RESEARCH ARTICLE

WILEY | Journal of Cellular Biochemistry

SB-216763, a GSK-3β inhibitor, protects against aldosterone-induced cardiac, and renal injury by activating autophagy

Yi-De Zhang[1] | Xiao-Jun Ding[2] | Hou-Yong Dai[1] | Wei-Sheng Peng[3] |
Nai-Feng Guo[1] | Yuan Zhang[1] | Qiao-Ling Zhou[3] | Xiao-Lan Chen[1] (iD)

[1] Department of Nephrology, Affiliated Hospital of Nantong University, Nantong, Jiangsu, China

[2] Department of Cardiology, Affiliated Danyang People's Hospital of Nantong University, Danyang, China

[3] Department of Nephrology, Affiliated Xiangya Hospital of Central South University, Changsha, Hunan, China

Correspondence
Xiao-Lan Chen, Department of Nephrology, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, China.
Email: chenxl8448@126.com

Abstract

Cardiovascular and renal inflammation induced by Aldosterone (Aldo) plays a pivotal role in the pathogenesis of hypertension and renal fibrosis. GSK-3β contributes to inflammatory cardiovascular and renal diseases, but its role in Aldo-induced hypertension, and renal damage is not clear. In the present study, rats were treated with Aldo combined with SB-216763 (a GSK-3β inhibitor) for 4 weeks. Hemodynamic, cardiac, and renal parameters were assayed at the indicated time. Here we found that rats treated with Aldo presented cardiac and renal hypertrophy and dysfunction. Cardiac and renal expression levels of molecular markers attesting inflammation and fibrosis were increased by Aldo infusion, whereas the treatment of SB-216763 reversed these alterations. SB-216763 suppressed cardiac and renal inflammatory cytokines levels (TNF-a, IL-1β, and MCP-1). Meanwhile, SB-216763 increased the protein levels of LC3-II in the cardiorenal tissues as well as p62 degradation, indicating that SB-216763 induced autophagy activation in cardiac, and renal tissues. Importantly, inhibition of autophagy by 3-MA attenuated the role of SB-216763 in inhibiting perivascular fibrosis, and tubulointerstitial injury. These data suggest that SB-216763 protected against Aldo-induced cardiac and renal injury by activating autophagy, and might be a therapeutic option for salt-sensitive hypertension and renal fibrosis.

KEYWORDS
aldosterone, autophagy, cardiac and renal injury, GSK-3β inhibitor

1 | INTRODUCTION

Aldosterone (Aldo) secreted from the adrenal cortex plays a crucial role in regulating renal sodium transport and electrolytic balance through the activation of

mineralocorticoid receptor (MR) in the kidney.[1,2] Clinical studies demonstrated that inhibition of MR could decrease the risk of both morbidity and mortality in patients with heart failure, and inhibit albumin excretion in hypertensive and diabetic patients.[3–5] In addition, MR antagonists also present a renoprotective effect in several experimental models of kidney disease.[6,7]

Aldo is implicated in cardiovascular and renal remodeling by inducing inflammation, oxidative stress, fibrosis, and hypertrophy.[2,8,9] Previous studies showed that chronic inflammation has a critical role in the pathogenesis of hypertension,[10,11] and renal inflammation is correlated with the development and progression of renal damage.[12,13] These findings suggest that Aldo-induced inflammation might be used as a potential therapeutic target for treating salt-sensitive hypertension and renal fibrosis.[14]

Glycogen synthase kinase 3β (GSK3β) is a multifunctional serine/threonine kinase. GSK3β is involved in the growth of the heart during development and in response to stress.[15] However, its role in regulating cardiac and renal injury remains unclear. GSK3β has a broad range of substrates, and regulates inflammatory response, cell differentiation, and survival.[16,17] GSK3β is an important positive regulator of inflammatory process.[18–21] GSK3β-deficient cells become more sensitive to tumor necrosis factor α (TNF-α)-induced apoptosis.[22] Recent studies demonstrated that the therapeutic effect of GSK3β inhibitor is associated with suppressing inflammatory response. Inhibition of GSK3β results in decreased activation of the pro-inflammatory transcription factor NF-κB. Additionally, GSK3β inhibition contribute to produce anti-inflammatory cytokine IL-10.[23]

GSK3β inhibition triggers a profound autophagic response in cells under serum-free condition.[24] This phenomenon was also observed in vivo from ischemic mouse models.[25,26] However, the mechanism underlying GSK3β inhibition-triggered autophagy is not fully clear. Autophagy is a lysosome-mediated intracellular catabolic process by which cells remove their damaged organelles for the maintenance of cellular homeostasis.[27] Autophagy is induced in response to intracellular or extracellular signals, such as starvation, pathogen infection, and endoplasmic reticulum stress.[28,29] Emerging evidence has indicated that autophagy may have an essential role for the host during bacterial clearance and may also interact with inflammatory processes, which consequently may impact the outcomes of disease progression.[30,31]

Based on the above findings, we investigated whether GSK3β inhibition protects against Aldo-induced cardiac and renal injury by activating autophagy. Current data suggest that rats treated with Aldo present cardiac and renal injury. The treatment of SB-216763 reverses these alterations. SB-216763 suppressed cardiovascular and renal inflammation by activating autophagy in cardiac and renal tissues.

2 | MATERIALS AND METHODS

2.1 | Animal models

The study was approved by the Ethics Committee of Nantong University. Adult male Wistar rats were obtained from the Chinese Academy of Sciences (Shanghai, China), and maintained in a pathogen-free facility. The animals were divided into four groups ($n = 9$/group): (1) Vehicle infusion group treated with vehicle alone; (2) Aldo-salt group treated with an infusion of Aldo-salt (1 mg/kg/day diluted in sunflower oil and administered by subcutaneous injection); (3) Aldo-salt plus SB-216763 group treated with an infusion of Aldo-salt plus SB-216763 at 1.5 mg/kg/day (MCE, Princeton, NJ); and (4) SB-216763 group. This dose was chosen on the basis of previous studies reporting its anti-inflammatory role.[32,33] After 4 weeks of treatment, urine was collected in metabolic cages, and hemodynamic parameters were assayed.[34] For example, blood pressure (BP) was measured in conscious but restrained animals, prewarmed to 34°C for 20 min. For each group, BP was measured three times on 3 separate days, and the mean value of all readings was taken as the average for the rat. Then blood samples, heart and kidney tissues were collected under sedation with sodium pentobarbital anesthesia.

