Published in final edited form as:

J Ren Nutr. 2015 July ; 25(4): 388–392. doi:10.1053/j.jrn.2015.01.019.

The effects of parenteral amino acid therapy on protein carbamylation in maintenance hemodialysis patients

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Abstract

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 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 March 2013 – 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 250cc of AAs 3-times per-week post-dialysis 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 $\pm$ SE 9.5 $\pm$ 2.4 and 9.3 $\pm$ 1.3 mmol/mol, respectively; $P=0.61$). The treated arm showed a significant reduction in C-Alb compared to controls at 4 weeks (8.4% reduction in the

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Disclosures:

Provisional applications for U.S. and International patents related to the contents of this manuscript have been filed by AHB, SAK, RT, and their affiliated institutions. RT is a consultant to Fresenius Medical Care North America.

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treated arm vs. 4.3% increase in controls; $P=0.03$) and the effect was greater by 8 weeks (15% reduction in the treated vs. 1% decrease in controls; $P=0.01$).

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

Introduction

Protein carbamylation describes the non-enzymatic 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 homocitruline, 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 through the dialysis procedure itself.^{11–13} We recently reported 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 employed 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 comprised age between 18 and 80 years old and no overt indication of malnutrition: BMI <20kg/ m², body weight loss within 6 months >10%, serum albumin <3.0mg/dl. Exclusion criteria were weekly dialysis time <12h, urea Kt/V <1.2, and co-morbidities compromising 1-year survival prognosis. For this open label pilot study, 14 prevalent hemodialysis subjects were enrolled to undergo thrice weekly post dialysis AA infusions over 8 weeks. 2 subjects withdrew from the study: one due to loss of interest in participation and the other due to unrelated medical reasons. 11 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 IRB.

Treatment and outcomes

Individuals receiving treatment were given 250cc of AA infusions at the end of dialysis. Each 250cc dose contained 14 g of essential AAs yielding the recommended daily intake of essential AAs (precise composition can be found in Supplemental Table 1; FDA IND 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 was measured by high performance liquid chromatography and tandem mass spectrometry using standard methods as previously described (coefficient of variation 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 dietician. No specific dietary instructions were given though subjects were asked to refrain from taking any additional nutritional supplements during the study period.

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 verses control groups for carbamylated albumin level as well as other pre-specified clinical indices (pre-dialysis urea level, serum albumin, hemoglobin, cardiac and inflammatory markers, and average essential AA levels). Within group comparisons employed Friedman and Wilcoxon Signed Rank tests as appropriate. $P < 0.05$ was considered statistically significant. $P < 0.15$ was considered a trend towards significance. All values are expressed as the mean (SE) or a percentage. Baseline AA levels were compared to baseline carbamylation levels using Pearson correlations. All statistics were performed using SAS (v9.2, SAS Institute, Cary, N.C., USA).

Results

The baseline characteristics of the treated and control groups were similar (Table 1) and baseline AA levels, on average, were negatively correlated to baseline carbamylated albumin levels (average Pearson correlation coefficient for all essential AAs = -0.25 ; Supplemental Table 2 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; $P=0.61$; Table 2). The treated arm showed a significant reduction in carbamylated albumin level compared to the control arm at 4 weeks (8.4% reduction in the treated arm vs. 4.3% increase in controls; $P=0.03$) and the effect was greater by 8 weeks (15% reduction in treated individuals vs. a 1% decrease in controls; $P=0.01$; Figure 1 and Table 2). There were no observed adverse events related to the study protocol.

Blood samples, all collected pre-dialysis, were tested for additional effects of AA supplementation. There were no significant changes in pre-dialysis urea, albumin, or hemoglobin levels (Table 2). There were also no changes in pre-dialysis AA concentrations, though notably blood samples were collected 44 hours after each previous AA infusion, allowing ample time for distribution and metabolism of the AA supplements.

Given prior associations of carbamylation, heart disease, and inflammation,^{1,3,17} in an exploratory analysis we investigated if any trends existed with cardiac and inflammatory biomarkers in response to decreased carbamylation. While BNP levels appeared to decrease in the treated arm to a greater degree than in controls, all P -values were non-significant in all exploratory analyses (Table 2). Conclusions regarding the effect of reduced carbamylation on such biomarkers remain limited given the small sample size of this study.

