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Original Article

Ginsenoside Rg1 protects mouse podocytes from aldosterone-induced injury *in vitro*

Nan MAO[1, #], Yuan CHENG[2, #], Xin-li SHI[3], Li WANG[5, 6], Ji WEN[1], Qiong ZHANG[4], Qiong-dan HU[4], Jun-ming FAN[1, 4, 6, *]

[1]Department of Nephrology, West China Hospital of Sichuan University, Chengdu 610041, China; [2]Department of Nephrology, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen 518035, China; [3]Department of Pathobiology and Immunology, Hebei University of Traditional Chinese Medicine, Shijiazhuang 050200, China; [4]Department of Nephrology, The Affiliated Traditional Chinese Medicine Hospital of Luzhou Medical College, Luzhou 646000, China; [5]The Research Center for Preclinical Medicine, Luzhou Medical College, Luzhou 646000, China; [6]State Key Laboratory of Biotherapy of Human Disease, West China Hospital, Sichuan University, Chengdu 610041, China

Aim: Aldosterone is elevated in many diseases such as hypertension, diabetic nephropathy and chronic kidney disease, *etc*. The aim of this study was to investigate the effects of aldosterone on intracellular ROS production and autophagy in podocytes *in vitro*, and to explore the possibility of ginsenoside Rg1 (Rg1) being used for protecting podocytes from aldosterone-induced injury.
Methods: MPC5 mouse podocyte cells were tested. Autophagosome and autophagic vacuole formation were examined under confocal microscopy with MDC and acridine orange staining, respectively. ROS were detected with flow cytometry. Malondialdehyde content and superoxide dismutase (T-SOD) activity were measured using commercial kits. The expression of LC3-II, beclin-1, SOD2 and catalase was measured by Western blotting.
Results: Treatment with aldosterone (10 nmol/L) significantly increased ROS generation and the expression of SOD2 and catalase in MPC5 cells. Furthermore, treatment with aldosterone significantly increased the conversion of LC3-I to LC3-II, beclin-1 expression and autophagosome formation. Co-treatment with rapamycin (1 ng/mL) or chloroquine (10 μmol/L) further increased aldosterone-induced autophagosome formation. Co-treatment with Rg1 (80 ng/mL) effectively relieved oxidative stress and increased T-SOD activity at the early stage and subsequently decreased autophagy in aldosterone-treated podocytes. Co-treatment with 3-MA (4 mmol/L) or NAC (50 mmol/L) exerted similar effects against aldosterone-induced autophagy in podocytes.
Conclusion: Aldosterone enhances ROS generation and promotes autophagy in podocytes *in vitro*. Ginsenoside-Rg1 effectively relieves aldosterone-induced oxidative stress, thereby indirectly inhibiting aldosterone-induced podocyte autophagy.

Keywords: podocyte; aldosterone; autophagy; oxidative stress; ROS; ginsenoside; Rg1; rapamycin; chloroquine; 3-methyladenine; *N*-acetylcysteine; kidney

Introduction

Podocytes are highly specialized, terminally differentiated, postmitotic epithelial cells that line the outer portion of the glomerular basement membrane (GBM) and are involved in glomerular barrier maintenance for protein filtration and GBM synthesis, providing structural support for the glomerular capillaries[1]. Because podocytes lack proliferative capacity in response to injury, once damaged, podocytopenia is followed by reorganization of the slit diaphragm and subsequent glomerulosclerosis[2]. The postmitotic podocyte is the primary glomerular target for both immune and metabolic toxins, which have the potential to induce reactive oxygen species (ROS) and/or injury in podocytes[3]. Podocyte damage plays a crucial role in the mechanisms of many glomerular diseases, including focal segmental glomerulosclerosis (FSGS), diabetic nephropathy (DN), minimal change disease (MCD), and membranous nephropathy (MN)[4–6].

Aldosterone, a mineralocorticoid, regulates electrolyte and blood pressure homeostasis by acting on the distal nephron of the kidney. Aldosterone levels increase in hypertension, DN, and chronic kidney disease[7–9]. In addition to its hemodynamic effects, the local effects of aldosterone on renal cells, including proximal tubular cells, mesangial cells, and podocytes, are currently being studied, and accumulating evidence suggests that aldosterone plays a pathogenic role in renal

injury, leading to severe proteinuria and glomerular injury[10]. Aldosterone-induced renal cell apoptosis is mediated by ROS[11, 12]. In podocytes, the major sources of cellular ROS are the mitochondrial electron transport chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Shibata *et al*[13] observed podocyte injury following increased NADPH oxidase activity in the kidney of aldosterone-infused rats. Another study found that mitochondria dysfunction was associated with higher levels of ROS production and was involved in aldosterone-induced podocyte injury[14, 15]. ROS are scavenged by a sophisticated antioxidant defense system that includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx). However, when ROS exceed the normal physiological coping range, aggregation of ROS leads to oxidative stress and, as a result, oxidation of DNA, proteins, and lipids, along with damage to organelles[16]. To degrade oxidized proteins and damaged organelles, autophagy may be triggered in podocytes.

Autophagy is an evolutionarily conserved catabolic process that involves the regulation of ROS by lysosome-mediated degradation of intracellular proteins and organelles to lessen oxidative injury and maintain cellular homeostasis and integrity[17]. This process involves the delivery of cytoplasmic components sequestered inside double-membrane vesicles to the lysosome. The formation of autophagosomes depends on several ATG (AuTophaGy) genes, including microtubule-associated protein 1 light chain 3 (MAP1-LC3, LC3), beclin-1, and phosphatidylinositol 3-kinase[18]. LC3-I, the cytosolic form of LC3, is specifically processed to LC3-II, the membrane-bound form, as podocytes differentiate. The LC3-II level is proportional to the number of autophagic vacuoles, which can also be estimated by scoring LC3-positive vesicles[19]. Beclin-1, as either an autophagic protein or a tumor suppressor protein, participates in autophagosome formation. Increased expression of beclin-1 suggests the occurrence of autophagy, which may trigger a rapid autophagic response under stress conditions[20]. Pharmacological inhibitors of autophagy, such as 3-methyladenine (3-MA), chloroquine, and bafilomycin (BAF), induce cell apoptosis, implying that autophagy is cytoprotective in a variety of cell injury models[21]. At the early stage, autophagy is a protective mechanism that promotes cell survival. By contrast, excessive or defective autophagy may cause an imbalance between autophagic stimulation and the cellular ability to develop autophagic degradation and restore essential cellular components, and at later stages, it can trigger cell injury and death[22]. Aldosterone-induced apoptosis of podocytes has been previously examined. However, it is still not known whether autophagy is induced by aldosterone and, if so, whether it plays a protective or deleterious role.

