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VASPIN reduces inflammation and endoplasmic reticulum stress of renal tubular epithelial cells by inhibiting HMGB1 and relieves renal ischemia-reperfusion injury

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Abstract. – OBJECTIVE: Renal ischemia-reperfusion injury (IRI) is a clinically common issue and the resulting acute kidney injury (AKI) seriously threatens the patient's life. Therefore, prevention and treatment of renal IRI are the key to alleviating AKI in such patients. The purpose of this study was to explore the effects of VASPIN on mouse renal IRI and human renal proximal tubular epithelial cells (HK-2 cells) to provide a new direction for the treatment of clinical renal IRI.

MATERIALS AND METHODS: C57/BL6 mice were used to construct a renal IRI model and recombinant mouse VASPIN was subcutaneously injected to determine whether VASPIN can alleviate renal IRI in mice by histological examination and detection of mouse urine and serum related indicators. In addition, HK-2 cells were cultured and an IRI model was constructed at the cellular level by hypoxia reoxygenation to examine the effect and mechanism of VASPIN on endoplasmic reticulum stress (ERS) in HK-2 cells.

RESULTS: Results revealed that in VASPIN-treated mice, edema of renal tubular epithelial cells was significantly improved and renal injury markers netrin-1 and L-FAPB were decreased in urine. In addition, VASPIN also reduced the expression of inflammatory factors in mouse serum and the level of oxidative stress in kidney tissue. The expression of ERS-related molecules (GRP78, ATF6, caspase12, and CHOP) in HK-2 cells treated with VASPIN was significantly reduced and VASPIN decreased the expression of the pro-inflammatory factor HMGB1. Moreover, VASPIN promoted the activity of the Nrf2/ARE/HO-1 signaling pathway and inhibited the NF-κB signaling pathway by inhibiting HMGB1.

CONCLUSIONS: VASPIN reduces inflammation and ERS levels in kidney tissue and attenuates renal IRI by activating the Nrf2/ARE/HO-1 signaling pathway and inhibiting the NF-κB signaling pathway *via* inhibition of HMGB1.

Key Words:
VASPIN, Inflammation, Endoplasmic reticulum stress, HMGB1

Introduction

Acute kidney injury (AKI), formerly known as acute renal failure, is characterized by a rapid decline in renal function in a short period of time, which usually occurs in hospitalized patients, especially in patients with multiple organ failure. The occurrence of AKI increases the risk of death for patients by 10-15 times[1]. Therefore, the adverse consequences of AKI have led researchers to explore new treatments to treat the disease.

Renal ischemia-reperfusion injury (IRI) is the main cause of AKI. IRI results in the destruction of the structure and function of renal vascular endothelial cells and renal tubular epithelial cells, thus leading to cell apoptosis, necrosis, inflammatory response, and the production of reactive oxygen species (ROS)[2]. Among them, apoptosis and inflammation play an important role in the pathophysiology of AKI. There is increasing evidence that pro-inflammatory cytokine release, inflammatory cell recruitment, and mitochondrial dysfunction-induced apoptosis are associated with renal disease progression[3]. Researchers have proposed several mechanisms of AKI-related apoptosis and inflammation. Endoplasmic reticulum stress (ERS) is one of the mechanisms of IRI-induced AKI that has received widespread attention in recent years[4].

VASPIN is a cytokine obtained by Japanese scholar Hida et al[5] during gene screening, which

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was up-regulated in visceral fat in obese OLETF rats (type 2 diabetes model rats) and down-regulated in non-obese LETO rats. VASPIN is a member of the serpin family of serine protease inhibitors, so it is called Visceral Adipose tissue-derived Serine Protease Inhibitor. Zieger et al[6] have found that VASPIN has significant anti-inflammatory and anti-oxidant effects in addition to regulating glucose metabolism in the body. It inhibits TNF-α-mediated apoptosis and inflammatory response of vascular smooth muscle cells by inhibiting ROS production, inhibiting the activation of Intercellular Adhesion Molecule-1 (ICAM-1) and NF-κB signaling pathway[7]. However, there is still a lack of research on the anti-inflammatory and anti-oxidative effects of VASPIN on mouse renal IRI.