Discussion

In this proof of concept pilot study, AA treatment was able to safely modulate protein carbamylation levels in maintenance hemodialysis patients over an 8-week period. There is growing evidence that protein carbamylation contributes to the pathophysiology of uremia, promoting atherosclerosis and inflammation by changing the functional properties of select proteins.¹ Given the established association between protein carbamylation and adverse outcomes in end stage renal disease (ESRD), the ability to effectively reduce protein carbamylation carries the potential to improve adverse uremic sequelae. Carbamylated albumin is a recently validated marker of carbamylation burden whose levels correlate with high urea and low amino acid levels, are strongly associated with mortality in patients on hemodialysis, and represent an indicator of protein carbamylation integrated over the life-span of serum albumin.^{2,5,9} We measured carbamylated albumin differences after 8 weeks as albumin would undergo 2 half-life cycles during this period allowing enough turnover to reflect changes to the carbamylation milieu.

The concept of administering intradialytic parenteral AAs is not new, though previous outcomes focused solely on protein and energy homeostasis. While these trials of AA therapy in hemodialysis patients have produced mixed results, all were limited to those with pre-existing malnutrition and none ever targeted carbamylation.^{14,15,18–21} Our intervention

was not designed to treat malnutrition directly, rather we sought to treat another mediator of adverse outcomes in dialysis patients—carbamylation. Importantly, 2 large observational studies recently demonstrated considerable improvements in overall survival with modest intradialytic nutritional support, yet the underlying mechanisms for this result were not entirely clear.^{22,23} Perhaps, those who benefited responded to restored AA concentrations and subsequent reduced protein carbamylation. Effect sizes might be greater and more consistent if studies included only subjects with significant AA deficiencies and accelerated protein carbamylation.

Because the catabolism of AAs, either as an energy source or resulting from protein degradation, can result in increases in urea,²⁴ we were initially concerned that AA supplementation could raise individuals' average urea levels, potentially blunting reductions in carbamylation. However, there were no significant changes in urea levels observed at the dosage used in this study. It is unclear how additional treatments or higher dosed treatments would affect the results. Similarly, it remains unclear if the percent reduction in carbamylation observed is sufficient to yield favorable clinical outcomes. Notably, prior work suggests that a carbamylation level below approximately 8.5 mmol/mol would associate with a significantly reduced risk for mortality as well as improved erythropoietin responsiveness, making the effect size observed in this pilot study meaningful.^{1,2,5}

The notion that excess urea itself is pathogenic in ESRD is controversial,²⁵ and given carbamylation is intrinsically linked to urea, one would expect reducing time averaged urea concentrations via increased dialysis could attenuate carbamylation. This certainly takes place to some degree; however, it is clear that the intermittent removal of urea via dialysis is inadequate to entirely prevent carbamylation from occurring in ESRD and that protein carbamylation can have deleterious effects.^{1,3,5} Furthermore, given the observed associations between carbamylated albumin, amino acid levels, and erythropoietin resistance, carbamylation measurements may integrate the effects of multiple pathophysiologic pathways: time-averaged urea as well as protein energy wasting, anemia, and chronic inflammation. Thus we sought to demonstrate a novel means of reducing carbamylation. While a small sample size precluded multi-variable adjustments in this study, the observed decrease in carbamylation in the AA treated individuals was unlikely attributable to a change in urea clearance as the average blood urea nitrogen (BUN), urea reduction ratio, and urea Kt/V did not change significantly during the study period. Nevertheless, frequent or longer session hemodialysis may in fact also reduce carbamylation load possibly explaining some clinical benefits observed when such dialytic approaches have been employed in other studies.²⁶

Our study has notable limitations. This proof of concept study, while achieving our objectives, represents a small sample size and the results will require further validation. Possible future expansion of this study in additional subjects will need to include additional pre- and post-infusion blood samples in order to perform more detailed AA measurements and quantify the intradialytic pharmacokinetic response to AA therapy. Lastly, this was not a randomized controlled study and the possibility for confounding cannot be sufficiently addressed.

Practical Application

The results of this study suggest that protein carbamylation as measured by carbamylated albumin levels can be attenuated using AA therapy. As protein carbamylation is increasingly viewed as a detrimental ramification of uremia, additional understanding of the mechanisms that underlie it and the means by which it can be modulated are of great interest. If even a small subset of individuals with the highest level of carbamylation burden could be helped with a simple therapeutic intervention such as AA supplementation, the impact on ESRD outcomes could be substantial. Additional study is necessary to determine the clinical utility of carbamylation targeted AA therapy in maintenance hemodialysis patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

S.K. received support from the National Kidney Foundation Young Investigator award and NIH award KL2TR001100; R.T. receives support from NIH award K24 DK094872.; S.A.K. receives support from the Howard Hughes Medical Institute; A.H.B. received support from the American Diabetes Association Junior Faculty Award.