Ginsenoside-Rg1 (Rg1) is an active ingredient isolated from panax notoginsenoside (PNS) and has extensive pharmacological actions, including antioxidant, anti-inflammatory, and anticancer properties[23, 24]. After either oral or intravenous administration of PNS, the Rg1 concentration in the kidney is high[25]. In our previous study, we showed that Rg1 prevented the damage caused by complement complex C5b-9 by suppressing intracellular ROS generation[26]. Therefore, we speculated that Rg1 could inhibit podocyte ROS generation and indirectly decrease aldosterone-induced podocyte autophagy.

In this study, mouse podocytes were used as a model to evaluate the effects of aldosterone on intracellular ROS generation and podocyte autophagy. In addition, we investigated the efficacy of Rg1 in protecting podocytes from aldosterone-induced injury.

Materials and methods

Cell culture

MPC5, a thermosensitive, SV-40-transfected, conditionally immortalized mouse podocyte cell line, was a gift from Peter MUNDEL (Mount Sinai School of Medicine, New York) and was used to perform our experiments as reported previously[27]. Cells were grown on type 1 collagen (BD Bioscience, MA, USA) at 33 °C and were treated with 10 U/mL mouse recombinant γ-interferon (PeproTech, USA) in RPMI-1640 (HyClone, USA) supplemented with 10% FBS (HyClone, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin. To induce differentiation, podocytes were maintained on a bed of type I collagen and grown without γ-interferon at 37 °C for 10 d to allow differentiation and conversion into arborized cells. All studies were performed after 10 d of culture under differentiating conditions.

Drug treatment

Aldosterone, *N*-acetylcysteine (NAC), rapamycin, 3-methyladenine (3-MA), and chloroquine (CL) were purchased from Sigma (USA). Rg1 (>98% pure) was purchased from DiDa Kexiang Biological Co, Ltd (Guizhou, China). To prepare a stock solution of Rg1, it was dissolved in 0.9% phosphate buffered saline (PBS) at a concentration of 1 μg/mL, which was then diluted with cell culture medium before use.

Podocytes were incubated with Rg1 at the same concentration used in our previous study[26]. Differentiated podocytes were divided into following groups and incubated for 24 h: control, aldosterone (10 nmol/L), H_2O_2 (100 μmol/L), 3-MA (4 mmol/L), 3-MA+aldosterone, chloroquine (10 μmol/L), chloroquine+aldosterone, rapamycin (1 ng/mL), rapamycin+aldosterone, NAC (50 mmol/L), NAC+aldosterone, Rg1 (80 ng/mL), and Rg1+aldosterone.

Transmission electron microscopy

The transmission electron microscopy method followed a previously described protocol[28], with some modifications. After incubation, cells were collected and fixed with 3% glutaraldehyde and then postfixed in 1% OsO_4, dehydrated in acetone, and embedded in Epon812. Serial ultra-thin sections (80 nm) were cut and stained with uranyl acetate/lead citrate and visualized using a Hitachi H-600IV electron microscope (Hitachi Instrument, Japan; ×10000).

Immunofluorescence

Cells were grown on 20-mm coverslips for immunofluorescence imaging. After treatment, cells were fixed, permeabi-

lized, blocked, and incubated with a primary rabbit polyclonal anti-LC3B antibody (1:400; CST, USA). Next, the cells were incubated with an affinity-purified biotinylated donkey anti-rabbit antibody (1:2000; CST, USA) in a dark room for 1 h. Then, the cells were incubated with rhodamine-phalloidin (Sigma, St Louis, MO, USA) for 1 h. Finally, the cells were stained with 4′,6-diamino-2-phenylindole (DAPI) for 3 min, mounted on slides using 95% glycerol, and then stored in the dark at 4 °C before observation under a fluorescence microscope (Olympus, Japan; ×630).

Monodansylcadaverine (MDC) staining
MPC5 cells were subcultured in 20-mm coverslip-bottom dishes and grown to 40% confluence. After treatment, monodansylcadaverine (MDC; Sigma, St Louis, MO, USA) was added to a final concentration of 50 μmol/L for 20 min. Then, the cells were immediately examined under a confocal microscope (Nikon Eclipse Ti, Japan; ×800) set for excitation at 405 nm and emission at 461 nm, and cell images were captured.

Acridine orange staining
MPC5 cells were subcultured in 20-mm coverslip-bottom dishes and grown to 40% confluence. After incubation, the cells were stained with acridine orange (0.05 μg/mL; Sigma, St Louis, MO, USA) in serum-free medium and Hoechst-33342 (1 μg/mL; Invitrogen, CA, USA) for 15 min at 37 °C. Finally, the cells were examined under a confocal microscope (×800) set for excitation at 488 nm and emission at 530 nm, and cell images were captured.

ROS determination
After treatment, the cells were washed and then incubated with 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Nanjing KeyGen Biotech Co Ltd, Nanjing, China) for 30 min at 37 °C in the dark. Cells were then washed 3 times and harvested with PBS. The 2′,7′-dichlorofluorescein (DCF) fluorescence distribution of 5×10^5 cells was detected by a flow cytometer (FACSAria, BD Biosciences, USA). Data were processed using the CellQuest program (BD Biosciences, USA).

Determination of malondialdehyde (MDA) concentration and superoxide dismutase (SOD) activity
The culture medium was obtained to measure malondialdehyde (MDA) concentration and superoxide dismutase (SOD) activity using assay kits (Jiancheng Bioengineering Institute, China). MDA was analyzed by TBA assay, and its concentration was determined by measuring the absorbance at 532 nm. SOD activity was measured as the absorbance at 550 nm.

