

studies, as noted above, have unraveled novel cellular and molecular insights into the lithium's effects on renal tubular cells in AKI and in NDI.

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DISCLOSURES

None.

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See related articles, "Delayed Administration of a Single Dose of Lithium Promotes Recovery from AKI," and "Lithium Causes G2 Arrest of Renal Principal Cells," on pages 488–500 and 501–510, respectively.

Arrestin(g) Podocyte Injury with Endothelin Antagonism

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Proteinuria is a major pathologic feature of a wide variety of progressive CKD. Even if it is accompanied by a normal GFR, proteinuria indicates significant kidney dysfunction and its presence has been incorporated into definitions for CKD.¹ Because proteinuria is an independent risk factor for CKD, it is imperative to determine the mechanisms leading to its development. Current antiproteinuric therapy relies mainly on renin-angiotensin inhibition, but this is only partially successful and novel treatments are urgently needed.²

In this issue of *JASN*, Buelli *et al.* examine how endothelin-1 (ET-1) mediates podocyte injury/dysfunction.³ The endothelins are a family of vasoactive peptides with a variety of roles in human disease, with ET-1 being the most relevant in kidney pathology. ET-1 binds to two receptors, endothelin A and endothelin B receptors (ET_AR and ET_BR, respectively). In general, ET_AR is responsible for vasoconstrictive and pathologic actions of ET-1, whereas ET_BR governs protective vasodilatory functions. Therefore, selective blockade of ET_AR with pharmacologic agents such as sitaxsentan and atrasentan has been explored as a renoprotective therapy, and positive results in reducing proteinuria were demonstrated in a variety of kidney diseases, including diabetic nephropathy.⁴

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ET-1 was previously shown to play a critical role in the development of metastatic ovarian cancer by promoting the formation of an ET_AR/ β -arrestin/Src kinase complex in an autocrine manner, which leads to stabilization and upregulation of β -catenin and Snail transcriptional regulators. Such actions resulted in an epithelial-to-mesenchymal transition of these cancer cells, increasing their invasive potential.⁵ These findings are relevant for proteinuric kidney disease because podocytes undergo a similar transition to a more motile phenotype in crescentic renal diseases.⁶ Drawing upon this comparison with tumor cell behavior, the authors examined the role of ET-1 in podocyte phenotypic changes. Utilizing *in vitro* podocyte cultures, they found that ET-1 does in fact induce an epithelial-to-mesenchymal transition–like event, with concomitant downregulation of the podocyte marker synaptopodin and upregulation of α -smooth muscle actin. These changes were associated with increased motility in a wound healing assay.

Turning to *in vivo* studies, the authors utilized the adriamycin nephropathy model in mice. This model recapitulates proteinuric renal disease, with features similar to human FSGS.⁷ The authors demonstrated a loss of Wilms tumor 1 positivity in glomeruli after adriamycin administration, and scanning electron microscopy revealed markedly abnormal foot processes and enlarged podocytes. Treatment with the ET_AR inhibitor sitaxsentan ameliorated these morphologic changes.

These results are perhaps not unexpected considering the antiproteinuric effect of ET_AR inhibition. The more interesting and novel finding in this study is the elucidation of the mechanism underlying this protection. It appears that, similar to ovarian cancer cells, ET-1–mediated podocyte injury involves the activation of a protein known as β -arrestin-1. The arrestins are ubiquitous proteins that participate in functions as diverse as G-protein signaling and endocytosis. They are also capable of acting as scaffold proteins that can gather various intracellular proteins into close proximity to initiate complex signaling pathways.⁸

Buelli *et al.* have performed a careful interrogation of the ET-1–triggered signaling events and demonstrate that ET-1 ligation to its receptor in podocytes leads to induction of β -arrestin-1 and promotes the formation of a multicomponent complex containing β -arrestin-1, ET_AR, and Src kinase. β -Arrestin-1 is also upregulated in hyperplastic lesions in samples from adriamycin-treated rodents and in human crescentic glomerular disease biopsies, suggesting clinical relevance for the activation of this signaling complex. Interestingly, assembly and activation of the ET_AR/ β -arrestin/Src complex results in the transactivation of the EGF receptor, which triggers downstream signaling that ultimately leads to the stabilization and increased activity of β -catenin.

β -Catenin is a key transcriptional regulator that is essential for normal kidney development, but its activity is largely silenced in adult kidneys. However, growing evidence implicates hyperactive β -catenin activity in the pathogenesis of a variety of kidney diseases. As a transcriptional regulator, β -catenin

controls, directly or indirectly, the expression of a battery of downstream target genes such as Snail1, plasminogen activator inhibitor-1, and matrix metalloproteinase-7, as well as multiple components of the renin-angiotensin system, all of which are highly relevant to kidney injury. Earlier studies showed that β -catenin activity is upregulated specifically in glomerular podocytes in various proteinuric kidney diseases such as FSGS and diabetic nephropathy and that genetic ablation of β -catenin in a podocyte-specific fashion protects kidneys against the development of proteinuria after adriamycin injury.^{9,10} Therefore, the trimeric complex of ET_AR/ β -arrestin-1/Src identified in this study mechanistically links ET-1 to the activation of β -catenin, a central player in mediating podocyte dysfunction.