Therefore, C57/BL6 mice were used to construct a renal IRI model to study the protective effect of exogenous VASPIN on the mouse kidney. Besides, human renal cortical proximal tubular epithelial HK-2 cells were cultured to study the effect and mechanism of VASPIN on inflammation and ERS of HK-2 cells.

Materials and Methods

Animals and Grouping

SPF male C57/BL6 mice (8 weeks old, 18-20 g) were purchased from Beijing Weitong Lihua Experimental Animals Co., Ltd. (Beijing, China) and housed in a constant temperature and humidity environment with temperature of 24 ± 2°C and relative humidity of 50%-60%. 12 h of an alternating cycle of light and clean feed and drinking water were artificially given to mice. Mice were randomized into 3 groups, namely, control group, renal IRI model group, and IRI+ VASPIN group. Mice in the IRI group and the IRI+VASPIN group were subjected to an IRI model, while those in IRI+VASPIN group were intraperitoneally injected with recombinant VASPIN (ProSpec, China) 250 mg/(kg*d) for one week before making IRI model. This investigation was approved by the Animal Ethics Committee of Shanxian Central Hospital Animal Center.

Operative Procedure and Treatment

We fasted the mice for 12 h before surgery. Then, mice received intraperitoneal injections of 4% chloral hydrate (10 μL/g). After the anesthesia worked, the mice were placed in a supine position on the dissection table and disinfected with alcohol, and scissors were used to gently cut the abdominal cavity and find bilateral renal arteries and veins. Then, a vascular clamp was used to clamp the bilateral renal arteries. Kidney ischemia was observed as the kidneys changed from bright red to dark red. After 40 min, the vascular clamp was loosened. It was observed that the kidney color turned bright red, indicating that the kidney IRI model was successfully constructed.

Cell Culture and Treatment

HK-2 cells were purchased from the American Type Culture Collection (ATCC) cell bank (Manassas, VA, USA). HK-2 cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, Rockville, MD, USA) 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin plus streptomycin (Gibco, Rockville, MD, USA). All cell experiments were performed in a sterile clean bench. After the cell growth density reached 80-90%, the cells were treated with hypoxia-reoxygenation (HRO), the cell culture medium and added sterile phosphate-buffered saline (PBS) were discarded. Then the cells were placed in an incubator filled with 95% N_2 for 4 h. Finally, the cells were taken, and the medium was changed again for further cell culture in the incubator.

Western Blot

HK-2 cells were taken and lysed on ice using the radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China). Then, the cell debris was removed by centrifugation to obtain the supernatant. After the protein concentration was measured by the bicinchoninic acid (BCA) method (Beyotime, Shanghai, China), 5 × loading buffer (Beyotime, Shanghai, China) was added to the lysate. An equal amount of protein was added to each well of the electrophoresis gel. Then, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the protein at 80 V. After the end of the electrophoresis, the protein was transferred to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) at constant current of 280 mA. After washing the PVDF membrane with Tris-Buffered Saline and Tween-20 (TBST), the PVDF membrane was blocked with 5% skim milk. Next, the PVDF membrane was incubated at 4°C overnight with primary antibody dilution (GRP78, 1:3000, Rabbit, Abcam, Cambridge, MA, USA, ATF6, 1:5000, Rabbit, Abcam, Cambridge, MA, USA, caspase12, 1:2000, Rabbit, Abcam, Cambridge, MA, USA, CHOP, 1:2000,

Rabbit, Abcam, Cambridge, MA, USA, HMGB1, 1:1000, Rabbit, Abcam, Cambridge, MA, USA, Nrf2, 1:3000, Rabbit, Abcam, Cambridge, MA, USA, ARE, 1:3000, Rabbit, Abcam, Cambridge, MA, USA, HO-1, 1:5000, Rabbit, Abcam, Cambridge, MA, USA, NF-κB p65, 1:3000, Rabbit, Abcam, Cambridge, MA, USA, β-actin, 1:3000, Rabbit, Abcam, Cambridge, MA, USA). After washing the PVDF membrane the next day, the PVDF membrane was incubated with secondary antibody dilution (Goat anti-rabbit, 1:3000, Abcam, Cambridge, MA, USA). Finally, enhanced chemiluminescence (ECL) was used to observe and analyze protein bands.

