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Renal effects of soluble guanylate cyclase stimulators and activators: A review of the preclinical evidence

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Direct stimulation of soluble guanylate cyclase (sGC) is emerging as a potential new approach for the treatment of renal disorders. sGC catalyzes the formation of cyclic guanosine monophosphate (cGMP), deficiency of which is implicated in the pathogenesis of chronic kidney disease (CKD). Therefore, new classes of drugs — sGC stimulators and activators — are being investigated in preclinical models under conditions where nitric oxide is deficient. In preclinical models with different etiologies of CKD, the sGC stimulators BAY 41-2272, BAY 41-8543, BAY 60-4552, riociguat and vericiguat and the sGC activators cinaciguat, ataciguat, BI 703704 and GSK2181236A have shown consistently renoprotective effects. Clinical trials are required to confirm these findings in humans, and to ascertain whether these agents could provide a future alternative to guideline-recommended treatments.

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Introduction

Chronic kidney disease (CKD), defined as a reduced estimated glomerular filtration rate (<60 ml/min/1.73 m^2), increased urinary albumin excretion (>30 mg/g of creatinine), or both, is a substantial cause of morbidity and mortality worldwide, and a growing public health concern [1,2]. The estimated global prevalence of CKD is 8–16% in the general population, rising to over 50% in high-risk subgroups [1,3]; however, few drugs for kidney disease have been successfully developed over the past 15 years [4]. While many therapeutic interventions have initially appeared effective in animal models, translation of these results into humans in the clinical setting has remained challenging [5]. There is a particularly high unmet medical need for new therapies in cases where guideline-recommended CKD treatments are unsuccessful. These patients are likely to have persistent high blood pressure and increased albumin excretion, which puts them at increased risk of rapid progression to end-stage renal disease (ESRD) [6–8].

Direct stimulation of soluble guanylate cyclase (sGC) is a novel therapeutic approach under investigation for various cardiovascular disorders associated with endothelial dysfunction. It has also been shown to have beneficial effects on renal tissue remodeling and organ function [9]. This review will focus on preclinical data on two classes of compounds that directly modulate sGC, sGC stimulators, and sGC activators. These compounds increase cyclic guanosine monophosphate (cGMP) formation under normal conditions and conditions where nitric oxide (NO) formation and bioavailability are impaired or NO tolerance has developed [10]. Given their non-overlapping mechanism of action compared with current therapies, activators and stimulators of sGC may offer an alternative option for patients who are unresponsive to available modalities and who therefore are at high risk of rapidly progressing to more serious disease.

Pathogenesis of CKD

Excessive accumulation of extracellular matrix occurs in virtually every type of CKD, leading to renal fibrosis [5]. Three key pathways have been identified in promoting tissue matrix expansion: firstly, elevated synthesis of extracellular matrix components; secondly, increased inhibition of matrix degradation; and finally, upregulated local expression of integrins [11]. This pathogenic process is progressive and ultimately leads to ESRD [5]. There are believed to be several pathways of progression toward ESRD at the cellular and molecular level; injury to the tubulointerstitium, the renal parenchyma comprising the tubules and bounded by the vasculature and nephrons, may be one of these pathways [12].

NO and cGMP deficiency are implicated in the pathogenesis of multiple organ systems, including the cardiovascular system and importantly, the renal system [10]. NO deficiency has been documented in numerous experimental and human renal diseases, including hypertension and diabetic nephropathy, glomerulosclerosis,

obstructive nephropathy, and chronic interstitial nephritis, as well as renal diseases due to cyclosporine A and radiocontrast media [13–21].

Pharmacological blockade of NO has been utilized to study the effects of NO inhibition in animal models of renal disease. In a rat model of obstructive nephropathy, increased fibrosis and apoptosis, and reduced blood flow and filtration rates were observed with the use of the NO synthase inhibitor L-NAME as compared with control animals [13]. In another study in rats, increased arterial pressure and reduced renal blood flow were observed as a result of treatment with L-NAME. The hemodynamic effects induced by NO inhibition were normalized by sGC activation, demonstrating that the function of NO is mediated by the cGMP pathway [22]. Together, this research has demonstrated that NO and cGMP deficiency contribute to the progression of CKD through both hemodynamic and direct pro-fibrotic effects. It also reveals a protective role of NO on renal function, interstitial fibrosis, and renal tubular apoptosis [13]. More than 80% of patients with CKD are hypertensive [23], and in most, if not all cases, the dominating pathohistological feature of their underlying kidney disorder is accumulation of extracellular matrix proteins [5].

Due to the established link between NO deficiency and vascular pathophysiology, targeting of the NO signaling pathway has become a therapeutic strategy; for example, organic nitrates have been used to treat cardiovascular disease for more than 150 years [24]. However, resistance to NO, non-specific, and cytotoxic effects and other limitations associated with such treatments have led to an interest in targeting the sGC pathway directly, resulting in the discovery and development of sGC stimulators and activators [24].

