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Protein Carbamylation in Kidney Disease: Pathogenesis and Clinical Implications

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Carbamylation describes a nonenzymatic posttranslational protein modification mediated by cyanate, a dissociation product of urea. When kidney function declines and urea accumulates, the burden of carbamylation naturally increases. Free amino acids may protect proteins from carbamylation, and protein carbamylation has been shown to increase in uremic patients with amino acid deficiencies. Carbamylation reactions are capable of altering the structure and functional properties of certain proteins and have been implicated directly in the underlying mechanisms of various disease conditions. A broad range of studies has demonstrated how the irreversible binding of urea-derived cyanate to proteins in the human body causes inappropriate cellular responses leading to adverse outcomes such as accelerated atherosclerosis and inflammation. Given carbamylation's relationship to urea and the evidence that it contributes to disease pathogenesis, measurements of carbamylated proteins may serve as useful quantitative biomarkers of time-averaged urea concentrations while also offering risk assessment in patients with kidney disease. Moreover, the link between carbamylated proteins and disease pathophysiology creates an enticing therapeutic target for reducing the rate of carbamylation. This article reviews the biochemistry of the carbamylation reaction, its role in specific diseases, and the potential diagnostic and therapeutic implications of these findings based on recent advances.
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INDEX WORDS: Carbamylation; uremia; posttranslational protein modification (PTM); pathophysiology; chronic kidney disease (CKD); end-stage renal disease (ESRD); cyanate; urea; inflammation; carbamoylation.

BACKGROUND

Proteins in the human body, in both health and disease, are exposed to chemical reactions capable of altering their structural and functional properties. Spontaneous posttranslational protein modifications are caused by the nonenzymatic attachment of reactive molecules to protein functional groups, as seen, for example, in glycation reactions. Because posttranslational modifications are capable of changing protein structure and function, they can create a mechanistic chemical link to the adverse pathophysiology underlying certain metabolic diseases.

Carbamylation is a protein modification that results from constant exposure to urea and its byproduct cyanate, which both increase as kidney function declines. Urea-driven carbamylation reactions occur not only on proteins, but also on free amino acids, and these targets may compete with each other for binding such that amino acid deficiency can exacerbate protein carbamylation. Furthermore, protein carbamylation may not be related solely to urea; recent work shows that cyanate also may be generated by myeloperoxidase (MPO) and peroxide-catalyzed oxidation of thiocyanate (derived from diet and smoking) at sites of inflammation.

Just as glycation is known to contribute to pathologic sequelae in conditions such as diabetes mellitus, carbamylation has been shown to change the properties of various enzymes, hormones, and other proteins, ultimately contributing to the deleterious effects of reduced kidney function. Similar to measurement of glycated hemoglobin for glucose monitoring in diabetes mellitus, measurement of protein carbamylation offers a time-averaged record of urea concentrations and amino acid deficiency, thus characterizing the duration and severity of kidney disease, as well as assessing the adequacy of kidney replacement therapies. This review describes the pathogenesis and clinical implications of protein carbamylation in kidney disease, paying special attention to recent advances made in the study of this process.

CASE VIGNETTE

A 58-year-old Hispanic man with end-stage renal disease (ESRD) resulting from chronic hypertensive nephrosclerosis was evaluated for worsening dyspnea on exertion. He had been adherent to his thrice-weekly hemodialysis treatment regimen over

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the past 4 years and his latest Kt/V was 1.3. He had been dialyzed to his estimated dry weight of 72 kg at his last treatment and his recent predialysis blood pressures averaged 145/85 mm Hg. The patient did not have a prior cardiac history, and a nuclear stress test performed 3 years earlier showed results within normal limits. There was no history of smoking or dyslipidemia. On examination, he was without pulmonary or lower-extremity edema. An electrocardiogram was significant for left ventricular hypertrophy. His cardiac troponin T level was elevated at 0.31 (reference range, <0.03) ng/mL, but failed to increase on serial remeasurements. A myocardial perfusion scan was positive for ischemia. Cardiac catheterization revealed severe left main, left anterior descending, and left circumflex coronary artery disease. The patient was referred for coronary artery bypass graft surgery. A blood sample drawn with the patient's informed consent for investigative purposes was tested for carbamylated albumin and shown to have 12 mmol of carbamylated albumin per mole of total albumin (1.2% serum carbamylated albumin). This amount is well above previously reported average values seen in hemodialysis patients (median serum carbamylated albumin, 0.77%; interquartile range, 0.58%-0.93%).[1,2]

