Preliminary Report

The influence of bicarbonate supplementation on plasma levels of branched-chain amino acids in haemodialysis patients with metabolic acidosis

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Abstract

Background. It has been hypothesized that correction of metabolic acidosis might improve the nutritional state of acidotic haemodialysis (HD) patients partly because of a reduced oxidation of branched-chain amino acids (BCAA).

Aim. We investigated whether bicarbonate (Bic) supplementation in acidotic HD patients results in increased plasma levels of BCAA.

Methods. In a longitudinal study (run-in period, 2 months; study period, 6 months), the effect of Bic supplementation on plasma levels of BCAA was studied in 12 acidotic HD patients (7 men, 5 women, mean age 54 ± 18 years) with a predialysis bicarbonate (Bic) concentration smaller or equal to 22 mmol/l. Bic was supplemented by increasing Bic concentration of the dialysate and by oral Bic supplementation.

Results. Predialysis Bic increased significantly during the study period (18.7 ± 2.7 vs 23.1 ± 11.5 mmol/l). There was no change in nutritional parameters. However, plasma levels of the BCAA valine, leucine, and isoleucine increased significantly.

Conclusions. In haemodialysis patients with metabolic acidosis, Bic supplementation over a 6-months period resulted in an increase in plasma levels of BCAA. Further study is needed to elucidate the mechanisms behind, and the clinical importance of the observed changes in plasma BCAA levels.

Introduction

Protein–calorie malnutrition is common in haemodialysis patients [1]. As recent studies suggest, it may be an important risk factor for mortality and morbidity in haemodialysis patients [2,3].

Malnutrition in patients with chronic renal failure is characterized by a reduction in muscle mass [4]. However, even in patients without clinical signs of protein–calorie malnutrition, abnormalities of amino-acid metabolism may already be present. For example, reduced extra- and intracellular levels of several essential amino acids have been observed in haemodialysis patients [5,6]. Especially the altered metabolism of branched-chain amino acids, which are important precursors for protein synthesis, might have important implications for the nutritional status of dialysis patients.

Recent evidence suggests that metabolic acidosis is an important catabolic factor in patients with chronic renal failure. Acidosis may lead to protein breakdown by stimulating ATP-dependent protein degradation via the ubiquitin pathway [7]. Moreover, acidosis could contribute to protein–calorie malnutrition by a direct effect on amino-acid metabolism. Acidosis increases the oxidation of branched-chain amino acids (BCAA) [8], which may explain the correlation between plasma bicarbonate (Bic) levels and intracellular valine in haemodialysis patients observed by Bergström et al. [5].

Therefore correction of acidosis may improve the nutritional status of haemodialysis patients both by a reduction in protein catabolism and a reduced oxidation of BCAA. The aim of the present study is to investigate the effect of Bic supplementation on plasma levels of BCAA.

Subjects and methods

Patient characteristics

Twelve haemodialysis patients with a predialysis plasma Bic concentration less than or equal to 22 mmol/l were studied (7 males; 5 females). The primary aetiology of renal disease was chronic glomerulonephritis in three patients, tubulointerstitial disease in two, renal cystic disease in three, renal vascular disease in two, and unknown in two patients. No patient had diabetes mellitus. Four patients still had residual
renal creatinine clearance (mean 2.40, SD 0.54, range 1.9–3.0 ml/minute). Eleven patients were administered phosphorus binders, and 10 patients erythropoietin. Two patients already used oral sodium bicarbonate before the start of the study. All patients were assigned to a diet of 1.0 g protein/kg/day. Clinical and laboratory characteristics of the patients at the start of the study are summarized in Table 1.

All patients were clinically stable and did not suffer from malignancies or other severe concurrent illness. During the study period, one patient suffered from depression after the death of her husband.

The study was approved by the ethics committee of the St Joseph Hospital in Veldhoven.

**Dialysis characteristics**

Fresenius 2008C or Monitrail DG-30® (Hospal) dialysis monitors and polyethyglycol (Asahi; Biowet®) membranes were used. Dialysis fluid contained a sodium concentration of 140 mmol/l, a potassium concentration of 2 mmol/l, a calcium concentration of 1.5 mmol/l and a glucose concentration of 7 mmol/l. Dialysate Bic concentration at the start of the study was 32 mmol/l. The dialysis frequency was twice weekly in two patients and three times weekly in the others.

**Study protocol**

The study was started after a baseline period of 2 months. The mean serum Bic level at the start of the study was 18.7 (SD 2.7, range 16–22) mmol/l (Table 1).

