

Pharmacological Profile of KBP-5074, a Novel Non-Steroidal Mineralocorticoid Receptor Antagonist for the Treatment of Cardiorenal Diseases

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Abstract

Objectives: Mineralocorticoid receptor antagonists (MRAs) have been demonstrated to reduce the risk of mortality in patients with heart failure and to decrease proteinuria in patients with chronic kidney disease (CKD). However, the use of existing MRAs in cardio renal disease is limited due to potential life-threatening hyperkalemia. The aims of this study are to determine the selectivity of KBP-5074 to bind to MR and to assess the efficacy of KBP-5074 in the attenuation of experimental hypertension and nephropathy in two commonly used animal models.

Results: KBP-5074 selectively bound to recombinant human MR with *in vitro* antagonistic activity (IC_{50} : 2.7 nM) superior than the other MRAs. This study demonstrated that KBP-5074 had better pharmacological activities than eplerenone (reference benchmark molecule) in the Dahl salt sensitive (DSS) rat and stroke-prone spontaneous hypertensive rat (SHRSP) models of hypertension and nephropathy. These activities included lowering blood pressure, decreasing urine albumin excretion, reducing kidney-body weight and heart-body weight ratios, reducing kidney small artery cross-sectional areas or renal injury scores, and elevating MR ligand aldosterone. KBP-5074 has also shown to have a better safety profile relative to eplerenone without the side effects on potassium, cholesterol, triglycerides and creatinine levels in animal toxicology studies.

Conclusion: KBP-5074 demonstrated excellent affinity and superior receptor selectivity for MR *in vitro* as compared to other MRAs. Based on the results from the DSS and SHRSP rat models, KBP-5074 may emerged as a promising next generation non-steroidal MRA for the treatment of CKD and cardiorenal diseases.

Keywords: Cardiorenal diseases; Chronic kidney disease; Dahl Salt Sensitive rat model; Mineralocorticoid receptor antagonists; Stroke Prone Spontaneously Hypertensive rat model; Systolic Blood Pressure

Introduction

A growing body of evidence suggests that over-activation of the aldosterone/mineralocorticoid receptor (MR) pathway plays an important role in the pathogenesis of cardiorenal diseases. Mineralocorticoid receptor antagonists (MRAs) have been demonstrated to reduce the risk of mortality in patients with heart failures and to decrease proteinuria in patients with chronic kidney disease (CKD). Pitt [1] reviewed the status regarding the safety of MRAs in heart failure with reduced ejection rate. However, the use of existing MRAs in cardiorenal disease has been less than optimal largely due to concerns about their safety, in large part due to the fear of hyperkalemia and renal dysfunction [2]. Although considerable progress has been made in the treatment of CKD, the development of a new therapeutic remains critical. Mineralocorticoid receptor (MR), a member of the steroid receptor family, has been shown to play a major pathophysiological role in the progression of kidney diseases. The inhibition of MR signaling considerably reduces proteinuria in patients with chronic kidney disease (CKD) [3]. CKD is a serious, progressive, life-threatening condition characterized by a gradual loss of kidney function over time. CKD affects more than 29 million people in the U.S. alone, becomes more prevalent with age, and is caused by numerous pathologic insults. CKD is associated with significant morbidity and mortality, and is strongly associated with poor health outcomes [4]. Adults with diabetes or hypertension are at greater risks of developing CKD and cardiovascular diseases. Individuals at the highest risk of progressive CKD are defined by a sustained decline in estimated glomerular filtration rate and/or the

presence of significant albuminuria/proteinuria. While some classes of drugs have been observed to ameliorate renal function decline, none are curative treatments or completely attenuate progression of renal function deterioration in patients with CKD. The majority individuals with CKD have an increased risk of cardiovascular diseases and die before they reach the end-stage renal disease.

Aldosterone acts on the renal cortical collecting ducts to affect the reabsorption of sodium and water in exchange for potassium. Unchecked excessive levels of aldosterone on the MR can lead to congestive heart failure, hypertension, and CKD while blocking the action of aldosterone with existing MRAs is limited due to the risk of hyperkalemia [2,5]. Eplerenone is steroidal MRA and a highly selective aldosterone blocker developed for the treatment of hypertension and heart failure and served as the reference benchmark molecule in this study. However, MR antagonism with eplerenone causes a dose-related increase in serum potassium concentrations [6]. Therefore, eplerenone is not recommended in patients receiving potassium-sparing diuretics, particularly in patients with renal insufficiency, diabetes, and microalbuminuria [7]. Preliminary data showed KBP-5074 selectively bound to recombinant human MR *in vitro* with a better pharmacological profile than eplerenone as a potential new drug for the treatment of CKD and cardiorenal diseases. The primary objectives of this study were to determine the selectivity of KBP-5074 to bind to MR *in vitro* and to assess the efficacy of KBP-5074 in the attenuation of hypertension in the SHRSP (Stroke prone spontaneous hypertensive rat) and DSS (Dahl salt sensitive) rat models of hypertension and nephropathy.

Material and Methods

Chemical description of KBP-5074

Chemical Name: 2-chloro-4-[(3S,3aR)-3-cyclopentyl-7-(4-hydroxypiperidine-1-carbonyl)-3,3a,4,5-tetrahydro-2H-pyrazolo[3,4-f]quinolin-2-yl] benzonitrile

Relative Molecular Mass: 504.02 g/mol

Appearance: Yellow or greenish-yellow crystalline powder

In vitro binding activity of KBP-5074

The antagonistic binding activity of KBP-5074 on 4 nuclear receptor super family members was conducted by Invitrogen Corporation, Madison, Wisconsin, USA. The proprietary Select Screen[®] Cell-Based Nuclear Receptor Profiling Service used the GeneBLazer[®] Beta-lactamase reporter technology to provide a reliable, rapid and sensitive method for analyzing the intracellular effect of a test compound on several important Nuclear Receptors (NRs). The GeneBLazer[®] Validated Assays for NRs have been tested and documented to show a high level of performance.

Experimental animals and housing

Animals were housed in a humidity-regulated (RH 60 ± 5%), temperature regulated (23 ± 1.5°C) and 12-hour light-dark cycle-regulated room (7:00-19:00). The animals had free access to food and drinking water. Animal food (Specific Pathogen Free rat chow was purchased from Trophic Animal Feed High-tech Company, Ltd., (Nantong, China) and drinking water were adhered to the international guidelines of GB14924.2-2001, GB14924.3-2011 and GB14925-2010. After one week of quarantine, animals were randomized into the designated groups for study. All animal procedures were conducted in accordance with the IACUC guidelines (IACUC Number AU-2013-013) and standard operating procedures for the care and use of laboratory animals of KBP Biosciences Company, Ltd. (Jinan, China).