2.2 | Quantitative real-time PCR (qPCR)

Total RNA was extracted from rat heart or kidney using Trizol reagent (Invitrogen, Carlsbad, CA). The reverse transcription (RT) was carried out using Oligo dT primer (Takara, Osaka, Japan). qPCR was performed using a standard protocol from the SYBR Green PCR kit (Toyobo, Osaka, Japan) on Applied Biosystems 7300 real-time PCR system. β-actin was used as internal control. The qPCR primers are as follows:

TNF-a forward, CTTCTGTCTACTGAACTTCGGG,
TNF-a reverse, GTTGTCTTTGAGATCCATGCC;
MCP-1 forward, GCTGACCCCAATAAGGAATG,
MCP-1 reverse, CTTGAGGTGGTTGTGGAAAAGA;
IL-1beta forward, ATGATGACGACCTGCTAGTGTGT,
IL-1beta reverse, TGGCTTATGTTCTGTCCATTGAG;
TGF-beta forward, CAACGCAATCTATGACAAAACC,
TGF-beta reverse, ACAAGAGCAGTGAGCACTGAAG;
Col1 forward, TCCTTCTGGTCCTCGTGGTCTCC,
Col1 reverse, TTCCCCATCATCTCCGTTCTTGC.

2.3 | Western blot and antibodies

Western blot analysis to assess rat LC3-I, LC3-II, p62, and β-actin protein expression was performed as previously described.[35] The anti- LC3-I/LC3-II/p62 primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). β-actin primary antibodies were purchased from Sigma (St. Louis, MO). Briefly, equivalent amounts of total protein

(150 µg) were electrophoresed through a 12% SDS-polyacrylamide gel, and then wet electro-transferred to 0.2 µm PVDF membranes (Bio-Rad, Richmond, CA). The blots were incubated at 37°C for 2 h with indicated antibodies, and then incubated with a goat anti-rabbit HRP-conjugated secondary antibody (1:5000, Jackson, Bar Harbor, ME). Protein signals were visualized by enhanced chemiluminescence detection (Pierce Biotechonology, Rockford, IL). The intensity of the selected bands was quantified using Image J.

2.4 | Enzyme-linked immunosorbent assay (ELISA)

The hearts and kidneys were removed and homogenized at the indicated time. Homogenates were sonicated for 30 s and then centrifuged at 2500g and 4°C. The supernatants were used for measurement of TNF-a, MCP-1, and IL-1β. ELISAs were performed using a TNF-a kit (R&D Systems, Minneapolis, MN), MCP-1 kit (R&D Systems), and IL-1β kit (R&D Systems) according to the manufacturers' protocols.

2.5 | Histological analysis

Rats were treated with Aldo or SB-216763 for 4 weeks, and kidney tissues or left ventricles were quickly fixed with buffered 4% paraformaldehyde, embedded in paraffin and cut into 4 µm-thick sections. Periodic acid-Schiff and Masson's trichrome staining were performed using serial sections. Tubulointerstitial fibrosis areas were semiquantified using image J software and expressed as a percentage of the total area. Perivascular fibrosis was assessed by calculating the percentage of Trichrome-stained collagen deposits surrounding the vessel to the total perivascular area using the software's color cube function.

2.6 | Fluorescence microscopy analysis

Transverse sections at 3 µm thickness were fixed with 3% paraformaldehyde, and subjected to immunocytochemistry as previously described.[32,33] The sections were briefly rinsed in PBS and blocked in a solution containing 5% BSA (Sigma) and 0.1% Triton X-100 (Sigma) for 1 h at room temperature. The slides were then immunostained with primary antibodies against LC3β (3868#, Cell Signaling Technology, Danvers, MA). To visualize the primary antibodies, slides were stained with FITC-conjugated secondary antibodies. The slides were also stained with 40, 6-diamidino-2-phenylindole (DAPI) to visualize the nuclei. After washed thrice in PBS samples were examined under a fluorescence laser scanning confocal FV1000 microscope (Olympus).

2.7 | Statistical analysis

All data are expressed as mean ± SEM, computed from the average measurements obtained from each group of animals. Results were analysed using unpaired Student's t-test or the Mann-Whitney U-test. Analyses were conducted using GraphPad Prism (4.0) (software, Inc. San Diego, CA). Differences were deemed statistically significant at $P < 0.05$.

3 | RESULTS

3.1 | SB-216763 suppresses aldo-induced cardiorenal inflammation

Aldo-treated rats present a significant increase in systolic blood pressure (SBP) and diastolic BP (DBP) (Table 1). Meanwhile, Aldo treatment results in an increase in ratio of heart weight to body weight and a decrease in heart rate (Table 1). Both cardiac dysfunction and hypertrophy are reversed by SB-216763, a GSK-3β inhibitor (Table 1). In addition, body weight, urine volume, serum creatinine, creatinine clearance, kidney weight/body weight ratio, and urinary protein excretion of each group at the end of the 4-week experiment are present in Table 2. Aldo-treated rats induce renal hypertrophy, increase glomerular filtration rate, and results in a significant increase in serum creatinine compared with the other groups. Both renal dysfunction and hypertrophy are prevented by SB-216763 treatment. We assayed the effect of SB-216763 on regulating GSK3β expression and activation. As shown in Figures 1A and 1B,

TABLE 1 Physiological and hematological parameters in Aldo-treated rats

	Control	Aldo	Aldo + SB216763
SBP, mm Hg	132 ± 0.8	151 ± 0.5*	138 ± 0.9**
DBP, mm Hg	94 ± 1.1	115 ± 0.7*	104 ± 1.3**
HR, beats/min	327 ± 4.3	277 ± 7.2*	311 ± 6.4**
HW/BW, mg/g	2.56 ± 0.03	2.88 ± 0.01*	2.67 ± 0.01**

Aldo, aldosterone; SBP, systolic blood pressure; DBP, diastolic blood pressure; HW, heart weight; BW, body weight.

Values are presented as mean ± SEM.

*$P < 0.05$ versus control.