Soluble guanylate cyclase

The enzyme sGC is a heterodimeric complex composed of two subunits (α and β), and is responsible, across various species and systems, for catalyzing cGMP formation from guanosine triphosphate. The prosthetic heme moiety at the β-subunit of sGC is essential for NO binding and subsequent enzyme activation [10]. NO is produced in response to physiological stimuli such as shear stress, and induces relaxation in the cardiovascular system through its modulation of sGC and cGMP levels, and consequent regulation of vascular tone and blood pressure. Under conditions where NO formation and bioavailability are impaired or NO tolerance has been established, two classes of compounds have been developed that can directly modulate sGC and increase cGMP formation: sGC stimulators and sGC activators [10].

Stimulators and activators of sGC target the enzyme in two different redox states: the NO-sensitive reduced (ferrous) enzyme and NO-insensitive oxidized (ferric) enzyme, and finally heme-free enzyme, respectively (Figure 1). Stimulators of sGC stimulate the reduced form of sGC directly and synergize with NO by stabilizing the nitrosyl–heme complex of reduced sGC [24]. Conversely, sGC activators increase the activity of the enzyme only when the heme iron is oxidized which subsequently lead to the heme-free enzyme. They bind to the unoccupied heme-binding complex, and produce only an additive effect with NO. In certain cases, sGC activators also protect sGC from oxidation-induced proteasomal degradation [24].

sGC stimulators

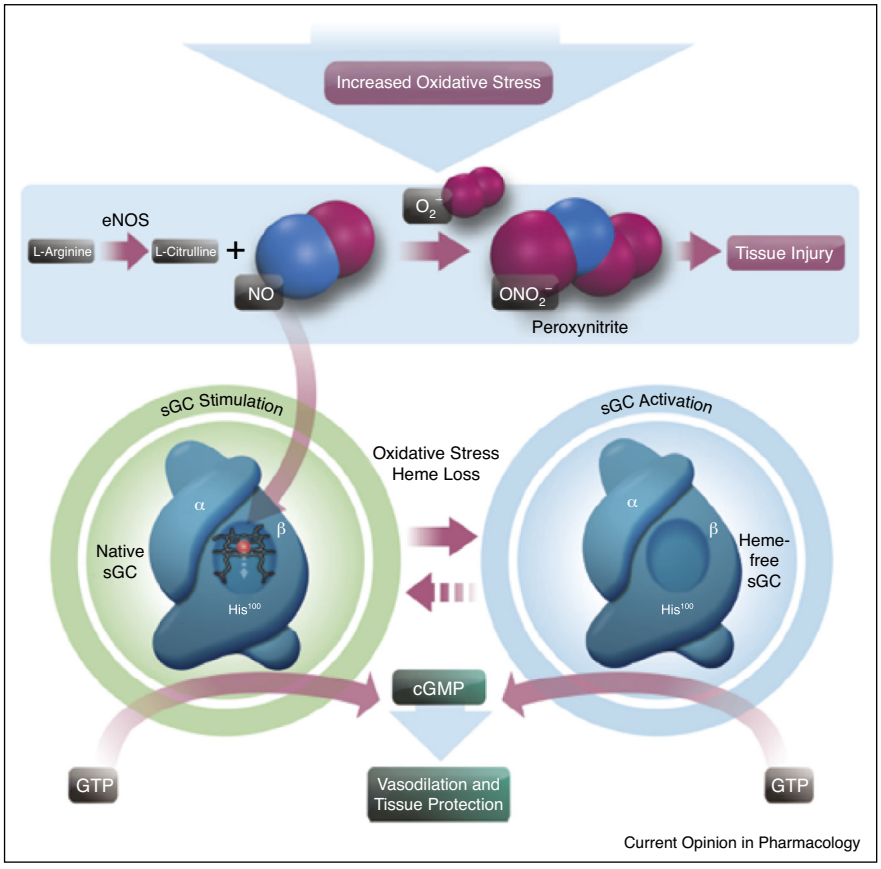
Effect of sGC stimulators in experimental kidney and cardiovascular disease

In 1994, scientists at Bayer started a screening for substances that could induce an increase in NO synthesis and thereby stimulate sGC in porcine endothelial cells. This screening led to the unexpected discovery of NO-independent sGC stimulators. At the same time it was reported that a benzyl indazole compound named YC-1 inhibited platelet aggregation by stimulation of cGMP synthesis. This compound was subsequently characterized as a direct NO-independent, but heme-dependent, sGC stimulator [10,25]. Kidney fibrosis induced by unilateral ureter ligation was inhibited by YC-1 via activation of cGMP-dependent protein kinase I [26]. However, along with its relatively weak sGC stimulating potency, it revealed a poor pharmacokinetic profile and a lack of specificity, as it was found to inhibit phosphodiesterases and to modulate many cGMP-independent effects [27]. Therefore, further optimization of potency, pharmacokinetic properties, and specificity was required to realize the full therapeutic potential of this novel class of drugs [25]. Since YC-1, the sGC stimulators BAY 41-2272, BAY 41-8543, BAY 60-4552, riociguat, and vericiguat have been developed, and their effects have been investigated in experimental models of kidney and cardiovascular disease [9,28–41,42•,43,44]. A summary of some of the results of these investigations is given below and in Table 1 and Figure 2.

