RESEARCH ARTICLE

Soluble guanylate cyclase stimulator praliciguat attenuates inflammation, fibrosis, and end-organ damage in the Dahl model of cardiorenal failure

Courtney M. Shea, Gavielle M. Price, Guang Liu, Renee Sarno, Emmanuel S. Buys, Mark G. Currie, and Jaime L. Masferrer

Cyclerion Therapeutics, Cambridge, Massachusetts

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Shea CM, Price GM, Liu G, Sarno R, Buys ES, Currie MG, Masferrer JL. Soluble guanylate cyclase stimulator praliciguat attenuates inflammation, fibrosis, and end-organ damage in the Dahl model of cardiorenal failure. Am J Physiol Renal Physiol 318: F148–F159, 2020. First published October 14, 2019; doi:10.1152/ajpregn.00247.2019.—Reduced nitric oxide (NO) and a decrease in cGMP signaling mediated by soluble guanylate cyclase (sGC) has been linked to the development of several cardiorenal diseases. Stimulation of sGC is a potential means for enhancing cGMP production in conditions of reduced NO bioavailability. The purpose of our studies was to determine the effects of praliciguat, a clinical-stage sGC stimulator, in a model of cardiorenal failure. Dahl salt-sensitive rats fed a high-salt diet to induce hypertension and organ damage were treated with the sGC stimulator praliciguat to determine its effects on hemodynamics, biomarkers of inflammation, fibrosis, tissue function, and organ damage. Praliciguat treatment reduced blood pressure, improved cardiorenal damage, and attenuated the increase in circulating markers of inflammation and fibrosis. Notably, praliciguat affected markers of renal damage at a dose that had minimal effect on blood pressure. In addition, liver fibrosis and circulating markers of tissue damage were attenuated in praliciguat-treated rats. Stimulation of the NO-sGC-cGMP pathway by praliciguat attenuated or normalized indicators of chronic inflammation, fibrosis, and tissue dysfunction in the Dahl salt-sensitive rat model. Stimulation of sGC by praliciguat may present an effective mechanism for treating diseases linked to NO deficiency, particularly those associated with cardiac and renal failure. Praliciguat is currently being evaluated in patients with diabetic nephropathy and heart failure with preserved ejection fraction.

cardiorenal; Dahl; fibrosis; hypertension; inflammation; praliciguat; soluble guanylate cyclase stimulator

INTRODUCTION

Nitric oxide (NO) binds and activates soluble guanylate cyclase (sGC) to increase the production of cGMP. Regulation of the NO-sGC-cGMP pathway is critical for normal cellular activity and physiological homeostasis (25). Increased oxidative stress and impaired NO signaling are associated with increased blood pressure (21), inflammation, and fibrosis in organs including the kidney (39), heart (30, 34), and liver (31). The inflammation and fibrosis associated with deficient NO signaling are linked to the pathogenesis of many renal, cardiovascular, and hepatic diseases.

Pharmacological interventions that augment NO signaling (including NO donors and inhibition of cGMP-specific phosphodiesterase-5) have been widely and successfully used in the clinic (14, 16). The potential for therapeutically targeting the NO-sGC-cGMP pathway in several disease states has resulted in the development of two classes of agonists: sGC “activators” and “stimulators” (5, 11). sGC activators activate heme-free sGC independently of NO. Oxidation of sGC and loss of the heme group, resulting in NO-insensitive sGC, may occur in pathological conditions associated with oxidative stress. sGC stimulators require the NO-binding heme iron of sGC to be in a reduced, ferrous state for full activity. sGC stimulators, by acting in concert with endogenous NO, may preserve the temporal and spatial features of NO-sGC-cGMP signaling.

The Dahl salt-sensitive (DSS) rat is an established model of hypertension and cardiorenal failure. DSS rats fed a high-salt (HS) diet develop systemic hypertension, chronic renal failure, heart failure with preserved ejection fraction (HFpEF), and liver dysfunction (13, 27, 29, 43). These pathologies are driven, in part, by reduced NO bioavailability and impaired cGMP signaling (43, 45). In previous studies, treatment with sGC activators and stimulators attenuated HS-induced mortality and renal and liver dysfunction in DSS rats as well as prevented increases in the expression of fibrotic and inflammatory markers in the kidney and heart (15, 23). These data suggest that the NO-sGC-cGMP signaling pathway is a potential target for altering the pathophysiology of cardiorenal diseases and their associated comorbidities.