Although well appreciated, the marked excess in cardiovascular burden observed in patients with chronic kidney disease (CKD) remains poorly understood and is not entirely explained by traditional risk factors. Novel disease biomarkers may provide insights into the mechanisms of uremic cardiac morbidity and mortality in addition to offering new therapeutic targets. Protein carbamylation recently has been implicated in several disease pathways of uremia, including atherosclerosis and cardiovascular disease. As the mechanisms of protein carbamylation are becoming better understood, approaches to treating increased carbamylation burden and its sequelae are emerging, carrying the potential for meaningful clinical applications into the future.

PATHOGENESIS

Biochemistry of Carbamylation and Its Link to Kidney Disease

In 1828, Friedrich Wöhler[3] discovered that urea could be synthesized by reacting cyanate with ammonia, and in 1895 it was found that under physiologic conditions, urea slowly dissociates into cyanate and its tautomer isocyanate.[4] Isocyanate is a highly reactive electrophile that quickly reacts with nucleophilic groups such as primary amines and free sulfhydryls, and by 1949, F. Schutz[5] suggested that urea-derived cyanate could react with the amine and sulfhydryl groups on proteins and free amino acids. This claim was shown to be true in 1960 when George Stark et al[6] observed that when ribonuclease is incubated with concentrated urea, a modified form accumulates that is less positively charged. Subsequent experiments found that lysines in the protein had been irreversibly modified to *N*(6)-carbamoyl-L-lysine (homocitrulline). Stark and others demonstrated that cyanate can produce irreversible modifications of primary amines and reversible modifications of thiols, hydroxyls, phenols, and imidazole groups.[6-10] The net result of these reactions, referred to as carbamylation, is the addition of a "carbamoyl" moiety ($-CONH_2$) to a functional group. Note that some authorities prefer the term carbamoylation to describe this reaction, but for this review, we use the term more commonly used throughout the biomedical literature, carbamylation.[11,12]

Because carbamylations of amines are stable, proteins may accumulate these on their amino-terminal α-amino groups or the ε-amino groups of lysine side chains throughout their lifespan. Free amino acids also may be carbamylated on their α-amino group or on nucleophilic groups on their side chains.[8,13] Theoretically, carbamylation can occur on any nucleophilic amino acid side chain moiety, depending on its solvent accessibility, nucleophilicity, and the pK_a of the group (carbamylation can occur on amines only in their uncharged state).[14] As a result, carbamylations have been detected on multiple lysines within proteins. However, due to varying susceptibilities, the relative degree of carbamylation at different sites varies widely.[2,15] Furthermore, because of differences in pK_a, α-amino groups of free amino acids react about 100 times faster than lysine side chain ε-amino groups on proteins at physiologic pH.[8,16]

The spontaneous dissociation of urea into cyanate and ammonium depends on pH and temperature. Under physiologic conditions, the equilibrium between urea and cyanate favors urea, with the cyanate-urea ratio averaging less than 1:100.[12,17,18] Nevertheless, because urea levels in the body are relatively high compared with many other biomolecules, significant amounts of cyanate can be generated. When urea concentrations increase with declining kidney function, so does the generation of cyanate, creating a pathologic state that promotes protein carbamylation. The plasma concentration of isocyanate in healthy individuals is ∼45 nmol/L, and in uremic patients, it reaches 140 nmol/L.[19] While this concentration may seem low compared with other biomolecules, recall that urea dissociation is constantly generating more cyanate, which rapidly binds to nearby proteins and amino acids, and thus the rate of protein and amino acid carbamylation may become significant. Kraus et al[20] demonstrated that serum concentrations of many carbamylated free amino acids in patients with ESRD actually exceed the concentrations of their unmodified precursors.

