The role of cGMP and its signaling pathways in kidney disease

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Submitted 1 February 2016; accepted in final form 10 July 2016

Shen K, Johnson DW, Gobe GC. The role of cGMP and its signaling pathways in kidney disease. Am J Physiol Renal Physiol 311: F671–F681, 2016. First published July 13, 2016; doi:10.1152/ajprenal.00042.2016.—Cyclic nucleotide signaling pathways are an emerging research field in kidney disease. Activated cell surface receptors transduce their signals via intracellular second messengers such as cAMP and cGMP. There is increasing evidence that regulation of the cGMP-cGK1-PDE signaling pathway may be renoprotective. Selective PDE5 inhibitors have shown potential in treating kidney fibrosis in patients with chronic kidney disease (CKD), via their downstream signaling, and these inhibitors also have known activity as antithrombotic and anticancer agents. This review gives an outline of the cGMP-cGK1-PDE signaling pathways and details the downstream signaling and regulatory functions that are modulated by cGK1 and PDE inhibitors with regard to antifibrotic, antithrombotic, and antitumor activity. Current evidence that supports the renoprotective effects of regulating cGMP-cGK1-PDE signaling is also summarized. Finally, the effects of icariin, a natural plant extract with PDE5 inhibitory activity, are discussed. We conclude that regulation of cGMP-cGK1-PDE signaling might provide novel, therapeutic strategies for the worsening global public health problem of CKD.
cGMP; cGMP kinase 1; cGK1; phosphodiesterase inhibitors; PDE; kidney disease; signaling
the CVD-associated benefits of PDE5 inhibitors and the potential for coexisting bleeding risk in CKD patients are needed. PDE5 inhibitors may also exert beneficial anticancer effects. These effects are generally based on the ability of the inhibitors to promote apoptosis (125), increase cell permeability to chemotherapeutic agents (11), and reverse multidrug resistance (MDR) (22). There is, however, little information on the use of PDE5 inhibitors for treating kidney cancer, the incidence of which has been rising over the past two decades independently of its increased incidental diagnosis from increased abdominal imaging (24). Most kidney cancers are a form of renal cell carcinoma (RCC), and some common subtypes, namely, clear cell RCC (46), demonstrate increased expression of MDR proteins (for example, MDR proteins MRPI and MRP2) (105). PDE5 inhibitors may, therefore, be useful in targeting MDR-induced resistance of RCC to cancer therapies. Kidney cancer is also strongly associated with impaired kidney function (74). Compared with the general population, the risk of RCC is increased approximately 2-fold in patients with an estimated glomerular filtration rate (eGFR) <30 ml·min⁻¹·1.73 m⁻² (CKD stages 4–5), 3.6-fold in patients on hemodialysis (76), and at least 15-fold in kidney transplant recipients (69). Kidney cancer has an increasing prevalence among patients with end-stage kidney disease (ESKD) before and after kidney transplantation (31, 52). As a corollary, CKD is also an outcome of kidney cancer treatment (21), with incidence increasing in patients who have had radical or partial nephrectomies (77). The use of PDE5 inhibitors, therefore, may show promise as an RCC therapy that may also reduce the development of CKD associated with the cancer.

Although research into cGMP-PDE signaling and the value of the use of selective PDE inhibitors in the treatment of kidney disease and its attendant complications have been reported for more than 40 years (33), the use of these agents has not become mainstream in the field of nephrology. The aim of this review is to outline cGMP-cGK1-PDE signaling pathways and their role in antifibrotic, antithrombotic, and antitumor outcomes, and the potential therapeutic role of PDE inhibitors, including the bioactive compounds obtained from a natural plant extract, in CKD and RCC.

Activation and Modulation of Guanylate Cyclases

sGC. cGMP is converted from GTP by either sGC or particulate (membrane-bound) guanylate cyclase (pGC) (109). sGC is a nitric oxide (NO) sensor. When NO binds to sGC heme, cGMP is generated after increased GTP cyclase activity (37). cGMP then serves to regulate several cell signaling functions, notably for this review those that act in the regulation of vascular tone and platelet function. sGC may also be activated in a NO-independent manner. Heme-dependent sGC activators include BAY 41-2272, BAY 41-8543, YC-1, CFM-1571 and A-350619; and the heme-independent sGC activators include HMR-1766 and BAY 58-2667 (34). With regard to kidney disease, preclinical studies have demonstrated that sGC stimulators effectively delay renal failure and modulate glomerulosclerosis in inflammatory glomerular disease (130).

