New Treatments for CKD—New Insights into Pathogenesis

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Albuminuria is a standard surrogate endpoint for many clinical studies examining therapies for diabetic nephropathy and other kidney diseases. This standard is based on the assumption that a reduction in albuminuria corresponds to a reduction in glomerular damage and therefore, amelioration of CKD. Albuminuria, however, can also result from decreased proximal tubular uptake. Because proximal tubule reabsorption of albumin is associated with an inflammatory response, inhibition of albumin absorption presents another potential target for therapy of CKDs.

The article by Reisman et al.† in JASN sheds light on such a target while showing the dosisociability of proteinuria and progression of kidney disease. The article by Reisman et al.1 shows that yearlong treatment of cynomolgus monkeys with bardoxolone methyl, a triterpenoid activator of the Nrf2 pathway, led to a decrease in serum creatinine and improvement in estimated GFR, despite an increase in albuminuria. The kidneys from the treated animals showed normal histology. Immunohistochemistry showed a significant decrease in the expression of megalin and increase in Nrf2-stimulated cytoprotective proteins. The article by Reisman et al.1 concludes that bardoxolone methyl-induced decrease in megalin resulted in decreased proximal tubule albumin uptake, leading to a decrease in the inflammatory response and improvement in kidney function.

For over 25 years, the production of reactive oxygen species has been linked to diabetic pathology in general and diabetic nephropathy in particular.2 Investigators have focused primarily on mechanisms for and consequences of production of reactive oxygen species resulting from hyperglycemia-dependent processes. More recently, interest has turned to possible endogenous responses that protect cells from oxidative injury. In this context, Nrf2 emerges as a central regulator of endogenous responses that protect cells from oxidative injury. In this context, Nrf2 emerges as a central regulator of endogenous antioxidant enzymes. Transgenic studies establish that kidneys...


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from Nrf2−/− animals are more susceptible to streptozotocin-induced diabetes, whereas compounds that increase Nrf2 activity, such as antroquinolol, tert-butyldihydroquinone, and triterpenoids, may be renoprotective.3,4 The mechanistic connection between increased Nrf2 activation and decreased megalin expression reported in the work by Reisman et al.1 is not entirely clear.

The decreased expression of megalin protein was not associated with a decrement in levels of mRNA encoding megalin, suggesting degradation of the megalin protein. Although accelerated proteolytic degradation of megalin may occur, a more intriguing hypothesis is that bardoxolone-induced megalin activation might stimulate regulated intramembrane proteolysis of megalin into membrane bound fragments that translocate to the nucleus and activate gene transcription and soluble megalin fragments that shed into the urine.5 These mechanistic issues, not explored in the current study, should be the subject of additional investigation. The correlation of increased Nrf2 activation, downregulated megalin expression, and improved renal function suggests the hypothesis that decreasing megalin expression prevents additional damage to the kidney resulting from reabsorbed protein as well as oxidized lipids. Megalin-mediated endocytosis of protein can trigger apoptosis in proximal tubule cells6 as well as activate a series of proinflammatory genes and probiotic genes. However, the literature does not uniformly describe a protective role for decreased megalin expression in proteinuric states depending on the cause of decreased megalin and the type of proteinuria.7,8 The possible renoprotective effect of decreased megalin expression induced by bardoxolone methyl is a novel finding that warrants additional investigation.

Several other aspects of this study are worth noting. The use of a primate model system is a real strength, because metabolism of bardoxolone methyl differs between humans and rodents. Thus, the finding that the drug has no discernible adverse effect after 1 year of high-dose treatment is reassuring for human application. The absence of histologic kidney abnormalities after 1 year of treatment is consistent with the findings in the megalin-deficient mouse.9 Likewise, the absence of an effect on serum electrolytes is not surprising, because the megalin-deficient mouse showed only low molecular weight proteinuria and not full Fanconi syndrome.

Megalin-deficient mice do exhibit urinary losses of both vitamin D-binding protein and 25 hydroxycholecalciferol, the precursor steroid that is reabsorbed into proximal tubule cells and undergoes activation to 1,25 dihydroxycholecalciferol. Reisman et al.1 comment in their discussion that bone histology was normal, although these data were not presented. Coupled with the normal serum calcium, this finding suggests that functional vitamin D deficiency did not occur in the animals subjected to treatment for 1 year with bardoxolone methyl, but more robust monitoring of vitamin D metabolism and analysis of bone histology would be needed before drawing firm conclusions. Development of vitamin D deficiency could blunt the salutary effects of bardoxolone methyl by permitting activation of the renin–angiotensin system, an increased inflammatory state, and/or effects on insulin secretion and sensitivity.10 In humans, this process could require years to manifest and thus, would not be described in a study even as long as 1 year.