Western blot analysis
Cells were harvested with ice-cold PBS containing 5 mmol/L EDTA. Proteins were extracted using a commercial assay kit (Nanjing KeyGen Biotech Co Ltd, Nanjing, China). The protein concentration of the lysate was determined using the BCA protein assay kit (Nanjing KeyGen Biotech Co Ltd, Nanjing,

China). Equal amounts of total protein (30 μg/lane) were separated by SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane (Millipore, Germany). The blot was then incubated with the following primary antibodies: LC3B (1:1000; CST, USA), beclin-1 (1:1000; CST, USA), SOD2 (1:2000; Epitomics, USA), and catalase (1:1000; CST, USA). It was then incubated with horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit antibodies (1:5000; Golden Bridge, China) for 1 h. After being washed, the blot was incubated in chemiluminescent substrate (Millipore, USA) for 1 min and finally exposed to X-ray film (Eastman Kodak, NY, USA). Specific protein bands were scanned and normalized to β-actin (1:500; Bioss, China) to quantitate protein levels.

Statistical analysis
Data are presented as means±SD. The significance of differences between groups was determined by one-way analysis of variance (ANOVA). P-values less than 0.05 were considered statistically significant. All results were analyzed using SPSS 13.0 statistical software.

Results
Aldosterone promotes podocyte autophagy through ROS generation
To morphologically demonstrate the induction of autophagy in aldosterone-treated podocytes, we performed an ultrastructural analysis. Morphological changes induced by aldosterone were observed by transmission electron microscopy. We noted the formation of double- and multiple-membrane encapsulated portions of the cytoplasm and/or organelles (autophagosomes). The number of autophagosomes was higher in the aldosterone-treated group than that in the control group, and rapamycin treatment further enhanced aldosterone-induced autophagosome formation (Figure 1A). A total of 20 cells were counted for each variable. Quantitative autophagosome data were collected in 3 sets of experiments (Figure 1B). Western blot analysis also showed that aldosterone promoted the conversion of LC3-I to LC3-II and the expression of beclin-1. H_2O_2, as a positive control for autophagy, promoted the accumulation of LC3-II and the expression of beclin-1 in MPC5 cells (Figure 1C). These findings indicate that oxidative stress promotes autophagy in MPC5 cells. However, the effect of different concentrations of aldosterone (1 μmol/L, 10, and 0.1 nmol/L) on autophagy was similar to the effect of H_2O_2. The expression of LC3-II was negatively correlated with aldosterone concentration.

Rg1 inhibits aldosterone-induced autophagy in podocytes
To further confirm the effect of aldosterone on podocyte autophagy, MPC5 cells were incubated with an anti-LC3B antibody and examined for the presence of autophagosomes (green fluorescent puncta) under fluorescence microscopy. We found that aldosterone-, chloroquine-, and rapamycin-treated cells showed bright green fluorescence, and this aldosterone-mediated effect was further enhanced by rapamycin and chloroquine. After pretreatment with 3-MA, NAC, or
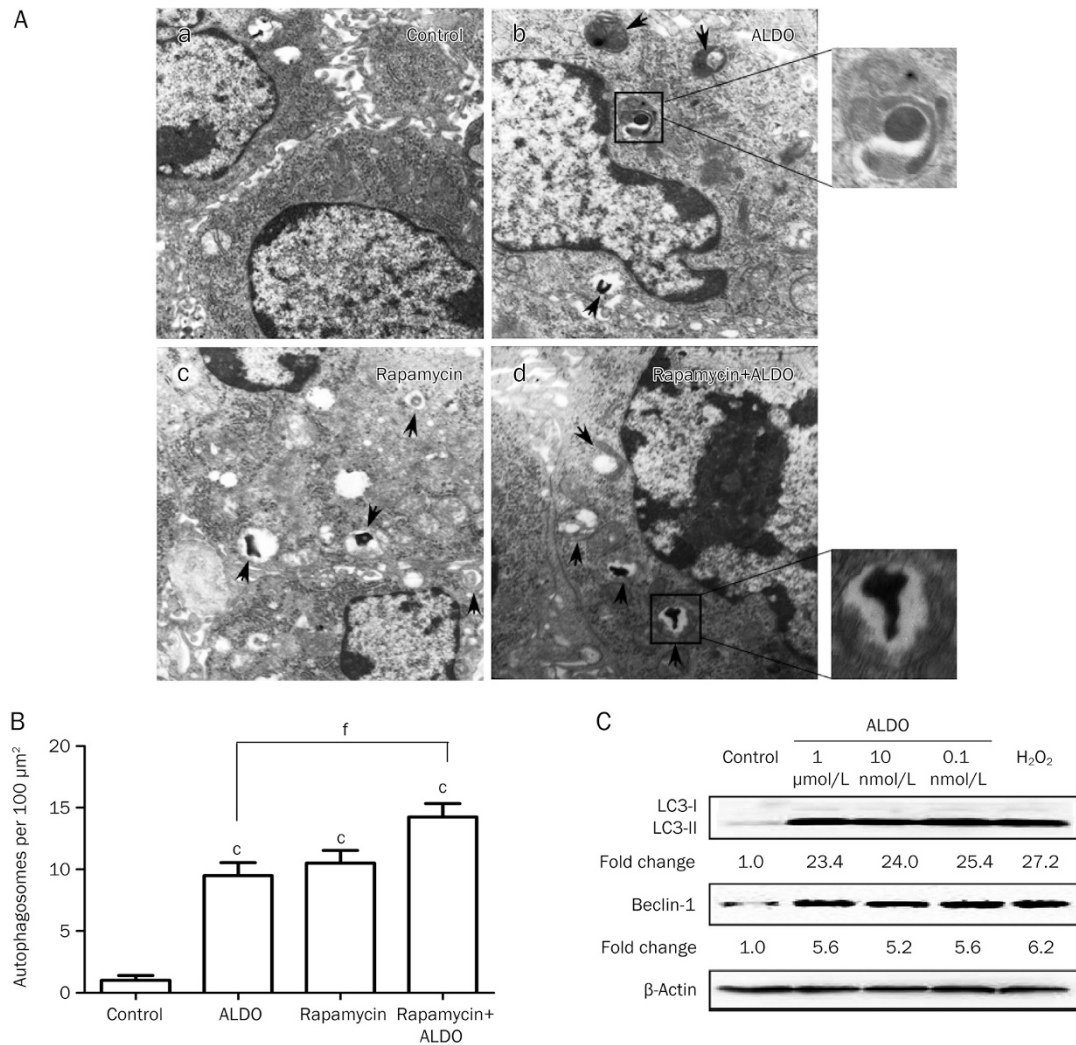
Figure 1. Aldosterone promotes podocyte autophagy through ROS generation. (A) MPC5 cells were incubated with buffer (Control), aldosterone (ALDO, 10 nmol/L), rapamycin (1 ng/mL), or rapamycin+aldosterone for 24 h. Under transmission electron microscope, we observed the formation of double- or multiple-membrane encapsulated portions of the cytoplasm and/or organelles. (B) A total of 20 cells were counted for each variable. Quantitative data for autophagosomes were obtained from 3 sets of experiments. ^{c}P<0.01 *vs* the control group. ^{f}P<0.01 *vs* the aldosterone group. The number of autophagosomes increased in the aldosterone-treated group, and rapamycin further enhanced aldosterone-induced autophagosome formation. (C) Western blot for LC3-II and beclin-1. β-Actin was used as a loading control.