pGC. Activation of pGC occurs on stimulation with natriuretic peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (59, 83). To date, seven subtypes of pGC receptors have been found (pGC-A—pGC-G) (42). Cross-activation of pGC with specific peptides modulates divergent functions of the pGC-cGMP axis, some of which may be related to kidney function and fibrosis in CKD, and also kidney cancer. For example, ANP-BNP-pGC-A signaling is involved in the critical regulation of blood pressure and also represses fibrogenesis by perturbing profibrotic gene expression driven by transforming growth factor-β (TGF-β) (55, 70, 124). CNP-pGC-B signaling is necessary for embryonic bone formation (6) but may also act in fibrogenesis via bone morphogenic protein and TGF-β; pGC-C, located in the intestines and kidneys, is bound to guanylin and uroguanylin to form an activated pGC-C/guanylin/uroguanylin complex that stimulates urinary and intestinal excretion of fluid and electrolytes (18). Loss of guanylin (for example, in guanylin-null mice) and resultant loss of pGC activity increased proliferation of colonic epithelia and so played a role in susceptibility to intestinal adenoma formation or progression (120). No change in apoptosis was demonstrated in this study. In contrast, Lin et al. (72) found that the activation of pGC-C played a proapoptotic and proinflammatory role in ischemia-reperfusion (I/R)-induced renal injury, since wild-type mice suffered greater kidney functional decline after bilateral I/R compared with pGC knockout (pGC−/−) mice. Thus the underlying pathological process may determine the role of pGC in disease, and this should be considered carefully in planning trials of the inhibitors that modulate pGC.

Role of cGMP

Degradation of cGMP via PDEs. The cellular concentration of cGMP requires a coordinated synthesis and degradation process. As enzymes that catalyze the degradation of cGMP, PDEs maintain intracellular cGMP levels at basal concentrations. The PDE superfamily contains 11 gene families (subtypes PDE1—PDE11), which are highly genetically specific. Each of the PDE subtypes encompasses one to four distinct genes, which have different Km to either cGMP or cAMP (75). PDE5, PDE6, and PDE9 are specific hydrolytic enzymes for cGMP, whereas PDE4, PDE7, and PDE8 are specific for cAMP. Other PDEs can hydrolyze both cAMP and cGMP. Castro et al. (20) demonstrated that cGMP, synthesized from different origins, was hydrolyzed by different subtypes of PDE, probably owing to the movement of specific types of PDEs and GCs within the cell (100). For example, cGMP synthesized by pGC is mainly hydrolyzed by PDE2, whereas PDE5 mainly controls the hydrolysis of cGMP, catalyzed by sGC.

Accumulating evidence suggests suppressing the hydrolysis of cGMP by PDE inhibitors ameliorates kidney diseases, including kidney cancer. For example, suppressing PDE5 RNA expression using PDE5 small interfering RNA (siRNA) induced apoptosis of OS-RC-2 human RCC cells (96); PDE5 inhibitors attenuated renal tubuloointerstitial fibrosis in a rat model of unilateral ureteral obstruction (UOU) (27); edema associated with nephrotic syndrome was ameliorated (128); and acute kidney injury induced by renal transplant surgery (73) or cisplatin (67) was prevented.

Regulatory role of downstream signaling of cGMP. Figure 1 summarizes the NO-natriuretic peptide-GMP-GK signaling pathway and its regulatory role. After being synthesized by GCs, cGMP stimulates cGK (also known as PKG) (19). cGK exists as two main subtypes (cGK1 and cGK2). Prominently

AJP-Renal Physiol • doi:10.1152/ajprenal.00042.2016 • www.ajprenal.org
Kidney fibrosis is a hallmark of progressing CKD. For example, cGMP-cGK1 signaling has antifibrotic potential in treating kidney disease. Modulation of the cGMP signaling pathway has antifibrotic potential in treating kidney disease. For example, cGMP-cGK1 inhibits kidney fibrosis by antagonizing Ras homolog gene family member A (RhoA)-RhoA kinase (RhoA-ROCK) signaling and transforming growth factor-β (TGF-β) profibrotic pathways. cGK1α, activated by cGMP, ameliorated fibrosis induced by UUO (107). In this research in rats, the expression of sGC and cGK1α increased significantly 7 days after UUO compared with the healthy kidney. The mRNA expression of markers of fibrosis, such as TGF-β and fibronectin, was decreased after treatment with the sGC activator YC-1. The result indicates that the stimulation of cGMP might be antifibrotic, at least after UUO. Additionally, immunohistochemistry was used to demonstrate the different locations of the two cGK1 isoforms. GK1α was present, and GK1β was absent, in medullary fibroblasts. This may suggest a more important role for GK1α than GK1β in suppressing kidney fibrosis, perhaps via its inhibition of RhoA-ROCK signaling (103).