BAY 41-2272 and BAY 41-8543

Administration of BAY 41-2272 to rats with an acute form of glomerulonephritis attenuated renal dysfunction, as determined by significant reductions in proteinuria and systolic blood pressure (Figure 2a) as compared with untreated and control animals [36]. This effect was accompanied by decreased transforming growth factor beta (TGFβ) production, matrix deposition, and macrophage infiltration, which was found to occur independently of changes in blood pressure [36]. In another study, using the same model, BAY 41-2272 elevated cGMP levels in mesangial cells, thereby reducing their proliferation and matrix production compared with placebo-treated animals. Again, treated rats had significantly lower proteinuria levels than controls and the effects were found to

Figure 1



Soluble guanylate cyclase stimulators and activators target two different redox states of the enzyme (reproduced from [24,25]). Soluble guanylate cyclase (sGC) stimulators and activators target two different states of sGC: the nitric oxide (NO)-sensitive reduced, native sGC, and the NO-insensitive oxidized, heme-free sGC. Stimulators of sGC stabilize the nitrosyl–heme complex of the reduced sGC (shown left) and exhibit a strong synergism with NO. Activators of sGC bind to the unoccupied heme-binding complex (shown right) or displace the prosthetic heme of sGC and protect sGC from proteasomal degradation. cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase.

have a direct pressure-independent component [30•]. Furthermore, BAY 41-2272 was shown to significantly limit the course of chronic glomerulonephritis in a rat model [41]. Improvements in kidney function, as assessed by lower plasma creatinine and urea levels, were observed. A further study found that BAY 41-2272 protected the kidney from progressive sclerosis and matrix deposition by limiting TGFβ expression [42•].

Both BAY 41-2272 and BAY 41-8543, when administered orally, produced dose-dependent vasodilation and markedly improved survival, compared with untreated controls, in rat models of hypertension, without causing tolerance [39,40]. In aged spontaneously hypertensive rats, BAY 41-2272 rapidly reversed existing pathological cardiac changes, for example by decreasing fibrosis levels to those of age-matched normotensive rats [31].

Studies in L-NAME-induced low-NO rat models of hypertension demonstrated that BAY 41-8543 had a renal protective effect (decreased plasma levels of urea and creatinine compared with untreated controls) [40], while BAY 41-2272 attenuated cardiac fibrosis (Figure 2b) and hypertrophy [44]. In other studies, and at a dose below that affecting blood pressure, BAY 41-2272 nevertheless attenuated cardiac fibrosis in rodent models of hypertension induced by infusion of angiotensin II [33] or suprarenal aortic constriction [34]. These findings suggest that sGC stimulation may protect against organ injury independently of its effects on vascular tone.

Increased cGMP production and subsequently enhanced renal recovery after unilateral ureteral obstruction relief was observed with sGC stimulation by BAY 41-8543 in a rat model, compared with obstruction relief alone [43]. This suggests that BAY 41-8543 may serve as a novel treatment approach to restore or preserve renal structure and function in cases of obstructive kidney disease [45].

BAY 60-4552

Limited published data are currently available for the other sGC stimulator, BAY 60-4552. However, in

Table 1

Summary of studies investigating sGC stimulators in experimental models of kidney and cardiovascular disease [9,28–41,42•,43,44]

