Recent Advances in Understanding the Pathogenesis of Atherosclerosis in CKD Patients

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A need exists for developing new therapies to improve cardiovascular outcomes in end-stage kidney disease. Three new areas that address novel pathophysiological mechanisms and/or therapeutic approaches toward cardiovascular events in chronic kidney disease patients include the use of an anti-inflammatory agent, the role of catalytic iron, and protein carbamylation. In preliminary studies, hydroxychloroquine, which has multiple anti-inflammatory properties, preserved vascular compliance for the aorta and major vessels, as well as reduced the extent of severity of atherosclerosis in ApoE−/− mice. The ability of iron to rapidly and reversibly cycle between 2 oxidation states makes iron potentially hazardous by enabling it to participate in the generation of powerful oxidant species. We have shown that high catalytic iron in the general population is associated with a 4-fold increase in prevalent cardiovascular disease (CVD), even after accounting for traditional risk factors. In addition, the highest levels of catalytic iron are present in dialysis patients and, more specifically, patients with prevalent CVD have several-fold higher catalytic iron levels compared with controls without CVD. These data suggest the utility of iron chelators for preventing and treating CVD in patients with chronic kidney disease and should be further investigated. Carbamylation of proteins results from nonenzymatic chemical modification by isocyanic acid derived from urea and an alternative route, the myeloperoxidase-catalyzed oxidation of thiocyanate. We have shown carbamylated low-density lipoprotein to have all the major biological effects relevant to atherosclerosis including endothelial cell injury, increased expression of cell adhesion molecules, and vascular smooth muscle cell proliferation. In 2 separate clinical studies, plasma levels of carbamylated protein independently predicted an increased risk of CVD and death.

IT IS NOW well established that chronic kidney disease (CKD) increases the risk for cardiovascular disease (CVD), and that end-stage kidney disease (ESKD) is associated with a 10 to 30 times increase in CV risk compared with an age- and gender-matched general population.[1] Therapies such as statins which are generally effective in preventing CV morbidity and mortality in the general population have had limited or no survival advantage in patients with ESKD (e.g., AURORA, 4D trials).[2, 3] Thus, there is a pressing need for developing new therapies possibly aimed at nontraditional risk factors to improve CV outcomes in ESKD. Three new areas that address novel pathophysiological mechanisms and/or therapeutic approaches toward cardiovascular events in CKD patients are the use of an anti-inflammatory agent, the role of catalytic iron, and the role of protein carbamylation.

Hydroxychloroquine for CVD in CKD

The process of atherogenesis has been considered for many years to consist largely of the accumulation of lipids within the artery wall. However, as pointed out by Russell Ross, one of the pioneers in the field, in the vessel wall, the atherosclerotic lesion represents what is best described as an inflammatory lesion rather than an accumulation of lipids. This notion is further supported by the close association of inflammatory markers with atherosclerosis and cardiovascular events. CKD is known to be a highly inflammatory state which is believed to be important in causing many complications such as atherosclerosis, erythropoietin resistance, and mortality.

Hydroxychloroquine (HCQ), an antimalarial agent with anti-inflammatory properties, is routinely used as a standard of care in many active rheumatologic disorders with high CVD burden and mortality, primarily believed to result from a high burden of inflammation. Over the last 2 decades, in multiple cohort-based analyses from different parts of world, it has been observed that use of HCQ is associated with a significant survival advantage.[4–7] However, HCQ has never been prospectively studied to determine if it reduces cardiovascular events in patients with normal renal function or CKD. As a first approach, we examined the effect of HCQ in an Apo-E deficient

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mouse model of atherosclerosis. CKD was surgically induced and HCQ was administered orally in a dose of 10 mg/kg/day for 16 weeks. Atherosclerosis was assessed functionally using noninvasive ultrasound visualization of the vessels as well as histopathologic analysis: pathologic *en face* staining with Sudan IV of whole aorta and Oil Red O staining of the atherosclerotic plaques.[8]

In a CKD model, HCQ reduced the progression of atherosclerosis and better preserved vascular compliance for the aorta (0.37 ± 0.01 vs. 0.29 ± 0.01, $P < .05$) and major vessels on serial ultrasound visualization. Histopathologic studies at the end of 16 weeks of therapy showed a significant reduction in the area of atherosclerosis (*en face* staining of whole aorta; treatment mice ($34 \pm 3\%$), placebo ($49 \pm 3\%$, $P < .0001$) as well as the severity of atherosclerosis (Oil Red O examination). Similar results were seen in non-CKD mice. We conclude that HCQ reduces the extent and severity of atherosclerosis in CKD and provides evidence for a novel approach toward the therapy of CKD-associated CVD. HCQ has several effects relevant to atherosclerosis. These include anti-inflammatory effects (e.g., stabilization of lysosomes), effects on the innate immune response (e.g., inhibition of toll-like receptors), effects on the adaptive immune system and regulatory cells, and effect on endothelial cells. Human studies to examine the utility of HCQ in CKD patients to prevent cardiovascular complications as well as additional studies to better understand the pathophysiological effects of HCQ appear to be warranted.

Role of Iron

The role of iron in atherosclerosis was first suggested by pathologist Jerome Sullivan when he was a junior faculty member in an article published as a hypothesis article in *The Lancet* in 1981 entitled *Iron and the Sex Difference in Heart Disease*.[9] He postulated that the reason why women of child-bearing age do not get cardiac events is not because of estrogens but because of the loss of iron during menstruation. This hypothesis formulated about 30 years ago has led to a large number of studies, often with conflicting data. In a review article in which 55 studies on the relationship between iron and CVD were analyzed, the authors reported that 27 supported the hypothesis, 20 studies found no evidence to support it, and 8 were contrary to the iron hypothesis.[10] We believe that these conflicting data are due to the reliance on total body iron (or measures of it), rather than catalytic iron.