RNA Isolation and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from cells. After discarding the medium, the cells were washed with PBS and added TRIzol to obtain cell suspension. After removing the precipitate by centrifugation, a spectrophotometer was used to detect the RNA concentration, and the SuperScript kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize complementary deoxyribose nucleic acid (cDNA). Later, primers for amplifying cDNA were constructed by Shanghai Jierui Biotechnology Co., Ltd. (Shanghai, China), and the cDNA using the SYBR Green kit (Invitrogen, Carlsbad, CA, USA) was amplified using different primers. With the expression of endogenous GAPDH in cells as control, the relative expression level of RNA is represented by $2^{-\Delta\Delta CT}$. Primers for qRT-PCR are shown in Table I.

Enzyme-Linked Immunosorbent Assay (ELISA)

HK-2 cells were passaged into 6-well plates, and then the cells were transfected or stimulated with VASPIN. The cell medium was taken, and the precipitate was removed by centrifugation. After that, the Scr ELISA kit (Lianke, Hangzhou, China), BUN ELISA kit (Lianke, Hangzhou, China), IL-1β ELISA kit (Lianke, Hangzhou, China) and TNF-α ELISA kit (Lianke, Hangzhou, China) were used to detect the contents of Scr, BUN, IL-1β, and TNF-α in the cell supernatant. The detection of indicators in mouse serum and urine is similar to the detection of cell supernatants.

Detection of Myeloperoxidase (MPO), Malonaldehyde (MDA), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) Activity

Mouse kidney tissues were taken and pulverized into powder at low temperature. Then, PBS

Table I. qRT-PCR primers.

Name	Sense/anti-sense	Sequence (5'-3')
GRP78	Sense	GCAGCCACGCCATGTGTGACCA
	Anti-sense	CGCGGCATATCGTACGTAACGT
ATF6	Sense	AGCTACGTACACGATGCTGCA
	Anti-sense	GCTACGTACGTACTCAGCTAGTG
Caspase12	Sense	AGTCCACGACTTACGTGCTCGATG
	Anti-sense	GCTACTGACCCATGAGCTGATCAG
CHOP	Sense	GGTTACCACGTGACTTGGATCCA
	Anti-sense	CGAGAGGTCTCTATGAGCGAGCTA
HMGB1	Sense	GGCGCGATATTCCTGAAGTCGA
	Anti-sense	AAGCTCGACTGATATACTCGA
IL-1β	Sense	GGTATAGCTAGCAATACTCTAG
	Anti-sense	AGCCGAGCTACTGTCGAGAGCT
TNF-α	Sense	AGCTCGATCGACGTCGACTGGCTA
	Anti-sense	GTCGTGGCAGCATCTGACGTACTG
Nrf2	Sense	GTCTCCTCGAGAGACGATCATGCGC
	Anti-sense	GCATGAGCACAGCTACGACTGAC
ARE	Sense	GGAGAGTCTCAGCGATCACGACG
	Anti-sense	AGCTGCTAGCGACTGACGAGCT
HO-1	Sense	GAGCTCGATCGACGACGTACCTG
	Anti-sense	GAGCGTACGTACTGACGAGCGT
NF-κB p65	Sense	GACTGTGCACGACGTATCCAGCA
	Anti-sense	GACTGCTACACGTACTGACTGC
GAPDH	Sense	GAGCTCGACGTACTGACTGACTGA
	Anti-sense	GCGACTACGACGTACGAGGCACTG

was used to dissolve the powder and remove the precipitate by centrifugation. MPO kit (Lianke, Hangzhou, China), MDA kit (Lianke, Hangzhou, China), SOD kit (Lianke, Hangzhou, China), and GSH-Px kit (Lianke, Hangzhou, China) were used to detect the contents of MPO, MDA, SOD, and GSH-Px in solution.