Connective tissue-based contracture is a key factor in the process of fibrosis, with remodeling via stress fiber contraction and the pericellular collagen network (122). The phosphorylation state of the myosin light chain (MLC) is crucial for keeping myofibroblasts in sustained contractility (85). Vasconestrictors such as angiotensin II and thromboxane A2 activate RhoA-ROCK, which subsequently phosphorylates the myosin binding subunit (MBS), thereby inhibiting the MLC phosphatase and so maintaining the phosphorylation state of MLC (58). This generates the contractility of stress fibers in the myofibroblasts (56). Hence, inhibition of RhoA-ROCK signaling modulated by cGK1α (103, 104, 121) restricts connective tissue-based contracture, which is promising in retarding the progression of fibrosis (Fig. 2).

cGK1α also disrupts the transcriptional activity of TGF-β-Smad signaling (70). Pretreatment with cGMP (1 mM) and ANP (1 μM) inhibited TGF-β1 (1 ng/ml)-stimulated myofi-
broblast transformation and collagen synthesis in cardiac cells, whereas pretreatment with a cGKIα inhibitor, KT5823, blocked these effects (70). The antifibrogenic mechanism of ANP-cGMP-cGKIα is suggested to be as follows. In the pathogenesis of fibrosis, TGF-β1 receptor (TβR1) kinase phosphorylates Smad3. Phosphorylated Smad3 then combines with Smad4 to form the Smad complex. Nuclear translocation of this complex is crucial in initiating mRNA transcription that mediates inflammation and collagen formation (78). However, increased intracellular cGMP activated by ANP stimulates cGKIα, which phosphorylates Smad3 on sites different from phosphorylation by TβR1 kinase. This phosphorylation process induced by ANP-cGMP-cGKIα signaling hinders the nuclear translocation of the Smad complex, which attenuates the proliferation and differentiation of the mesenchymal cells. The mechanism of renal fibrosis mediated by TGF-β-Smad3 (63) is demonstrated in Fig. 3. Smad2 physically interacts with Smad3 to competitively inhibit TβR1-induced phosphorylation of Smad3, which in return inhibits kidney fibrosis (82). Smad7 is another renoprotective mediator. Secreted in the transcription process induced by the Smad complex, Smad7 negatively regulates TGF-β-Smad3 signaling (81). Since the mechanism of the counterregulatory roles of ANP-cGMP-cGKIα and TGF-β-Smad signaling remains unexplored in kidney fibrosis, future research is needed to comprehensively examine the influence of cGKIα on Smad signaling.

**Antithrombotic effects of cGMP-cGKI signaling in kidney disease.** Compared to patients with stages 2 and 3 CKD, the plasma of patients diagnosed with CKD stage 4 shows increased accumulation of the coagulation factor fibrinopeptide-A, which suggests an increased prothrombotic tendency with an increasing stage of CKD (112). Impaired hemostasis in CKD is also suggested by a higher incidence of pulmonary embolism in patients with CKD compared with people who have normal kidney function (60). Increased expression of platelet stimuli, such as tissue factor (TF) (50, 91), fibrinogen (114), and decreased expression of tissue-type plasminogen activator (tPA) (50) result in platelet hyperactivity in CKD. For example, platelets from uremic patients showed an increase in phosphatidylserine and caspase-3 activity, which are both associated with thrombophilic tendency (12). As CKD advances to its final stage, the metabolism of platelets undergoes further changes. In ESKD, enhanced thrombophilic tendency coexists with aberrant platelet function (57). For example, patients on dialysis have a higher risk of upper gastrointestinal hemorrhage than patients with normal renal function (61). In a flow cytometry-based analysis, platelets of ESKD patients had a blunted response to platelet agonists compared with patients with normal renal function (128a). The cause of platelet dysfunction in patients with ESKD is multifactorial, but one contributing factor is the impaired function of integrin-αIIbβ3 (also known as GPIIbIIIa) (39), which is the major fibrinogen transmembrane receptor of platelets. The increased affinity of integrin-αIIbβ3 for fibrinogen is the main mechanism of platelet adhesion and aggregation (113). Another factor is the increased secretion of tPA during hemodialysis, which disrupts the process of clotting (101). Impaired integrin-αIIbβ3 and upregulated tPA in the setting of hemodialysis increase the risk of hemorrhage.