Biochemical Rationale for the Pathologic Effects of Protein Carbamylation

A number of studies published over the past several decades have demonstrated that carbamylation causes changes in the physical properties of proteins, thus suggesting its involvement in molecular and cellular dysfunction.[6,21] A major chemical effect of carbamylation is neutralization of positively charged lysines, which changes protein-water interactions and alters ionic interactions on the protein surface.[12] Such changes can alter secondary and tertiary structures, leading to functional conformational changes. In the

1960s, the first enzyme shown to be inactivated by carbamylation was glutamate dehydrogenase.[22] At the same time, several other groups were able to demonstrate the wide-ranging effects of protein carbamylation.[16] For example, investigators demonstrated that carbamylation of pepsinogen transforms the positively charged ε-NH_2 groups on its lysine side chains into uncharged homocitrulline side chains.[23] This alteration results in changes in electrophoretic mobility and altered enzymatic activity.[23] More recent studies demonstrated that carbamylation of type I collagen induces conformational changes within its triple helix structure, altering collagen interactions with inflammatory cells and sensitivity to collagenases.[24,25] Others have demonstrated that carbamylated erythropoietin loses its erythropoietic and angiogenic effects, yet maintains several of its tissue-protective properties.[26-28] Further research has capitalized on this finding, testing whether carbamylated erythropoietin may be used as a nonhematopoietic tissue-protective agent in ischemic injuries.[29] Dozens of studies through the years have shown similar results implicating carbamylation in changes in protein charge,[30] conformation,[31,32] stability,[33] enzyme and hormone activity,[34-38] binding properties,[39-41] receptor-drug interaction,[10,42] and cellular expression and responses.[43-48] Last, tissue culture and animal model studies of the effects of carbamylated albumin have suggested that carbamylation gives albumin nephrotoxic and neutrophil-inactivating properties, providing further evidence that carbamylation of even a minor fraction of a normal protein may still confer significant pathogenic properties to the protein.[43,44,49]

The prevalence of protein carbamylation throughout the body recently was illustrated in a study by Pietrement et al.[50] In a CKD animal model, the authors were able to quantitatively demonstrate using liquid chromatography–tandem mass spectrometry that carbamylation occurs in a diverse set of tissues, including aorta, kidney, bone, skin, liver, and heart, with greater accumulations in the long-lived extracellular matrix proteins. Although there appeared to be a basal level of carbamylation occurring in control mice without kidney disease, there was a 2-fold increase in carbamylation burden in 75% nephrectomized mice at 20 weeks in all the aforementioned tissues. Given the ubiquity of urea in all tissue compartments and the potential resulting effects of carbamylation on all the body's organs, the disease implications of carbamylation seem far reaching.

In addition to the effects of carbamylation on proteins, this chemical modification may have consequences on the bioavailability and pharmacologic properties of free amino acids. When the α-amino group of a free amino acid is irreversibly carbamylated, it cannot participate in protein synthesis or be