Dahl Salt Sensitive (DSS) rat model

90 Male Dahl salt sensitive rats, 8-9 weeks old, were divided into eight groups (Control group, n=10; Model group, n=12; Excipient group, n=12; Eplerenone group, n=12; KBP-5074: 0.3 mg/kg/day group, n=11; KBP-5074: 1 mg/kg/day group, n=11; KBP-5074: 3 mg/kg/day group, n=11; KBP-5074: 10 mg/kg/day group, n=11). All the rats were marked with ID number on the tails, and cages were marked with ID on the cage cards. Rats in the control group were fed a low-sodium chow (0.4%) and served as negative controls. Other rats were fed with high salt chow (4% sodium chloride) for the induction of high blood pressure. From day 1 of high salt chow feeding, rats in treatment groups were dosed orally via gavage with solid dispersions of KBP-5074 in vehicle (sterile water for injection) at 0.3, 1, 3 and 10 mg/kg/day, twice a day (0.15, 0.5, 1.5 and 5 mg/kg/dose). Rats in the Model group received sterile water for injection (Vehicle). Excipient group animals received oral gavage of the excipients used in the formulation of different KBP-5074 dosing concentrations. For comparison, eplerenone was dosed at 120 mg/kg/day mixed in rat chow. The treatment lasted for 44 days. SBP was monitored using tail-cuff method, once a week for 6 weeks. Additional measurement was done on day 39. 24-hour urine samples were collected using metabolism cage once a week. Samples were then analyzed for albumin using Urine Albumin Detection Kit. On day 42, blood samples at 0 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 24 h post the last dose were collected from the tail vein of animals in KBP-5074-treated groups.

Stroke-Prone Spontaneously Hypertensive Rat (SHRSP) model

The effects of KBP-5074 on systolic blood pressure (SBP) and kidney function was evaluated in stroke-prone spontaneously hypertensive rat

(SHRSP) model. 70 Male rats (10-12 weeks old) were divided into six groups. (1) Normal control group, n=10; (2) Disease model group, n=12; (3) Eplerenone 100 mg/kg/day group, n=12; (4) KBP-5074 5 mg/kg/day group, n=12; (5) KBP-5074 15 mg/kg/day group, n=12; (6) KBP-5074 50 mg/kg/day group, n=12. Treated animals were fed high salt (4% sodium chloride) Specific Pathogen Free (SPF) rat chow. Starting on Study Day 1, all treated rats (12/sex/group) were administered orally twice a day with solid dispersion of KBP-5074 at 0 (vehicle control, distilled water for injection), 5, 15 or 50 mg/kg/day. For comparison, eplerenone was mixed in SPF rat chow and administered to a group of 12 male rats at 100 mg/kg/day. A separate normal control group (n=10) were fed low sodium (0.4% sodium chloride) diet. The treatment lasted for 35 days. SBP was measured once a week for 5 weeks using the tail-cuff method. Urine samples (24-hour) were collected using metabolism cage once a week for 5 weeks.

Blood collection, terminal sacrifice and histopathology

On day 44 in DSS rat model and on day 35 in SHRSP rat model, blood samples were collected for plasma or serum preparation. Rats were anesthetized by pentobarbital sodium and exsanguinated using a 25-gauge needle insert into abdominal aorta. Blood samples were immediately transferred to vacutainer collection tubes with anti-coagulant for plasma or tubes without anti-coagulant for serum preparation. Plasma and urine chemistry (e.g., creatinine, blood urea nitrogen and electrolytes) were analyzed with automated diagnostic clinical chemistry analyzer (Hitachi 7168, Japan) according to standard operating procedures. Urinary albumin was measured using a rat albumin ELISA kit (Bethyl Laboratories, Inc., Montgomery, Texas, USA). Urinary total protein was measured by BCA kit (Sigma, St. Louis, Missouri, USA). Plasma aldosterone was determined using a RIA kit by the Nuclear Medicine Institute, Shandong Medical College (Jinan, Shandong, China). At the end of the study, kidneys and hearts were collected and fixed in 10% buffered formalin for histopathological examination. Kidney damage was semi-quantitatively analyzed based on the degree of glomerulonephritis (proliferative, crescents/sclerosis), protein cast severity, interstitial inflammatory response, and perivascular inflammatory infiltration. The heart damage was analyzed by the measurement of left ventricular wall thickness. Kidneys and hearts were processed for histopathology staining, including hematoxylin and eosin (H&E), periodic acid Schiff (PAS) for the detection of glycogen, and Masson trichrome staining for the detection of collagen fibers in tissues.

Statistical analysis

All data are expressed as mean ± SEM unless otherwise indicated. Statistical comparisons of parameters between groups were made using one-way ANOVA followed by Tukey test; p values <0.05 was considered statistically significant. Data analysis was conducted using Graph Pad Prism 5.0 software.

Results

In vitro binding activity of KBP-5074

The antagonistic binding activity of KBP-5074 on 4 nuclear receptor super family members was compared Eplerenone and Spironolactone. *In vitro* study showed the IC₅₀ (2.7 nM) of KBP-5074 against the mineralocorticoid receptor (MR) was the lowest relative to the other compounds tested in table 1. KBP-5074 selectively bound to the MR and blocked the binding of aldosterone to MR, and KBP-5074 had little or no binding affinity to the glucocorticoid, progesterone and androgen receptors.

The efficacy of KBP-5074 in DSS rat model

Efficacy in preventing elevation of SBP in DSS rat model: After 41 days of high salt diet feeding, animals in DSS rat model and excipient

control groups showed continuous increase in SBP. Treatment of animals with KBP-5074 ameliorated the increase of SBP. Compared to DSS rat model group on day 41, KBP-5074 treated groups at 0.3, 1, 3 and 10 mg/kg significantly attenuated SBP by 9%, 19%, 22% and 22%, respectively (Figure 1).

Efficacy in preventing elevation of urine albumin in DSS rat model: After 36 days of salt feeding, DSS rats showed continuous increase in urinary excretion of albumin. Animals treated with excipient or eplerenone had higher levels of albumin excretion in the urine than the DSS rat model group. Rats dosed with KBP-5074 at 0.3 mg/kg/day showed no significant attenuation of elevated albumin excretion. Treatment with KBP-5074 at 1, 3 and 10 mg/kg/day significantly reduced the 24-hour urinary albumin excretion by 35%, 48% and 57%, respectively (Figure 2).

Efficacy in preventing elevation of urine total protein in DSS rat model (Figures 3 and 4): Figure 4 shows that on day 36, total protein levels increased significantly in the DSS rat model group relative to the low sodium control group. Treatment with excipient and eplerenone resulted in elevation of total protein excretion in the urine. Treatment with KBP-5074 resulted in apparent decreases of urinary total protein relative to the DSS rat model group. The reduction of urine total protein did not appear to be dose-dependent. At 3 mg/kg/day, KBP-5074 significantly ameliorated the elevation of urine total protein excretion when compared with the DSS rat model group (Figure 3).