**$P < 0.05$ versus Aldo group.

TABLE 2 Physiological and renal parameters in Aldo-treated rats

	Control	Aldo	Aldo + SB216763
Creatinine clearance, mL/min	1.21 ± 0.16	1.34 ± 0.52	1.19 ± 0.66
Urine volume (mL/day)	11.5 ± 2.3	$36.2 \pm 5.7*$	$24.6 \pm 5.9**$
Serum creatinine (mg/dL)	0.74 ± 0.19	$1.36 \pm 0.32*$	$1.06 \pm 0.42**$
KW/BW, mg/g	2.81 ± 0.01	$3.93 \pm 0.02*$	$3.01 \pm 0.01**$

Aldo, aldosterone; KW, kidney weight; BW, body weight.

Values are presented as mean $\pm$ SEM.

*$P < 0.05$ versus control.

**$P < 0.05$ versus Aldo group.

SB-216763 increases phosphor-GSK-3βS9 (PSer9) levels, but not total GSK-3β levels in the heart and kidney.

Previous studies demonstrated that inflammation plays an important role in the pathogenesis of hypertension, and the development and progression of renal fibrosis.[13,36,37] We then assayed the expression of various proinflammatory cytokines by qPCR and ELISA. Figure 1C-F showed that the cardiac mRNA and protein levels of TNF-a, MCP-1, and IL-1β are markedly increased by Aldo infusion, whereas SB-216763 treatment inhibits cardiac inflammatory cytokines levels (Figure 1C-F).

Similarly, the renal inflammatory cytokines levels (TNF-a, MCP-1, and IL-1β) are enhanced after Aldo treatment, whereas the expression of these genes is inhibited by SB-216763 (Figure 2A-D).

3.2 | SB-216763 suppresses aldo-induced cardiorenal fibrosis

To evaluate cardiorenal fibrosis, we assayed the expression of collagen type I (Col I) and transforming growth factor-β (TGF-
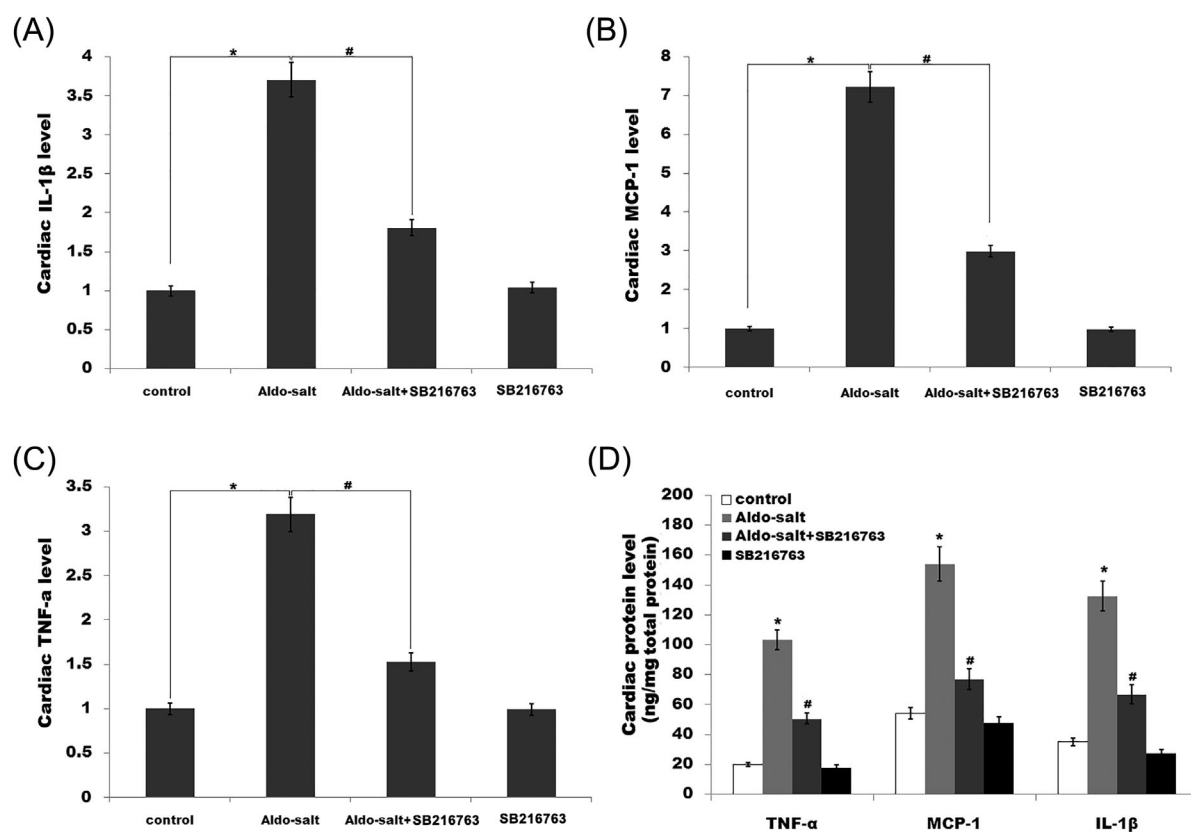