Rg1, the cells showed fewer green fluorescent puncta than the aldosterone-treated group (Figure 2A, 2B). Western blot examination also showed that aldosterone promoted the conversion of LC3-I to LC3-II and the expression of beclin-1, and rapamycin and chloroquine further enhanced the expression of LC3-II and beclin-1 in aldosterone-treated cells. The expression of LC3-II and beclin-1 was lower in NAC- or Rg1-pretreated MPC5 cells (Figure 2C). These results suggest that aldosterone promoted autophagy in MPC5 cells and that this effect was ameliorated by NAC or Rg1.

Effects of 3-MA, rapamycin, NAC, and Rg1 on aldosterone-induced autophagosome formation in podocytes by monodansyl-cadaverine (MDC) staining

To quantitate the occurrence of autophagy, cells were stained with MDC and examined under confocal microscopy. After treatment, MPC5 cells were incubated with MDC for 20 min, washed, and visualized by confocal microscopy. MDC staining showed bluer punctate staining in aldosterone-treated cells than in the control cells; 3-MA, NAC, or Rg1 inhibited autophagosome formation; and rapamycin further enhanced aldosterone-induced autophagosome formation (Figure 3A). A total of 50 cells were counted for each variable. Quantitative analysis of positive cells with blue punctate staining is shown in Figure 3B.