Urine chemistry analysis in DSS rats: Figure 4 shows DSS rats in model group had significantly elevated urinary creatinine levels when compared to control (low sodium diet) group. Treatment with KBP-5074 showed no attenuation of this creatinine elevation. No changes of urine potassium levels were observed in DSS rats after oral administration of KBP-5074. Urine sodium and chloride levels in all DSS rats were significantly elevated ($p < 0.001$) relative to low sodium control group. Treatment with KBP-5074 and bench mark molecule eplerenone showed no amelioration of sodium elevation. Similarly, BUN levels were significantly increased in DSS rats

relative to control (low sodium diet) group. Oral administration of KBP-5074 up to 10 mg/kg/day for 36 days showed no attenuation of BUN elevation. Urinary albumin/creatinine ratios (UACR) on Day 36 showed a dose-related decrease from 21.58 mg/mg to 14.89, 8.91, 8.12 and 6.28 mg/mg at 0.3, 1, 3 and 10 mg/kg/day KBP-5074 treatment groups, respectively.

Blood chemistry analysis in DSS rats: Figure 5 shows no elevation of blood creatinine levels were observed in DSS rats when compared with low sodium control group. However, treatment with KBP-5074 resulted in a decreasing trend of creatinine levels at all dose groups except the 10 mg/kg/day group (Tukey test, $p < 0.05$). The amelioration of creatinine elevation by KBP-5074 was apparently not dose-related. DSS rats showed no changes in BUN and sodium. Treatment with KBP-5074 resulted in no changes in BUN and serum sodium levels. Treatment with either KBP-5074 or eplerenone resulted in elevation of blood potassium concentrations when compared to DSS control group (21%, $p < 0.001$). No changes in blood chloride levels were observed the excipient control group and DSS rats when compared to rats on a low sodium diet. Treatment with eplerenone and different KBP-5074 doses resulted in approximately 4% increase ($p < 0.001$) of blood chloride levels compared to DSS rat model group.

Plasma aldosterone in DSS rats: Aldosterone is the cognate ligand of mineralocorticoid receptor (MR). Blocking the binding of aldosterone to MR would lead to elevation of free aldosterone in circulation, probably due to feedback up regulation. The levels of plasma aldosterone at the end of the study (Day 44) were summarized in figure 6. Treatment with KBP-5074 resulted in significant elevation of plasma aldosterone in a dose-dependent manner. The percent increase of aldosterone at 0.3, 1, 3 and 10 mg/kg/day were 47%, 61%, 165% and 692%, respectively.

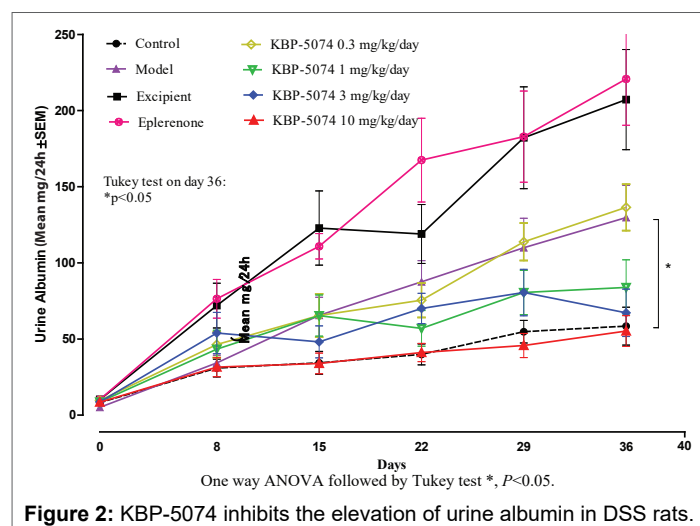
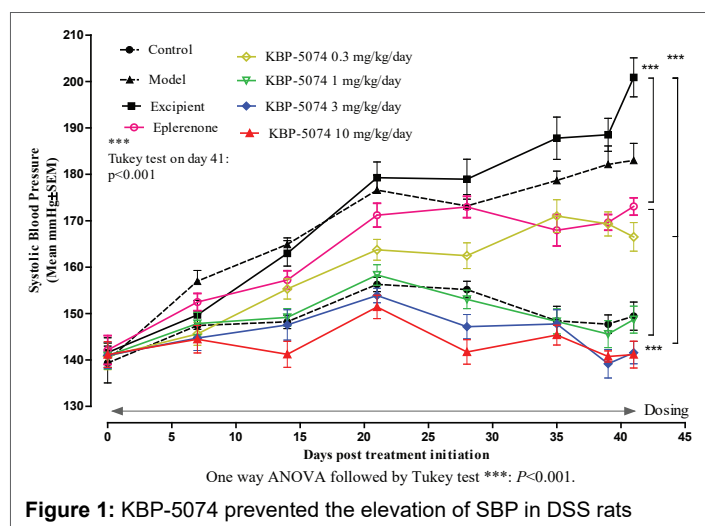
Kidney-body weight and heart-weight ratios in DSS rats: Kidney and heart to body weight ratio reflects pathological or physiological changes of these organ. Increase ratio suggests the inflammation and/or fibrosis in the organ. At the end of the study (day 44), the organs were collected and the organs and body were weighed. The organ/body weight ratios

Table 1: MR Antagonist Activity (IC_{50}) and Selectivity

| Compound | Mineralocorticoid Receptor (nM) | Glucocorticoid Receptor (nM) | Progesterone Receptor (nM) | Androgen Receptor (nM) |
|---------------------------|---------------------------------|------------------------------|----------------------------|------------------------|
| KBP-5074 | 2.70 | 2410 | 122 | no activity |
| Eplerenone ^a | 268 | >10000 | >10000 | 2090 |
| Spirolactone ^b | 14.3 | 1950 | 906 | 30.7 |

^aEplerenone is a more selective MR antagonist marketed for the treatment of high blood pressure.

^bSpirolactone is an old aldosterone antagonist with additional anti-androgen properties and has been used primarily as a diuretic and antihypertensive.