The NO-GMP signaling pathway interferes with platelet activation and aggregation via phosphorylation of diverse substrates of cGKI, for example, inositol 1,4,5-trisphosphate receptor (IP3R)-associated cGMP kinase substrate (IRAG), vasodilator-stimulated phosphoprotein (VASP), and Ras-related protein Rap1b, also known as GTP-binding protein smg p21B (115). Elevation of intracellular Ca2+ in platelets is one of the stimuli for platelet activation. Similar to the case of Ca2+-dependent contraction, a triple complex containing cGK1β,
IRAG, and IP3RI is also present during platelet activation (2). Binding directly with IRAG, cGK1β is targeted to IP3RI, so IRAG is the important intermediate between cGK1 and IP3RI (3). Through phosphorylation of IRAG, cGK1β induces IP3RI-induced Ca2+ release from IP3-sensitive stores in platelets (2), which as a result inhibits elevation of intracellular Ca2+. The substrate of cGK1, VASP, is another mediator of platelet aggregation and is abundantly expressed in platelets (93). The C-terminal domain of VASP is essential for anchoring the molecule at focal adhesion sites (44). In intact platelets, cGK1 impairs the integrity of the C-terminal domain of VASP by phosphorylation (116), which is then regulated by cGMP-cGK1 signaling to inhibit the aggregation of platelets (7, 9).

Rap1b is abundantly expressed in platelets and is a crucial mediator for thrombosis mediated by fibrinogen-integrin αIIbβ3 signaling (113). For example, Rap1b knockout mice exhibit prolonged tail bleeding time, although without spontaneous bleeding; and lack of Rap1b gene expression protects mice from arteriosclerosis (25). Similarly, inhibition of Rap1b activity may decrease cardiovascular risk for patients with CKD. Rap1b may modulate the interaction of integrin with the platelet actin cytoskeleton (10); however, the precise mechanism of the increased affinity of platelet integrin for fibrinogen, regulated by Rap1b, remains largely unknown (10). Danielewski et al. (28) demonstrated that addition of the specific activator of sGC, NO, and a cGK1-specific analog suppressed Rap1b activation induced by platelet agonists. The reduction of Rap1b was in parallel with the increased phosphorylation state of VASP, which is a sign of the activity of cGK1. Moreover, the cGK1 inhibitor Rp-8pCPT abolished the effect of NO-induced Rap1b inhibition. These results indicate NO-cGMP-cGK1 inhibits platelet aggregation via inhibiting Rap1b activity. Phosphorylation of Rap1b by cGK1 may be the mechanism underpinning inhibited Rap1b activity (84).

Although questions remain on the roles of cGK1β in platelet aggregation, it has been generally accepted that the cGMP-induced platelet response has two phases (115): in the first phase, cGK1 stimulates the activation of platelets to foster a short-lived aggregation; and in the second phase, aggregation of platelets is inhibited by cGK1 (71). Li et al. (115) reported that elevated platelet cGMP levels occurred after physiological activation of platelets in wound healing. In comparison, elevated cGMP was found to limit the size of the primary platelet plug. The net effect of cGK1 on the bleeding risk in patients with CKD remains uncertain, although the kinase does have the potential to augment the bleeding risk by further suppressing the already reduced levels of integrin-αIIbβ3 found in ESKD.

