determined as indicated previously$^8$. The globin synthesis was measured according to a method published earlier$^9$. Statistical evaluations were carried out using variance analysis.

Results and Discussion

At the beginning of treatment ($t_0$) the lowest plasma creatinine and urea concentrations were found in the control group. Neither treatment had any influence on the plasma creatinine or urea concentrations. The rise of creatinine concentration in the control group is not significant. From our
results we have no evidence that keto acids augment the reutilisation of nitrogen containing waste metabolites (Table I).

Though the plasma transferrin levels in all groups were significantly lower than normal, neither essential amino acids nor keto acids caused an improvement in the transferrin concentration. The serum albumin concentration was not altered either.

<table>
<thead>
<tr>
<th>TABLE I. For explanation, see text. ( t_s ) = beginning of treatment. ( t_t ) = after 3 months treatment. KA = group treated with keto acids. EAA = group treated with essential amino acids</th>
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<tbody>
<tr>
<td>Control ( n = 11 )</td>
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</tr>
<tr>
<td>( t_s )</td>
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<tr>
<td>Urea (mg%)</td>
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<td>Creatinine (mg%)</td>
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<tr>
<td>Albumin (g/l)</td>
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<tr>
<td>Transferrin (g/l)</td>
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In a preceding paper it had been demonstrated that plasma amino acid concentrations showed minor alterations in our dialysis patients compared with healthy subjects. The amino acid pattern was not influenced by treatment with essential amino acids or keto acids.

Summarising, none of the measured parameters involved in protein metabolism was affected by either treatment (Table II).

During the experimental period the haemoglobin concentration rose insignificantly in all patients whether supplemented or not. Therefore the improved haemoglobin levels cannot be attributed to the treatment. In accordance with that conclusion, the haematocrit values were not influenced in any group.

Neither synthesis of porphobilinogen nor the conversion of porphobilinogen to porphyrins was affected by essential amino acids or keto acids.

Only the level of globin synthesis by peripheral red blood cells was stimulated by essential amino acids and keto acid supplementation. Our assay system was independent of the plasma amino acid pattern, because all the amino acids required for optimal protein synthesis are added to the incubation mixture. Therefore it is assumed that treatment with essential amino acids or keto acids affects the enzymes involved directly, the underlying
<table>
<thead>
<tr>
<th></th>
<th>Control n = 11</th>
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<th>KA n = 9</th>
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<th>EAA n = 10</th>
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<tr>
<td></td>
<td>t₀</td>
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<tr>
<td><strong>Haemoglobin (g/100 ml)</strong></td>
<td>8.2 ± 2.5</td>
<td>10.7 ± 2.1</td>
<td>6.6 ± 1.2</td>
<td>8.0 ± 1.9</td>
<td>7.8 ± 1.6</td>
<td>9.5 ± 2.0</td>
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<td><strong>Haematocrit (%)</strong></td>
<td>23.1 ± 2.6</td>
<td>27.0 ± 5.4</td>
<td>22.3 ± 3.2</td>
<td>22.1 ± 5.9</td>
<td>24.0 ± 7.0</td>
<td>27.0 ± 9.0</td>
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<td><strong>Δ-ALA-dehydrase (nmoles PBG/h x 10⁷ reticulocytes)</strong></td>
<td>10 ± 3.5</td>
<td>11 ± 3.8</td>
<td>9.5 ± 4</td>
<td>12.0 ± 3.8</td>
<td>10.1 ± 3</td>
<td>13.2 ± 4</td>
</tr>
<tr>
<td><strong>PBG-desaminase (nmoles porphyrins/h x 10⁷ reticulocytes)</strong></td>
<td>12 ± 4</td>
<td>11 ± 4.1</td>
<td>9.1 ± 3</td>
<td>11 ± 4</td>
<td>12 ± 3.0</td>
<td>9.5 ± 3</td>
</tr>
<tr>
<td><strong>Globin Synthesis (dpm/10 mg Globin x min)</strong></td>
<td>4.200±810</td>
<td>4.300±780</td>
<td>4.000±900</td>
<td>6.200±880</td>
<td>3.500±700</td>
<td>5.000±650</td>
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p < 0.01: + against o
biochemical mechanism being uncertain. Since the degree of anaemia was not influenced, despite an increase of globin synthesis it is concluded that globin synthesis is not the rate limiting step in haemoglobin synthesis. This is in agreement with Giordano's earlier findings that Histidine supplementation could improve globin synthesis without affecting the degree of anaemia. Obviously the impairment of porphyrin synthesis is a more important factor in the regulation of haemoglobin synthesis, than globin synthesis.

From our results it is concluded:
1 Although a significant stimulation of globin synthesis was observed after treating patients with essential amino acids or keto acids, no improvement of anaemia occurred. This finding supports the assumption that globin synthesis is not the rate limiting step in haemoglobin synthesis.
2 After treating dialysis patients on a free protein intake with essential amino acids or keto acids no clinically important alteration was observed; sub-normal transferrin levels were not influenced.
3 Finally, uraemic patients on an adequate protein and food intake do not require supplements of essential amino acids or keto acids.

References

3 Kopple, JD and Swendseid, ME (1977) *Nephron*, 18, 1

Open Discussion

GURA (Israel) We have been giving intravenous amino acids to four patients on chronic haemodialysis and we have quite different results. We had a decrease in predialysis K levels in all our four patients and we had an increase in haemoglobin in 3 out of 4 of our patients, therefore resulting in a decrease in the blood transfusion requirements that was quite substantial after the second month of treatment. Phosphate levels decreased in all four patients and one of them even developed hypophosphataemia which required treatment. One patient was crippled and bed-ridden because of severe soft-tissue calcifications. After three months he was walking with a stick and after six months he was walking freely and using his stick to beat his wife. I would rather conclude that if you choose your patients correctly, there are some that might enjoy treatment with amino acid supplementation.
LEBER We were discussing your results in Würzburg last week. There was a marked difference from our patients. You had patients with haemoglobin levels down to about 5.2 - 5.5 if I read your slides correctly. We did not have such patients. I agree that there might be patients who are not able to ingest adequate food intake or protein intake for whom this supplement, especially as infusions, might be useful, but I repeat that in our opinion the well-nourished patients do not need these supplements.

MATTHEI (Göttingen) Dr Ebert from our department of gastroenterology has observed that α-keto isocaproic acid is a stimulator of insulin release in isolated islets. Might the stimulated insulin output after keto acids explain the increase of globin synthesis observed in your experiments?

LEBER I do not think so. This measurement of globin synthesis is done in vitro. They are washed blood cells, incubated in an adequate medium with the amino acids required, and we are measuring the incorporation of histidine. This could only be an indirect effect, that the blood cells are positively influenced by increased insulin release. I cannot imagine this.