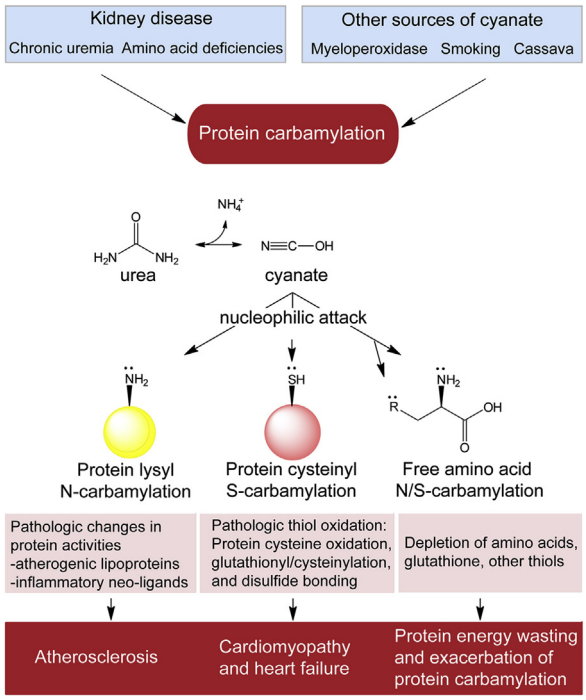

Figure 1. The chemical pathophysiology of carbamylation in uremia and the hypothesized pathogenesis of carbamylation-associated disease complications.

catabolized as an energy source.[18,20] In patients with kidney disease, the proportion of carbamylated plasma free amino acids that accumulate may be significant,[18,20] thus raising the possibility that functional amino acid depletion by carbamylation exacerbates the protein-energy wasting associated with kidney disease and long-term kidney replacement therapies.[51-53] Last, S-carbamylation of free sulfhydryl groups on cysteine and glutathione has been shown to interfere with their antioxidant functions, raising the possibility that carbamylation contributes to the thiol oxidative stress associated with uremic states.[54-60]

Mechanistic Role of Carbamylation in Specific Disease Processes

Heart Disease

Recent studies from tissue culture and animal models give compelling evidence that protein carbamylation is mechanistically involved in the excessive cardiovascular disease burden associated with kidney failure. Multiple studies have suggested that carbamylation can contribute to atherosclerosis and cardiovascular risk by its effects on lipoproteins, collagen, fibrin, proteoglycans, and fibronectin (Fig 1; Table 1).[61]

Carbamylation of low-density lipoprotein (LDL) may enhance its atherogenic properties partly by decreasing its binding to the LDL receptor[62] and

Table 1. Carbamylation of Proteins Promoting Atherosclerosis

Carbamylated Substrate	Effect	References
LDL	Diminishes LDL-receptor recognition/clearance of LDL	62, 65
	Triggers macrophage scavenger recognition	62, 69
	Promotes foam cell formation	62
	Promotes endothelial/monocyte adhesion	72
	Induces endothelial apoptosis/endothelial progenitor cell senescence	62, 71, 72, 128
	Stimulates vascular smooth muscle proliferation	62, 71, 73
	Increases oxidative stress	128
	Promotes auto-antibodies	96
HDL	Promotes foam cell formation	74
Collagen	Decreases ability to polymerize into normal fibrils	24
	Increases release of matrix metalloproteinase 9 by monocytes	48
	Increases adhesion of monocytes	48
Fibrin	Increases basement membrane thickening	61
Proteoglycans, fibronectin	Alters ECM by altered collagen and basement membrane affinity	61

Abbreviations: ECM, extracellular matrix; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

preventing its clearance from circulation (a phenomenon seen in animal models of chronic kidney failure[63,64] and in uremic patients).[65-68] Carbamylated LDL also has been shown to readily bind macrophage scavenger receptors, facilitating foam cell formation and inflammatory signaling.[62,69,70] Moreover, carbamylated LDL can induce endothelial cell apoptosis[71,72] and stimulate vascular smooth muscle proliferation,[73] while carbamylated collagen increases the release of matrix metalloproteinases by monocytes, possibly stimulating remodeling of the extracellular matrix.[24,48] Carbamylation of high-density lipoprotein similarly appears to confer atherogenic properties,[74] and there are studies suggesting that cyanate itself has direct pathologic effects on endothelial cells.[75,76] Most recently, epidemiologic studies have further established protein carbamylation as an independent risk factor for all-cause mortality and death from cardiac causes in both uremic and nonuremic populations (see Fig 2 and Recent Advances).[2,62,77]