Effects of 3-MA, rapamycin, NAC, and Rg1 on aldosterone-induced autophagic vacuole formation in podocytes by acridine orange staining
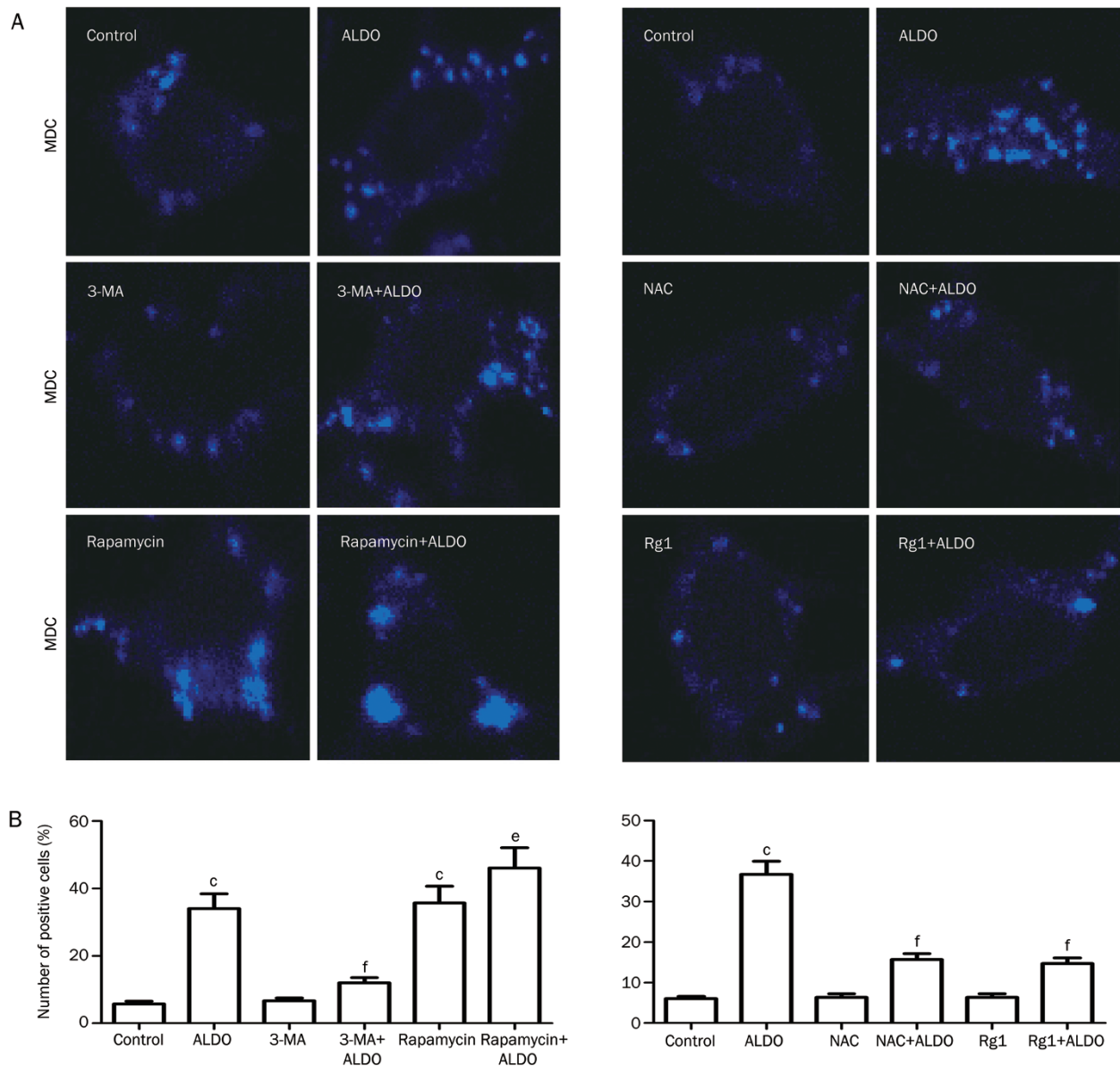
MPC5 cells were stained with acridine orange to determine
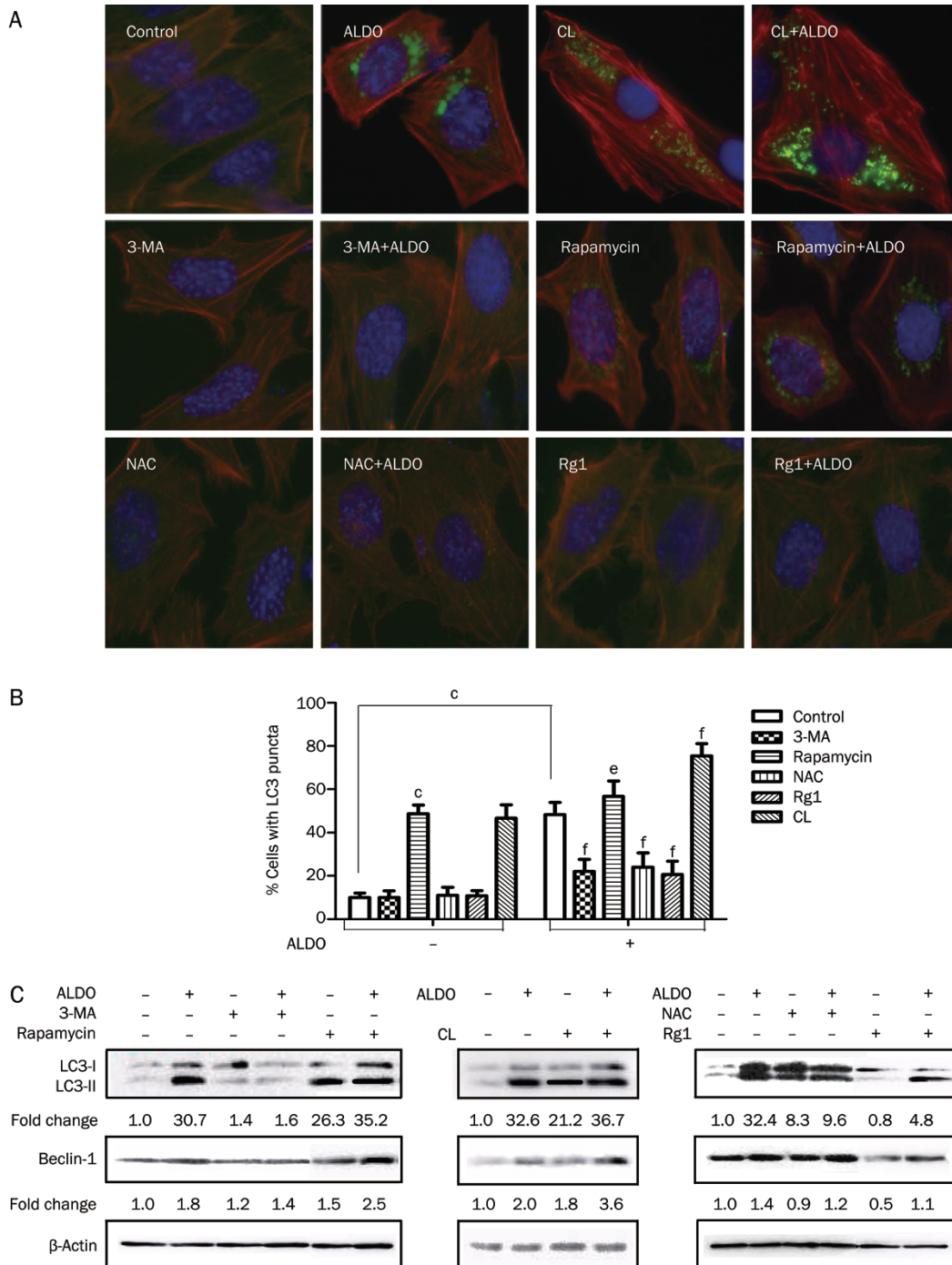
Figure 2. Effect of Rg1 on aldosterone-induced autophagy in podocytes. (A) After treatment with the indicated drugs for 24 h, cells were incubated with an anti-LC3B primary antibody, and the presence of autophagosomes (green fluorescent puncta) was assessed by fluorescence microscopy. (B) Fifty cells were counted for each variable. Quantitative analysis of autophagosomes with green-fluorescent puncta. $n=3$. $^cP<0.01$ *vs* the control group. $^eP<0.05$, $^fP<0.01$ *vs* the aldosterone group. (C) Western blot for LC3-II and beclin-1. β-Actin was used as a loading control.