At the start of the seven study patients had a predialysis plasma Bic level of less than 20 mmol/l and five patients had predialysis plasma Bic levels 20–22 mmol/l. The goal was to increase the predialytic Bic level to at least 22 mmol/l and 24 mmol/l respectively. In order to achieve this, dialysate Bic was increased to 35 mmol/l (Monitrail SC-30) or 36 mmol/l (Fresenius 2008-C). When the treatment goal was not achieved after 3 months, oral sodium Bic supplementation was started (6 patients) at a dose ranging from 500 to 1000 mg t.i.d. respectively.

Measurements were performed at the start of the baseline period (t = −2 months), at the start of the study (t = 0 months), and 3 and 6 months later.

In order to prevent the effect of haemodilution, blood samples for albumin and transferrin measurements were taken after a haemodialysis session because of clearance during haemodialysis [9].

Blood samples for the measurement of plasma Bic were obtained after the insertion of the dialysis needle and at the end of haemodialysis. Bic levels were assessed in anaerobic serum using a colorimetric technique (Kodak®). Albumin was measured using the bromcresol green method. Blood samples for analysis of BCAAs were taken after an overnight fast. For this purpose, ice-chilled heparin blood, drawn from the fistula of the patient, was centrifuged for 5 min (11 500 r.p.m.) at 4°C. One hundred microlitres of plasma was stirred in Eppendorf tubes with sulphosalicylic acid, to deproteinize the sample. Thereafter the fluid was stored at −80°C until the sample was analysed. It has been shown that this type of storage does not influence amino-acid determination [10]. BCAAs were measured by a HPLC method using a Spherisorb ODS II column. The coefficient of variation of this method is below 3% [11].

Urea kinetics were calculated using a three-sample, single-pool, variable-volume model for estimation of protein catabolic rate (PCR) and Kt/V [12]. PCR was corrected for actual body-weight. Protein and calorie intake were assessed using dietary interviews conducted by an experienced dietician. These values were also corrected for actual body-weight. Anthropometric and bio-impedance measurements were performed after haemodialysis at dry weight. Volume status was assessed by echography of the inferior vena cava [13].

The following anthropometric variables were measured: triceps skinfold thickness as an index of body fat and mid-arm muscle circumference (calculated as mid-arm circumference – nTSF) as index of muscle mass. Anthropometric measurements were obtained using standard techniques by a single experienced observer [14].

Bioimpedance (BIA) measurements were performed using a Xitron® 4000B multifrequency bioimpedance analyser. The electrodes were placed contralateral to the site of vascular access. A range of frequencies between 5 and 500 kHz was used. Estimations of extracellular and intracellular volume and fat-free and fat mass were calculated by equations provided by the manufacturer. Because these estimations have only been validated in healthy subjects, the absolute values for intra- and extracellular resistance are presented. Intra-day coefficient of variation for two subsequent measurements was below 3%.

**Statistical analysis**

For all parameters, overall differences between the various points in time (t = −2, t = 0, t = 3 and t = 6 months) were analysed using Friedman’s analysis of variance for non-parametric data. When this result was statistically significant (P < 0.05), variables were included in the further statistical analysis.

In addition, Friedman’s analysis of variance was implemented to assess the differences among t = −2, t = 3 and t = 6 months and among t = 0, t = 3 and t = 6 months, this in order to prevent false-positive results. Differences between two time points were analysed using the Wilcoxon test.

Parameters, which are graphically displayed in Figure 1, are shown as box-plots (the box encompasses the 25th, 50th and 75th percentile points, whereas the horizontal lines outside the box mark the extreme values.

**Results**

Table 2 displays the results of all data at the various time points. Both pre- and postdialysis plasma Bic levels increased significantly during the period of acid-base balance with a maximum plasma Bic level of 27.8 mmol/l (SD 1.1, range 24.0–30.6 mmol/l) at 6 months.

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or Median [Range]</th>
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<tr>
<td>Age (years)</td>
<td>53.7 (SD 17.8) [26–74]</td>
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<tr>
<td>Sex (M : F)</td>
<td>7:5</td>
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<tr>
<td>Time on dialysis (months)</td>
<td>71.4 (SD 81.6) [5–264]</td>
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<tr>
<td>Dry body-weight (kg)</td>
<td>66.2 (SD 8.0) [55.2–77.0]</td>
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<tr>
<td>Percentage of dry body-weight1 (%)</td>
<td>93.7 (SD 12.0) [76.8–123.8]</td>
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<tr>
<td>Body mass index (mm²/m2)</td>
<td>22.7 (SD 2.2) [19.3–22.7]</td>
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<tr>
<td>nProtein catabolic rate (g/kg/day)</td>
<td>1.03 (SD 0.29) [0.81–1.23]</td>
</tr>
<tr>
<td>Kt/V (week)</td>
<td>3.95 (SD 1.13) [2.38–5.79]</td>
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<tr>
<td>Serum albumin (g/l)</td>
<td>40.2 (SD 2.6) [37.1–45.1]</td>
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Values presented as mean (SD) or median [range]. 1Percentage of dry body-weight derived from NHANES data [19].
Branched-chain amino acids (BCAA)

The thickness did not change significantly during the study analysis, the results of the statistical analysis did not

in body composition, as assessed by BIA. Protein Moreover, no statistically significant di

tferences were

study period, which was due to depression after the death of

Fig. 1.