Praliciguat (IW-1973) is a sGC stimulator from a novel pyrazole-pyrimidine heterocyclic structural class. The pharmacological characterization of praliciguat has been described in preclinical models of disease (41). The objectives of the present studies were to explore the effects of praliciguat in the DSS model, where the primary outcomes included hemodynamics and kidney function and the secondary outcomes included circulating biomarkers, mRNA expression, and liver injury. In this article, we report the effects observed with praliciguat after chronic dosing in the DSS rat model on blood pressure and HS-induced inflammation, fibrosis, and end-organ failure. These data were used for pharmacokinetic and pharmacodynamic modeling to help select doses used in phase 2 studies for diabetic nephropathy (ClinicalTrials.gov Identifier NCT03217591) and HFpEF (ClinicalTrials.gov Identifier NCT03254485).
MATERIALS AND METHODS

Drugs

The sGC stimulator praliciguat was synthesized at Ironwood Pharmaceuticals, which was later spun off to Cyclerion Therapeutics. Praliciguat was formulated in 8% NaCl rodent chow (open standard diet with 8% NaCl, catalog no. D14041201, Research Diets, New Brunswick, NJ) for the treatment groups as follows: 8.3, 25, and 83 mg praliciguat/kg chow (equivalent to 1, 3, and 10 mg·kg⁻¹·day⁻¹, respectively).

Animal Model

Male DSS rats (7 wk old, weight ranging from 230 to 270 g) were purchased from Harlan Laboratories. The characteristics of this rodent model of hypertension, kidney failure, and heart failure have been previously described (8, 38). All rats were singly housed in a room with controlled temperature (17.7–26.7°C), a relative humidity of 30–70%, and a 12:12-h light-dark cycle and allowed free access to food and water. All animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited, United States Department of Agriculture-registered, and Office of Laboratory Animal Welfare-assured animal research facility (Gateway Pharmacology Laboratories, St. Louis, MO). All animal use protocols were reviewed and approved by the Institutional Animal Care and Use Committee before commencement.

Experimental Design, Dietary and Drug Regimens, and Sample Collection

Two studies were performed in DSS rats. Study 1 was an 8-wk study to investigate the hemodynamic, renal, and hepatic effects of praliciguat by echocardiography. Study 2 was a 7-wk study to further examine the cardiovascular effects of praliciguat by echocardiography.

Study 1. Telemetry transmitters (Data Sciences) were surgically implanted in 48 DSS rats to continuously monitor systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate. A laparotomy was performed to expose the abdominal aorta. The catheter tip of a telemetry transmitter was inserted into the abdominal aorta and secured with a suture (5-0 silk, Ethicon), and the body of the transmitter NS was placed in the abdominal cavity and secured to the abdominal wall. The abdominal incision was closed with uninterupted suture (4-0 vicryl, Ethicon). Buprenorphine (0.05 mg·kg⁻¹, day⁻¹) was administered subcutaneously for postoperative pain relief. After recovery from anesthesia, rats were returned to their home cages, and the cages were placed on radio signal receivers (Data Sciences). Rats recovered for 7–14 days before the initiation of the study. Telemetry data were collected continuously except for when the rats were removed from the radio signal receivers for 24-h urine collection.

For the duration of study 1, rats were fed a normal salt (NS) diet (open standard diet with 0.3% NaCl, catalog no. D11112201, Research Diets), a HS diet (open standard diet with 8% NaCl, catalog no. D14041201, Research Diets), or a HS diet containing praliciguat beginning at week 2 after first receiving the HS diet alone (Fig. 1A). Rats were randomly divided into the following five groups (n = 8 for each): NS group; HS control group; HS group (1 mg·kg⁻¹·day⁻¹) (PRL1 group; only n = 7 for analyses), HS + praliciguat (3 mg·kg⁻¹·day⁻¹) (PRL3 group), and HS + praliciguat (10 mg·kg⁻¹·day⁻¹) (PRL10 group). Blood and 24-h urine samples were collected at week 2, before drug treatment, for a comparison between the start and end of the treatment period. At the end of the study, rats were weighed and anesthetized, and the heart was perfused with heparin (1 U/mL in PBS) containing 1 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich). The whole heart was weighed, the right ventricular free wall was then removed, and the left ventricular (LV) free wall plus ventricular septum (LV + S) was weighed. Other organs were weighed and halved; one-half of each organ was fixed in 10% formalin for histopathology, and the other half was snap frozen in liquid nitrogen for the determination of praliciguat concentration and gene expression analyses.