From Sickle Cell Disease to Cataracts

The first demonstration of the deleterious effects of carbamylation in vivo came in the 1970s when it was proposed that sickle cell crisis could be prevented by treating patients with sickle cell disease with urea or cyanate. The rationale for this novel therapy was based on the fact that carbamylated hemoglobin S had a higher affinity for oxygen and did not sickle as readily as unmodified hemoglobin S.[78-83] Unfortunately, patients being treated with cyanate were observed to later develop cataracts, and carbamylation soon was implicated.[84] Subsequent studies of humans and animals confirmed the finding and demonstrated that cyanate treatment of lens proteins (crystallins) caused them to form interchain disulfide bonds and protein aggregates, thus explaining how carbamylation modifications made these proteins cataractogenic.[32,85-92]

Arthritis

Although much of the literature has focused on carbamylation and its effects on the cardiorenal disease axis, an additional consequence of protein carbamylation appears to be the induction of autoantibody

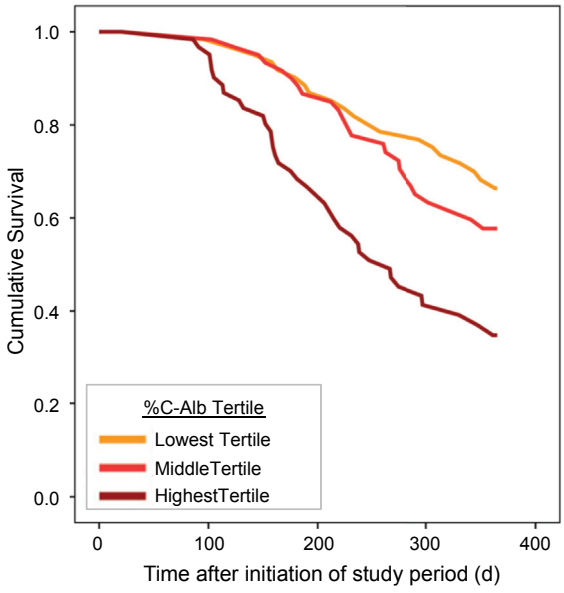

Figure 2. Kaplan-Meier curve estimates of the incidence of all-cause mortality over 12 months in incident hemodialysis patients in the ArMORR (Accelerated Mortality on Kidney Replacement) cohort. Participants (n = 187) were categorized into upper, middle, and lower tertiles according to percent serum carbamylated albumin (%C-Alb) values measured at the outset of the study. Adapted from Berg et al.[2]

responses. Protein carbamylation creates potential neo-epitopes that induce multiple autoantibody responses.[93-96] Rheumatoid arthritis (RA) is a disease associated with multiple types of autoantibodies, and when investigators examined whether anti–carbamylated protein antibody levels are elevated in patients with RA, they found that anti-homocitrulline antibodies are present and at a higher level in individuals with RA and arthralgias compared with healthy controls. They also showed positive associations with joint damage and could predict disease development in "pre-RA" patients independent of established biomarkers.[95,97] Similar results have been seen in patients with juvenile idiopathic arthritis.[98]

Other Diseases

Because the carbamylation reaction theoretically can occur on any protein, it is not surprising that the reaction has been implicated in many additional disease processes. For example, carbamylated proteins at concentrations present in uremia have been shown to activate glomerular mesangial cells to a profibrogenic phenotype and stimulate collagen deposition.[99] Thus, accelerated carbamylation due to kidney failure conceivably could further accelerate the progression of kidney disease, leading to a detrimental positive feedback loop. Examples exist in other diseases as well: carbamylation of the neurologically important τ protein has been reported to result in significantly diminished ability to induce tubulin assembly into microtubules, promoting abnormal polymers that are found in Alzheimer disease.[100] When carbamylated albumin was added to mesangial cells, expression of microRNAs associated with human kidney cell carcinoma was found to markedly increase.[43] Carbamylation of ceruloplasmin impairs ferroxidase and thus its antioxidant activity, with additional cardiovascular implications as a possible consequence.[101]

Last, urea dissociation is not the only source of cyanate in humans: cyanate also may be derived from the consumption and metabolism of cassava root. Cassava contains cyanogens that are metabolized to cyanate, and it recently has been shown that cassava consumption is associated with increased protein carbamylation and neurologic disease (ie, the paralytic disease Konzo).[102-105] The connection between cassava, carbamylation, and neurologic disease is intriguing because it raises the possibility that carbamylation could be a contributor to neurologic complications associated with chronic uremia.