**Antitumor Pathways Regulated by cGK1 and PDE Signaling**

Upregulation or activation of cGK1 may have clinically relevant antitumorigenic effects in treating various cancers. For example, activation of cGK1α and cGK1β induced apoptosis in the human colon cancer cell line SW-480, especially for cGK1β deleted (1–93; constitutively activated mutant with a deleted autoinhibitory domain), which induced a twofold increase in apoptosis, which was associated with inhibition of the transcriptional activity of the cyclin D1 promoter, thereby interrupting the cell cycle (29). Hou et al. (49) gained more evidence for the role of cGK1 by observing dramatically downregulated expression of cGK1 (both α and β isoforms) in various human tumor tissues, including the liver, pancreas, lung, thyroid, and colon. In immunodeficient mice overexpressing cGK1β, colon cancer xenografts decreased in size compared with xenografts in wild-type mice (49). However, there is some ambiguity in terms of the role of cGK1α in treating cancer (134). For example, the sGC inhibitor ODQ and...
cGK1α gene knockout inhibited ovarian cancer cell proliferation, indicating antiapoptotic effects of cGMP-cGK1α signaling in the pathogenesis of ovarian cancer (68). Thus it may be necessary to evaluate spontaneous tumorigenesis while using cGK1 activators for treating existing cancers.

The MEKK1-SEK1-JNK1 signaling pathway plays a key role in inducing apoptosis (4, 136). cGK1β activates this pathway via phosphorylating MEKK1 (117). Impairing the integrity of MEKK1 may enhance tumor-killing abilities of drugs which disrupt the cytoskeleton, as the position of the “plant homeodomain” PHD on the N terminal of MEKK1 is crucial for the initiation of ubiquitination, which inhibits JNK-dependent apoptosis (126, 133). Whether the integrity of PHD is disrupted during phosphorylation of MEKK1 by cGK1β remains unknown.

Except for activating signaling that induces cell death, the cGMP-cGK1-PDE signaling also exerts antitumorigenesis effects via inhibiting signaling that maintains tumor cell survival. Cancer may be associated with structural damage and cell proliferation accompanying chronic inflammation (150). During inflammation, eicosanoids, the substrates of arachidonic acid, are catalyzed by cyclooxygenase (COX), which contributes to inflammation, but also to carcinogenesis (129). Nonsteroidal anti-inflammatory drugs (NSAIDs) may also be used as tumoricidal agents (127). Booth et al. (14) demonstrated that celecoxib combined with the PDE5 inhibitor sildenafil, at physiological dosages, could act synergistically to kill multiple cancer cell types. They found that PDE5 was overexpressed in cancers of the mammary gland, liver, and lung compared with nontumor tissues harvested from the same organs. Mechanistically, the antitumor effect of knocking down PDE5 was equal to the combined effects of celecoxib and sildenafil, whereas knocking down COX did not promote the lethality of the two drugs, which indicates a NO synthase-dependent, COX-independent, pathway (14). The COX-independent mechanism in which the lethality of NSAIDs and sildenafil was synergized was further demonstrated when they found that sildenafil, combined with OSU-03012, a derivative of celecoxib which does not inhibit COX, exerted greater antineoplastic effects than celecoxib/sildenafil in treating glioblastoma (15). Other studies have demonstrated that PDE5 inhibitors synergized the tumoricidal effects of routine chemotherapy (for example, doxorubicin) in killing cancer cells of the gastrointestinal/genitourinary system (13) and the central nervous system (97). Moreover, sildenafil therapy was highly specific to cancer cells and did not induce apoptosis of normal cells (102), which made it an effective antitumor agent. In kidney cancer, Ren et al. (96) indicated that suppressed gene expression of PDE5 by PDE5 siRNA promoted apoptosis in OS-RC-2 human renal carcinoma cells. To our knowledge, there has been no other preclinical research targeting the effect of selective PDE5 inhibitors in treating kidney cancer. Further translational research is necessary to prove the synergistic effects of PDE5 inhibitors and OSU-03021 in treating kidney cancer. Additionally, future research regarding how different subtypes of kidney cancers would respond to PDE5 inhibitors is warranted.

Therapeutic Implications of Selective PDE5 Inhibitors in Treating Kidney Disease

Among the known hydrolytic enzymes specific to cGMP, inhibitors of PDE5 have received the most research and have exhibited the greatest renoprotective potential (Fig. 4). PDE5 is a cGMP-specific enzyme that has three isoforms. PDE5A1 and PDE5A2 are widely expressed in tubular epithelial cells of the renal proximal tubule and medullary collecting duct, as well as in vascular smooth muscle cells, platelets, brain, and lung. PDE5A3 is only expressed in vascular smooth muscle cells (8). As well as their use for erectile dysfunction (26) and pulmo-
vascular hypertension (41, 51), selective PDE5 inhibition is now attracting research interest in the field of kidney disease (1).