Anthropometry

MAMC (cm)

TSF (cm)

Serum proteins

Albumin (g/l)

Transferrin (g/l)

Bioimpedance anthropometry

REC
t (Ω)

Rt
 (Ω)

VECV (litre)

VICV (litre)

Dietary intake

PCRn (g

/kg/day)

Protein intake (g

/kg/day)

Calorie intake (kcal

/day)

Fluid status

Vena cava diameter (mm)

osmosis correction. The values obtained at t = 3 and 6 months were significantly greater than those at t = −2

and t = 0 months. Values obtained for predialysis plasma Bic levels at t = 0, 3 and 6 months are shown as box-plots in Figure 1.

Dry body-weight, MAMC, and triceps skinfold thickness did not change significantly during the study period. In addition, no significant change was observed in body composition, as assessed by BIA. Protein catabolic rate (nPCR), as a marker of protein intake,

and Kt/V also did not differ between the various points in time, nor did protein and calorie intake, as assessed by dietary interviews (Table 2). In one patient, protein intake and nPCR decreased markedly during the study period, which was due to depression after the death of her husband. When this patient was excluded from the analysis, the results of the statistical analysis did not change.

Moreover, no statistically significant differences were obtained for serum levels of albumin and transferrin,
nor for predialytic urea levels and haemoglobin during the various points in time (Table 2).

However, plasma levels of the BCAA leucine, isoleucine, and valine increased significantly during the study period, an effect which was already present after 3 months of acidosis correction (Table 2). There was no significant correlation between the plasma bicarbonate concentration and plasma levels of branched-chain amino acids at any point in time. The change in the cumulative plasma levels of BCAA during the study period is shown in Figure 1.

Discussion

In this prospective study we assessed the effect of Bic supplementation on plasma levels of BCAA over a 6-month period in patients with a predialysis Bic plasma concentration less than or equal to 22 mmol/l.

Bic was supplemented by increasing the Bic concentration of the dialysate and oral administration of Bic. Due to a lack of acidic patients in our dialysis population, it was not possible to construct a control group. Therefore, a 2-month baseline period was used to correct for spontaneous variations in plasma levels of BCAA. Plasma levels of BCAA remained fairly constant between the start and end of the baseline period.

As expected, plasma Bic levels increased significantly after Bic supplementation. Moreover, postabsorptive plasma levels of the BCAA valine, leucine, and isoleucine increased significantly, an effect which was already observed after 3 months. A drawback of our study is the fact that only plasma levels of BCAA were studied, whereas muscular levels of BCAA are probably more representative for BCAA metabolism at the intracellular level. However, the rise in plasma levels of BCAA after Bic supplementation probably reflects an important effect of acidosis correction on protein metabolism in dialysis patients. BCAA are important precursors for protein synthesis [15], which might explain the relationship between markers of nutritional status and plasma levels of valine, observed by Young et al. [16].

In patients with CRF, decreased plasma and muscle levels of BCAA have been well described. Although haemodialysis treatment partly reverses these abnormalities, decreased plasma and muscle levels of BCAA (especially valine) appear to be a general finding even after the start of renal replacement therapy [5,6]. It is well known from experimental studies that metabolic acidosis has an important effect on BCAA metabolism. BCAA are mainly metabolized by deamination to ketoanalogues followed by oxidation. In uraemic rats, acidosis-induced catabolism of BCAA is due to stimulation of branched-chain ketoacid dehydrogenase [17]. Therefore the increase in plasma levels of BCAA after acidosis correction can very well be explained by decreased oxidation. In clinical studies, further arguments for a relationship between metabolic acidosis and the metabolism of branched-chain amino acids was found by Bergström et al., who observed a significant correlation between intracellular valine levels and plasma bicarbonate in haemodialysis patients [5]. Moreover, Löfberg et al. observed an increase in intracellular levels of branched-chain amino acids in haemodialysis patients after correction of acidosis [18].

During this study we did not observe a change in markers of body composition and serum proteins. This is, however, not surprising in view of the fact that most of the patients were not severely malnourished and the degree of acidosis was generally mild.

In conclusion, plasma levels of BCAA increased during a period of acidosis correction in haemodialysis patients. Larger prospective studies in malnourished dialysis patients with uraemic acidosis are needed to elucidate the clinical importance of the effect of acidosis correction on nutritional status.

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References

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