Mineralocorticoid Receptor Antagonism in AKI:
A New Hope?

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AKI is a leading cause of morbidity and mortality in hospitalized patients, particularly among the critically ill, and its incidence is increasing.1,2 This has piqued the interest of renal researchers, leading to a massive surge in our knowledge of its pathogenesis, systemic effects, and clinical course. Consequently, there has been a paradigm shift in our appreciation of AKI. Classically, AKI was assumed to be a renal–specific, limited, reversible condition easily supported by dialysis. Conversely, we now recognize it as a systemic entity that modulates the underlying disease process and causes dysfunction of other organs.3,4 Moreover, survivors of AKI are at increased risk of long–term adverse outcomes, including CKD, ESRD, cardiovascular events, gastrointestinal bleeding, bone fractures, and premature death.5,6 Hence, identifying effective therapeutic targets in AKI is of paramount importance. In search of effective therapies, we have vastly increased our mechanistic understanding of AKI; however, we have been less successful at translating this knowledge into clinically relevant therapies, perhaps because of its intricate pathophysiology.

AKI is often instigated by a drop in renal perfusion to a level that prevents renal cells from producing sufficient ATP to maintain essential processes. This ischemic injury initiates a response that increases generation of reactive oxygen species, cytokines, chemokines, and leukocyte activation and decreases nitric oxide (NO), thus propagating renal injury. Injury of the endothelial cells leads to microvascular dysfunction characterized by diminished vasodilation and enhanced vasoconstriction.7 This imbalance between vasoconstriction and vasodilation contributes to the persistent hypoperfusion of the outer medullary region, causing extension of renal injury.8 Consequently, strategies that diminish this imbalance have attracted much interest. Vasodilators that preferentially increase medullary perfusion, such as NO, may be particularly effective. NO is especially appealing because of its antioxidant, anti-inflammatory, and other beneficial effects. Indeed, agents that augment NO activity (e.g., endothelial nitric oxide synthase [eNOS] –expressing surrogate cells, nitrite, and phosphodiesterase 5 inhibitors) protect against ischemia-reperfusion–induced AKI (I/R-AKI).9 Alternatively, the imbalance can be improved by blocking vasoconstrictors (e.g., angiotensin II and endothelin [ET]). Because aldosterone has vasoconstrictor, profibrotic, and proinflammatory properties, it may also be an attractive target.

In this issue of JASN, Barrera-Chimal et al.8 provide compelling evidence that BR-4628, a third generation mineralocorticoid receptor (MR) antagonist, is highly protective against experimental I/R-AKI. Moreover, Barrera-Chimal et al.8 uncover a novel mechanism by which it extends its protection; it prevents AKI-induced inactivation of the ETB receptor, thus normalizing generation of NO. The concept that MR antagonists may be effective against AKI is not novel. Indeed, Bobadilla and colleagues9,10 previously reported that blocking the aldosterone/MR cascade by either spironolactone or adrenalectomy protects against AKI. These maneuvers decreased oxidative stress and increased the expression and activity of eNOS, thus resulting in increased NO and normalization of renal blood flow (RBF). However, the mechanism by which aldosterone blockade restored NO was not fully elucidated. Of particular relevance was their finding that I/R-AKI increased prepro-ET as well as the ET receptor subtypes ETA and ETB.10 Of note, despite the 2-fold increase in both receptors, RBF and NO were reduced by approximately 50%, suggesting that, in the presence of aldosterone, ET predominantly activates ETA. Adrenalectomy not only prevented the increase in ETA but also, further increased ETB, resulting in normalization of RBF and NO. Thus, the beneficial effect of aldosterone exclusion may be because of normalization of ETA and/or upregulation of ETB.