Selective PDE5 inhibitors and erectile dysfunction in kidney disease. Sexual dysfunction, including erectile dysfunction, is a common comorbidity among patients with CKD (90), and is an initial indicator of cardiovascular dysfunction (111). The PDE5 inhibitor sildenafil (also known as Viagra) safely improved the erectile function of patients on dialysis or after renal transplantation (64). Until now, the effects of PDE5 inhibitors in improving sexual function have not been tested among patients undergoing conservative CKD therapy.

Antifibrotic potential of selective PDE5 inhibitors. Progressive kidney fibrosis is a common pathway to ESKD. As discussed before, cGMP-cGK1-PDE signaling is critical in the pathogenesis of fibrosis. Prior studies have evaluated the effects of selective PDE5 inhibitors in treating kidney fibrosis. The effects of PDE5 inhibition in treating mesangial proliferative glomerulonephritis (GN) were first demonstrated in a preclinical model by Hohenstein et al. (48) in 2008. They used the anti-Thy1 model of GN to evaluate the antiproliferative and antifibrotic effects of vardenafil (another selective PDE5 inhibitor) and found significant antifibrotic potential, which suppressed the expression of proteins associated with TGF-β signaling (for example, thrombospondin-1 and p-Smad-2/3). Fibroblast activation plays a predominant role in diabetic nephropathy (54), which has become the most common cause of CKD and ESKD in many countries. A study using streptozotocin (STZ)-induced diabetes in rats showed that 8-wk treatment of vardenafil decreased expression of TGF-β1 and fibronectin. Similar to the renoprotection in the anti-Thy1 GN model, vardenafil significantly ameliorated the accumulation of inflammatory cells, glomerular remodeling, and tubulointerstitial lesions (36). Proteinuria was significantly decreased, a result ascribed to the increased cGMP level in podocytes.

While mostly supportive of these results, other reports of the antifibrotic effects of PDE5 inhibitors have been conflicting. In experimental diabetes after alloxan injection in rats, administration of vardenafil 6 mo after alloxan injection produced significant renoprotective effects, as demonstrated by increased eGFR and reduced glomerular remodeling (65). Using the subtotal (%) nephrectomy model of CKD, pretreatment with sildenafil postponed the deterioration of kidney function and attenuated glomerulosclerosis and tubulointerstitial damage, whereas delivery of sildenafil 4 wk after the surgery failed to show any antifibrotic action (98).

PDE5 inhibition may decrease risk of CVD in patients with CKD. Recent preclinical research has found that sildenafil is efficacious in treating resistant hypertension associated with narrowing or occlusion of the renal artery (32, 35). The mechanism of sildenafil in ameliorating renovascular hypertension is associated with its antisueroxide potential, which protects the endothelial cells of the blood vessels (66). Previous research has demonstrated that sildenafil restored endothelial function via enhancing NO bioavailability (5). This may lead to beneficial effects on atherosclerosis, which is common among patients with CKD (80). Atherosclerosis is associated with enhanced oxidative stress, decreased NO activity, and endothelial dysfunction (45). Whether PDE5 inhibition can decrease the risk of CVD for patients with CKD requires further evaluation.

Sildenafil also inhibits platelet activation and aggregation (110). As discussed previously, GSKβ inhibits Ca^2+ release in platelets by phosphorylating IRAG and IP3R1, thereby inhibiting platelet activation and aggregation. Wilson et al. (132) demonstrated that NO donors and sildenafil produced a synergistic effect to inhibit platelet aggregation. Sildenafil alone, however, merely inhibited thrombin-induced Ca^2+ release without inhibiting platelet aggregation. The investigators found that PDE5 tethering to the intraplatelet complex of cGSKβ-IRAG-IP3R1 coordinated the phosphorylation of IP3R1 by cGSKβ, which is a novel hypothesis of the anticoagulant effects of PDE5 inhibitors. To date, there has been no clinical trial to test the antiplatelet effects of selective PDE inhibitors in patients with mild-to-moderate renal impairment. Comprehensive evaluation of their effects on decreasing CKD-associated CVD mortality and the risk of bleeding should be carried out before they are widely used clinically.