the effect of Rg1 on aldosterone-induced autophagic vacuole formation. After treatment, cells were stained with acridine orange and Hochest-33342 for 15 min and then washed and examined under confocal microscopy. Aldosterone and rapamycin promoted the formation of autophagic vacuoles (indicated by green staining). The aldosterone-mediated effect was further enhanced by rapamycin, whereas 3-MA inhibited autophagic vacuole formation, and NAC and Rg1 reduced aldosterone-induced autophagic vacuole formation (Figure 4A). A total of 50 cells were counted for each variable. Quantitative analysis of autophagic vacuole formation is shown in Figure 4B.
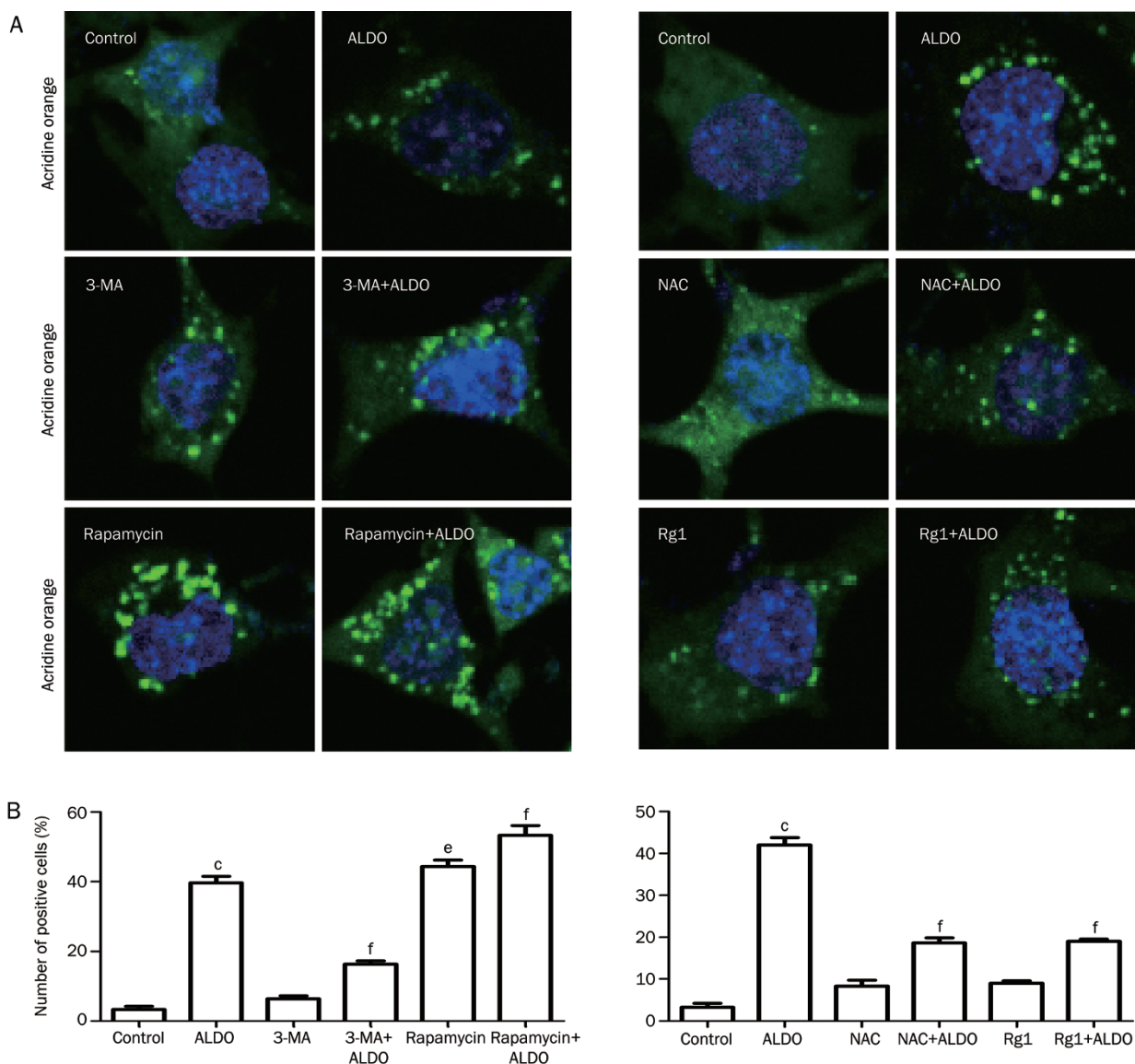
A

B

Figure 3. Effects of 3-MA, rapamycin, NAC, and Rg1 on aldosterone-induced autophagosome formation. (A) After treatment with the indicated drugs for 24 h, MPC5 cells were incubated with monodansylcadaverine (MDC, 50 µmol/L) for 20 min, washed, and then examined under confocal microscopy. (B) Fifty cells were counted for each variable. Quantitative analysis of positive cells with punctate blue staining. $n=3$. $^cP<0.01$ *vs* the control group. $^eP<0.05$, $^fP<0.01$ *vs* the aldosterone group.

Rg1 alleviates the oxidative stress induced by aldosterone in podocytes

MPC5 cells were stained with DCFH-DA to determine the effect of Rg1 on aldosterone-mediated ROS generation in podocytes. After treatment, cells were stained with DCFH-DA and analyzed using flow cytometry. Intracellular ROS generation in podocytes was significantly increased after treatment with aldosterone for 24 h. Pretreatment with NAC or Rg1 markedly reduced the elevation of ROS. DCF fluorescence was measured in the different groups [Figure 5A; means±SD ($n=3$), Figure 5B]. In addition, we studied the effect of aldosterone (1 µmol/L, 10, or 0.1 nmol/L) and Rg1 (80 ng/mL) on the

expression of SOD_2 and catalase by Western blotting (Figure 5C). Cells treated with aldosterone or H_2O_2 showed higher SOD_2 and catalase protein levels than the control group, suggesting that aldosterone enhances ROS generation. In addition, cells that were pretreated with Rg1 showed lower SOD_2 and catalase protein levels after aldosterone treatment for 24 h than the single aldosterone treatment group, indicating that Rg1 can inhibit ROS generation.