This study by Barrera-Chimal et al.8 extended on this notion; Barrera-Chimal et al.8 found that a novel MR antagonist also protected against I/R-AKI and showed that this beneficial effect was linked to the ETB receptor. Ischemia/reperfusion–induced oxidative stress led to a cysteine sulfenic acid modification of the ETB receptor, causing its inactivation and resulting in decreased eNOS activity. BR-4628 prevented AKI-induced sulfonation of the ETB receptor, thus resulting in preserved eNOS activity, NO levels, and RBF, thereby attenuating renal injury. Barrera-Chimal et al.8 also showed that an ETB receptor antagonist could negate the
protective actions of BR-4628, implying that the beneficial effects of the MR antagonist were a direct consequence of $\mathrm{ET}_A$ activation and not an epiphenomenon. Taken together, these studies suggest that the MR is an important factor in determining the balance between $\mathrm{ET}_A$ and reactive oxygen species and $\mathrm{ET}_B$/NO, making it an attractive therapeutic target in AKI.

Despite the potential benefit of MR antagonism in cardiovascular and renal diseases, the available steroidal MR antagonists have shortcomings that curb their use. Spirolonolactone is potent but lacks selectivity, resulting in side effects related to activation of other oxosteroid receptors; eplerenone has higher selectivity but less MR affinity, rendering it less potent. Both also increase plasma levels of renin, angiotensin II, and aldosterone, which may diminish their effectiveness (through nongenomic effects in the case of aldosterone). They cannot be used in patients with the S810L mutation (a gain-of-function mutation associated with early-onset and gestational hypertension). Most importantly, they can cause hyperkalemia and renal dysfunction. These shortcomings stimulated the development of novel nonsteroidal MR blockers with renal-sparing properties. Several are proving to be as efficacious as the steroidal antagonists but with decreased side effects. The fortuitous discovery that various dihydropyridine calcium channel blockers compete with aldosterone binding to the MR led to the development of dual MR/calcium channel blockers as well as MR-selective antagonists devoid of calcium channel blocking properties. BR-4628 is one such dihydropyridine–based MR antagonist with low calcium channel binding activity. It exhibits greater sensitivity and potency toward the MR and thus far, has been reported to lack most of the adverse effects of spironolactone and eplerenone, including hyperkalemia. Indeed, in this study by Barrera-Chimal et al., serum potassium levels were not elevated by BR-4628. Although this finding is very promising, we must interpret it with caution, because potassium content in rat chow may be more controlled than in human diet and also, because food intake is usually reduced during the 24-hour posts ischemia period. Hence, we cannot exclude the possibility that there was insufficient time for the rats to develop hyperkalemia. In this respect, it is important to note that, in the previously mentioned study, spironolactone also did not cause hyperkalemia. Consequently, additional studies are needed to confirm that BR-4628 will not increase the risk of hyperkalemia.

The efficacy of the MR antagonist is not limited to reducing the severity of I/R-AKI. Administering spironolactone before or soon after ischemia also enhanced recovery of I/R-AKI and blunted its progression to CKD. Although this may primarily be caused by a reduction in AKI severity, it is tempting to speculate that it also prevents progression through its effects on the ET system. Evidence for this is provided by the work by Zager et al., which reported that unilateral ischemia increased renal ET and $\mathrm{ET}_A$ immediately after ischemia and that they continued to rise so that their levels were dramatically higher at 2 weeks. In contrast, $\mathrm{ET}_B$ was not initially increased and only minimally elevated at 2 weeks. This profound imbalance between the $\mathrm{ET}_A$ and $\mathrm{ET}_B$ receptors suggests that ET may be critical in promoting the ongoing renal vasoconstriction and consequently, the ensuing local hypoxia, activation of inflammatory and profibrotic signaling pathways, and thereby, tissue damage. Indeed, blocking the $\mathrm{ET}_A$ receptor with atrasentan prevented the structural changes and likely arrested progression to CKD. This efficacy of atrasentan makes the MR antagonists even more appealing. That is, because atrasentan blocks the $\mathrm{ET}_A$ receptor, ET may be shunted toward the $\mathrm{ET}_B$ receptor; however, activation of this receptor is likely diminished because of sulfonilation-induced inactivation. MR antagonists can, therefore, exert their beneficial effects by favorably affecting both sides of the $\mathrm{ET}_A$/$\mathrm{ET}_B$ balance. They prevent the ischemia-induced increases in $\mathrm{ET}_A$ and decreases in $\mathrm{ET}_B$ as well as the sulfonilation of the $\mathrm{ET}_B$. Thus, blocking the MR may be particularly effective at preventing AKI from progressing to CKD, and it may also facilitate the use and enhance the effectiveness of $\mathrm{ET}_A$ blockers.