Study 2. For the duration of the 7-wk study, rats were maintained on a NS diet, HS diet, or HS diet with praliciguat incorporated into the chow beginning at week 2 (Fig. 1B). Rats were randomly divided into the following three groups: NS group (n = 8), HS group (n = 21, only n = 12 analyses), and HS + praliciguat (10 mg·kg⁻¹·day⁻¹) (PRL10 group; n = 8). At week 7, body weight was measured, urine was collected over a 24-h period, blood samples were collected, and echocardiographic measurements of LV function and geometry were obtained. At the end of the study, rats were anesthetized, and the heart was harvested as described above.

Urine, Serum, and Plasma Analysis

Rats were placed in metabolic cages with free access to food and water, and urine was collected over 24 h. Urine samples were centrifuged at 2,000 rpm for 10 min at 4°C to remove debris and transferred to clean tubes. For serum samples, blood was collected in serum separator tubes, kept at room temperature for at least 30 min, and then centrifuged at 6,000 rpm for 10 min. For plasma samples, blood was collected in plasma separator tubes containing EDTA and then centrifuged at 3,500 rpm for 10 min at 4°C. Terminal plasma samples were transferred to Eppendorf tubes containing 5 µL of 100 mM IBMX. Urine, serum, and plasma samples were stored at −80°C until analysis.

Urine and serum samples were assessed using a Randox Daytona Clinical Chemistry Analyzer. All assessments were performed in accordance with the manufacturer’s instructions. Serum osteopontin (OPN), tissue inhibitor of metallopeptidase 1 (TIMP-1), monocyte chemoattractant protein-1 (MCP-1), and plasma TNFα-terminal-pro-bradykininogen (NT-proBNP) levels were assessed with biomarker rat assay kits (Mouse/Rat OPN Quantikine ELISA Kit MOST00 and Rat TIMP1 Quantikine ELISA Kit SRTM100, R&D Systems, and Rat MCP1 Ultra-Sensitive Kit K153AYC and Rat NT-proBNP Kit K153JKD, Meso Scale Discovery) in accordance with the manufacturer’s instructions.

Histopathology

Fixed kidney tissue samples were embedded in paraffin blocks and sectioned for histopathology. Sections were stained with hematoxylin and eosin as well as periodic acid-Schiff. Tissues were scored by a blinded pathologist for glomerulosclerosis, interstitial inflammation, interstitial fibrosis, and vascular alterations using a scale from 0–4, where 0 represents minimal damage (<25% of the area affected), 2 represents 26–50% damage, and the maximum score of 4 represents 75–100% of the tissue damaged. Glomerulosclerosis was scored based on periodic acid-Schiff-positive material in the glomeruli, and interstitial fibrosis was scored based on the severity of matrix deposition (22). Interstitial inflammation was indicated by the presence of lymphocytes and plasma cells. Vascular alterations included changes in blood vessels, such as vessel necrosis, inflammation of the vessel (indicated by the presence of neutrophils), and thrombosis of the vessels.

Quantification of Biomarker Gene Expression

Frozen kidney and liver tissues were pulverized, homogenized, and processed using a QuantiGene sample processing kit (Thermo Scientific/Affymetrix) following the manufacturer’s instructions. The gene expression of TIMP-1 (Timp1), α-smooth muscle actin (Acta2), collagen type III-α1 (Col3α1), IL-6 (Il6), NF-E2 subunit 1 (Nfkβ1), TNF-α (Tnfa), MCP-1 (Mcp1), VCAM-1 (Vcam1), ICAM-1 (Icam1), transforming growth factor-β1 (Tgfβ1), and matrix metallopeptidase (MMP)-9 (Mmp9) was measured using a custom-designed Quanti-
Gene 2.0 Plex Assay kit (Thermo Scientific/Affymetrix) and detected in a MAGPIX multiplex platform (Luminex). Median fluorescence intensity (MFI) was generated for each gene target and normalized to housekeeping genes. DNA-directed RNA polymerase II subunit RPB1 and peptidylprolyl isomerase B were chosen to match the target transcript abundance in the kidney, whereas hypoxanthine phosphoribosyltransferase-1 and peptidylprolyl isomerase B were chosen for the liver.

**Quantification of Praliciguat Concentrations in Tissue and Plasma**

Tissues were pulverized, weighed, and diluted in 20% acetic acid containing 100 μM IBMX. Standards were prepared in naive plasma and each respective naive tissue. Plasma samples, diluted tissue samples, and standards were plated. Plasma samples were precipitated with 100% acetonitrile containing internal standard and centrifuged at 4,000 g at 4°C for 10 min, and the supernatant was transferred to a clean injection plate. Tissue samples were plated onto a Phenomenex Phree phospholipid removal plate with 3× acetonitrile containing internal standard. Tissue and plasma samples were both dried under nitrogen at 55°C and resuspended in 0.1% formic acid.