FIGURE 1 SB-216763 suppresses cardiac inflammation and fibrosis caused by Aldo-salt. Cardiac (A) and renal (B) protein expression of total GSK3β and p-GSK3β were assayed in cardiorenal tissues treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt. (C-E) Cardiac mRNA levels of TNF-α, MCP-1, and IL-1β in rats treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt. (F) Cardiac protein levels of TNF-α, MCP-1, and IL-1β in rats treated with Aldo-salt or with Aldo-salt plus SB-216763 were assayed using ELISA. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt
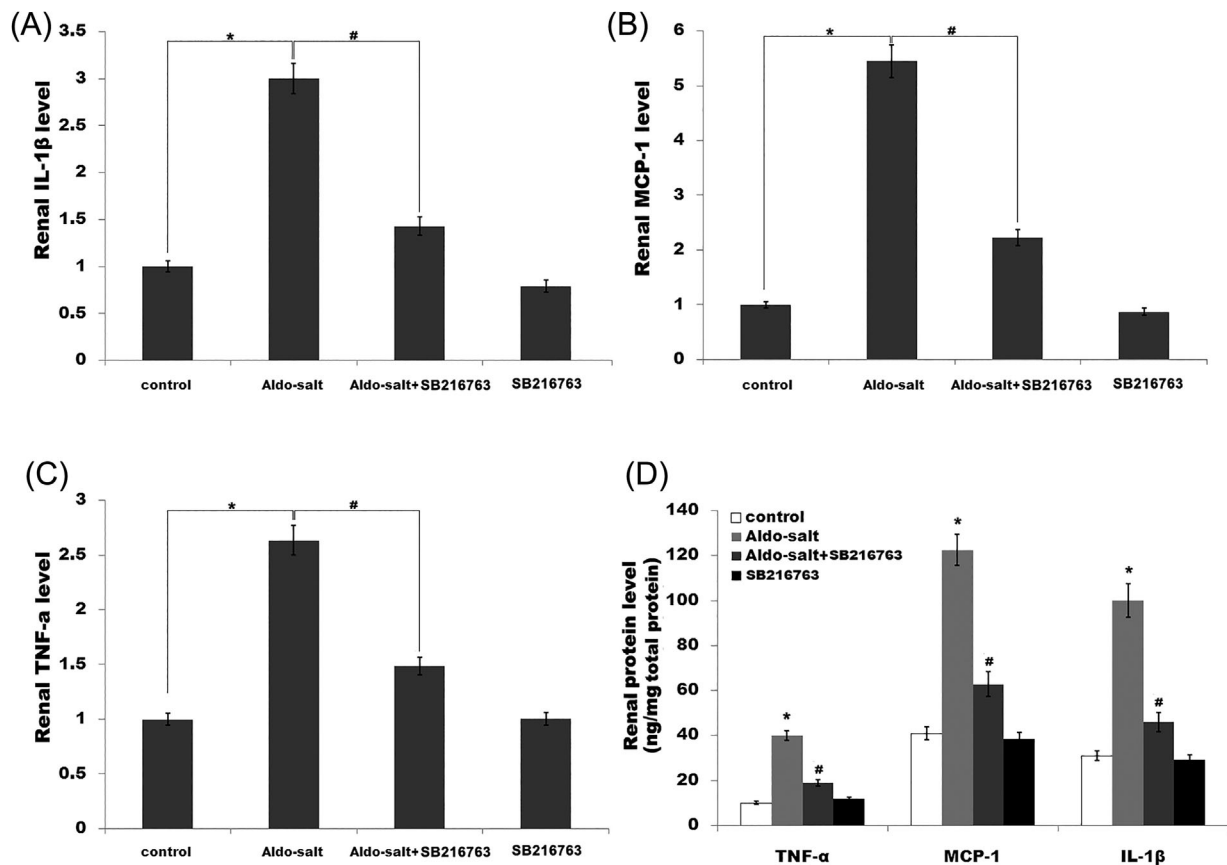
FIGURE 2 SB-216763 suppresses renal inflammation and fibrosis caused by Aldo-salt in vivo. (A-C) Renal mRNA levels of TNF-α, MCP-1, and IL-1β in rats treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt. (D) Renal protein levels of TNF-α, MCP-1, and IL-1β in rats treated with Aldo-salt or with Aldo-salt plus SB-216763 were assayed using ELISA. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt

β), which are extracellular matrix protein and profibrotic marker, respectively. Although the Aldo-treated group shows an increase of Col I and TGF-β in rat heart, the expression Col I and TGF-β is inhibited by SB-216763 treatment (Figure 3A). As in the case of heart, Aldo treatment upregulates the expression of renal Col I and TGF-β, and SB-216763 suppresses this increase (Figure 3B). Perivascular fibrosis in the left ventricle was assessed by deposition of collagen around the vasculature. Figure 3C presents representative images of collagen deposition and quantitation of fibrosis. SB-216763 treatment markedly suppresses Aldo-induced perivascular fibrosis. Meanwhile, periodic acid Schiff-stained sections revealed that SB-216763 inhibits Aldo-induced tubulointerstitial damage (Figure 3D). These results suggest that SB-216763 treatment inhibits cardiorenal inflammation and fibrosis induced by Aldo.