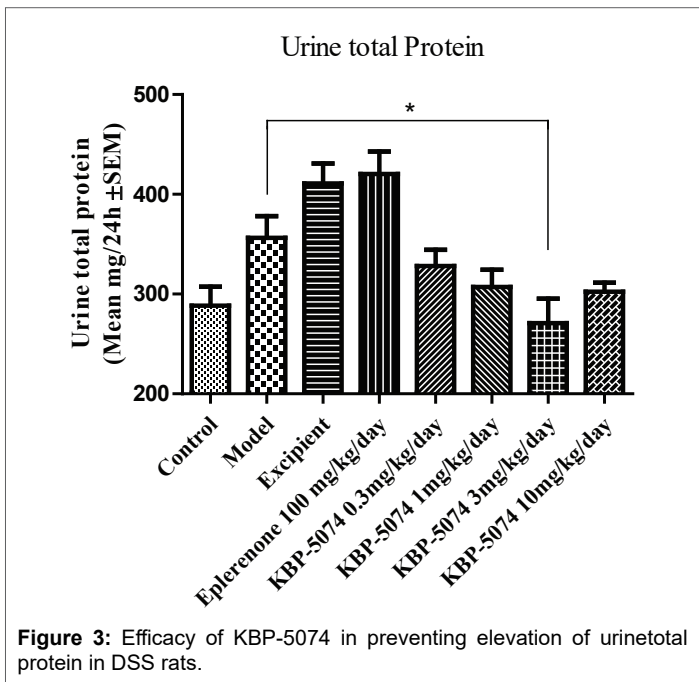
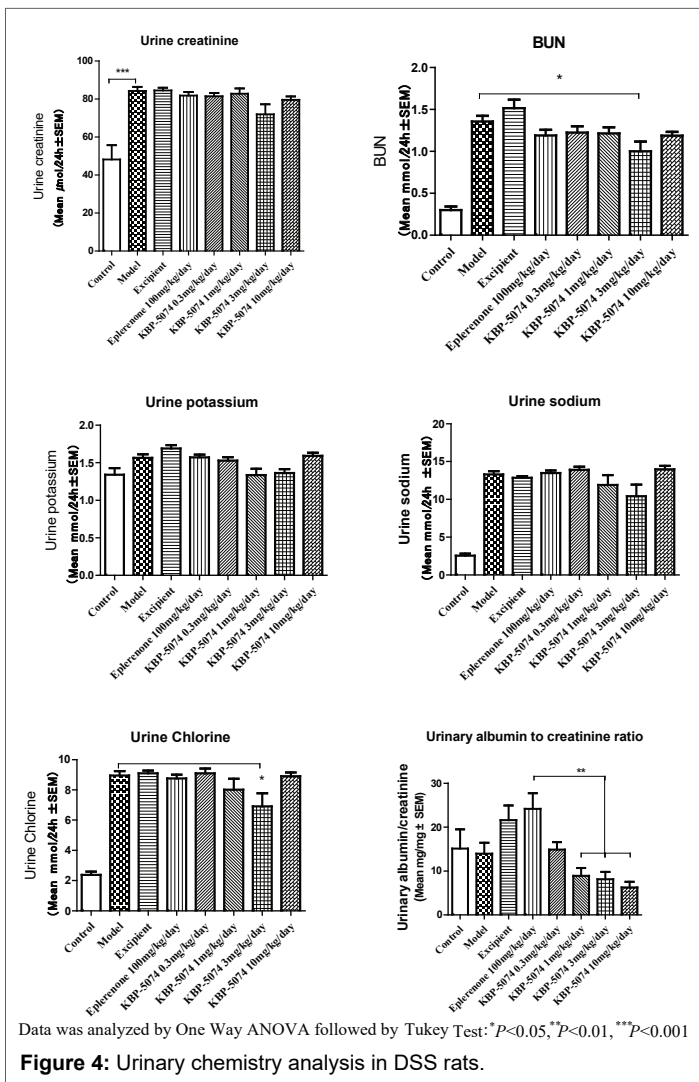
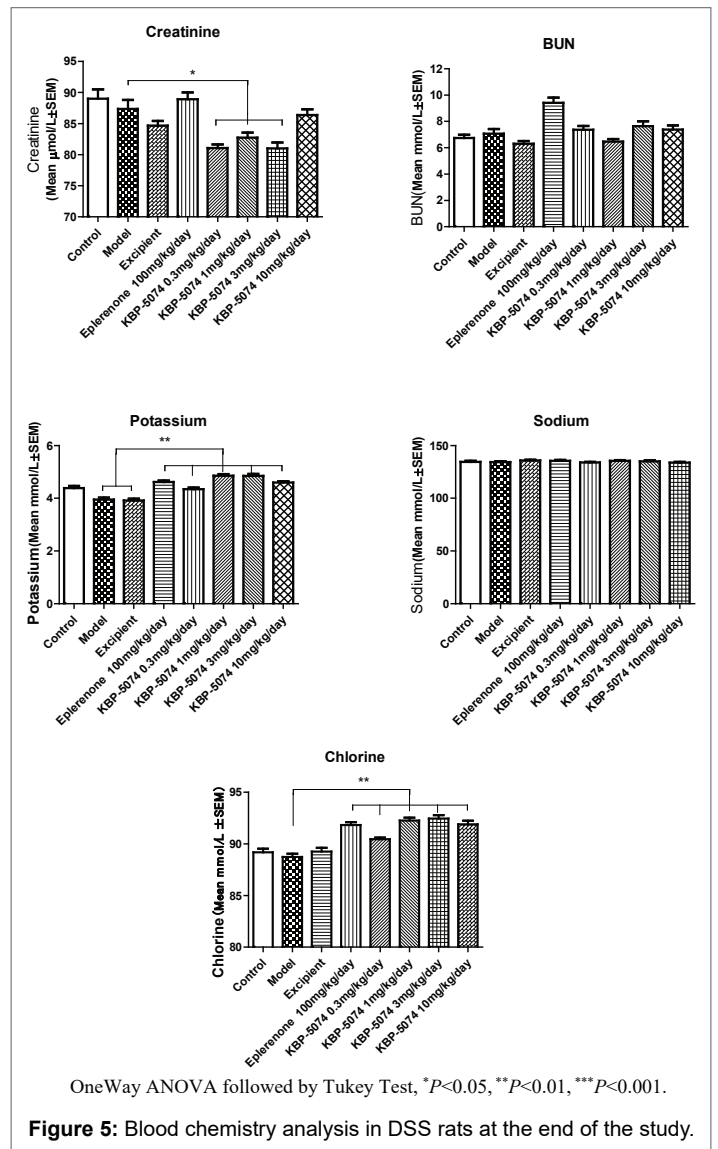


Figure 3: Efficacy of KBP-5074 in preventing elevation of urinetoal protein in DSS rats.



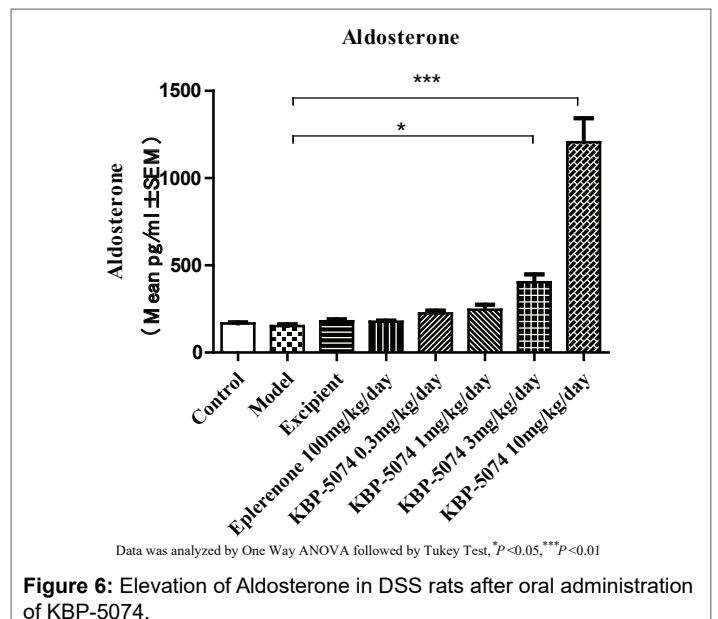
Data was analyzed by One Way ANOVA followed by Tukey Test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 4: Urinary chemistry analysis in DSS rats.