Carbamylated Hemoglobin as a Biomarker of Uremia

Analogous to hemoglobin A_{1c} in diabetes mellitus, when urea-derived cyanate stably attaches to a protein, its half-life is defined by that of the protein. In this sense, carbamylated proteins can give not only a sense of the total body "carbamylation burden," but also a measure of urea concentrations averaged over the lifespan of the protein. Given the possibility that carbamylation contributes directly to the pathogenesis of cardiovascular and other diseases associated with uremia, measurements of carbamylated proteins should behave as ideal biomarkers because of their direct, quantitative, and mechanistic relationships to urea concentrations and disease pathophysiology. A number of studies published over several decades have shown this to be true, beginning with studies of carbamylated hemoglobin in patients with kidney disease.

In 1981, Flückiger et al[106] observed that a subfraction of hemoglobin A_1 was carbamylated and that the proportion of carbamylated hemoglobin was related to a patient's blood urea concentration. Several subsequent studies have confirmed this.[107-110] It later was shown in a small cohort of dialysis patients ($n = 55$) that carbamylated hemoglobin levels are higher in individuals with $Kt/V < 1.1$ than in those with Kt/V greater than this; also, as predicted, carbamylation level correlates negatively with both Kt/V and urea reduction ratio (URR).[111] Others similarly have demonstrated that carbamylated hemoglobin could be a more accurate indicator of average urea levels between hemodialysis sessions than the standard indexes of dialysis adequacy, Kt/V, and URR.[110,112] In contrast, in a study limited by sample size ($n = 7$), Balion et al[46] found significant variability over time in individuals' carbamylated hemoglobin levels, with little association with markers of dialysis adequacy.

A drawback to past studies of carbamylated hemoglobin and dialysis adequacy is that none of the studies went so far as to show that carbamylated hemoglobin values were associated with disease outcomes. Without such outcomes studies, it is difficult to discern the significance of carbamylated hemoglobin as a clinical biomarker. Furthermore, measurement of carbamylated hemoglobin in patients with ESRD may be compromised by the significant variability in the age of erythrocytes in this patient population, in the same way that erythrocyte aging influences hemoglobin A_{1c} levels.[113,114] The age of erythrocytes in patients with ESRD depends on their degree of ESRD-related anemia, dialysis-related red blood cell loss, and erythropoietin prescription and whether they have received a transfusion recently.[113-116] Thus, in ESRD, higher carbamylated hemoglobin levels might represent urea load or simply might indicate an older population of circulating red blood cells.

In addition to the application of carbamylated protein measurements as indicators of the adequacy of kidney replacement therapy, these time-averaged

biomarkers may be useful in discriminating between acute and longer term increases in urea levels.[117-119] When looking at individuals with acute, acute-on-chronic, or stable CKD, matched for degree of decrease in kidney function, carbamylated hemoglobin levels could sufficiently discern between groups, offering utility in identifying individuals with acute and possibly reversible kidney injury from those with chronic and likely stable kidney disease.[120,121] Interestingly, in kidney transplant recipients, carbamylated hemoglobin concentration has been reported to decrease by 19.7% within 2-3 weeks posttransplantation, but this occurs with an average hemoglobin level increase of 25%, making the true in vivo kinetics less clear.[121]