The decrease in serum phosphate seen in the bardoxolone methyl-treated animals is of interest and may or may not be related to the observed megalin reduction. Acute inhibition of megalin function by injection of receptor-activated protein produces a marked decrease in renal brush border membrane expression of Npt2a, the major sodium phosphate cotransporter expressed along the proximal tubule, which would result in renal phosphate wasting.11 Alternatively, Nrf2 activation may directly decrease sodium phosphate cotransporter expression, also causing lower serum phosphate concentrations. A decrease in active vitamin D resulting from urinary losses could lead to decreased intestinal absorption of phosphate and therefore, lower serum phosphate concentrations. Also, the decrease in serum phosphate may simply be a function of a higher GFR. Immunoblot or immunohistochemical determination of renal sodium phosphate cotransporter expression would likely distinguish between these possibilities, because decreased intestinal absorption of phosphate stimulates renal Npt2a expression. Because serum phosphate levels correlate with cardiovascular and CKD risk, it is tempting to speculate that some of the protective effects of bardoxolone methyl in human kidney disease may be related to an effect on phosphate homeostasis.

The observed decrease in megalin expression may also have a salutary effect on the progression of kidney by decreasing the proximal tubule reabsorption of potential nephrotoxins, such as aminoglycosides or cis-platinum. A recently published clinical trial documented the safety and efficacy of bardoxolone methyl in the treatment of diabetic patients with CKD and a wide range of proteinuria. Similar to the findings in the work by Reisman et al.1, the work by Pergola et al.12 reported that serum BUN, uric acid, and phosphate decreased in subjects taking bardoxolone methyl, suggesting an effect on proximal tubule transport that may be directly or indirectly caused by improved GFR. Although these alterations in solute content and the effects on megalin point to a predominating effect on proximal tubule function, the decrease in serum magnesium suggests potential effects of bardoxolone methyl in more distal segment transport processes. If so, then perhaps the measured increase in GFR could result from alterations in tubuloglomerular feedback mechanisms. The role for Nrf2 activation in these effects has not been determined.

Although our understanding of the mechanisms for the observed effects of bardoxolone methyl on kidney function is woefully incomplete, the addition of bardoxolone methyl to the repertoire of treatments for CKD, including diabetic nephropathy, is a welcome event. The mechanism of action is complementary to our established therapies, particularly inhibition of the renin–angiotensin–aldosterone (RAAS) axis, which has been the mainstay for the past three decades. The clinical use of RAAS inhibitors has been accompanied by
substantive improvement in kidney survival. Research into this pathway uncovered the pleiomorphic effects of the RAAS system on kidney function beyond hemodynamics and highlighted the role of chronic inflammation in the progression of CKD. Bardoxolone methyl may yet be found to have hemodynamic effects, but these effects are likely to be a minor aspect of its renal benefits. This agent targets a specific anti-inflammatory pathway that clearly plays a major role in kidney function. Unlike many other anti-inflammatory agents, bardoxolone methyl does not simply antagonize an established inflammatory response or mop up inflammatory metabolites. It is proactive and not reactive. Additionally, the bardoxolone methyl-induced inhibition of megalin expression will decrease proximal tubule uptake of angiotensinogen, thus lessening the intrarenal production of angiotensin II.\(^\text{13}\) Used with RAAS inhibitors, bardoxolone methyl may blunt the GFR-lowering effect and augment with the anti-inflammatory effects of RAAS inhibitors, yielding a potentially highly synergistic approach to the treatment of CKD. Thus, bardoxolone methyl is not likely to supplant RAAS inhibition but is likely to be only the first salvo into an exciting new arena of renal therapeutics.

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See related article, “Bardoxolone Methyl Decreases Megalin and Activates Nrf2 in the Kidney,” on pages 1663–1673.

**Connecting the Segments**

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Tubular networks lined by epithelial cells, which are ancient structures found in all metazoan organisms, are vital components of many of our organs, including the gastrointestinal tract, lung, and kidney. Although there is commonality to the overall structure of tubes, their size, specific cell composition, and function are diverse, suggesting that they underwent distinct morphogenesis. Consistent with this hypothesis, different tube segments in branched organs have separate

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