OneWay ANOVA followed by Tukey Test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5: Blood chemistry analysis in DSS rats at the end of the study.



Data was analyzed by One Way ANOVA followed by Tukey Test, * $P < 0.05$, *** $P < 0.01$

Figure 6: Elevation of Aldosterone in DSS rats after oral administration of KBP-5074.

were summarized in figure 7. In DSS rat model group, both kidney- and heart-body weight ratios were increased ($p < 0.001$). Treatment with KBP-5074 resulted in a significant dose-dependent decrease of kidney-body weight ratio. The percent of inhibition at 0.3, 1, 3 and 10 mg/kg/day were 7%, 15%, 16% and 23%, respectively. Similarly, treatment with KBP-5074 resulted in a significant decrease of heart-body weight ratio. However, the decreases were apparently not dose-dependent.

Histopathological evaluation of kidney and heart in DSS rats: As summarized in figure 8, diseased animals had more than 1-fold higher renal injury score than normal control ($p < 0.001$). Treatment with KBP-5074 showed strong trend of prevention of kidney injury. At 0.3 mg/kg/day, no prevention of kidney injury was observed. At 1 mg/kg, the kidney injury score was significantly lower than diseased animals ($p < 0.001$). The average of kidney injury scores at 3 and 10 mg/kg/day were lower than diseased animals. However, the difference was not statistically significant. Eplerenone showed no prevention of kidney injury. In heart damage analysis, diseased animals had trend of increased left ventricular wall thickness. Treatment with KBP-5074 resulted in dose-dependent prevention of the increase of the heart wall thickness. At 0.3, 1, 3 and 10 mg/kg/day, decreases of left ventricular wall thickness were 4% ($p > 0.05$), 6% ($p < 0.05$), 7% ($p < 0.05$), and 6% ($p < 0.05$), respectively. Eplerenone showed a 5% reduction ($p > 0.05$) of heart wall thickness increase (Figure 8). Photomicrographs of kidneys and hearts stained with H&E, Masson or PAS were included in Appendix A (Supplementary Data File).

Efficacy of KBP-5074 in SHRSP model

Efficacy in preventing elevation of SBP in SHRSP rats: After 33 days of high salt diet feeding, animals in vehicle groups showed continuous increase in systolic blood pressure. Treatment of animals with KBP-5074 inhibited the increase of blood pressure. Compared to disease model group on day 33, KBP-5074-treated groups at 5, 15, and 50 mg/kg/day prevented the elevation of blood pressure of 13% ($p < 0.01$), 12% ($p < 0.01$) and 20% ($p < 0.001$) respectively (Figure 9). The bench mark molecule eplerenone showed no significant attenuation of blood pressure elevation.

Efficacy on urinary albumin excretion in SHRSP rat: The levels of urinary albumin were monitored once a week for four weeks. Rats treated with vehicle showed continuous increases in urinary albumin excretion up to Day 29. Rats dosed with KBP-5074 at 5, 15, and 50 mg/kg/day reduced the elevation of urinary albumin excretion. The percent reduction of urinary albumin was 63% ($p < 0.05$), 62% ($p < 0.05$) and 51% ($p > 0.05$) after oral administration of KBP-5074 at 5, 15 and 50 mg/kg/day, respectively. Bench mark molecule eplerenone showed no reduction of urinary albumin excretion in the early time points but showed a 39% decrease of urinary albumin excretion on Day 29 (Figure 10).

Effects of KBP-5074 on urine chemistry: At the end of the study, urinary creatinine, BUN, chlorine, sodium, potassium, and urinary albumin/creatinine ratio were analyzed. The urinary albumin/creatinine ratios (UACR) in the SHRSP animals were 2.4-fold higher than in normal animals ($p > 0.05$). Treatment with KBP-5074 at 5, 15 and 50 mg/kg/day resulted in 66% ($p < 0.05$), 64% ($p < 0.05$) and 53% ($p > 0.05$) reduction of the albumin/creatinine ratio, respectively. Treatment with the bench mark molecule eplerenone resulted in trend of 34% ($p < 0.05$) reduction. Urinary excretion of BUN, sodium, potassium, and chloride were increased in the SHRSP animals. Treatment with either KBP-5074 or eplerenone did not diminish the increases of the above parameters. Urinary creatinine in the SHRSP animals showed no changes when compared to normal animals (Figure 11).

Effects of KBP-5074 on blood chemistry: Comparing to normal rats, the SHRSP animals showed no significant changes in blood creatinine, BUN, sodium, potassium and chloride levels on Day 29. Treatments with

either KBP-5074 or the bench mark molecule eplerenone also showed no significant effects on these blood chemistry parameters (Figure 12).

Plasma aldosterone: Blocking the binding of aldosterone to MR will lead to elevation of free aldosterone in circulation, probably due to feedback up regulation. The levels of aldosterone in animal plasma at the end of the study (Day 35) were summarized in figure 13. Treatment with KBP-5074 at 5 and 15 mg/kg/day showed no affects in plasma aldosterone levels in this model. At 50 mg/kg/day, plasma aldosterone levels were significantly increased (Tukey test, $p < 0.01$). The bench mark molecule eplerenone did not affect the aldosterone levels after dietary administration of KBP-5074 at 100 mg/kg/day for 35 days.

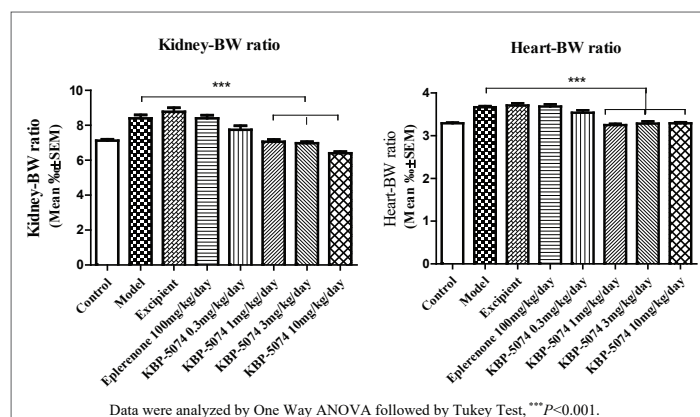


Figure 7: Kidney-body weight and heart-body weight ratios in DSS rats.