Citation	Compound	Treatment groups	Key findings
Peters *et al.*, 2004 [36]	BAY 41-2272	PBS control OX-7 only OX-7 + BAY 41-2272	Administration of BAY 41-2272 to rats with an acute form of glomerulonephritis induced by OX-7 antibody injection attenuated renal dysfunction, as determined by the presence of proteinuria. This effect was accompanied by decreased TGFβ production, fibronectin, PAI-1, matrix deposition, and macrophage infiltration. In general, the fibrotic response in this model was not altered by blood pressure lowering alone
Hohenstein *et al.*, 2005 [30•]	BAY 41-2272	OX-7 + vehicle OX-7 + BAY 41-2272	BAY 41-2272 elevated cGMP levels in mesangial cells, thereby reducing their proliferation and matrix production in experimental glomerulonephritis
Wang *et al.*, 2005 [41]	BAY 41-2272	Nonnephrectomy + PBS Uninephrectomy + PBS Uninephrectomy + anti-thy1 Uninephrectomy + anti-thy1 + BAY 41-2272 Uninephrectomy + anti-thy1 + hydralazine	Bay 41-2272 limited the progressive course of anti-thy1-induced chronic glomerulosclerosis toward tubulointerstitial fibrosis and impaired renal function, at least in part in a blood pressure-independent manner, suggesting that sGC stimulation counteracts fibrosis and progression in chronic renal disease
Wang *et al.*, 2006 [42•]	BAY 41-2272	Nonnephrectomy + PBS Uninephrectomy + PBS Uninephrectomy + anti-thy1 Uninephrectomy + anti-thy1 + BAY 41-2272 Uninephrectomy + anti-thy1 + pentoxifylline	In a chronic model of glomerulonephritis, BAY 41-2272 protected the kidney from progressive sclerosis and matrix deposition by limiting TGFβ expression; BAY 41-2272 administration resulted in marked reductions of glomerular and tubulointerstitial histological matrix accumulation, expression of TGFβ1 and fibronectin, macrophage infiltration, and cell proliferation, as well as improving renal function. The protective effect achieved by elevating cGMP via direct sGC stimulation with BAY 41-2272 was superior to that produced by preventing degradation of cGMP using the non-specific PDE inhibitor pentoxifylline
Zanfolin *et al.*, 2006 [44]	BAY 41-2272	Untreated control L-NAME only L-NAME + BAY 41-2272 BAY 41-2272	In a low-NO rat model of hypertension BAY 41-2272 attenuated cardiac fibrosis and hypertrophy
Masuyama *et al.*, 2006 [33]	BAY 41-2272	Placebo control BAY 41-2272 low (2 mg/kg/day) BAY 41-2272 high (10 mg/kg/day) Ang II + placebo Ang II + BAY 41-2272 low (2 mg/kg/day) Ang II + BAY 41-2272 high (10 mg/kg/day)	In a rat model of hypertension, BAY 41-2272 ameliorated angiotensin II-induced cardiovascular remodeling, and the effects on the extracellular matrix may have been exerted partially via cGMP, independently of blood pressure
Masuyama *et al.*, 2009 [34]	BAY 41-2272	Surgery control Pressure overloaded Pressure overloaded + BAY 41-2272	In a rat model of hypertension, BAY 41-2272 had no effects on blood pressure, but decreased aortic constriction-induced collagen accumulation in the left ventricle, inhibiting the number of myofibroblasts and gene expressions of TGFβ1 and type 1 collagen
Jones *et al.*, 2009 [31]	BAY 41–2272	BAY 41-2272 BAY 58-2667 Age-matched normotensive	In aged spontaneously hypertensive rats, BAY 41-2272 rapidly reversed existing pathological cardiac changes, for example by decreasing fibrosis levels to those of age-matched normotensive rats
Stasch *et al.*, 2001, 2002 [39,40]	BAY 41-8543 and BAY 41–2272	Untreated control BAY 41-2272 Vehicle control BAY 41-8543	Oral BAY 41-2272 and BAY 41-8543 produced dose-dependent vasodilation without causing tolerance and significantly improved survival in rat models of hypertension
Wang-Rosenke *et al.*, 2011 [45]	BAY 41-8543	Unilateral ureteral obstruction with obstruction relief Unilateral ureteral obstruction with obstruction relief + BAY 41-8543	Administration of BAY 41-8543 to untreated obstructed rats showing mildly increased systolic blood pressure, marked tubular atrophy and apoptosis, tubulointerstitial macrophage infiltration, and fibrosis significantly increased plasma cGMP. This was paralleled by significant decreases in systolic blood pressure, renal tubular diameter, apoptosis, and renal macrophage infiltration. sGC stimulation decreased tubulointerstitial fibrosis, as shown by tubulointerstitial volume, matrix protein accumulation, α-smooth muscle actin expression, collagen IV deposition, and TGFβ1 mRNA expression

Table 1 (*Continued*)

