RESEARCH BRIEF

The Effects of Parenteral Amino Acid Therapy on Protein Carbamylation in Maintenance Hemodialysis Patients

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Objective: Protein carbamylation is a urea-driven post-translational protein modification associated with mortality in dialysis patients. Free amino acids (AAs) are competitive inhibitors of protein carbamylation and animal studies suggest increasing AA concentrations reduces carbamylation burden. We hypothesized that AA therapy in maintenance hemodialysis patients would reduce carbamylation, carrying the potential to improve clinical outcomes.

Design: Prospective pilot clinical trial (NCT1612429).

Setting: The study was conducted from March 2013 to March 2014 in outpatient dialysis facilities in the Boston metropolitan area.

Subjects and Intervention: We enrolled 23 consecutively consenting hemodialysis subjects, infusing the first 12 individuals with 250 cc of AAs 3 times per week postdialysis over 8 weeks. The remaining 11 subjects served as controls.

Main Outcome Measure: Change in carbamylated albumin (C-Alb), a measure of total body carbamylation burden, between baseline and 8 weeks was the primary outcome.

Results: The treated and control groups had similar clinical characteristics and similar baseline C-Alb levels (mean ± SE 9.5 ± 2.4 and 9.3 ± 1.3 mmol/mol, respectively; $P = .61$). The treated arm showed a significant reduction in C-Alb compared with controls at 4 weeks (8.4% reduction in the treated arm vs. 4.3% increase in controls; $P = .03$) and the effect was greater by 8 weeks (15% reduction in the treated vs. 1% decrease in controls; $P = .01$).

Conclusion: In this pilot study, AA therapy appeared safe and effective at reducing C-Alb levels in hemodialysis patients compared with no treatment. The impact of reduced protein carbamylation on clinical outcomes should be further investigated.

Introduction

PROTEIN CARBAMYLATION DESCRIBES the nonenzymatic binding of urea-derived cyanate to free amino groups on proteins and, as might be predicted, protein carbamylation accumulates in patients with reduced kidney function.[1] A broad range of studies have demonstrated how carbamylation is capable of changing the charge, structure, and functional properties of specific proteins in the human body.[1] Such protein modifications, in turn, have been shown to trigger inappropriate molecular and cellular responses resulting in adverse clinical outcomes such as accelerated atherosclerosis and erythropoietin resistance.[2-4] Recently, using measurements of total-body carbamylation burden such as carbamylated albumin and homocitrulline, we and others have shown strong associations between excess protein carbamylation and mortality in several distinct hemodialysis cohorts.[5,6] Thus, protein carbamylation has been implicated as an important contributor to the excess morbidity and mortality observed in patients with chronic kidney disease.

Carbamylation modifications of amines are irreversible and proteins can accumulate these on their N-terminal α-amino groups or the ε-amino groups of lysine side chains throughout the lifespan of the protein. Similarly, *free* amino acids (AAs) can become carbamylated on their α-amino group or on the nucleophilic groups of their side-chains.[7-9] Cyanate's affinity for the α-amino groups on free AAs is far greater than that of lysine side-chains on proteins, and thus free AAs can act as natural ambient scavengers of carbamylation, in essence shielding proteins from undergoing the carbamylation modification.[7,10] The link between AA balance and carbamylation is particularly noteworthy in the hemodialysis population as free AAs can become depleted due to protein-energy wasting and

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through the dialysis procedure itself.[11-13] We recently reported that protein carbamylation in dialysis patients is strongly and inversely correlated with free AA levels.[5] Through in vivo mouse model experiments we have further shown that urea-induced protein carbamylation is significantly attenuated by AA supplementation in mice with AA deficiencies.[5] We therefore hypothesized that AA therapy, in select individuals undergoing routine hemodialysis, may reduce protein carbamylation burden. If so, such targeted therapy could be leveraged to reduce the risks of uremic complications that remain unacceptably high in patients on maintenance hemodialysis. To test this hypothesis, we used the first proof-of-concept investigation of AA therapy aimed at reducing protein carbamylation in hemodialysis patients (ct.gov NCT1612429).

