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See related article, "Mitochondria Protection after Acute Ischemia Prevents Prolonged Upregulation of IL-1β and IL-18 and Arrests CKD," on pages 1437–1449.

The Use of Sildenafil for Glomerular Disease

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Transient receptor potential channel 6 (TRPC6) is a Ca^{2+} ion transport channel associated with the slit diaphragm of podocytes, and is indispensable for regulation of structural components of podocytes and renal function.[1] Since the discovery of a TRPC6 gain-of-function mutation that causes familial FSGS, TRPC6 activation has been associated with several progressive glomerular diseases.[2,3] In fact, TRPC6 in the context of kidney disease has become quite ubiquitous, and its activity is being considered by many as an indicator of podocyte vulnerability and progressive kidney disease.[4] It is of no surprise that researchers began investigating blocking TRPC6 activity, thereby reducing deleterious Ca^{2+} influx in an effort to adjust the podocyte cytoskeleton. In the current issue of the *Journal of the American Society of Nephrology*, Sonneveld *et al.*[5] found sildenafil (*i.e.*, Viagra) has antiproteinuric effects *via* a mechanism involving peroxisome proliferator-activated receptor γ (PPAR-γ) in mouse podocytes, and may be an effective regulator of TRPC6 signaling for use in treating glomerular disease.

As a successful erectile dysfunction drug, sildenafil occupies the active site of phosphodiesterase type 5 (PDE5), giving rise to cGMP, which initiates smooth muscle relaxation and increased blood flow. Although sildenafil is a well known PDE5 blocker in penile and cardiac tissue, its role in podocytes is poorly defined. Sonneveld *et al.* contribute to ongoing research efforts that have demonstrated the renoprotective effects of sildenafil by more clearly defining a pathway in which sildenafil blocks PDE5 leading to cGMP accumulation, protein kinase G-1 (PKG-1) activation, and in turn, PPAR-γ activation to downregulate TRPC6 expression.[5] In podocytes, cGMP accumulation is known to suppress renal disease,[6] and podocyte responsiveness to PDE5 blockers has opened the door to cGMP regulation as a means of treating podocyte injury. Several groups have extensively studied components of pathways activated by cGMP accumulation in podocytes, and a great deal of evidence supports TRPC6 inactivation as a mechanism to treat kidney disease. For example, cGMP accumulation has been linked to podocyte contractility, mobility, and cytoskeletal structure[7]; PKG-1 is associated with poor clinical outcome in renal cell carcinoma[8]; PPAR-γ agonists have been shown to protect podocytes from nephropathies[9]; and overexpression of TRPC6 alone is sufficient to cause podocyte damage and subsequent glomerulopathies.[10,11] Collectively, these findings highlight the importance of crosstalk among cGMP, PKG-1, PPAR-γ, and TRPC6, and demonstrate the importance of TRPC6 signaling in kidney disease.

Sonneveld *et al.* found PDE5 is expressed in podocytes, and sildenafil can have antiproteinuric effects through PPAR-γ to decrease TRPC6 expression levels, and ultimately minimize deleterious Ca^{2+} influx.[5] Transcriptional downregulation of TRPC6 *via* PPAR-γ in response to sildenafil ameliorated podocyte injury, and was deemed more important than affecting TRPC6 channel functionality directly, as previously reported.[12] cGMP accumulation had no effect on Ca^{2+} influx in the presence of PPAR-γ antagonists, suggesting PKG-1–mediated binding of PPAR-γ to the TRPC6 promoter is the pivotal interaction regulating Ca^{2+} homeostasis. In the context of clinically approved drugs, this study is a reminder of the potential benefits of repurposing approved drugs. After all, sildenafil was not intended as an erectile dysfunction drug and is, in essence, a side effect drug itself. Complex pathways with

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influential secondary messengers, like cGMP, need to be broken down to be fully understood. Although feedback mechanisms and TRPC6 channel dynamics in response to cGMP accumulation in podocytes remain unclear, studying each component of the cGMP–PKG-1–PPAR-γ–TRPC6 pathway individually, and in combination, helped define functional roles in podocytes. Blocking PDE5 with sildenafil clearly initiates a signaling cascade that downregulates TRPC6 transcription *via* PPAR-γ, subsequently reducing Ca^{2+} influx to reduce podocyte injury and presumably glomerular disease. Despite this newly discovered mechanism by which sildenafil operates in podocytes, there are some limitations to the study. There does not seem to be substantial evidence to support the notion that PPAR-γ–mediated transcription of TRPC6 is the principal mechanism regulating TRPC6 activity in response to sildenafil treatment, given TRPC6 channel dynamics were not evaluated. Without considering the phosphorylation state of TRPC6 at Thr69, a recently reported response to sildenafil that inhibits TRPC6 channel function, the number of channels at the membrane is only problematic in the context of podocyte injury if the channels are open and allowing Ca^{2+} influx.[12] Thus, activation of each component of the sildenafil-initiated signaling cascade should be compared directly before determining the most crucial step. Is reducing TRPC6 expression or inhibiting TRPC6 channel activity (or both) the mechanism of PDE5-mediated renoprotection? The interplay between mechanical deactivation of TRPC6 *via* phosphorylation and TRPC6 membrane expression (*i.e.*, number of active channels) has yet to be explored to provide insight on channel conformation, activity, and openness probability in response to sildenafil treatment, and the contribution of each to Ca^{2+} homeostasis and podocyte injury. A compensatory or cooperative response of other TRPC channels also merits consideration. TRPC channel expression can influence activity of other TRP channels, which may have adverse effects in podocytes. In this study, TRPC1 was also downregulated in podocytes treated with sildenafil, and Kiso *et al.*[13] showed sildenafil decreases TRPC1, TRPC3, and TRPC6 expression in cardiomyocytes; both of which draw concern because of the undefined role of TRPC1 in glomerular disease. Of note, TRPC6 protein levels were not assessed by Western blot, making it difficult to evaluate the outcomes of decreased transcriptional activity of the TRPC6 promoter. As stated, an increase in TRPC6 transcription may cause functional effects, but promoter activity does not translate to functional activity of a protein. Caution should be taken when assuming an increase in transcription leads to an increase in translational products that are effective within a given pathway. Post-translational modification, especially with a channel-forming, membrane-associated protein, has to be monitored. Additionally, sildenafil is associated with increased blood flow and GFR, although Sonneveld *et al.* suggest antiproteinuric effects of renal vasodilation are nonspecific because of the absence of TRPC6 in renal vasculature.[5] Although transcriptional regulation of TRPC6 *via* PPAR-γ may be important in regulating Ca^{2+} influx, the TRPC6-mediated mechanisms and the effectiveness of sildenafil in treating glomerulopathies remain unclear.