All samples were analyzed using a SCIEX API 5500 liquid chromatography-tandem mass spectrometry in positive ionization (ESI) mode linked to an Acquity UPLC. Samples were loaded onto a Phenomenex Kinetex XB C18, 3.0 × 100 mm, 5-μm column. The mobile phases consisted of aqueous 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). The flow rate was 1 mL/min. The gradient was held at 100% mobile phase A for 0.5 min, ramped to 0% mobile phase A by 2 min, and returned to 100% mobile phase A by 2.01 min. The total run time was 4 min/sample.

**Echocardiography**

Echocardiography was performed at the end of study 2. Rats were placed under light anesthesia (2% isoflurane), and echocardiographic images were obtained using the Vevo 770 high-resolution micro-ultrasound system (VisualSonics). A two-dimensional parasternal short-axis view of the LV was captured in M-mode using a RMV-710B scan head (25 MHz) at a frame rate of 80–100 frames/min. LV end-systolic internal diameter (LVIDs) was calculated using the system’s cardiac measurement package. LV ejection fraction (EF) was calculated from LV end-diastolic and end-systolic volumes (LVEDV and LVESV, respectively) with the following formula: EF (in %) = (LVEDV − LVESV)/LVEDV × 100.

**Data and Statistical Analysis**

Any animals that lost more than 20% body weight or did not survive until the end of the study were excluded from all analyses. In total, 10 rats were excluded, which included 1 rat in the PRL1 group...
(study 1) and 9 rats in the HS group (study 2). Unless noted otherwise, data are presented as means ± SE. SBP and DBP were averaged over 24 h, and data analyzed by mixed-effect analyses. Blood pressure data from days 0, 14–15, 35–36, 48–49, and 53–54 were excluded as animals were in metabolic cages. Relative tissue weights were reported as grams of tissue weight divided by total body weight in kilograms. Albuminuria was defined as grams of urine albumin per gram of creatinine. For albuminuria and serum uric acid, the percent increase was calculated as \((\text{week 8} - \text{week 2})/\text{week 2} \times 100\) and the percent decrease was calculated as \((\text{week 2} - \text{week 8})/\text{week 2} \times 100\).

**Fig. 2. Study 1: effect of praliciguat on systolic blood pressure (SBP; A), diastolic blood pressure (DBP; B), and heart rate [HR, in beats/min (bpm); C] in Dahl salt-sensitive rats fed a high-salt (HS) diet. NS, normal salt; PRL1, PRL3, and PRL10, HS + praliciguat at 1, 3, or 10 mg·kg\(^{-1}\)·day\(^{-1}\), respectively. Data points are presented as means ± SE. Data from \(n = 8\) rats/group were analyzed by mixed-effect analyses. *\(P < 0.05\) vs. the HS control group; #\(P < 0.05\) vs. the PRL10 group.**
Starting by respectively, blood pressure increased in the HS group (to 167 mmHg) and remained below 105 mmHg for the duration of the study. SBP and DBP in the NS diet, average SBP remained below 144 mmHg and DBP below 80 mmHg, respectively; Fig. 2, A and B). For rats maintained on the NS diet, average SBP remained below 144 mmHg and DBP below 105 mmHg for the duration of the study. SBP and DBP increased in the HS group (to 167 ± 1 and 125 ± 1 mmHg, respectively, by day 13) and were higher than the NS group starting by day 3, before the initiation of praliciguat treatment.

Treatment with praliciguat dose dependently decreased SBP and DBP in DSS rats fed the HS diet. At baseline (week 0), SBP and DBP were similar among all rats (134 ± 1 and 99 ± 1 mmHg, respectively; Fig. 2, A and B). For rats maintained on the NS diet, average SBP remained below 144 mmHg and DBP below 105 mmHg for the duration of the study. SBP and DBP increased in the HS group (to 167 ± 1 and 125 ± 1 mmHg, respectively, by day 13) and were higher than the NS group starting by day 3, before the initiation of praliciguat treatment. Throughout the remainder of the study, SBP and DBP of the HS group steadily increased, reaching 222 ± 4 and 170 ± 6 mmHg, respectively, at day 56. At the end of the 8-wk study, treatment with praliciguat (1 mg·kg⁻¹·day⁻¹) had little effect on blood pressure compared with the HS control group. However, by the end of the study, treatment with praliciguat (3 mg·kg⁻¹·day⁻¹) attenuated the HS-induced increase in SBP to 193 ± 3 mmHg and DBP to 149 ± 5 mmHg, 14% and 12% lower, respectively, than in the HS group. Both SBP and DBP decreased immediately after treatment with praliciguat (10 mg·kg⁻¹·day⁻¹) to 169 ± 5 and 128 ± 6 mmHg, respectively, and remained ~25% lower than the HS group throughout the study. Immediately after the treatment start, SBP and DBP were lower in rats that received praliciguat (10 mg·kg⁻¹·day⁻¹) than rats that received praliciguat (1 mg·kg⁻¹·day⁻¹); this dose-dependent effect persisted throughout the study. Heart rate changes measured over 24 h were not different among groups at any point during the study (Fig. 2C).