Iron is the most common transitional metal in the body and participates in many important biological functions. Critical to these functions is its ability to undergo redox cycling. This precise property ultimately makes iron dangerous because it can result in the formation of highly reactive oxygen metabolites. The term catalytic (or labile) iron is used to denote iron that can participate in redox cycling.[11] It is generally associated with low-molecular–weight chelates and constitutes only a tiny fraction of total cellular iron.

There is limited but important information suggesting the role of catalytic iron in cardiovascular events. In a study carried out by Dr. Rajapurkar et al.,[12] they measured catalytic iron in approximately 500 subjects which included healthy subjects and individuals with CKD and CVD. Subjects who had catalytic iron in the upper tertile had a 10-fold higher probability of having CVD. Even after adjustments for age, sex, and the Framingham criteria, there was a 4-fold higher probability.[12]

In a smaller study consisting of patients with ESKD on dialysis, Rajapurkar et al. reported that, compared with normal subjects, dialysis patients had a much higher level of catalytic iron. Of the 59 patients studied, 37 (63%) had no coronary artery disease (CAD) and had much lower levels (1.35 ± 0.34 μmol/L) of catalytic iron, whereas the remaining 22 (37%) patients with significant CAD had 8.92 ± 4 μmol/L levels.[13]

In the 22 patients with diabetes, 14 of whom had CAD had levels of 8 ± 4 μmol/L compared with <1 μmol in those without CAD.[13] Similar marked differences were seen in nondiabetic patients. These data show that the presence of high catalytic iron identifies patients who are very likely to have CVD. This test could be used in asymptomatic dialysis patients to identify patients who may have CAD.

We want to briefly mention the utility of serum catalytic iron in identifying patients with acute coronary syndrome. In a study by Lele et al., they measured catalytic iron in 250 healthy volunteers, 51 patients with chest pain without myocardial infarction (MI), and 127 with acute MI. Catalytic iron was elevated in all patients with MI and, at a cutoff of 0.3 μmol/L, the sensitivity was 84%, specificity 95%, and diagnostic accuracy was 92%.[14] These observations about the utility of catalytic iron in patients with acute coronary syndrome have been confirmed in 2 additional studies. Steen et al.[15] evaluated catalytic iron in 1,700 patients from the thrombosis in myocardial infarction trial and demonstrated that catalytic iron was associated with a stepwise increased risk of death, with the highest quartile at almost 4-fold risk compared with baseline.[15] In a prospective study involving 806 patients, the highest quartile of catalytic iron was associated with a 6-fold increase in mortality.[16] Collectively, these studies indicate the utility of catalytic iron for diagnosis and as potential therapeutic target.

Carbamylated Proteins

Carbamylation of proteins results from nonenzymatic chemical modification by the isocyanic acid derived from urea. More recently, Wang et al., in a study published in *Nature*, have demonstrated an alternative route for carbamylation—namely, by the myeloperoxidase-catalyzed oxidation of thiocyanate. They reported that, in 2 separate clinical studies involving 1,000 subjects, protein-bound

homocitrulline independently predicted the risk for acute coronary disease or stroke, frequency of death, and frequency of major cardiovascular events.[17] The Wang study was in patients with normal renal function. A study published in *JASN* last year by Koeth et al.[18] examined the relationship between protein carbamylation and major adverse cardiac events in 347 patients on hemodialysis. Protein carbamylation as measured by plasma homocitrulline levels was much higher in patients undergoing hemodialysis compared with controls.[18] The highest tertile of protein carbamylation was associated with a significant higher mortality and Kaplan–Meier analyses revealed a significant association between elevated protein carbamylation and death over a 5-year follow-up period. This study shows that serum protein carbamylation predicted an increased cardiovascular risk in patients with ESKD.

In another study, The percentage of carbamylated albumin was significantly higher in patients with CKD compared with normal subjects. In addition, the patients from the Accelerated Mortality on Renal Replacement (ArMORR) study who died within 12 months had significantly higher protein carbamylation compared with patients who survived the 12-month period.[19] To validate the major findings from the ArMORR cohort, Berg et al. tested whether baseline carbamylated albumin was associated with mortality in a second independent study of hemodialysis subjects (the 4D Trial). They found significant risk of death among 4D subjects with elevated carbamylated albumin similar to those observed in the ArMORR study. The concentration of urea in their blood was not different from that of ArMORR survivors, suggesting alternate pathways for carbamylation.[19] In a recent study, carbamylation was associated with a higher erythropoietin (EPO) resistance index and was a better predictor of mortality than the EPO resistance index.[20]

The studies indicate an association between carbamylation and adverse outcomes but do not provide any cause–effect relationship. We have carried out a number of studies that demonstrate that carbamylated LDL (cLDL) has effects that are relevant to atherosclerosis. We have shown that cLDL is taken up by endothelial cells through some specific receptors[21] and cLDL causes endothelial cell injury.[22] We have also shown that cLDL causes overexpression of adhesion molecules[23] and induces cell proliferation in vascular smooth muscle cells.[24] These biological effects are all relevant to atherosclerosis. In addition, we have carried out studies to demonstrate that high urea by itself is able to induce atherosclerosis in vivo.[25]

We have addressed 3 potential approaches to cardiovascular events in CKD patients. It is likely that there is at least some interrelationship between these pathophysiological processes. A better understanding of these mechanisms may help to develop new modalities of treatment targeted toward removing catalytic iron, use of inflammatory agents such as HCQ, and approaches to reduce carbamylated proteins. These new therapeutic modalities may also prove beneficial in the general population.

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