References

1. Kalim S, Karumanchi SA, Thadhani RI, et al. Protein Carbamylation in Kidney Disease: Pathogenesis and Clinical Implications. *Am J Kidney Dis*. 2014
2. Kalim S, Tamez H, Wenger J, et al. Carbamylation of serum albumin and erythropoietin resistance in end stage kidney disease. *Clin J Am Soc Nephrol*. 2013; 8:1927–34. [PubMed: 23970130]
3. Wang Z, Nicholls SJ, Rodriguez ER, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med*. 2007; 13:1176–84. [PubMed: 17828273]
4. Ok E, Basnakian AG, Apostolov EO, et al. Carbamylated low-density lipoprotein induces death of endothelial cells: a link to atherosclerosis in patients with kidney disease. *Kidney Int*. 2005; 68:173–8. [PubMed: 15954906]
5. Berg AH, Drechsler C, Wenger J, et al. Carbamylation of serum albumin as a risk factor for mortality in patients with kidney failure. *Sci Transl Med*. 2013; 5:175ra29.
6. Koeth RA, Kalantar-Zadeh K, Wang Z, et al. Protein carbamylation predicts mortality in ESRD. *J Am Soc Nephrol*. 2013; 24:853–61. [PubMed: 23431074]
7. Stark GR. Reactions of cyanate with functional groups of proteins. 3. Reactions with amino and carboxyl groups. *Biochemistry*. 1965; 4:1030–6. [PubMed: 5839993]
8. Stark GR. Modification of proteins with cyanate. *Methods Enzymol*. 1972; 25:579–84. [PubMed: 23014442]
9. Kraus LM, Kraus AP Jr. Carbamoylation of amino acids and proteins in uremia. *Kidney Int Suppl*. 2001; 78:S102–7. [PubMed: 11168993]
10. Carreras, J.; Chabas, A.; Diederich, D. Physiological and clinical implications of protein carbamylation. In: Grisolia, S.; Baguena, R.; Major, F., editors. *The Urea Cycle*. New York: John Wiley & Sons, Inc; 1976. p. 501-48.
11. Raj DS, Oladipo A, Lim VS. Amino Acid and protein kinetics in renal failure: an integrated approach. *Semin Nephrol*. 2006; 26:158–66. [PubMed: 16530607]
12. Raj DS, Zager P, Shah VO, et al. Protein turnover and amino acid transport kinetics in end-stage renal disease. *Am J Physiol Endocrinol Metab*. 2004; 286:E136–43. [PubMed: 13129859]
13. Carrero JJ, Stenvinkel P, Cuppari L, et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). *J Ren Nutr*. 2013; 23:77–90. [PubMed: 23428357]

14. Dukkipati R, Kalantar-Zadeh K, Kopple JD. Is there a role for intradialytic parenteral nutrition? A review of the evidence. *Am J Kidney Dis.* 2010; 55:352–64. [PubMed: 19854550]
15. Cano NJ, Fouque D, Roth H, et al. Intradialytic parenteral nutrition does not improve survival in malnourished hemodialysis patients: a 2-year multicenter, prospective, randomized study. *J Am Soc Nephrol.* 2007; 18:2583–91. [PubMed: 17656473]
16. Rose WC. II. The sequence of events leading to the establishment of the amino acid needs of man. *Am J Public Health Nations Health.* 1968; 58:2020–7. [PubMed: 5748871]
17. Tang WH, Shrestha K, Wang Z, et al. Protein carbamylation in chronic systolic heart failure: relationship with renal impairment and adverse long-term outcomes. *J Card Fail.* 2013; 19:219–24. [PubMed: 23582087]
18. Capelli JP, Kushner H, Camiscioli TC, et al. Effect of intradialytic parenteral nutrition on mortality rates in end-stage renal disease care. *Am J Kidney Dis.* 1994; 23:808–16. [PubMed: 8203363]
19. Eustace JA, Coresh J, Kutchev C, et al. Randomized double-blind trial of oral essential amino acids for dialysis-associated hypoalbuminemia. *Kidney Int.* 2000; 57:2527–38. [PubMed: 10844622]
20. Pupim LB, Flakoll PJ, Brouillette JR, et al. Intradialytic parenteral nutrition improves protein and energy homeostasis in chronic hemodialysis patients. *J Clin Invest.* 2002; 110:483–92. [PubMed: 12189242]
21. Navarro JF, Mora C, Leon C, et al. Amino acid losses during hemodialysis with polyacrylonitrile membranes: effect of intradialytic amino acid supplementation on plasma amino acid concentrations and nutritional variables in nondiabetic patients. *Am J Clin Nutr.* 2000; 71:765–73. [PubMed: 10702171]
22. Weiner DE, Tighiouart H, Ladik V, et al. Oral intradialytic nutritional supplement use and mortality in hemodialysis patients. *Am J Kidney Dis.* 2014; 63:276–85. [PubMed: 24094606]
23. Lacson E Jr, Wang W, Zebrowski B, et al. Outcomes associated with intradialytic oral nutritional supplements in patients undergoing maintenance hemodialysis: a quality improvement report. *Am J Kidney Dis.* 2012; 60:591–600. [PubMed: 22632807]
24. Morris SM Jr. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr.* 2002; 22:87–105. [PubMed: 12055339]
25. Meyer TW, Hostetter TH. Uremia. *N Engl J Med.* 2007; 357:1316–25. [PubMed: 17898101]
26. Diaz-Buxo JA, White SA, Himmele R. Frequent hemodialysis: a critical review. *Semin Dial.* 2013; 26:578–89. [PubMed: 23834104]