### Results

#### Effect of Praliciguat on Blood Pressure (Study 1)

After 6 wk of treatment, average plasma concentrations of praliciguat were 56 ± 7, 259 ± 30, and 531 ± 28 nm in the PRL1, PRL3, and PRL10 groups, respectively. Free fraction concentrations in plasma were 0.2 ± 0.0, 1.0 ± 0.1, and 2.1 ± 0.1 nm, respectively. Total tissue-to-plasma ratios were similar across dose groups in the heart (9.7 ± 3, 6.7 ± 1, and 9.7 ± 3, respectively) and kidney (6.9 ± 1, 8.5 ± 3, and 5.9 ± 0, respectively; Fig. 3). Liver-to-plasma ratios suggested a dose-related increase (49.1 ± 13, 73.68 ± 20, and 98.99 ± 12), which was not significant by one-way ANOVA.

#### Effect of Praliciguat on Cardiac Remodeling and Stress (Study 1)

At the end of study 1, body weight was lower in the HS group than in the NS group (384 ± 19 vs. 486 ± 7 g); body weight was higher in the PRL10 group than in the HS group (435 ± 8 g; data not shown). The relative LV+S weight was higher in animals fed the HS diet than those fed the NS diet (3.7 ± 0.2 and 2.3 ± 0.0, respectively; Fig. 4A). Treatment with praliciguat attenuated LV hypertrophy associated with the HS diet. The LV+S weight was 3.2 ± 0.1, 3.1 ± 0.2, and 2.8 ± 0.1 for the PRL1, PRL3, and PRL10 groups, respectively. Similar results were observed in the whole heart weight...
analysis (data not shown). Plasma NT-proBNP, a marker of cardiac stress, was also measured (28). Plasma NT-proBNP was higher in the HS group than in the NS group (4,082 ± 1,297 vs. 546 ± 48 pg/mL; Fig. 4A). The HS-induced increase in plasma NT-proBNP levels was prevented in the PRL10 group (649 ± 62 pg/mL), whereas the levels trended toward lower than the HS group for the PRL3 group (1,818 ± 737 pg/mL, *P = 0.099).

Effect of Praliciguat on Cardiac Function (Study 2)

In study 2, echocardiography was performed to evaluate changes in cardiac function in DSS rats treated with praliciguat. As observed in study 1, body weight was lower in the HS group than in the NS group (355 ± 9 vs. 423 ± 4 g) and higher in the PRL10 group than in the HS group (400 ± 5 g; data not shown). Praliciguat (10 mg·kg⁻¹·day⁻¹) attenuated the increases in relative LV + S weight associated with HS, and the resultant relative LV + S weight was similar to that in the NS group (Fig. 5A). Additionally, praliciguat (10 mg·kg⁻¹·day⁻¹) prevented the HS-induced increase in LVIDs (3.5 ± 0.2, 4.6 ± 0.3, 3.5 ± 0.3 mm for the NS, HS, and PRL10 groups, respectively; Fig. 5B). Treatment with praliciguat also attenuated the HS-induced decrease in EF (82 ± 2%, 70 ± 3%, and 85 ± 2% in the NS, HS, and PRL10 groups, respectively; Fig. 5C). As was observed in study 1, plasma NT-proBNP levels were higher in the HS group than in the NS group; once again, treatment with praliciguat (10 mg·kg⁻¹·day⁻¹) prevented the HS-induced increase (Fig. 5D).