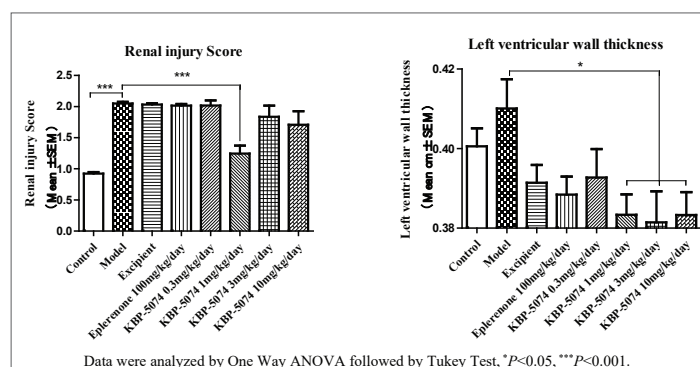


Figure 8: Histopathological evaluation of kidney and heart.

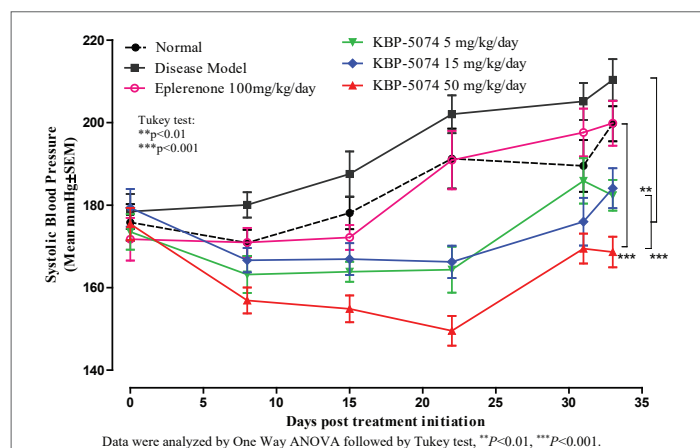


Figure 9: Efficacy of KBP-5074 in preventing elevation of blood pressure in SHRSP rats.

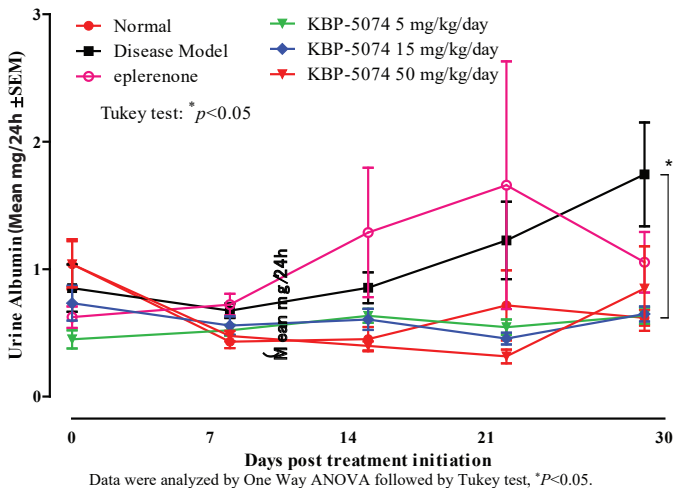


Figure 10: Efficacy of KBP-5074 on urinary albumin excretion in SHRSP rats.

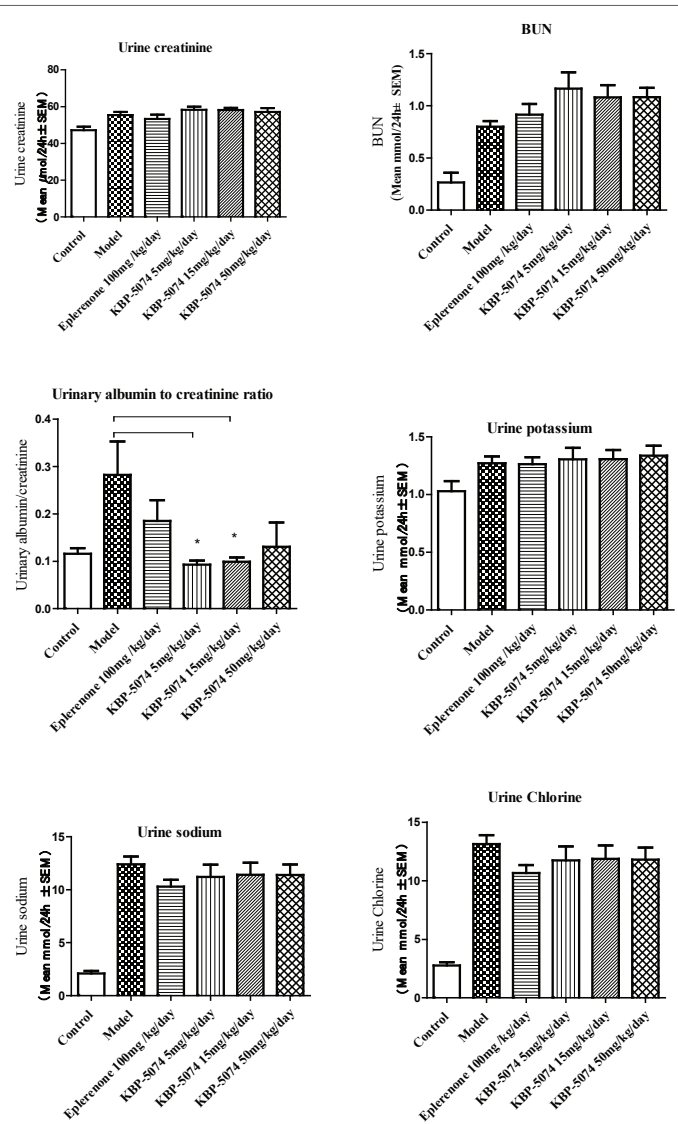


Figure 11: Effects of KBP-5074 on urinary chemistry in SHRSP rats.