Citation	Compound	Treatment groups	Key findings
Wang-Rosenke *et al.*, 2012 [43]	BAY 41-8543	Sham surgery control Unilateral ureteral obstruction with obstruction relief Unilateral ureteral obstruction with obstruction relief + BAY 41-8543	Administration of BAY 41-8543 to rats with unilateral ureteral obstruction increased cGMP production and led to significant amelioration of renal matrix protein expansion, macrophage infiltration, tubular apoptosis, and atrophy
Costell *et al.*, 2012 [29]	BAY 60-4552	Normal diet HSFD HSFD + GSK2181236A low (0.1 mg/kg/day) HSFD + GSK2181236A high (1.0 mg/kg/day) HSFD + BAY 60-4552 low (0.3 mg/kg/day) HSFD + BAY 60-4552 high (3.0 mg/kg/day)	In spontaneously hypertensive stroke-prone rats, a low dose of BAY 60-4552 decreased urine output and improved survival. A high dose also reduced urine output, and in addition reduced microalbuminuria and attenuated the increase in mean arterial pressure
Sharkovska *et al.*, 2010 [38•]	Riociguat	High renin study L-NAME only L-NAME + riociguat low (3 mg/kg/day) L-NAME + riociguat high (10 mg/kg/day) Low renin study Sham surgery control 5/6 nephrectomy 5/6 nephrectomy + riociguat	In two independent rat models of hypertension, riociguat reduced mortality and normalized blood pressure. Renal target organ damage was reduced in one model, as demonstrated by reductions in plasma creatinine and urea and less fibrosis. In the 5/6 nephrectomy model, creatinine clearance was improved and interstitial fibrosis reduced
Geschka *et al.*, 2011 [9]	Riociguat	Vehicle control Riociguat low (3 mg/kg/day) Riociguat high (10 mg/kg/day)	Riociguat-treated salt-sensitive rats fed a high-salt diet had improved survival and attenuated systemic hypertension and systolic dysfunction. Fibrotic tissue remodeling and degeneration were also enhanced in the heart and kidneys
Ott *et al.*, 2012 [35]	Riociguat	Vehicle control Telmisartan Riociguat Telmisartan + riociguat	Significant reductions in blood pressure and albuminuria were induced with combined riociguat and telmisartan treatment in diabetic eNOS knockout mice as compared with diabetic controls
JP Stasch *et al.*, unpublished	Vericiguat	Vehicle control Vericiguat	Vericiguat reduced mortality, improved systemic hypertension and tubulopathy, glomerulopathy, and vasculopathy in salt-sensitive rats fed a high-salt diet. In a low-NO/high-renin rat model of hypertension, vericiguat reduced right and left heart hypertrophy and mortality, and showed a positive effect on grading of kidney changes, and the incidence and severity of myocardial lesions

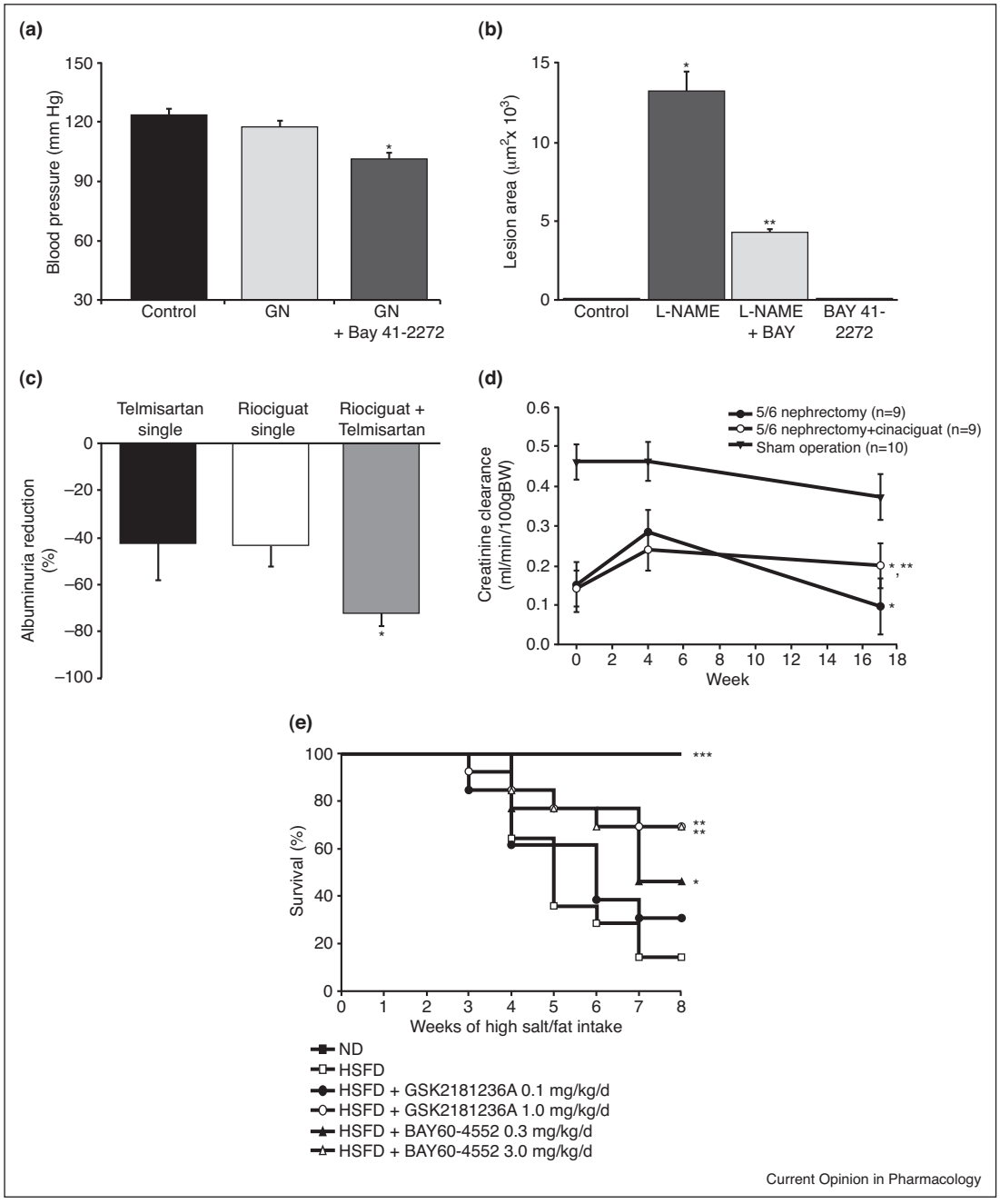
Ang, angiotensin; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; HSFD, high-salt/high-fat diet; L-NAME, L-NG-nitroarginine methyl ester; mRNA, messenger ribonucleic acid; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; PBS, phosphate buffered saline; PDE, phosphodiesterase; sGC, soluble guanylate cyclase; TGFβ, transforming growth factor beta.