Effect of Praliciguat on Renal Function and Fibrosis (Studies 1 and 2)

At the end of study 1, the susceptibility of salt-loaded DSS rats to kidney damage and dysfunction (26, 44) was demonstrated by an increase in levels of albuminuria and serum uric acid in salt-loaded rats (0.50 ± 0.09 vs. 0.02 ± 0.01 g/g for HS vs. NS groups and 2.9 ± 0.4 vs. 1.8 ± 0.1 mg/dL for HS vs. NS groups, respectively). Urinary albumin levels were the same in the NS and HS groups (0.04 ± 0.01 and 0.04 ± 0.01 g/g) at baseline, before the start of the HS diet. By week 2, urinary albumin levels had increased in all rats that received the HS diet, averaging 0.49 ± 0.03 g/g. Albuminuria in the NS group remained low throughout the entire study, with the
highest levels observed in week 2 (0.09 ± 0.01 g/g). After week 2, albuminuria remained consistently elevated and unchanged in the HS group for the remainder of the study. From week 2 to week 8, continuous treatment with praliciguat at 1 and 3 mg·kg\(^{-1}\)·day\(^{-1}\) significantly reduced albuminuria by an average of 41% and 39%, respectively, and praliciguat at 10 mg·kg\(^{-1}\)·day\(^{-1}\) reduced albuminuria by an average of 21%.

These reductions translated to the PRL3 and PRL10 groups having significantly lower albuminuria (0.26 ± 0.02 and 0.29 ± 0.03 g/g, respectively) than the HS group (Fig. 6A). Of note, by week 8, the 24-h urinary output for the HS group was higher than for the NS group (1.08 ± 1.10 and 0.08 ± 0.08 dL); praliciguat treatment had no effect on the HS-diet-induced increases in urinary volume, suggesting that changes in albu-
minuria in the treatment groups were not due to changes in urinary output. For all groups, serum uric acid levels increased from week 2 to week 8, including 101% in the NS group and 233% in the HS group. Similar to serum uric acid levels in the NS group, serum uric acid increased for the praliciguat-treated groups by 119% for the PRL1 group (P = 0.07 vs. the HS group), 119% for the PRL3 group (P = 0.08 vs. the HS group), and 79% for the PRL10 group (P < 0.05 vs. the HS group). By week 8, serum uric acid levels for all praliciguat-treated groups were significantly lower than for the HS group (1.9 ± 0.2 mg/dL for the PRL1 group, 1.9 ± 0.4 mg/dL for the PRL3 group, and 1.4 ± 0.2 mg/dL for the PRL10 group; Fig. 6B). After 8 wk, serum creatinine levels were higher in the HS group than in the NS group (1.03 ± 0.09 and 0.81 ± 0.03 mg/dL, respectively) and creatinine levels were lower in the PRL10 than in the HS group (0.74 ± 0.02 mg/dL; data not shown). At week 8, blood urea nitrogen, serum Na+, and serum K+ were similar across all groups (data not shown). Also, in study 2, beneficial effects of praliciguat on kidney function were observed as the HS-induced increase in albuminuria was attenuated in the PRL10 group (Fig. 6C).

At the end of study 1, mRNA expression levels of genes associated with kidney inflammation and fibrosis (32, 46) were higher in the HS group than in the NS group: Timp1 (4.8-fold), Acta2 (1.9-fold), Col3a1 (2.9-fold), Il6 (2.8-fold), Nfkb1 (1.5-fold), Tnfa (2.0-fold), Mcp1 (2.2-fold), Vcam1 (1.6-fold), and Icam1 (1.9-fold; Fig. 7). HS-induced upregulation for most of these genes was attenuated in the PRL3 group or the PRL10 group. The exceptions included Col3a1, where treatment with all doses attenuated upregulation of expression, and Acta2,

![Fig. 9. Study 1: effect of praliciguat on the kidney histology of Dahl salt-sensitive rats after 8 wk on a high-salt (HS) diet by hematoxylin and eosin (H&E) and periodic acid-Schiff staining. A—D: dot plots of histological scores for glomerulosclerosis (A), interstitial fibrosis (B), interstitial inflammation (C), and vascular alterations (D). NS, normal salt; PRL1, PRL3, and PRL10, HS + praliciguat at 1, 3, or 10 mg·kg⁻¹·day⁻¹, respectively. Data are shown as means ± SE; n = 8 rats/group. Data were analyzed using one-way ANOVA followed by a Dunnett’s multiple-comparison test versus the HS group, *P < 0.05. Plots with no error bars indicate no variations in score across all samples in that group. E–G: representative H&E stains of kidney sections from animals in the NS control group, HS control group, and PRL10 group. Scale bar = 25 μm.](#)

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where a nonsignificant trend was observed. Levels of serum markers associated with cardiorenal dysfunction and inflammation (MCP-1, TIMP-1, and OPN) (18, 24, 33) were also higher in the HS group than in the NS group (Fig. 8). Treatment with praliciguat at doses greater than 1 mg·kg\(^{-1}\)·day\(^{-1}\) lowered each serum marker.

