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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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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