stroke-prone, spontaneously hypertensive rats, BAY 60-4552 improved survival (Figure 2c), attenuated the development of hypertrophy, reduced urine output and microalbuminuria, and attenuated increases in blood pressure compared with untreated controls [29].

Riociguat

Riociguat (BAY 63-2521) is the most advanced of the sGC stimulators. It has been approved for use in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension [46,47], and is in clinical development for a number of other indications [48]. Riociguat is a heme-dependent sGC stimulator closely related to BAY 41-2272 and BAY 41-8543 [10]. In two independent rat models of hypertension, riociguat had a potent protective effect against cardiac and renal damage. In the first model (low-NO/high-renin), riociguat induced a marked dose-dependent decrease in renal interstitial fibrosis and normalized blood pressure versus control rats [38•]. In a subtotal nephrectomy rat model, riociguat-treated rats exhibited significantly lower blood pressure compared with untreated sham-operated animals, and had a significantly higher creatinine clearance compared with untreated rats [38•]. Furthermore, in a model of chronic volume and pressure overload using salt-sensitive Dahl rats fed a high-salt diet, riociguat significantly reduced glomerulosclerosis and interstitial and perivascular fibrosis, and significantly decreased mortality, compared with vehicle-treated rats [9]. In addition, riociguat in combination with telmisartan has been studied in a diabetic eNOS knockout mouse model, where animals were previously treated with an angiotensin II receptor blocker. Significant

Figure 2



(a) Example of effects of soluble guanylate cyclase stimulators on systolic blood pressure (BAY 41-2272) (reproduced from [36]). Effects of the pharmacologic soluble guanylate cyclase stimulator BAY 41-2272 (10 mg/kg/day) in rats with induced anti-thy1 glomerulonephritis (GN). Blood pressure was measured in conscious animals using a tail cuff method. *$P < 0.01$ versus GN. **(b)** Example of effect on fibrosis (BAY 41-2272) (reproduced from [44]). Area of fibrosis in rats treated with control, treated chronically with chronic N-nitro-L-arginine methyl ester (L-NAME; 20 mg/rat/day for eight weeks) alone or in association with BAY 41-2272 (10 mg/kg/day) or treated with BAY 41-2272 alone. Results are expressed as mean ± SEM. *$P < 0.01$ compared with control group, **$P < 0.01$ compared with L-NAME group. **(c)** Example of effect on survival (BAY 60-4552) (reproduced from [29]). Survival following chronic administration of BAY 60-4552 (0.3 or 3.0 mg/kg/day) or GSK2181236A (0.1 or 1.0 mg/kg/day) in spontaneously hypertensive prone Sprague rats on a high-salt/high-fat diet (HFSD) versus rats on HFSD alone or no diet (ND). Results are expressed as mean ± SEM. *$P < 0.001$ versus HFSD. **$P < 0.01$ versus HFSD. ***$P < 0.05$ versus HFSD. **(d)** Example of effect on albumin reduction (riociguat) (reproduced from [35]). Percentage reduction in urinary albumin excretion per day in diabetic, endothelial nitric oxide synthase knockout mice treated with riociguat (3 mg/kg/day), telmisartan (1 mg/kg/day), and both (3 mg/kg/day and 1 mg/kg/day) compared with diabetic controls. Results are expressed as mean ± SEM. *$P < 0.05$ compared with diabetic vehicle. **(e)** Example of effect on creatinine clearance upon 5/6 nephrectomy (cinaciguat) (reproduced from [32]). Change in creatinine clearance in male Wistar rats who were allocated to three groups: 5/6 nephrectomy, 5/6 nephrectomy treated with cinaciguat (~50 mg/day), and sham operation. Results are expressed as mean ± SD. *$P < 0.001$ versus sham operation. **$P < 0.05$ versus 5/6 nephrectomized rats.

reductions in urinary albumin excretion, an early sign of diabetic nephropathy, were observed compared with diabetic controls (Figure 2d) [35].

The combination of promising preclinical data and further positive clinical trials have led to the approval of riociguat (Adempas®), making it the first of the sGC stimulators to be approved for clinical use in any indication [48].