RECENT ADVANCES

Recent years have produced a number of important developments in the field of kidney disease and carbamylation. A first important technical advance was the application of mass spectrometry and proteomics to quantitatively measure global protein carbamylation and screen for individual carbamylation modifications on proteins in tissue and blood. Wang et al[62] demonstrated the use of amino acid analysis to allow quantification of protein-bound homocitrulline after protein hydrolysis. In this manner, tissues and plasma can be analyzed for their proportional amounts of lysine and its carbamylated form (homocitrulline), thus measuring global accumulated protein carbamylation in a given sample. This analytic method has allowed investigators to confirm the increases in tissue protein carbamylation in uremic states and correlate them with clinical outcomes. More advanced proteomic techniques also have made it possible to study the significance of carbamylation modifications at specific sites on specific protein targets.[15,50] Recently our group devised an assay using high-performance liquid chromatography and tandem mass spectrometry to measure the most abundant site of carbamylation on human serum albumin (the lysine at amino acid 549).[2] Development of these new methods for the quantitative analysis of carbamylated proteins has made possible several important clinical studies showing the association between carbamylation of plasma proteins and clinical outcomes in patients with kidney disease.

While the correlation between plasma protein carbamylation and time-averaged urea concentrations provides intriguing evidence of the potential clinical utility of carbamylation measures in people with kidney disease, a more noteworthy association recently elucidated is that carbamylated protein levels are strongly predictive of mortality risk in ESRD. Using measurements of carbamylated albumin or serum protein homocitrulline to lysine ratio as markers of protein carbamylation burden, recent studies of both incident and prevalent hemodialysis patients have shown associations between elevated protein carbamylation and all-cause and cardiovascular mortality in 3 distinct hemodialysis cohorts.[2,77] Direct comparisons of carbamylated albumin and homocitrulline as assays of total carbamylation burden are lacking, but may be instructive as the field moves forward.

New clinical correlation studies in patients with varying degrees of kidney disease may provide clues to the pathologic mechanisms underlying the link between protein carbamylation and mortality. In one study, serum protein carbamylation levels in patients with chronic systolic heart failure were shown to be correlated significantly with brain natriuretic peptide concentrations, possibly invoking an association between carbamylation and progressive ventricular dysfunction.[122] In a separate study, serum carbamylated albumin levels were associated strongly with erythropoietin resistance and mortality in incident dialysis patients after controlling for traditional risk factors such as markers of inflammation and iron indexes.[1] Together, these observations suggest multiple mechanistic pathways that may be contributing to cardiac death in patients with ESRD.

Another recently proposed concept regarding the pathophysiology of uremic carbamylation is the hypothesis that free amino acids compete with proteins for reaction with cyanate, in essence protecting proteins from the carbamylation reaction. Cyanate's affinity for the α-amino groups on free amino acids is far greater than that of lysine side chains on proteins, and thus free amino acids compete with proteins for binding to cyanate and can act as natural ambient scavengers for carbamylation. A corollary to this hypothesis is that amino acid deficiencies will make uremic patients susceptible to even greater degrees of carbamylation, which is noteworthy because free amino acids can become depleted in uremic patients due to protein-energy wasting and hemodialysis.[51,52,123] Recent observational work has found that protein carbamylation in dialysis patients is associated with free amino acid deficiencies.[2] Furthermore, it was confirmed using in vitro and in vivo mouse model experiments that urea-induced carbamylation is intensified by amino acid deficiencies.[2] Thus, it has been hypothesized that amino acid therapy, in select individuals, may reduce the risk of uremic complications by reducing protein carbamylation. This hypothesis currently is under investigation in a first-in-human clinical trial of amino acid therapy for the reduction of carbamylation in hemodialysis patients (ClinicalTrials.gov identifier NCT01612429).