In addition, the MDA level in the culture medium was used to measure the level of oxidative stress in the cells. As expected, culture medium from MPC5 cells exposed to aldosterone for 2 h contained a higher level of MDA than medium

Figure 4. Effects of 3-MA, rapamycin, NAC, and Rg1 on aldosterone-induced formation of autophagic vacuoles. (A) After treatment with the indicated drugs for 24 h, MPC5 were stained with acridine orange (0.05 µg/mL) and Hoechst-33342 (1 µg/mL) for 15 min, washed and then examined under confocal microscopy. (B) Fifty cells were counted for each variable. Quantitative analysis of autophagic vacuoles. n=3. ^{c}P<0.01 *vs* the control group. ^{e}P<0.05, ^{f}P<0.01 *vs* the aldosterone group.

from control cells. However, pretreatment with Rg1 significantly reduced the MDA in medium from MPC5 cells exposed to aldosterone (Figure 6A). T-SOD activity was decreased in MPC5 cells exposed to aldosterone for 2 h, indicating compensatory clearance of ROS in these cells. Rg1 pretreatment increased T-SOD activity in aldosterone-induced MPC5 cells (Figure 6B).

Taken together, these data suggest that Rg1 alleviates the oxidative stress induced by aldosterone in MPC5 cells.

Discussion

Aldosterone levels disproportionately increase in many disease conditions, such as hypertension, DN, and chronic kidney

disease[9, 29, 30]. We found that ROS generation and autophagosome formation were higher in aldosterone-treated podocytes than that in control podocytes. Aldosterone promoted the conversion of LC3-I to LC3-II and the expression of beclin-1 in podocytes. The effect of aldosterone (at all tested concentrations) on autophagy was similar to the effect of H_2O_2. Furthermore, the expression of LC3-II was negatively correlated with aldosterone concentration in podocytes. This suggests that low-level aldosterone-induced autophagy is a protective mechanism that promotes cell survival, whereas high levels of aldosterone may directly induce cell apoptosis or death. The mTOR inhibitor rapamycin and the lysosomal protease inhibitor chloroquine further enhanced aldosterone-induced

A

B

C
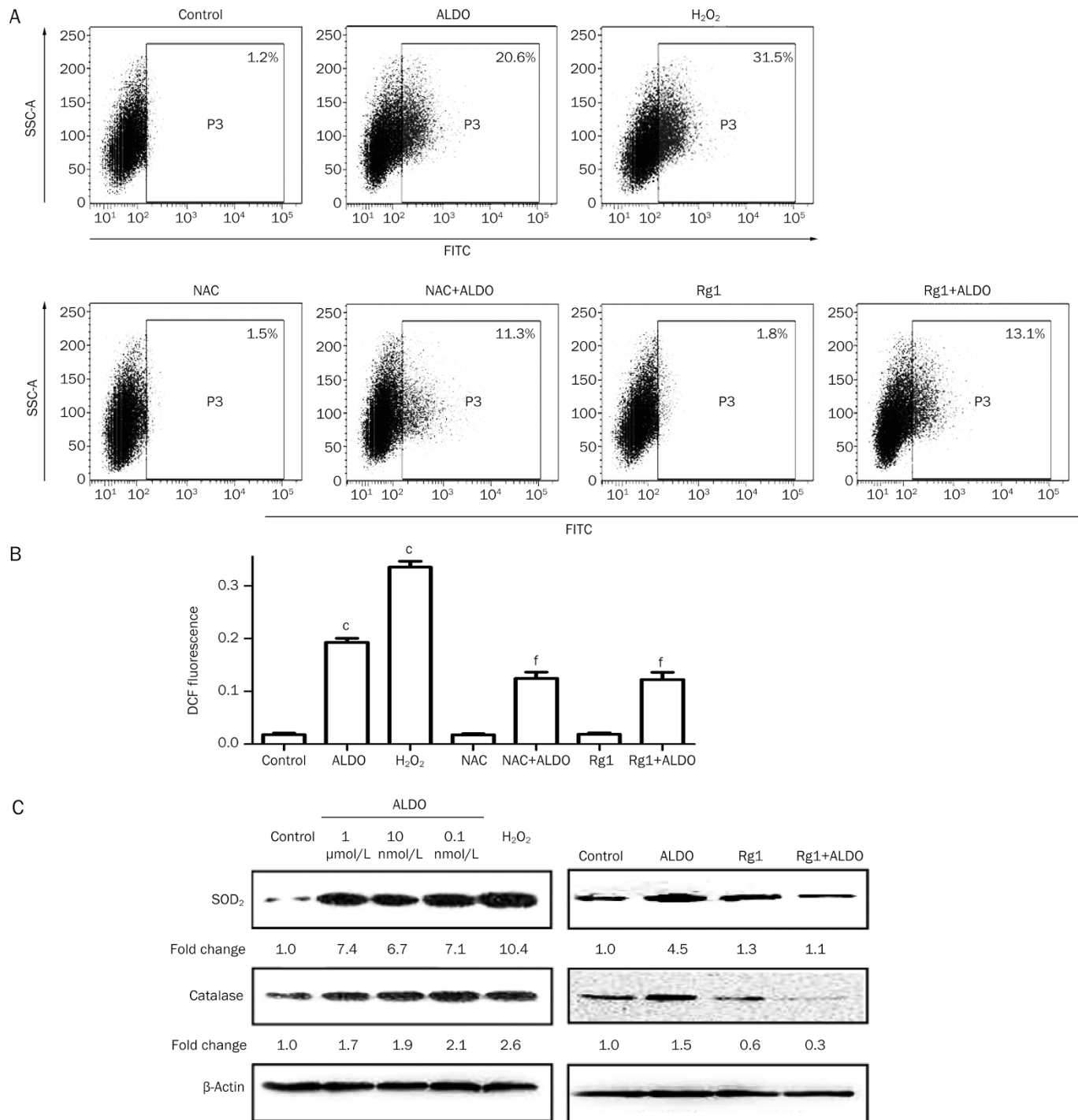
Figure 5. Effects of NAC and Rg1 on aldosterone-induced reactive oxygen species (ROS) generation in podocytes. (A) MPC5 cells were incubated with the indicated drugs for 24 h. Flow cytometry was used to measure DCF fluorescence after DCFH-DA staining. ROS generation increased after treatment with aldosterone for 24 h. Pretreatment with NAC or Rg1 reduced the elevation of ROS bursts. (B) Data are expressed as means±SD. Data were from 3 independent experiments. $^{c}P<0.01$ vs the control group. $^{f}P<0.01$ vs the aldosterone group. (C) Western blot for SOD_2 and catalase. β-Actin was used as a loading control.