Selective PDE5 inhibitors and edema associated with nephrotic syndrome. Overexpression of PDE5 in the inner medullary collecting duct may be responsible for the blunted natriuretic response to ANP in nephrotic syndrome. This was demonstrated in a rat model of nephrotic syndrome induced by adriamycin (128). The research found that intrarenal infusion of IBMX, a nonselective PDE inhibitor, and zaprinast (specific to PDE5, PDE6, PDE9, and PDE11) normalized the blunted response to ANP, which resulted in reduced sodium retention and volume overload in this animal model. The overexpression of PDE5 was again demonstrated in puromycin aminonucleoside-induced nephrotic syndrome and anti-Thy-induced GN rat models (92). However, to date the efficacy of selective PDE5 inhibition in alleviating edema in nephrotic syndrome remains largely unexplored.

Beneficial effects of PDE5 inhibition in treating acute kidney injury. PDE5 inhibition has some beneficial effects in treating acute kidney injury. Sildenafil and tadalafil ameliorated I/R kidney injury via enhancing ERK activation (23) and decreasing leukocyte infiltration (43, 89). The enhanced tolerance to ischemic injury indicates that PDE5 is a potential target in preventing acute kidney injury associated with surgery (62). In drug-induced acute kidney injury, sildenafil was able to mitigate cisplatin-induced apoptosis in kidney tubular epithelial cells (67). In this study, sildenafil significantly attenuated the activation of caspase-3 and reversed the increased proapoptotic ratio of Bax/Bcl-2, leading to protection of the kidney against cisplatin-induced acute renal failure.

A Promising Herbal PDE5 Inhibitor in Treating Kidney Disease

Traditional Chinese herbal medicine is a rich therapy resource which has received relatively little rigorous scientific evaluation to date. Where rigorous evaluation has occurred, there are well-known success stories. For example, arsenic trioxide (As2O3) and artemisinin, which are extracted from herbs and minerals, have become first-line medications in treating leukemia and malaria (118, 131). Herbal PDE inhibitors have been described by Roja et al. (95). The genus Epimedium (also known as “Yin Yang Huo” in Chinese) has long been used in traditional Chinese medicine to promote reproduction and treat sexual dysfunction based on the theory of nourishing and warming the “Yang” in the body. The major
active compound of *Epimedium*, icariin, is a strong PDE5 inhibitor (IC$_{50}$ for PDE5A1 5.9 μM) (79, 87). Dell’agili et al. (30) modified the molecular structure of icariin to form a new compound, 3,7-Bis (2-hydroxyethyl) icaritin. Compared with icariin, the inhibitory ability of icaritin to PDE5A1 was enhanced 80-fold (IC$_{50}$ 75 nM). Preclinical research had demonstrated icariin as renoprotective in treating experimental diabetic nephropathy (94), cispaltin-induced nephrotoxicity (123), and I/R kidney injury (137). However, none of these investigations focused on the role of icariin in regulating cGMP-PDE signaling. Future research is needed to evaluate the effects of icariin and icaritin in treating kidney fibrosis based on their PDE5-inhibitory ability.

### Conclusion

By virtue of their high efficiency in enhancing the intracellular concentration of cGMP, PDE5 inhibitors show considerable promise in the treatment of kidney disease and its complications. cGK1 regulates the phosphorylation processes of numerous substrates, leading to interference in the TGF-β, RhoA-ROCK, and PKG-MEKK1-SEK1-JNK1 pathways, which in turn inhibits kidney fibrosis and tumorigenesis. Most research to date has been carried out in preclinical models, and only limited clinical studies have demonstrated that selective PDE inhibitors may retard progressive kidney scarring and failure in CKD, reduce cardiovascular risk, ameliorate erectile dysfunction and fluid retention in CKD, and prevent ischemic and nephrotic acute kidney injury. One concern is that PDE5 inhibitors may heighten the bleeding risk. Future research is needed to bridge gaps in our understanding of the effects of PDE5 inhibitors in treating kidney disease, including kidney cancer, and in alleviating edema related to the nephrotic syndrome. Large-scale clinical trials are warranted to assess the overall impact of PDE5 inhibitors on CKD progression, acute kidney injury, cardiovascular and all-cause mortality, quality of life, and potential dysfunction in hemostasis.

### GRANTS

K. Shen has been supported at the Centre for Kidney Disease Research, University of Queensland, Queensland, Australia, and by a scholarship from the Guangzhou University of Chinese Medicine, Baiyun District, Guangzhou, China.

### DISCLOSURES

D. W. Johnson has received consultancy fees, research funds, speaking honoraria and travel sponsorships from Janssen-Cilag, Amgen, Pfizer, and Roche.

### AUTHOR CONTRIBUTIONS


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