Methods

The primary objective of this study was to test if parenteral AA therapy, using doses safely incorporated into intradialytic parenteral nutrition in other studies,[14,15] could decrease carbamylation burden as measured by carbamylated albumin.

Subjects

Twenty-five subjects were recruited from local outpatient dialysis centers. All subjects were maintenance hemodialysis patients initially identified as appropriate for the study by their treating physicians. Because the parameters of carbamylation response to AA therapy were unknown, baseline carbamylation levels were not part of the inclusion criteria. Rather, inclusion criteria included age between 18 and 80 years old and no overt indication of malnutrition: body mass index (BMI) <20 kg/m^2, body weight loss within 6months $>$ 10%, and serum albumin <3.0 mg/dL. Exclusion criteria were weekly dialysis time <12 h, urea Kt/V $<$ 1.2, and comorbidities compromising 1-year survival prognosis. For this open label pilot study, 14 prevalent hemodialysis subjects were enrolled to undergo thrice weekly postdialysis AA infusions over 8 weeks. Two subjects withdrew from the study: one due to loss of interest in participation and the other due to unrelated medical reasons. Eleven additional patients were then recruited to serve as controls, receiving no treatment and only having carbamylated albumin levels measured at designated time points. All participants provided written informed consent. This study was approved by the Partner's Human Research Committee Institutional Review Board.

Treatment and Outcomes

Individuals receiving treatment were given 250 cc of AA infusions at the end of dialysis. Each 250 cc dose contained 14 g of essential AAs yielding the recommended daily intake of essential AAs (precise composition can be found in Table 1; Food and Drug Administration Investigational New Drug exemption granted).[16] Our primary outcome was change in carbamylated albumin (mmol/mol) over 8 weeks representing a period of greater than 2 half lives of albumin, theoretically allowing enough albumin turnover to reflect a new carbamylation environment. Carbamylated albumin and free amino acids were measured by high-performance liquid chromatography and mass spectrometry using methods as previously described (coefficient of variation for the carbamylated albumin assay of 4.2%).[5] The investigator assaying blood samples was blinded to the subjects' treatment or control status. All subjects received routine nutritional counseling from a registered dietitian. No specific dietary instructions were given though subjects were asked to refrain from taking any additional nutritional supplements during the study period.

Table 1. Amino Acid Content per 250 cc Infusion

Amino Acid	Amount per Treatment (g)	Minimum Recommended Daily Allowance (g/d)
Tryptophan	0.50	0.25
Phenylalanine	2.20	1.10
Lysine	1.60	0.80
Threonine	1.00	0.50
Methionine	2.20	1.10
Leucine	2.20	1.10
Isoleucine	1.40	0.70
Valine	1.60	0.80
Histidine	0.63	–
Cysteine	0.04	–

Statistics

Given the small sample size, statistical evaluation of our data was performed using Mann-Whitney U tests at baseline, 4 weeks, and 8 weeks comparing the AA treated versus control groups for carbamylated albumin level as well as other prespecified clinical indices (predialysis urea level, serum albumin, hemoglobin, cardiac and inflammatory markers, and average essential AA levels). Within group comparisons used Friedman and Wilcoxon signed-rank tests as appropriate. $P<.05$ was considered statistically significant. $P<.15$ was considered a trend towards significance. All values are expressed as the mean (standard error) or a percentage. Baseline AA levels were compared with baseline carbamylation levels using Pearson correlations. All statistics were performed using SAS (v9.2, SAS Institute, Cary, NC).

Results

The baseline characteristics of the treated and control groups were similar (Table 2) and baseline AA levels, on average, were negatively correlated to baseline carbamylated albumin levels (average Pearson correlation coefficient for all essential AAs $= -0.25$; Table 3 for complete data). The treated and control groups had similar baseline carbamylated albumin levels (9.5 ± 2.4 and 9.3 ± 1.3 mmol/mol, respectively; $P = .61$; Table 4). The treated arm showed a significant reduction in