autophagosome formation, whereas 3-MA, NAC, and Rg1 inhibited aldosterone-induced autophagy. Pretreatment with Rg1 markedly reduced the elevation of ROS bursts and MDA level and increased T-SOD activity at the early stage of aldosterone treatment. These findings demonstrate that aldosterone promotes podocyte autophagy through ROS generation. Rg1 could relieve the oxidative stress and autophagy induced by aldosterone in podocytes.
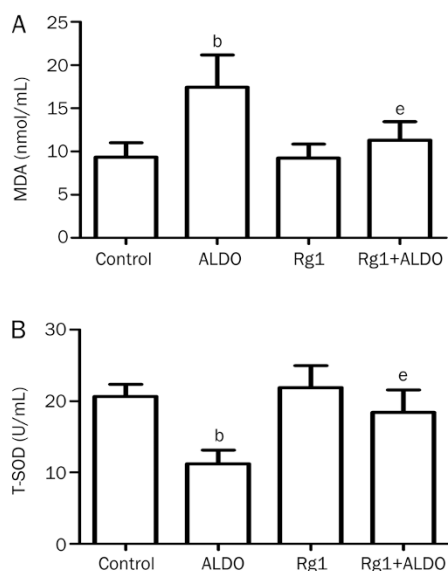
Figure 6. Effect of Rg1 on aldosterone-induced oxidative stress in MPC5 cells exposed to aldosterone. MPC5 cells were exposed to aldosterone for 2 h in the presence or absence of 10 nmol/L aldosterone and 80 ng/mL Rg1. The culture media were collected to measure MDA content (A) and T-SOD activity (B). $n=3$. [b]$P<0.05$ *vs* the control group. [e]$P<0.05$ *vs* the aldosterone group.

The local effects of aldosterone on podocytes play an important pathogenic role in renal injury, which results in severe proteinuria, glomerulosclerosis, arteriopathy, and renal fibrosis[10, 31]. Growing evidence suggests that aldosterone-induced renal cell apoptosis is mediated by ROS. ROS are essential signals that activate autophagy in a variety of conditions, such as cancer, ischemia/reperfusion, and neurodegenerative diseases[32, 33]. During the past few years, research on autophagy in cancer and neurodegenerative diseases has rapidly progressed. However, the role of autophagy in renal pathophysiology is poorly understood. We presume that the incremental production of endogenous aldosterone in podocytes may contribute to the ongoing oxidative stress and autophagy. In return, autophagy serves to reduce oxidative damage and remove damaged components. Therefore, we speculate that aldosterone induces podocyte autophagy through a ROS signaling pathway. However, when oxidative damage exceeds a certain threshold, it will lead to cell death.

Autophagy is an early response to cell injury in the pre-apoptotic lag phase that allows the cell to adapt to the changing environmental stimuli and then persists during injury[34]. At the early stage of cell injury, autophagy has a cytoprotective role and degrades oxidized proteins and damaged organelles. At the later stage, when the cell injury becomes too severe, excessive autophagy may cause an imbalance between autophagic stimulation and the cell's ability to promote autophagic degradation and may trigger cell injury and cell death[35]. Autophagy has been implicated in the pathology of various human diseases. Under non-stress conditions, autophagy is maintained at a low basal level. However, upon stress or stimulation, autophagy can be strongly induced. The conversion of LC3-I to LC3-II indicates the increased formation of autophagic vacuoles. Therefore, we assayed LC3-II production in podocytes incubated in medium containing aldosterone, Rg1, and either an autophagic inducer or an autophagic inhibitor to evaluate the actual autophagy flux. Pretreatment with Rg1 decreased the formation of autophagosomes, suggesting that Rg1 inhibits podocyte autophagy by relieving oxidative stress.

Recent findings implicate ROS in the regulation of autophagy. Conversely, autophagy can also suppress ROS production. To remove cellular ROS, antioxidative enzymes such as manganese superoxide dismutase (MnSOD, SOD_2) and catalase are elevated to protect podocytes from ROS-mediated injury. Our study shows that aldosterone increased ROS generation, MDA content, T-SOD activity, and the expression of SOD_2 and catalase in podocytes. Interestingly, we found that Rg1 reduced ROS production and MDA level and increased T-SOD activity in aldosterone-treated podocytes.

Ginseng, the best-known and most popular herbal medicine[36], plays an important role in the treatment of kidney diseases in China. Its major component, Rg1, has been increasingly studied and has some beneficial actions on kidney injury, including membranous nephropathy, acute renal failure, and renal interstitial fibrosis[26, 37, 38]. Rg1 inhibits ROS production and stimulates NO production in renal cells[26, 37]. Zhang *et al*[39] showed ginsenoside-Rg1 inhibits autophagy in H9c2 cardiomyocytes exposed to hypoxia/reoxygenation. However, the pharmacological effects of Rg1 on autophagy in podocytes have not been previously reported. In our study, Rg1 significantly decreased the serum level of ROS produced by podocytes, increased T-SOD activity, and indirectly inhibited aldosterone-induced autophagy.

We conclude that aldosterone promotes autophagy in podocytes by promoting intracellular ROS generation. Rg1 significantly relieves this oxidative stress and protects podocytes from aldosterone-induced injury, thereby indirectly inhibiting aldosterone-induced podocyte autophagy and evading cell death. These findings provide new insight into the renoprotective effects of Rg1 and may aid in the development of new agents for the prevention and treatment of kidney diseases. However, further studies are needed to clarify the exact signaling pathway through which Rg1 inhibits aldosterone-induced autophagy in podocytes.

Author contribution
Nan MAO and Yuan CHENG designed the research, performed the experiments, analyzed the data, and co-wrote the manuscript; Xin-li SHI, Li WANG, and Ji WEN provided technical help and analyzed the data; Li WANG, Qiong ZHANG,

Qiong-dan HU, and Jun-ming FAN provided language help and revised the paper.

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