The extent of fibrosis, inflammation, and damage in the kidneys was assessed histologically by scoring glomerulosclerosis, interstitial fibrosis, interstitial inflammation, and vascular alterations (Fig. 9). After 8 wk, the HS diet induced visible inflammatory cell infiltration, tubule damage, and fibrosis, resulting in higher histological scores in kidney tissue from the HS group than from the NS group. Treatment with praliciguat attenuated fibrosis and inflammation at all doses tested, with the kidneys completely protected from the HS-induced damage at 10 mg·kg\(^{-1}\)·day\(^{-1}\) praliciguat (Fig. 9, E–G).

**Effect of Praliciguat on Liver Weights and Markers of Fibrosis (Study 1)**

Expression of the profibrotic genes Mmp9, Timp1, and Tgfb1 (37) was upregulated in the livers of the HS group (Fig. 10, A–C). Gene expression levels of Mmp9, Timp1, and Tgfb1 were lower in livers from praliciguat-treated rats than from HS rats. Praliciguat treatment attenuated the HS-induced increase in gene expression at all doses for Mmp9, in PRL3 and PRL10 groups for Tgfb, and in the PRL10 group for Timp1.

The relative weight of the liver was higher in the HS group (38.2 ± 1.3 g/kg) than in the NS group (32.9 ± 0.5 g/kg; Fig. 10D). Relative liver weights were lower in the PRL3 and PRL10 groups than in the HS groups (34.8 ± 0.4 and 32.2 ± 0.4 g/kg, respectively).

**DISCUSSION**

cGMP and its downstream pathways are important for normal organ function, and an increase in cGMP is anti-inflammatory and antifibrotic (1, 35). Hypertension, cardiorenal impairment, vascular disease, and hyperlipidemia are pathologies directly linked to dysfunction in the NO-sGC-cGMP signaling pathway. In patients with hypertension and heart failure, there is reduced NO bioavailability (36, 42). Oxidation of the heme Fe\(^{2+}\) in sGC leads to loss of the heme; this prevents sGC activation by NO and decreases cGMP production. The present studies add to the growing body of evidence that sGC stimulators can improve pathologies in diseases associated with impaired NO signaling; moreover, these effects by sGC stimulators are not completely inhibited by the possible reduction in the proportion or amount of “heme-intact sGC.”

Exposure of DSS rats to a HS diet, a well-established and widely used model of cardiovascular and renal dysfunction, is typically associated with severe pathophysiology (e.g., severe and rapid onset of hypertension and albuminuria) and mortality. Previous studies have implicated deficient NO-cGMP signaling as an important driver of hypertension and organ dysfunction in salt-loaded DSS rats, for example, oral administration of the NO precursor L-arginine, but not of D-arginine (a form of arginine that is not a NO precursor), inhibited the development of hypertension in the DSS model (6). In addition, cGMP levels are lower in multiple organs, such as the aorta and placenta, of DSS rats fed a HS diet than those fed a NS diet (3, 40).

In these studies, we advanced the pharmacology of praliciguat by describing the results of two comprehensive preclinical studies in the DSS model of hypertension and of cardiorenal failure. Praliciguat has unique pharmacological characteristics including high protein binding (99.6%) and volume of distribution (10.5 L/kg), and its multidimensional pharmacology and mechanism(s) of action in the vasculature and in tissues expressing sGC have been previously reported (17, 41). The effects of praliciguat on SBP and DBP, including fast onset and a sustained reduction, were observed at very low (nanomolar) free plasma levels, with the largest decreases occurring with the highest dose of praliciguat. In both studies,

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**Fig. 10. Study 1: effect of praliciguat on markers of liver fibrosis in Dahl salt-sensitive rats after 8 wk on a high-salt (HS) diet. NS, normal salt; PRL1, PRL3, and PRL10, HS + praliciguat at 1, 3, or 10 mg·kg\(^{-1}\)·day\(^{-1}\), respectively. A–C: dot plots of expression of genes [matrix metallopeptidase-9 (Mmp9), tissue inhibitor of metallopeptidase 1 (Timp1), and transforming growth factor-β1 (Tgfb1)] associated with liver fibrosis relative to housekeeping genes. D: dot plots of liver weight relative to body weight for each group. Data are shown as means ± SE; n = 8 rats/group. Data were analyzed using one-way ANOVA followed by a Dunnett’s multiple-comparison test versus the HS group. *P < 0.05.**
one study in which rats were evaluated via telemetry and the other study via echocardiography, praliciguat protected rats from the HS-induced structural and functional changes in the heart, including heart hypertrophy (LV + S and LVIDs), decreased EF, and abnormal levels of NT-proBNP. These measures are relevant to clinical evaluations of cardiac dysfunction, which include LV hypertrophy assessed by echocardiography, a well-known abnormality in patients with HFpEF (20), and increased plasma NT-proBNP, an indicator of heart stress and a guideline-recommended diagnostic marker of heart failure (7).