Figure 1.
Carbamylated albumin levels through time for the study participants

Table 1

Baseline characteristics of the study population.

Variable	Treated (n=12)	Control (n=11)
Demographic		
Age (year)	61.4 ±2.8	62.3 ±3.2
Male (%)	8 (67)	8 (73)
White (%)	9 (75)	9 (82)
Clinical		
BMI (Kg/m ²)	30.9 ±1.3	33.4 ±2.2
Systolic BP (mmHg)	149 ±6.0	141 ±6.5
Diastolic BP (mmHg)	78 ±2.9	77 ±4.3
Dialysis dose in Kt/V	1.7 ±0.1	1.5 ±0.1
Catheter access (%)	1 (8)	4 (36)
Dialysis vintage (year)	1.9 ±0.5	1.6±0.1
Cause of ESRD*		
Diabetes (%)	7 (58)	6 (55)
Hypertension (%)	2 (17)	4 (36)
GN (%)	1 (8)	3 (27)
Other (%)	3 (25)	3 (27)

Data are expressed as mean ±SE or count (percent).

BMI, body mass index; ESRD, end stage renal disease; GN, glomerulonephritis; BUN, blood urea nitrogen.

* Some individuals reported multiple causes of their kidney disease

Table 2

Laboratory values at baseline and 8 weeks.

	Baseline	Week 8	Percent change
C-Alb (mmol/mol)			
Control	9.3 ±1.3	9.2 ±0.9	-1.1%
Treated	9.5 ±2.4	8.1 ±1.7 ^A	-15.0%
<i>P</i> value	0.61	0.01 *	
Albumin (g/dL)			
Control	3.8 ±0.1	3.9 ±0.1	2.6%
Treated	3.8 ±0.2	3.6 ±0.1	-5.3%
<i>P</i> value	0.72	0.15	
BUN (mg/dL)			
Control	49 ±5	48 ±4	-2.0%
Treated	48 ±5	47 ±3	-2.1%
<i>P</i> value	0.87	0.77	
Hemoglobin (g/dL)			
Control	11.3 ±0.4	11.7 ±0.4	3.5%
Treated	11.1 ±0.2	11.2 ±0.3	-0.9%
<i>P</i> value	0.66	0.23	
Troponin-T (µg/L)			
Control	0.067 ±0.022	0.066 ±0.018	-1.5%
Treated	0.120 ±0.086	0.103 ±0.071	-14.2%
<i>P</i> value	0.45	0.23	
BNP (pg/mL)			
Control	13402 ±6534	10877 ±5071	-18.8%
Treated	17272 ±7520	10283 ±4783 ^A	-40.5%
<i>P</i> value	0.70	0.93	
IFN-gamma (pg/mL)			
Control	0.8 ±0.2	2.9 ±1.8	262.5%
Treated	2.6 ±1.1	2.9 ±1.6	11.5%
<i>P</i> value	0.03 *	0.63	
TNF-alpha (pg/mL)			
Control	10.1 ±1.1	10.1 ±0.9	0%
Treated	12.8 ±1.1	12.9 ±1.5	0.8%
<i>P</i> value	0.09	0.07	

C-Alb, Carbamylated albumin.

Data are expressed as mean ±SE.

*
p significant at <0.05^A
Within-group trend to significance (P <0.15).