Vericiguat

Vericiguat (BAY 1021189), a more recent sGC stimulator, improved survival, systemic hypertension and tubulopathy, glomerulopathy, and vasculopathy in salt-sensitive

Table 2

Summary of studies investigating sGC activators in experimental models of kidney and cardiovascular disease [22,27,29,32,37,53,54•,55,56]

Citation	Compound	Treatment groups	Key findings
Kalk *et al.*, 2006 [32]	Cinaciguat	Sham surgery control 5/6 nephrectomy 5/6 nephrectomy + cinaciguat	Cinaciguat treatment of 5/6 nephrectomized rats reduced blood pressure, left ventricular weight, cardiac arterial wall thickness, and cardiac myocyte diameter compared with control rats. Creatinine clearance, glomerulosclerosis, and fibrosis were also improved with cinaciguat treatment
Fang *et al.*, 2012 [53]	Cinaciguat	Untreated control Cinaciguat	In type-1 diabetic rats treated with cinaciguat the urinary protein:creatinine ratio was attenuated, and there was reduced glomerular sclerosis and tubular damage. Cinaciguat treatment also significantly decreased the elevated TGFβ mRNA levels seen in the diabetic rats and the desmin expression of podocytes
Chen *et al.*, 2014 [55]	Cinaciguat	Vehicle ACE-i + CCB ACE-i + cinaciguat	In a rat model of type-2 diabetic nephropathy (ZSF1), administration of cinaciguat in combination with an ACE-i resulted in a significant reduction in the incidence of glomerulosclerosis and tubulointerstitial lesions compared with vehicle, with benefits sustained beyond treatment discontinuation. Such an effect was not observed in the ACE-i + CCB group, demonstrating its dissociation from MAP lowering.
Hoffmann *et al.*, 2014 [27]	Cinaciguat	High-salt diet only High-salt diet + cinaciguat	Long-term cinaciguat treatment of salt-sensitive rats on a high-salt diet resulted in significantly improved survival and reductions in blood pressure and heart rate compared with control animals. Urea and protein levels in urine and uric acid levels in plasma were also reduced with cinaciguat treatment. Furthermore, biomarkers of inflammation and fibrosis in the kidney and left ventricle were also lower with treatment. Likewise, cardiac function and fibrotic remodeling were improved
Dautzenberg *et al.*, 2014 [22]	Cinaciguat	Vehicle control Cinaciguat	Under NO-deficient conditions, cinaciguat treatment of intact anaesthetized rats at least partly stabilized the resultant hypertension and renal vasoconstriction, and re-established the modulation of renal blood flow autoregulation
Benz *et al.*, 2007 [54•]	Ataciguat	Sham surgery control Subtotal nephrectomy Subtotal nephrectomy + ataciguat Subtotal nephrectomy + ACE-i	In subtotal nephrectomized rats, ataciguat treatment ameliorated the increases in urinary albumin and glomerular cell number induced by the surgery and observed in the untreated rats. In addition, relative kidney and left ventricular weight were reduced
Schafer *et al.*, 2010 [37]	Ataciguat	Sham surgery control Left coronary arterial ligation + placebo Left coronary arterial ligation + ataciguat	In a model of chronic heart failure, ataciguat-treated rats demonstrated improved vasomotor function and reduced platelet activation
Harrison *et al.*, 2014 [56]	BI 703704	Vehicle BI 703704 at doses of 0.3, 1, 3, and 10 mg/kg/day	In a rat model of type-2 diabetic nephropathy (ZSF1) BI 703704 treatment resulted in significant reductions in proteinuria and the incidence of glomerulosclerosis and interstitial lesions compared with vehicle. Beneficial effects were observed at doses that did not significantly alter MAP and HR
Costell *et al.*, 2012 [29]	GSK2181236A	Vehicle GSK2181236A 0.1 mg/kg GSK2181236A 1.0 mg/kg	Models used were: Sprague Dawley rats during coronary artery ischemia/reperfusion; and, spontaneously hypertensive stroke prone rats on HSFD Low dose GSK2181236A attenuated the development of cardiac hypertrophy, while the high dose also improved survival. Vasodilatory responses to GSK2181236A were unaltered by HSFD

ACE-i, angiotensin-converting-enzyme inhibitor; CCB, calcium channel blocker; HR, heart rate; HSFD, high-salt/high-fat diet; MAP, mean arterial pressure; mRNA, messenger ribonucleic acid; NO, nitric oxide; sGC, soluble guanylate cyclase; TGFβ, transforming growth factor beta; ZSF1, male ZSF1 obese.