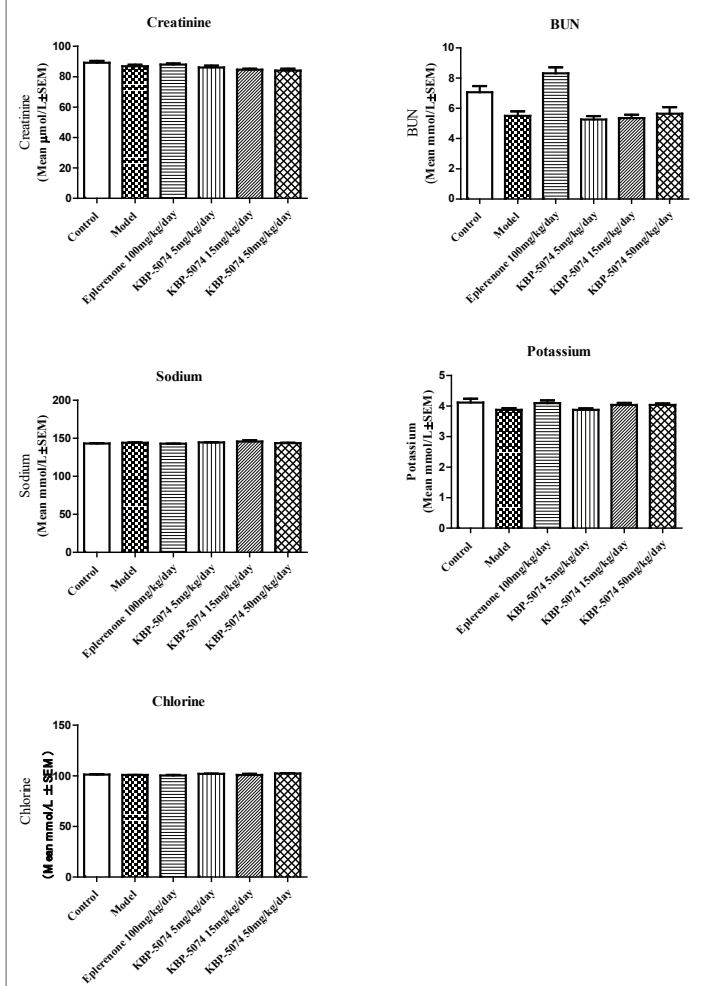
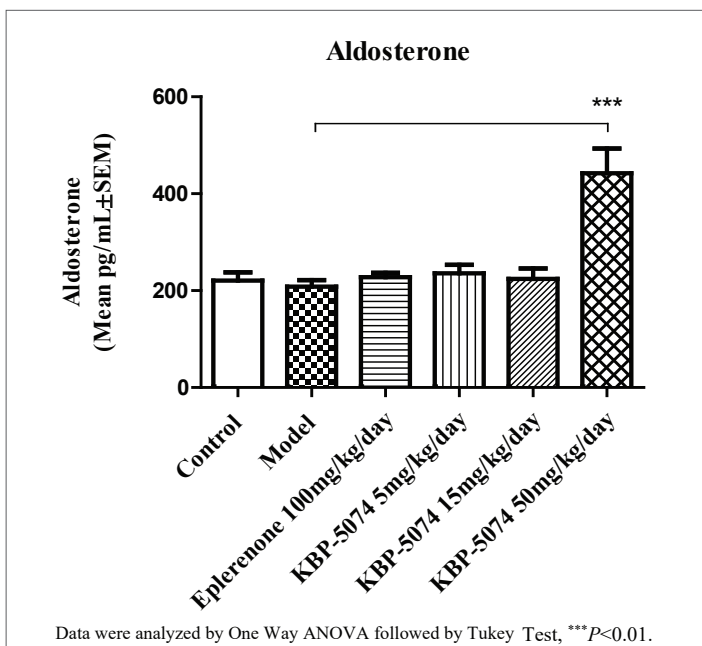


Figure 12: Activity of KBP-5074 in blood chemistry.



Data were analyzed by One Way ANOVA followed by Tukey Test, *** $P < 0.01$.
Figure 13: Plasma aldosterone in SHRSP rats was increased after oral administration of KBP-5074 at 50 mg/kg/day.

Kidney-body weight and Heart-body weight Ratios in SHRSP rat model: At the termination of this SHRSP study on Day 35, kidneys and hearts were harvested and the organ weights and body weights were measured. The organ-body weight ratios are summarized in figure 14. Treatment with KBP-5074 resulted in a significant reduction of kidney-body weight ratios when compared to the SHRSP model group. The percent of decrease after oral administration at 5, 15, and 50 mg/kg/day were 12%, 14% and 16% ($p < 0.001$), respectively. Similarly, treatment with KBP-5074 resulted in a significant decrease of heart-body weight ratio ($p < 0.001$) when compared to the SHRSP model group. The percent of decrease at 5, 15, and 50 mg/kg/day were 6%, 3% and 8% ($p < 0.01$), respectively (Figure 14).

Histopathological Evaluation of Kidney and Heart in SHRSP rats: The morphological change of the small artery in affected organ and tissue reflects pathological remodeling of arteries that contributes to the development and complications of hypertension. At the end of the study, the cross-section area of the small arteries in the kidney and heart were semi-quantitatively analyzed using Leica camera Qv3 software. The average cross-section area of randomly selected four small arteries in each section was presented in figure 15. SHRSP animals had 5% increased kidney artery cross-section area ($p < 0.001$). Treatment with KBP-5074 at 5, 15 and 50 mg/kg/day resulted in significantly smaller kidney artery cross-sectional area (6%, 9% and 10%, respectively) than the SHRSP model group ($p < 0.001$). Furthermore, treatment with KBP-5074 at 15 and 50 mg/kg/day also showed a significantly 2% and 3% smaller cross-sectional kidney artery area than eplerenone ($p < 0.05$, $P < 0.001$ respectively). No differences in heart small artery cross-sectional areas were observed in any groups.

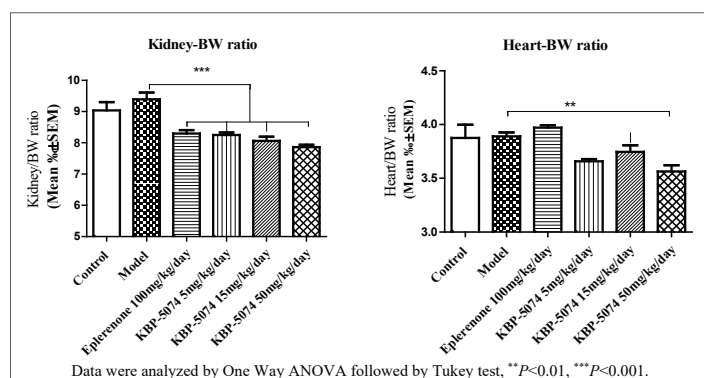


Figure 14: Kidney and Heart body weight ratios were significantly decreased in SHRSP rats after oral administration of KBP-5074.

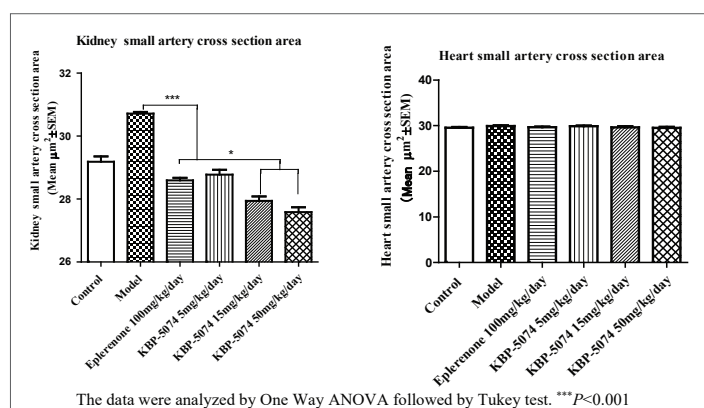


Figure 15: Kidney and heart small artery cross sectional areas.