3.3 | SB-216763 increases autophagy activation in cardiorenal tissues

Recent studies demonstrated that GSK3β functions as a key regulator coordinating cellular homeostasis by suppressing autophagy in physiological and pathological processes such as cancer,[38] axonal degeneration,[39] and diabetes.[40] Moreover, autophagy plays a crucial role in inflammation and fibrosis. Saitoh et al[41] demonstrated that loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. Knockdown of autophagy increases the immune response in hepatitis C virus-infected hepatocytes.[42] The p62 protein, also called sequestosome 1 (SQSTM1), binds directly to LC3 and GABARAP family proteins via a specific sequence motif. The protein is itself degraded by autophagy and may be used as a marker to study autophagic flux.[43] Here we found that the Aldo treatment slightly increases the protein levels of LC3-II (the marker of autophagy activation) in the cardiorenal tissues as well as p62 degradation, whereas SB-216763 treatment results in marked activation of autophagy (Figures 4A and 4B and 5A and 5B). The fluorescence analysis was carried out to further verify the activation of autophagy after Aldo or Aldo plus SB-216763 treatment. Figure 4C and Figure 5C showed that following SB-216763 treatment, there is a significant increase of LC3 green puncta representing autophagic vacuoles and an accumulation of LC3-II in cardiorenal tissues, indicating that autophagy is activated.
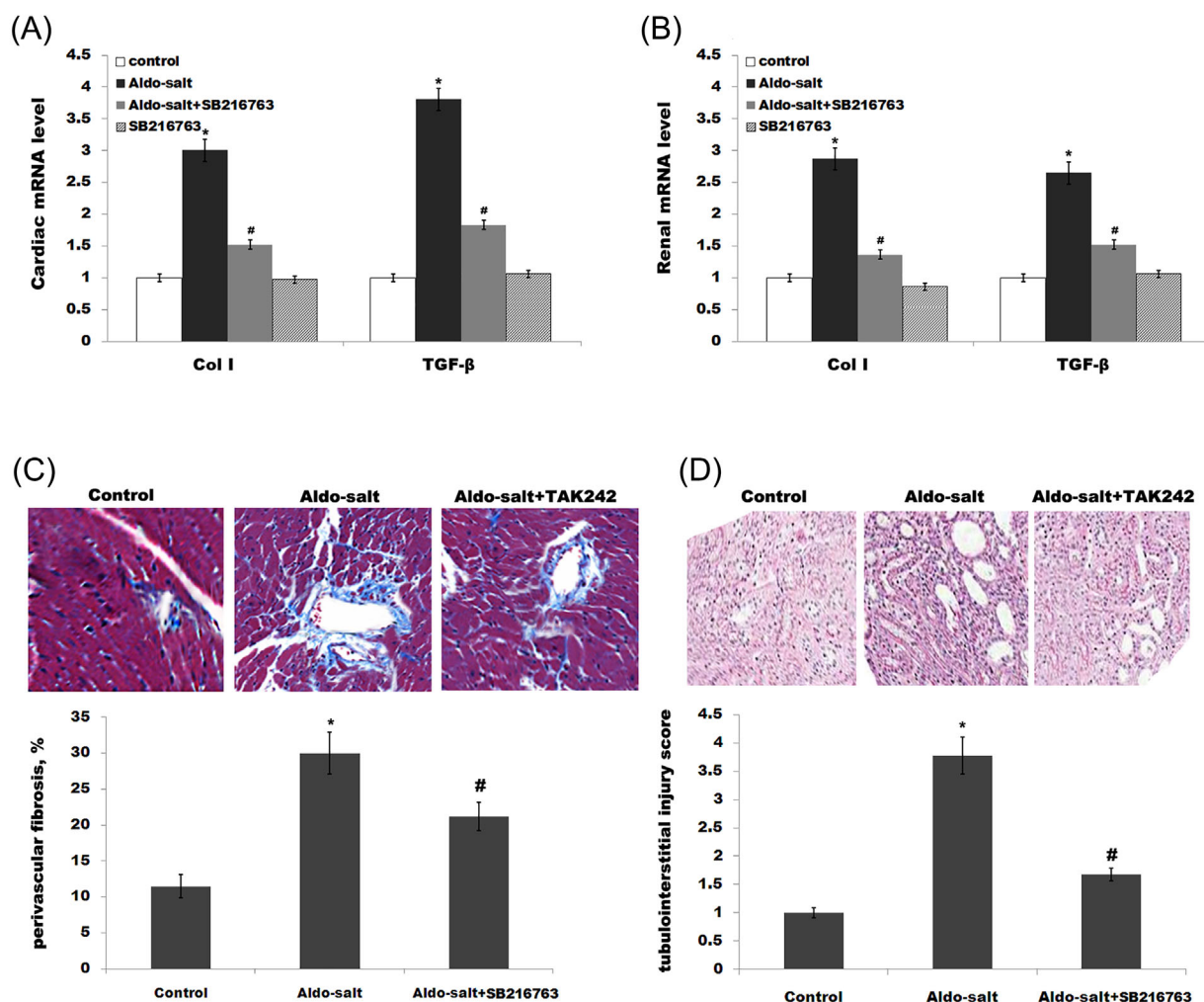
FIGURE 3 SB-216763 suppresses Aldo-induced cardiorenal fibrosis. Cardiac (A) or renal (B) mRNA levels of Col I and TGF-β in rats treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, [#]$P < 0.05$ versus Aldo-salt. (C) Representative images and quantitation of perivascular fibrosis in left ventricles of rats treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, [#]$P < 0.05$ versus Aldo-salt. (D) Histological staining with periodic acid Schiff (PAS), also showing tubulointerstitial damage of rats treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, [#]$P < 0.05$ versus Aldo-salt. $n = 9$, $200 \times$ magnification

3.4 | SB-216763 suppresses aldo-induced cardiorenal injury by regulating autophagy

SB-216763 suppresses Aldo-induced cardiorenal injury and activates autophagy, and autophagy plays important role in inhibiting proinflammatory response. Therefore, we next investigated whether SB-216763 suppresses Aldo-induced cardiorenal injury by activating autophagy. A 3-methyladenine (3-MA), which inhibits the formation of autophagosomes, attenuates LC3-II upregulation. A 3-MA is usually used to inhibit and study the mechanism of autophagy.[44] As shown in Figures 6A and 6B, the level of perivascular fibrosis and tubulointerstitial injury score increases after Aldo treatment, whereas SB-216763 can reverse the alteration. More important, pharmacological inhibition of autophagy by 3-MA markedly inhibits the role of SB-216763 in suppressing perivascular fibrosis

and tubulointerstitial injury. These results demonstrate that SB-216763, a GSK-3β inhibitor, protects against Aldo-induced cardiac, and renal injury by activating autophagy.

4 | DISCUSSION

In this study, we investigated the potential role of SB-216763, a GSK3β inhibitor, in treating salt-sensitive hypertension, and renal fibrosis. The current data demonstrate that: (i) SB-216763 inhibits Aldo-induced cardiorenal inflammation; (ii) SB-216763 inhibits Aldo-induced cardiorenal fibrosis; (iii) SB-216763 activates cardiorenal autophagy induced by Aldo; and (iv) SB-216763 inhibits cardiorenal injury by activating autophagy. These results reveal the important role of SB-216763 and autophagy in regulating salt-sensitive
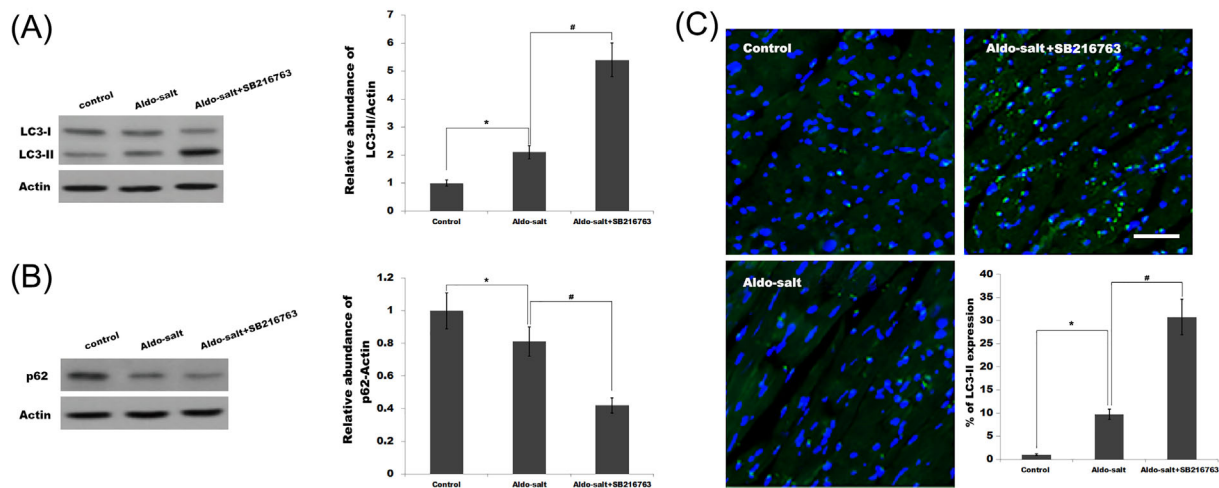
FIGURE 4 SB-216763 activates cardiac autophagy. Cardiac protein expression of LC3-I, LC3-II (A), and p62 (B) were assayed in cardiac tissues treated with Aldo-salt or with Aldo-salt plus SB-216763. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt. Representative micrographs (C) showing immunofluorescent staining for LC3-II for among different groups as indicated. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt

hypertension and renal fibrosis, and suggest SB-216763 might be used as a potential therapeutic target for treating salt-sensitive cardiorenal injury.

Renal and cardiovascular fibrosis is found to be linked to inflammation in Aldo-treated models.[45] Inhibition of inflammatory cytokines ameliorates renal and cardiac injury in several experimental models.[46] Here we demonstrate that Aldo treatment induces the production of pro-inflammatory cytokines (such as TNF-a, IL-1β, and MCP-1), whereas SB-216763 markedly reverses these alterations in kidney and heart.

Autophagy is generally considered to be a cell survival mechanism that functions in response to various stress

conditions and plays a critical role in human physiology and diseases, especially in inflammation and immunity.[47,48] Autophagy or autophagy-related proteins could control inflammatory signaling by regulating inflammatory transcriptional responses. For example, upregulated p62 in autophagy-deficient cells activate the pro-inflammatory transcription factor NF-κB.[49] Autophagy also prevents tissue inflammation due to its role in apoptotic corpse clearance.[30] Recent studies reported that impaired or deficient autophagy is believed to contribute to renal and cardiovascular disease as described in previous studies that focused on the role of autophagy in cardiac-renal disease,[50,51] but the mechanism is not understood clearly. GSK3β can regulate autophagy, but its
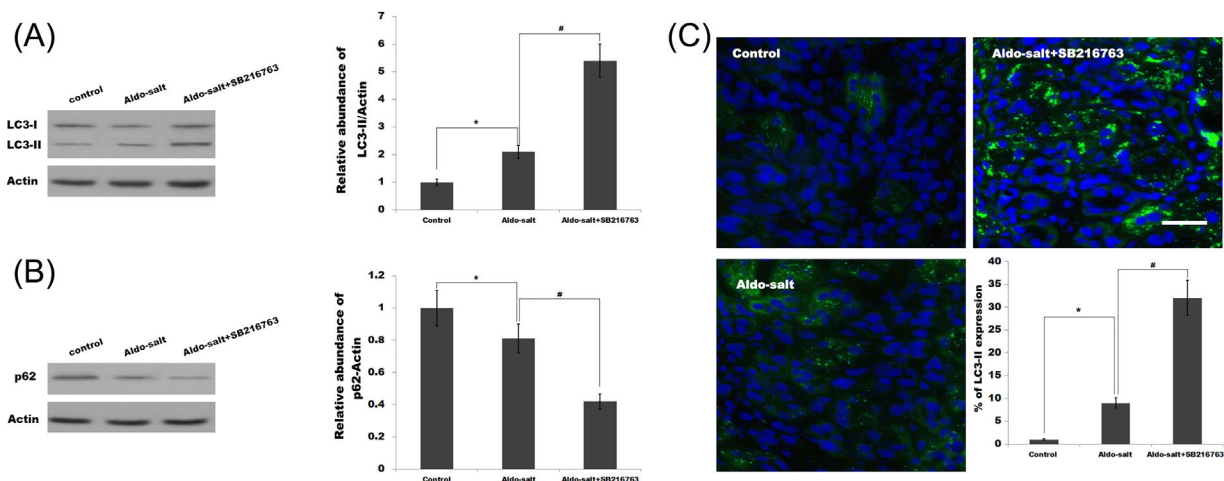

FIGURE 5 SB-216763 activates renal autophagy. Renal protein expression of LC3-I, LC3-II (A) and p62 (B) were assayed in renal tissues treated with Aldo-salt or with Aldo-salt plus SB-216763. SB-216763 increased the Aldo-salt-induced autophagy. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt. Representative micrographs (C) showing immunofluorescent staining for LC3-II for among different groups as indicated. *$P < 0.05$ versus control, #$P < 0.05$ versus Aldo-salt
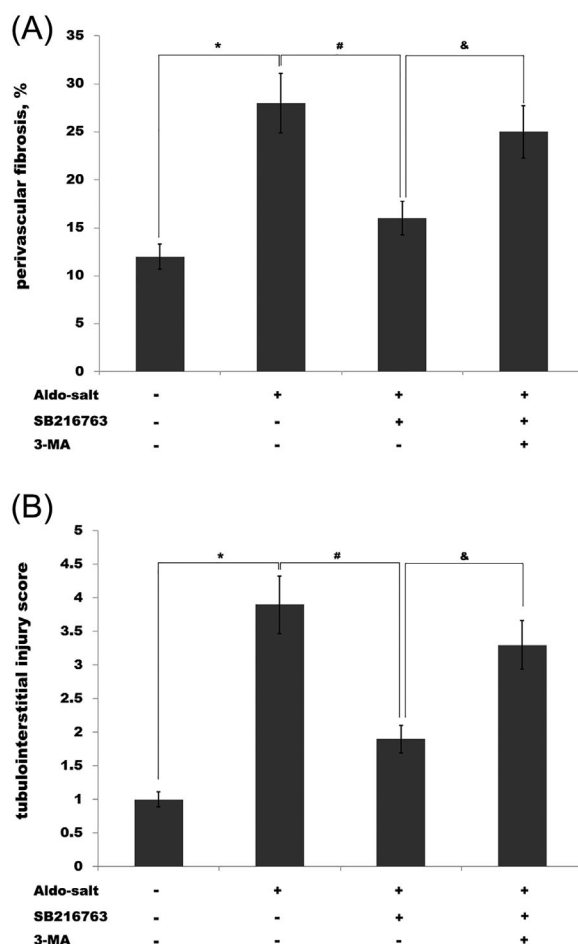
FIGURE 6 SB-216763 suppresses Aldo-induced cardiorenal injury by regulating autophagy. Rats were treated with Aldo (1 mg/kg/day), SB-216763 (1.5 mg/kg/day) or 3-MA (800 nmol) for 4 weeks, and the levels of perivascular fibrosis and tubulointerstitial injury were assessed using Periodic acid-Schiff and Masson's trichrome staining. Quantitation of perivascular fibrosis in left ventricles (A) or tubulointerstitial damage in kidney tissues (B) was shown. [*#&]$P < 0.05$. $n = 9$. Inhibition of autophagy by 3-MA partially destroys the role of SB-216763 in inhibiting Aldo-induced cardiorenal injury