The findings in this report are significant for many reasons. First, Buelli *et al.* clearly demonstrate that ET-1 has profound effects on podocyte phenotype and morphology including foot process architecture, which is associated with proteinuria. Second, the data provide evidence that ET_AR activation and β -arrestin-1/Src recruitment lead to increased β -catenin activity primarily through an ET-1–driven, Wnt-independent pathway. Recent data have implicated β -catenin stabilization through ligation of Wnt to frizzled receptors on the cell surface *via* the so-called canonical pathway.¹¹ The identification of the novel ET-1–driven ET_AR/ β -arrestin/Src pathway suggests that multiple pathways can lead to β -catenin activation in podocytes. This important finding carries significant therapeutic implications.

This study does, however, have limitations. In spite of the evidence showing favorable effects of ET_AR blockade on podocyte phenotype and intracellular signal transduction, this study was unable to demonstrate a significant effect on proteinuria, which is the ultimate goal of podocyte protection. In separate studies, we also could not improve proteinuria in adriamycin nephropathy when atrasentan was given either before or after the initiation of injury.¹² These observations are in harmony with the fact that although ET-1 was induced at late time points, no significant change in ET_AR expression was found in this model of podocyte injury and proteinuria.¹²

The lack of effect of ET_AR inhibition on proteinuria could suggest that adriamycin may cause proteinuria by a mechanism that is independent of ET-1 regulation, although the ET_AR/ β -arrestin complex is clearly involved in regulating other aspects of podocyte biology such as migration and crescentic formation. The authors point out that the pores that form in the glomerular barrier may not be affected by ET_AR blockade.¹³ Of note, ET_AR inhibitors have been successful in reducing proteinuria in diabetic nephropathy and other renal injuries in which there is increased intraglomerular pressures.¹⁴ However, there is evidence that adriamycin nephropathy is not characterized by these increased intraglomerular pressures, perhaps explaining the lack of efficacy of ET_AR on proteinuria in this model.¹⁵ Furthermore, adriamycin nephropathy is often considered to be a model of human FSGS with characteristic features of podocyte depletion, but it is not

traditionally utilized to study crescentic glomerular disease which is the type of renal disease characterized by podocyte migration. In this regard, it would be interesting to examine ET_AR blockade in the antiglomerular basement membrane model of kidney disease, which more closely mimics crescentic glomerular disease.

Taken together, the study by Buelli *et al.* has identified a signaling cascade that leads to impaired podocyte phenotype and function, thereby providing significant insights into our understanding of podocyte injury in proteinuric glomerular disease. The novel associations made between ET-1, ET_AR, β -arrestin-1, EGF receptor, and β -catenin now provide a number of promising targets for therapeutic intervention and will require further study in the future.

DISCLOSURES

None.

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See related article, " β -Arrestin-1 Drives Endothelin-1-Mediated Podocyte Activation and Sustains Renal Injury," on pages 523–533.

Cardiovascular Events after AKI: A New Dimension

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The possibility that AKI is a risk factor for subsequent cardiovascular disease is an intriguing one. Several studies have shown that survivors of AKI are at increased risk of death and CKD. Because cardiovascular disease is the leading cause of death in many countries, it would be useful to understand whether there is increased risk of cardiovascular events after an episode of AKI. Therefore, prospective studies, such as the National Institutes of Health–National Institute of Diabetes and Digestive and Kidney Diseases-sponsored ASessment, Serial Evaluation, and Subsequent Sequelae of Acute Kidney Injury study, have specified *a priori* adjudicated cardiovascular events as outcomes of interest.¹

Although CKD and ESRD are now well accepted risk factors for cardiovascular disease, little is known about the connection between AKI and cardiovascular events. There are several potential pathophysiological mechanisms by which AKI may directly contribute to increased risk of cardiovascular disease, independent of its impact on CKD progression. There are limited data (mostly from children²) that AKI survivors have higher levels of BP, consistent with animal models showing that postischemic rats develop salt-sensitive hypertension.³ As discussed by Wu *et al.*, there is a growing literature linking AKI to abnormalities in mineral metabolism, especially elevations in levels of fibroblast growth factor-23,^{4,5} a novel biomarker that has garnered great interest recently as likely being directly cardiotoxic.⁶ Finally, AKI is associated with acute increases in inflammatory cytokine levels.^{7–9} As has been shown in other acute conditions characterized by inflammation (*e.g.*, acute

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