We still do not have effective therapies against AKI. An ideal therapy would prevent development of AKI, limit its severity and systemic sequelae, and promote recovery as well as prevent its chronic sequelae, including progression to CKD. Because of the recent recognition that the MR has a variety of wide-reaching mechanisms and thus, may affect the distinct parameters mentioned above, targeting the MR might be particularly effective in AKI. However, as with chronic cardiac and renal diseases, the use of available MR antagonists has been limited because of their side effects. The study by Barrera-Chimal et al. in this issue of JASN provides new hope for MR antagonism in AKI. Because of the multifaceted mode of action and favorable safety profile of these antagonists, these agents may not only interrupt the transition of AKI to CKD but also, provide a therapeutic strategy in CKD in general. However, it must be remembered that, because of the complexities of each stage of AKI, it is likely that MR antagonism will need to be part of a fluid multi-pronged approach to make a significant effect on this disease. We await advancement toward translational studies that are needed to address their effectiveness and safety profile in human AKI.

DISCLOSURES
None.

REFERENCES
NADPH Oxidase 4 at the Nexus of Diabetes, Reactive Oxygen Species, and Renal Metabolism

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NADPH oxidases (NOXs) catalyze the transfer of electrons from NADPH to molecular oxygen to produce superoxide and/or hydrogen peroxide, two major reactive oxygen species (ROS).\(^1\) Clarity on in vivo function and relevance to human disease are best exemplified by NOX2, which is responsible for generating the respiratory burst (i.e., large quantities of microbial ROS) in phagocytes. In turn, NOX2 deficiency is a cause of chronic granulomatous disease, an inherited disorder characterized by recurrent and persistent infections. Whereas NOX2 and its function within phagosomes were the first to be discovered and are the most widely recognized, in total, seven NOXs have been identified (NOX1, -2, -3, -4, and -5 and dual oxidase 1 and -2) with differences in tissue distribution, intracellular localization, and regulation. In addition to their potential contribution to oxidative stress, NOX ROS production is now recognized to play a fundamental role in cell signaling, with diverse effects on cell growth, differentiation, motility, and survival.\(^2\) These insights have motivated significant interest in nonphagocytic NOX in human health and disease.

NOX4 is the predominant NOX isoform expressed in the kidney.\(^3,4\) Unlike other isoforms, it is constitutively expressed with basal ROS production implicated in several normal renal physiologic functions.\(^5\) NOX4 expression is increased in tubular epithelial cells, mesangial cells, and podocytes cultured in high-glucose media and kidney tissue from different rodent models of diabetes.\(^6–10\) The deleterious effects of NOX4 in these contexts are numerous and extend beyond free radical/oxidant–mediated tissue injury. For example, in addition to reducing ROS levels, transient NOX4 knockdown has been shown to reduce Akt/PKB and extracellular signal–regulated kinase-1/2 phosphorylation in cortical homogenates from diabetic mice\(^6\) and inhibit TGF-\(\beta1\)–induced renal myofibroblast activation.\(^11\) Furthermore, the NOX1/NOX4 inhibitors GKT136901 and GKT137831 have been shown to attenuate ROS production, albuminuria, renal fibronectin and TGF-\(\beta\) content, and glomerular hypertrophy in \(\text{db/db}\) and OVE25 mice, respectively.\(^12,13\) Finally, global deletion of Nox4 attenuated albuminuria and glomerular injury in streptozotocin–treated \(\text{ApoE}^{-/-}\) mice.\(^14\) Collectively, these data

See related article, “Sulfenic Acid Modification of Endothelin B Receptor is Responsible for the Benefit of a Nonsteroidal Mineralocorticoid Receptor Antagonist in Renal Ischemia,” on pages 398–404.