underlying mechanism remains unclear. Moreover, the different studies have given a different conclusion about the role of GSK3β in regulating autophagy. Sarkar et al[52] reported that lithium (an inhibitor of GSK3β activity) can induce autophagy by inhibiting inositol monophosphatase, but another study showed that lithium can reduce autophagy and apoptosis after neonatal hypoxia-ischemia.[53] Inhibition of GSK3β activity using SB-216763 or knockdown of GSK3βpromotes autophagy to reduce cadmium-induced apoptosis.[54] In the study we found that SB-216763 suppresses cardiovascular and renal inflammation and activates autophagy in cardiac and renal tissues. More important, inhibition of autophagy increases perivascular fibrosis and tubulointerstitial injury in the SB216763-treated group. These results demonstrate that SB-216763 protects against Aldo-induced cardiac and renal injury by activating autophagy. As shown in Figures 4 and 5, Aldo treatment can slightly increase the

protein levels of LC3-II in the cardiorenal tissues as well as p62 degradation, indicating Aldo slightly activates autophagy. The results suggest that autophagy might be a protective mechanism following occurrence of cardiorenal injury. Unfortunately, the current level of autophagy activation is not competent to resist cardiorenal injury induced by Aldo. Therefore, additional autophagy activation induced by SB-216763 plays an important role in inhibiting Aldo-induced cardiorenal injury.

5 | CONCLUSION

The current study demonstrated that a GSK-3β inhibitor, SB-216763, suppresses aldosterone-induced cardiac, and renal injury in by increasing autophagy activation, thus offering a new target for prevention of cardiac and renal injury.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

AUTHORS' CONTRIBUTION

Planned experiments: X-LC; Performed experiments: Y-DZ, X-JD, H-YD, W-SP, and N-FG; Analyzed data: Q-LZ; Contributed reagents or other essential material: Y-DZ and W-SP; Wrote the paper: X-LC.

ORCID

Xiao-Lan Chen http://orcid.org/0000-0003-0732-713X

REFERENCES

1. Brown NJ. Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis. *Nat Rev Nephrol*. 2013;9:459–469.
2. Irita J, Okura T, Jotoku M, et al. Osteopontin deficiency protects against aldosterone-induced inflammation, oxidative stress, and interstitial fibrosis in the kidney. *Am J Physiol Renal Physiol*. 2011;301:F833–F844.
3. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med*. 1999;341:709–717.
4. Williams GH, Burgess E, Kolloch RE, et al. Efficacy of eplerenone versus enalapril as monotherapy in systemic hypertension. *Am J Cardiol*. 2004;93:990–996.
5. White WB, Duprez D, St Hillaire R, et al. Effects of the selective aldosterone blocker eplerenone versus the calcium antagonist amlodipine in systolic hypertension. *Hypertension*. 2003;41:1021–1026.
6. de las Heras N, Ruiz-Ortega M, Miana M, et al. Interactions between aldosterone and connective tissue growth factor in vascular and renal damage in spontaneously hypertensive rats. *J Hypertens*. 2007;25:629–638.
7. Brown NJ, Nakamura S, Ma L, et al. Aldosterone modulates plasminogen activator inhibitor-1 and glomerulosclerosis in vivo. *Kidney Int*. 2000;58:1219–1227.
8. Rafiq K, Hitomi H, Nakano D, Nishiyama A. Pathophysiological roles of aldosterone and mineralocorticoid receptor in the kidney. *J Pharmacol Sci*. 2011;115:1–7.
9. Zhang YD, Dai HY, Xie HL, Zhou QL, Liu ZH. The role of renal damage on cardiac remodeling in patients with diabetic nephropathy. *Clin Nephrol*. 2013;80:249–255.
10. Savoia C, Schiffrin EL. Inflammation in hypertension. *Curr Opin Nephrol Hypertens*. 2006;15:152–158.
11. Li JJ, Chen JL. Inflammation may be a bridge connecting hypertension and atherosclerosis. *Med Hypotheses*. 2005;64:925–929.
12. Lee SB, Kalluri R. Mechanistic connection between inflammation and fibrosis. *Kidney Int Suppl*. 2010;119:S22–S26.
13. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. *Nat Rev Nephrol*. 2014;10:493–503.
14. Doi T, Doi S, Nakashima A, et al. Mizoribine ameliorates renal injury and hypertension along with the attenuation of renal caspase-1 expression in aldosterone-salt-treated rats. *PLoS ONE*. 2014;9: e93513.
15. Webb IG, Nishino Y, Clark JE, et al. Constitutive glycogen synthase kinase-3alpha/beta activity protects against chronic beta-adrenergic remodelling of the heart. *Cardiovasc Res*. 2010;87: 494–503.
16. Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. *Nat Rev Drug Discov*. 2004;3:479–487.
17. Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A. Glycogen synthase kinase 3: more than a namesake. *Br J Pharmacol*. 2009;156:885–898.
18. Martin M, Rehani K, Jope RS, Michalek SM. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat Immunol*. 2005;6:777–784.
19. Beurel E, Michalek SM, Jope RS. Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3). *Trends Immunol*. 2010;31:24–31.
20. Wang H, Garcia CA, Rehani K, et al. IFN-beta production by TLR4-stimulated innate immune cells is negatively regulated by GSK3-beta. *J Immunol*. 2008;181:6797–6802.
21. Beurel E, Jope RS. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. *Prog Neurobiol*. 2006;79:173–189.
22. Schwabe RF, Brenner DA. Role of glycogen synthase kinase-3 in TNF-alpha-induced NF-kappaB activation and apoptosis in hepatocytes. *Am J Physiol Gastrointest Liver Physiol*. 2002;283: G204–G211.
23. Klamer G, Song E, Ko KH, O'Brien TA, Dolnikov A. Using small molecule GSK3beta inhibitors to treat inflammation. *Curr Med Chem*. 2010;17:2873–2881.
24. Yang J, Takahashi Y, Cheng E, et al. GSK-3beta promotes cell survival by modulating Bif-1-dependent autophagy and cell death. *J Cell Sci*. 2010;123:861–870.
25. Zhai P, Sciarretta S, Galeotti J, Volpe M, Sadoshima J. Differential roles of GSK-3beta during myocardial ischemia and ischemia/reperfusion. *Circ Res*. 2011;109:502–511.
26. Zhou X, Zhou J, Li X, Guo C, Fang T, Chen Z. GSK-3beta inhibitors suppressed neuroinflammation in rat cortex by activating autophagy in ischemic brain injury. *Biochem Biophys Res Commun*. 2011;411:271–275.
27. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. *Science*. 2000;290:1717–1721.
28. Dreux M, Chisari FV. Viruses and the autophagy machinery. *Cell Cycle*. 2010;9:1295–1307.
29. Virgin HW, Levine B. Autophagy genes in immunity. *Nat Immunol*. 2009;10:461–470.
30. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature*. 2011;469:323–335.
31. Choi AJ, Ryter SW. Autophagy in inflammatory diseases. *Int J Cell Biol*. 2011;2011:p. 732798.
32. Sha T, Iizawa Ii YM. Combination of imipenem and TAK-242, a Toll-like receptor 4 signal transduction inhibitor, improves survival in a murine model of polymicrobial sepsis. *Shock*. 2011;35:205–209.
33. Yao L, Kan EM, Lu J, et al. Toll-like receptor 4 mediates microglial activation and production of inflammatory mediators in neonatal rat