Although the aforementioned studies reported that protein carbamylation remains tightly linked with

average serum urea nitrogen level, perhaps more significant is the fact that carbamylation is a stronger predictor of mortality than predialysis urea, Kt/V, or URR values,[2] suggesting that it potentially is a more sensitive marker to urea load and clearance than simply measuring pre- and postdialysis urea measures (a practice fraught with limitations).[124,125] Although these various measures of dialysis adequacy are all interrelated, there are several reasons why carbamylated albumin measurements may be associated more tightly with outcomes. Rather than depending on single measurements of pre- and postdialysis urea levels (Kt/V or URR), carbamylated albumin values represent a measure of average urea level over an extended period, thus better accounting for all the variations that occur through time. Furthermore, given the observed association between carbamylated albumin and amino acid levels and erythropoietin resistance, carbamylation measurements may integrate the effects of multiple pathophysiologic pathways: time-averaged uremia as well as protein-energy wasting, anemia, and chronic inflammation. These advantages raise the possibility that measurements of circulating carbamylated proteins may represent a better indicator of the adequacy of dialysis than Kt/V or URR. If urea-induced carbamylation is a significant contributor to the pathologic sequelae of kidney disease, as recent studies have suggested, patients receiving standard regimens of intermittent hemodialysis who still have excessive carbamylation may benefit from more intensive kidney replacement therapies, such as nocturnal hemodialysis.

Although studies showing associations between carbamylated protein levels and mortality have focused on patients receiving hemodialysis, the same pathophysiologic connections between chronic uremia, protein carbamylation, and resulting end-organ damage presumably also are present in patients on peritoneal dialysis therapy and those with earlier stages of CKD. Additional studies are needed to determine the relationship between carbamylated protein levels and clinical outcomes in different patient populations and whether the applications for these tests in therapeutic monitoring remain robust.

Finally, in addition to studies of uremia-associated protein carbamylation, new insights into alternative mechanisms that underlie carbamylation also have been made. Wang et al[62] and others[126] have demonstrated that beyond the effects of decreased kidney function and hyperuremia, inflammation can create a unique source of cyanate and carbamylation by MPO. MPO, which is stored primarily in granules of neutrophils, monocytes, and certain tissue macrophages, can react with hydrogen peroxide and environmentally derived thiocyanate to generate cyanate and thus promote the carbamylation reaction to occur. Thiocyanate derived from foods, smoke exposure, or even air pollution[127] can be oxidized through the MPO-catalyzed reaction with hydrogen peroxide, yielding hypothiocyanate and cyanate. Given the marked increased levels of MPO at sites of inflammation, it has been demonstrated that MPO can further promote carbamylation independent of urea load at sites of inflammation and atherosclerotic plaque. Although the study by Wang et al[62] suggested that tobacco smoking may contribute to MPO-catalyzed carbamylation, more recent studies have suggested that MPO may not be as significant a source in patients with kidney disease. In a study of incident hemodialysis patients, no correlation between serum carbamylated albumin levels and MPO enzyme activity could be found.[2] Furthermore, in a study of patients with heart failure and variably preserved kidney function, it was shown that carbamylation, as measured by protein-bound homocitrulline, appears to relate primarily to kidney indexes (even minor deviations), with no significant association between protein-bound homocitrulline and systemic MPO measurement.[122]

SUMMARY

A rich literature reflecting decades of investigation demonstrates the mechanistic and pathophysiologic involvement of protein carbamylation in the adverse outcomes of kidney disease, which remain a major public health burden. There is evidence that select carbamylated proteins could serve as useful biomarkers of disease and therapeutic response in kidney failure. For example, by integrating information on urea clearance, amino acid balance, and inflammation, elevated carbamylation measurements potentially could identify dialysis patients at the greatest risk for uremic complications. This subset of patients may stand to benefit from interventions precisely targeted at reducing carbamylation through modified dialysis prescriptions, nutritional support, or anti-inflammatory therapies. Identifying at-risk patients such as the one in the opening clinical vignette of this report and treating them with dialytic or nutritional interventions to attenuate carbamylation could work to counter the all too common complications that individuals with kidney disease currently must endure. Improvements in analytics and a new growth of well-executed clinical studies have shown that carbamylation is both an important marker and effector of uremia and one that is worthy of additional investigation.

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