There is an interdependence between cardiac and renal functions, and kidney dysfunction is a common comorbidity in patients with chronic heart failure (4). Previously, we (41) reported the benefit of praliciguat on proteinuria; here, we explored the effects of praliciguat on the progression of kidney dysfunction by tracking albuminuria throughout the study. Treatment with praliciguat protected kidney function, as shown by attenuated development of albuminuria and lower renal expression of proinflammatory and profibrotic genes than in the HS control group. Interestingly, praliciguat protected against histologically assessed glomerular damage, inflammation, and fibrosis at the lowest dose tested (1 mg·kg⁻¹·day⁻¹), a dose that had minimal effect on blood pressure in these rats. These data suggest that the renal effects of praliciguat are not solely due to a reduction in blood pressure and support investigation of praliciguat as a potential treatment for kidney diseases, such as diabetic nephropathy, in which hypertension may not be the key driver of inflammation, fibrosis, and decline in renal function. The reduction in albuminuria between the start and end of treatment did not reach statistical significance in the high-dose praliciguat (10 mg·kg⁻¹·day⁻¹) group, probably because all rats in this group had low levels of albuminuria at week 2. However, this dose had significant effects in all other evaluations of kidney function and damage.

Beyond the cardiorenal effects, praliciguat treatment attenuated the hypertrophy and expression of genes associated with fibrosis and inflammation observed in the livers of salt-loaded DSS rats. We previously demonstrated in mouse models of liver fibrosis that praliciguat treatment restored hepatic cGMP levels and was anti-inflammatory and antifibrotic. In these models of liver fibrosis, praliciguat treatment also reduced adipocyte size within white adipose tissue, which may play a role in the mechanism of action of praliciguat in models of liver disease and may explain the attenuation in liver hypertrophy (12). In the clinic, liver damage has been associated with heart failure (2) and adipocyte size is a marker of steatosis (10).

The extensive tissue distribution of praliciguat may contribute to its multiorgan effects. In the DSS model, praliciguat concentrations in the kidney, heart, and liver were at least fivefold greater than in the plasma; similar fold differences were observed in Sprague-Dawley rats (41). In mice, concentrations in liver were 40-fold greater than in plasma (12). The anti-inflammatory and antifibrotic effects of praliciguat in the DSS model were not restricted to the kidney and liver but were also observed for circulating markers. Administration of vaso- dilators like oral systemic sGC stimulators could have systemic pharmacological effects, including hypotension. However, the effects of sGC stimulators on blood pressure is dose responsive and can be minimized by adjusting the dose and dosing regimen. Praliciguat’s hemodynamic and cardiorenal protective effects and tissue distribution properties support exploration of praliciguat in the clinic. In a 14-day study in patients with type 2 diabetes and hypertension, praliciguat reduced blood pressure, improved metabolic parameters, and reduced asymmetric dimethylarginine [a marker of endothelial dysfunction (19)]. Currently, praliciguat is under evaluation in phase 2 studies for diabetic nephropathy (ClinicalTrials.gov Identifier NCT03217591) and HFpEF (ClinicalTrials.gov Identifier NCT03254485).

In conclusion, praliciguat reduced hypertension and ameliorated pathologies associated with NO deficiency in multiple target organs in the DSS rat, a model of cardiorenal failure with reduced NO. Some beneficial effects of praliciguat were observed at a dose that had minimal effects on systemic blood pressure. Based on these preclinical data, the sGC stimulator praliciguat has the potential to provide multiple benefits in diseases with low NO availability.

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DISCLOSURES

All authors were or are employed by and may own stock/stock options in Cyclerion Therapeutics.

AUTHOR CONTRIBUTIONS

C.M.S. and J.L.M. conceived and designed research; C.M.S., G.L., and R.S. performed experiments; C.M.S., G.L., and R.S. analyzed data; C.M.S., G.M.P., G.L., R.S., E.S.B., and J.L.M. interpreted results of experiments; C.M.S. and G.M.P. prepared figures; C.M.S., G.M.P., and E.S.B. drafted manuscript; C.M.S., G.M.P., E.S.B., and J.L.M. edited and revised manuscript; C.M.S., G.M.P., G.L., R.S., E.S.B., M.G.C., and J.L.M. approved final version of manuscript.

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