Evaluation of sildenafil treatment in the context of glomerular disease has been conducted with some success. In a clinical setting, administration of sildenafil improves kidney function, prevents disease progression, reduces proteinuria, and restores GFR in patients with conditions ranging from pulmonary hypertension to diabetic nephropathy (reviewed by Vasquez *et al.*[14]). In a laboratory setting, sildenafil treatment has been largely beneficial in reducing proteinuria, inflammation, oxidative stress, fibrosis, hypertension, and general renal damage in several kidney injury models (see Schinner *et al.*[6] for a full review). Although the potential use of sildenafil to treat glomerulopathies is attractive, being the first and most well studied PDE5 blocker, mild but common side effects and adverse reactions to sildenafil treatment have been observed in some patients.[14] In this regard, it is difficult to overlook the potential use of other approved PDE5 inhibitors or PPAR-γ agonists. Sonneveld *et al.* have identified an alternative mechanism for Viagra, and revealed a multistep pathway to downregulate TRPC6 and prevent harmful Ca^{2+} influx in podocytes.[5] In so doing, they have uncovered other potential therapeutic entry points, such as the use of direct-acting PPAR-γ agonists like pioglitazone, with fewer side effects than PDE5 blockers. The data presented shows that pioglitazone is at least, if not more effective in all assays as a renoprotective agent than sildenafil. This result is likely because of the resource investment necessary for a sildenafil-initiated response requiring sequential responses from PDE5, cGMP, and PKG-1 to activate PPAR-γ. Using pioglitazone, TRPC6 is more efficiently and specifically blocked, having no effect on other TRPC channels, unlike sildenafil (example, TRPC1).

Moving forward, the best option to reduce susceptibility of podocyte injury by maintaining Ca^{2+} homeostasis may still be by blocking TRPC6 directly. Although some groups have already identified potential TRPC6 inhibitors, *in vivo* efficacy has been inadequate.[15] Thus, future research efforts will likely identify drugs that will be more effective than current treatment options, which downregulate TRPC6 activity by targeting distant upstream mediators. In fact, several regulators of TRPC6 in glomeruli have already been proposed.[3] One would anticipate such drugs to come in the form of a small molecule or microRNA routed agent that targets structural components of the TRPC6 channel, thereby regulating mechanical processes that reduce Ca^{2+} influx and regulate the glomerular filter.

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DISCLOSURES

J.R. is cofounder of TRISAQ Inc., a biotech company dedicated to developing novel therapies for renal disease. He stands to gain royalties for commercialization of these products.

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See related article, "Sildenafil Prevents Podocyte Injury *via* PPAR-γ–Mediated TRPC6 Inhibition," on pages 1491–1505.

Genetic Complexities of the HLA Region and Idiopathic Membranous Nephropathy

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The first human disease associations of the major histocompatibility (MHC) genes were reported over half a century ago, providing important insights into the genetic basis of autoimmunity. Since then, numerous association studies have examined the effect of specific MHC variants, also referred to as the human leukocyte antigen (HLA) alleles, on the risk of autoimmune conditions. These initial studies, however, were frequently flawed by low resolution of HLA typing, small cohort sizes, and spurious associations due to the extended linkage disequilibrium across this region. The modern era of genome-wide association studies (GWAS) provided robust validation of the pivotal role of this region in genetic susceptibility to many autoimmune and inflammatory disorders, including SLE, multiple sclerosis, inflammatory bowel disease, type 1 diabetes, rheumatoid arthritis, and many others. In the kidney field, GWAS provided strong evidence for HLA involvement in susceptibility to common forms of immune-mediated glomerular diseases, such as IgA nephropathy (IgAN)[1–4] and membranous nephropathy (MN).[5] Suggestive HLA signals have also been reported in recent studies of steroid-sensitive nephrotic syndrome[6] and lupus nephritis.[7] The described effects of HLA are consistently large, with per allele disease risk increasing from 50% to >400% above baseline, highlighting the critical role of HLA in the pathogenesis of these disorders.

The HLA region resides on chromosome 6p21.3 and is among the most gene-dense portions of DNA, with gene products ranging from antigen-binding molecules and receptors to signaling factors. This region is also among the most polymorphic in humans, with 21 core HLA genes coding for proteins critical for the human immune response to infectious pathogens. The region can be subdivided into classic class I, II, and III and extended class I and II. Class I encompasses HLA-A, -B, and -C that function as presenters of peptides to cytotoxic T cells, and class II consists of HLA-DR and HLA-DQ molecules that present epitopes to CD4-positive T cells. The currently

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