Dahl rats fed a high-salt diet (JP Stasch *et al.*, unpublished). In a low-NO/high-renin rat model of hypertension, vericiguat reduced right and left ventricle hypertrophy and mortality, and showed a positive effect on grading of kidney changes, and the incidence and severity of myocardial lesions [49–53,54•]. The potentially beneficial effects of vericiguat on cardiac function are supported by the findings from recently completed phase I studies, which found that this agent leads to improvement in important cardiologic parameters, including: cardiac output/index, systemic vascular resistance, and stroke volume [49]. Clinical phase IIb trials are now underway in patients with heart failure who have reduced or preserved ejection fraction (SOCRATES, NCT01951625, and NCT01951638) [52].

sGC activators

Effect of sGC activators in experimental kidney and cardiovascular disease

The effects of the sGC activators cinaciguat, ataciguat, BI 703704, and GSK2181236A have been investigated in experimental models of kidney and cardiovascular disease (Table 2).

Cinaciguat (BAY 58-2667)

Several studies have demonstrated that cinaciguat has a protective renal effect. In rats with subtotal nephrectomy, chronic cinaciguat treatment lowered blood pressure, preserved renal function, improved plasma levels of natriuretic peptides, reduced left ventricular hypertrophy and cardiac arterial wall thickness, and slowed renal disease progression compared with untreated controls (Figure 2e) [32]. Cinaciguat was further shown to inhibit podocyte damage, tubular damage, and glomerulosclerosis in a type-1 diabetic rat model of nephropathy [53]. In a rat model of type-2 diabetic nephropathy, administration of cinaciguat, together with the angiotensin-converting-enzyme inhibitor enalapril, resulted in significant and sustained reductions in glomerulosclerosis and tubulointerstitial lesions, compared with controls [55]. Under NO-deficient conditions, cinaciguat treatment of intact anesthetized rats at least partly stabilized the resultant hypertension and renal vasoconstriction, and re-established the modulation of renal blood flow autoregulation [22]. In a salt-sensitive rat hypertension model, long-term cinaciguat was associated with markedly improved survival, a lower increase in blood pressure, improvements in cardiac and renal function, and anti-fibrotic and anti-inflammatory effects compared with untreated controls [27].

Ataciguat (HMR1766)

A new structural class of sGC activators includes the novel anthranilic acid derivative, ataciguat [37]. In a rat model of congestive heart failure, chronic treatment with ataciguat improved vascular function and sensitivity to NO, and reduced platelet activation [37]. In a rat model of non-inflammatory renal failure, ataciguat showed beneficial blood pressure-independent renoprotective effects on kidney structure and urinary albumin excretion compared with untreated controls [54•].

BI 703704

Recently presented but limited data showed BI 703704 to have a significant renal protective effect in a rat model of diabetic nephropathy when compared with untreated controls, with reductions in proteinuria and the incidence of glomerulosclerosis observed at doses that did not significantly alter blood pressure or heart rate [56].

GSK2181236A

Limited published data suggest that GSK2181236A may provide partial benefit against hypertension-induced end-organ damage in rat models [29].

Taken together, these results suggest that sGC activators also have the potential to provide pressure-independent renal and cardiac protection.

Discussion and conclusion

In *in vivo* experimental models of kidney and cardiovascular disease, the sGC stimulators BAY 41-2272, BAY 41-8543, BAY 60-4552, riociguat, and vericiguat have demonstrated protection against renal target organ damage in experimental CKD models (Table 1). It is of note that this class of new drugs worked in the CKD models nearly independent of the underlying cause of CKD. We tested models of reduction of nephron numbers, malignant hypertension, immunological induced glomerulonephritis, and finally diabetic kidney disease were tested. Stimulation of sGC and subsequent cGMP production represents an important common pathway in the maintenance of renal function independent of the initial underlying cause of CKD, and therapeutic use of these sGC stimulators has been shown to produce a broad range of antifibrotic, antiproliferative, and antiproteinuric effects. It is of note that this new class of drugs can also improve blood pressure control at higher doses. This is of major clinical impact, since most patients with CKD suffer from hypertension which is an independent progression factor of CKD.

The results indicate that sGC stimulators may be capable of restoring physiological signaling in disorders where cGMP signaling has been disrupted. The sGC system has an important physiological role in both vascular and non-vascular tissues so the beneficial effects may extend to restoring the endothelium-mediated regulation of myocardial and renal function [51]. In addition, in *in vivo* models mirroring the pathophysiology of different subtypes of CKD and cardio-renal syndrome, the sGC activators cinaciguat, ataciguat, BI 703704, and GSK2181236A were capable of protecting and improving physiological renal function (Table 2).

In conclusion, preclinical data suggest that both sGC stimulators and sGC activators may offer therapeutic benefits for patients with CKD, on top of guideline-based renin–angiotensin system blockade. These novel drugs have the potential to address an unmet medical need in CKD, by providing an alternative for those patients in whom currently available guideline-recommended treatments alone are ineffective, and who are consequently at risk for rapid progression to ESRD. Clinical trials are required to confirm and extend these preclinical findings to humans.

Conflict of interest statement

Johannes-Peter Stasch is an employee of Bayer Pharma AG.

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