brain following hypoxia: role of TLR4 in hypoxic microglia. *J Neuroinflammation*. 2013;10:23.

34. Clemitson JR, Pratt JR, Frantz S, Sacks S, Samani NJ. Kidney specificity of rat chromosome 1 blood pressure quantitative trait locus region. *Hypertension*. 2002;40:292–297.

35. Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. MiR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *J Biol Chem*. 2010;285:25221–25231.

36. Parissis JT, Korovesis S, Giazitzoglou E, Kalivas P, Katritsis D. Plasma profiles of peripheral monocyte-related inflammatory markers in patients with arterial hypertension Correlations with plasma endothelin-1. *Int J Cardiol*. 2002;83:13–21.

37. Zhang Y, Peng W, Ao X, et al. Tak-242, a toll-like receptor 4 antagonist, protects against Aldosterone-induced cardiac and renal injury. *PLoS ONE*. 2015;10:e142456.

38. Azoulay-Alfaguter I, Elya R, Avrahami L, Katz A, Eldar-Finkelman H. Combined regulation of mTORC1 and lyso-somal acidification by GSK-3 suppresses autophagy and contributes to cancer cell growth. *Oncogene*. 2015;34:4613–4623.

39. Wakatsuki S, Tokunaga S, Shibata M, Araki T. GSK3B-mediated phosphorylation of MCL1 regulates axonal autophagy to promote Wallerian degeneration. *J Cell Biol*. 2017;216:477–493.

40. Wei H, Qu H, Wang H, et al. 1,25-Dihydroxyvitamin-D3 prevents the development of diabetic cardiomyopathy in type 1 diabetic rats by enhancing autophagy via inhibiting the beta-catenin/TCF4/GSK-3beta/mTOR pathway. *J Steroid Biochem Mol Biol*. 2017;168:71–90.

41. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature*. 2008;456:264–268.

42. Shrivastava S, Raychoudhuri A, Steele R, Ray R, Ray RB. Knockdown of autophagy enhances the innate immune response in hepatitis C virus-infected hepatocytes. *Hepatology*. 2011;53:406–414.

43. Bjorkoy G, Lamark T, Pankiv S, Øvervatn A, Brech A, Johansen T. Monitoring autophagic degradation of p62/SQSTM1. *Methods Enzymol*. 2009;452:181–197.

44. Zhou Y, Cai T, Xu J, et al. UCP2 attenuates apoptosis of tubular epithelial cells in renal ischemia/reperfusion injury. *Am J Physiol Renal Physiol*. 2017;313:F926–F937.

45. Briet M, Schiffrin EL. Aldosterone: effects on the kidney and cardiovascular system. *Nat Rev Nephrol*. 2010;6:261–273.

46. Elmarakby AA, Quigley JE, Olearczyk JJ, et al. Chemokine receptor 2b inhibition provides renal protection in angiotensin II − salt hypertension. *Hypertension*. 2007;50:1069–1076.

47. Ren J, Taegtmeyer H. Too much or not enough of a good thing-The Janus faces of autophagy in cardiac fuel and protein homeostasis. *J Mol Cell Cardiol*. 2015;84:223–226.

48. Deretic V, Levine B. Autophagy balances inflammation in innate immunity. *Autophagy*. 2017;17:1–29.

49. Moscat J, Diaz-Meco MT. P62 at the crossroads of autophagy, apoptosis, and cancer. *Cell*. 2009;137:1001–1004.

50. Leventhal JS, Wyatt CM, Ross MJ. Recycling to discover something new: the role of autophagy in kidney disease. *Kidney Int*. 2017;91:4–6.

51. Lenoir O, Tharaux PL, Huber TB. Autophagy in kidney disease and aging: lessons from rodent models. *Kidney Int*. 2016;90:950–964.

52. Sarkar S, Floto RA, Berger Z, et al. Lithium induces autophagy by inhibiting inositol monophosphatase. *J Cell Biol*. 2005;170:1101–1111.

53. Li Q, Li H, Roughton K, et al. Lithium reduces apoptosis and autophagy after neonatal hypoxia-ischemia. *Cell Death Dis*. 2010;1:56.

54. Wang SH, Shih YL, Kuo TC, Ko WC, Shih CM. Cadmium toxicity toward autophagy through ROS-activated GSK-3beta in mesangial cells. *Toxicol Sci*. 2009;108:124–131.