Specimens of hearts and kidneys were sectioned and stained with H&E, PAS and Masson. Overall, kidneys and hearts in SHRSP model group showed increased thickness in small vascular wall as well as increased perivascular gap, and enlarged myocardium and fibrinoid. Treatment with KBP-5074 resulted in trend of decreased perivascular gap, and myocardium and fibrinoid. Photomicrographs of histopathological sections of heart and kidney were shown in Appendix B (Supplementary Data File).

Discussion and Conclusion

Aldosterone is a steroid hormone produced primarily in the glomerular zone of the adrenal cortex. It has been implicated for many years to play an important role in the pathophysiology of cardiovascular and renal diseases. Elevated aldosterone concentrations have been documented in patients with hypertension and heart failure, leading to the use of aldosterone antagonists for the treatment of these conditions [8,9]. This study showed that KBP-5074 blocked the binding of aldosterone to the mineralocorticoid receptor (MR), and bound selectively to the MR with an IC_{50} of 2.7 nM. KBP-5074 also demonstrated superior *in vitro* antagonist activity against MR when compared to eplerenone and spironolactone. Pharmaceutical antagonism of the MR can protect against organ damage caused by elevated aldosterone levels in patients experiencing heart failure, CKD, and hypertension. While traditional steroid-based MR antagonists effectively reduced mortality rates and extended patient survival, their broad application was limited by significant side effects, most notably hyperkalemia [10]. The prevalence of hyperkalemia in CKD patients is considerably higher than in the general population. A recent review reports that hyperkalemia occurs in as much as 40-50% in the CKD population compared to 2-3% in the general population [11]. Current therapies for CKD include spironolactone and eplerenone which are effective diuretics and anti-hypertensives for patients who are not at risk for hyperkalemia. There is currently no safe MRA-based therapy available for patients with moderate to severe renal impairment (e.g., estimated glomerular filtration rate $< 30 \text{ mL/min/1.73 m}^2$) due to the risk of life-threatening hyperkalemia. For this reason, a new MR antagonist with improved efficacy and reduced adverse effects would contribute to the efforts in addressing this unmet medical need. Recently, BAY 94-8862 (Finerenone) was reported to significantly improve the survival of SHRSP rats at 10 mg/kg/day when compared with either eplerenone or spironolactone. Reduction in urinary protein/creatinine ratio after finerenone treatment suggested a beneficial impact on kidney health. This was supported by histopathological analysis demonstrating that finerenone treatment reduced vascular, glomerular, and tubule-interstitial damage. Clinical trials are ongoing to investigate the efficacy and safety of BAY 94-8862 in the treatment of cardiorenal diseases [12]. Esaxerenone (CS-3150) is another oral non-steroidal MRA currently undergoing clinical trials for the treatment of essential hypertension and cardiorenal diseases [13,14]. In DSS rats, CS-3150 prevented the increase in SBP in a dose-dependent manner without hyperkalemia. CS-3150 also suppressed proteinuria and renal hypertrophy induced by high salt diet [14].

The pharmacokinetics and pharmacodynamics relationship of KBP-5074 after oral administration to DSS rats was evaluated in our study. A reverse correlation between systemic drug exposure (AUC) and SBP, urinary excretion of albumin (24-hour) and kidney-body weight ratio were observed. More than 50% reduction in SBP and approximately 50% reduction of kidney-body weight ratio as well as absence of proteinuria were observed after oral administration of KBP-5074. In addition, a correlation between KBP-5074 and increases of plasma aldosterone concentrations has been demonstrated. Oral administration of KBP-5074 to DSS rats resulted in significant increases in plasma aldosterone levels.

Eplerenone apparently showed no significant effect on aldosterone levels after dietary administration of 100 mg/kg/day for 44 days to DSS rats. Eplerenone was administered orally to rats as the positive control group via the dietary route by mixing eplerenone in the rat chow to target the dose ingested by the rats was as close to 100 mg/kg/day as possible [15-17]. Study from Merck Research Laboratories [18] reported that dietary administration of eplerenone at 100 mg/kg/day resulted in no significant increase in either serum aldosterone or potassium concentration in DSS rats. It was plausible that administration of eplerenone with this dosing regimen failed to achieve the effective blood levels required for the stimulation of aldosterone release.

SHRSP rats are characterized by severe hypertension and associated endothelial dysfunction and vascular damage. Exact molecular mechanisms contributing to these processes remain unclear but aldosterone appears to play a role. Blocking the binding of aldosterone to MR would lead to elevation of free aldosterone concentration in systemic circulation, probably due to feedback up regulation. Aldosterone binds to the MR which has an important regulatory role in body fluid and electrolyte balance. Aldosterone also influences a variety of different cell functions such as oxidative stress, inflammation and organ fibrosis [19,20]. Harvey et al. [21] demonstrated that plasma aldosterone was increased in SHRSP animals. Significant elevation of aldosterone was observed after oral administration of KBP-5074 to SHRSP rats in our study. Also, KBP-5074 showed dose-dependent amelioration of kidney and heart damage, and reduction of the increases in kidney-body weight and heart-body weight ratios. In addition, prevention of the increase in the kidney small artery cross-sectional areas and absence of proteinuria were observed after KBP-5074 administration to SHRSP rats. The effects of KBP-5074 after oral administration to SHRSP and DSS rats have been remarkably similar. Neither hyperkalemia nor other overt clinical signs of toxicity were observed after oral administration of KBP-5074 to SHRSP and DSS rats.

Zhang et al. [22] recently showed KBP-5074 was safe and well-tolerated in Phase I single and multiple ascending dose pharmacokinetics and safety studies in healthy subjects. Preliminary Phase II results in subjects with mild to moderate impairments revealed that KBP-5074 demonstrated similar pharmacological effects on aldosterone, SBP, UACR and potassium levels as those observed in SHRSP and DSS rat models of hypertension. Preclinical findings in animals as well as early phase clinical data in healthy human volunteers and patients with CKD demonstrated KBP-5074 treatment resulted in reduction of blood pressure, suggesting a potential role of KBP-5074 in the management of hypertension and cardiorenal diseases. Also, KBP-5074 administration to hypertensive rats resulted reduction of UACR, kidney-body weight ratio, and kidney small artery cross-section area or renal injury scores, suggesting KBP-5074 may have a potential renal protective effect. In addition, KBP-5074 increased aldosterone levels, providing evidence for its pharmacological mechanism of action through binding to the MR.

In conclusion, KBP-5074 demonstrated excellent affinity and superior receptor selectivity for MR *in vitro* as compared to other MRAs. The greater potency of KBP-5074 at MR may translate to an effective clinical dose of about 1 mg/day [22] as compared to the clinically approved 50 mg/day dose for eplerenone. This lower dose, in combination with greater receptor selectivity, is predicted to result in far less adverse effects as those observed with other MRAs. Preclinical and clinical data available to date suggest KBP-5074 may emerged as a promising next generation non-steroidal MRA with minimal off-target effects (e.g. hyperkalemia) while maintaining potent efficacy for the treatment of CKD and cardiorenal diseases.

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Supplementary Data

Supplementary information (Appendices A and B) to this article can be found along with this file.

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