Hematoxylin and Eosin (HE) Staining

After the mouse renal IRI model was completed, mice were sacrificed, and the kidneys of the mice were taken. After gently peeling the envelope of the mouse kidney, the kidney was placed in 4% paraformaldehyde for 24 h. Then, the mouse kidney was dehydrated to make paraffin sections. Paraffin sections of mouse kidney tissue were deparaffinized and hydrated and stained in hematoxylin stain for 5 minutes, followed by differentiation for 3 s in hydrochloric acid alcohol. After washing the paraffin sections with running water, the paraffin sections were stained in an eosin solution for 3 minutes. After the paraffin sections were dehydrated and sealed, the staining results were observed under a microscope.

Cell Transfection

Lenti-NC and Lenti-HMGB1 were constructed by Shanghai Jima Bio (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfect Lenti-NC and Lenti-HMGB1 into HK-2 cells. Lenti-NC was used as a negative control and Lenti-HMGB1 was used to increase the expression of HMGB1 in HK-2 cells.

Cell Counting Kit-8 (CCK8) Assay

HK-2 cells were passaged into 96-well plates, and then the cells were treated differently and set up 3 replicate wells. Then, 10 μL of CCK8 reagent (Dojindo Molecular Technologies, Kumamoto, Japan) was added to each well. The cells were placed in incubator and incubated for 4 hours. Then, the 96-well plates were taken and the absorbance at 450 nm of each well was measured using a microplate reader.

Immunofluorescence (IF) Staining

Cell slides were placed in 24-well plates and HK-2 cells were cultured. After the cells were treated differently, the 24-well plates were taken out and the medium was discarded. After washing the cells with PBS, the cells were fixed with 4% paraformaldehyde and treated with 0.5% Triton-PBS. After blocking the cells with 10% goat serum, the cells were incubated with primary antibody dilution (GRP78, 1:500, rabbit, Abcam, Cambridge, MA, USA, ATF6, 1:500, rabbit, Abcam, Cambridge, MA, USA, HMGB1, 1:500, rabbit, Abcam, Cambridge, MA, USA, Nrf2, 1:500, rabbit, Abcam, Cambridge, MA, USA, NF-κB p65, 1:500, rabbit, Abcam, Cambridge, MA, USA) at 4°C overnight. After washing the cells the next day, the cells were incubated with fluorescent secondary antibody dilution for 1 h in the dark. Then the slides were taken and fixed using closure containing 4',6-diamidino-2-phenylindole (DAPI). Finally, the staining results were observed and recorded using a fluorescence microscope.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 21.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis in this study. Mean ± standard deviation represented the measurement data. After performing a normality test and a homogeneity test of variance on the data, the appropriate statistical method was select for analysis. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc test (Least Significant Difference). All experiments were repeated more than 3 times. $p<0.05$ indicated that the difference is statistically significant.

Results

Exogenous VASPIN Reduces Mouse Kidney IRI

To determine if VASPIN has a protective effect on the kidney, a mouse renal IRI model was constructed and mice were treated with VASPIN. HE staining (Figure 1A) showed significant swelling of the renal tubular epithelial cells in the IRI group and inflammatory cell infiltration in the interstitial, while VASPIN significantly improved the morphology of the renal tissue. The results of ELISA (Figure 1B, 1C) showed that the expression of kidney injury markers netrin-1 and L-FAPB in the urine of VASPIN-treated mice was significantly lower than that of the IRI group. In the serum of mice in the IRI group, the expression of Scr, BUN, IL-1β, and TNF-α was significantly increased, indicating that IRI causes kidney dam-

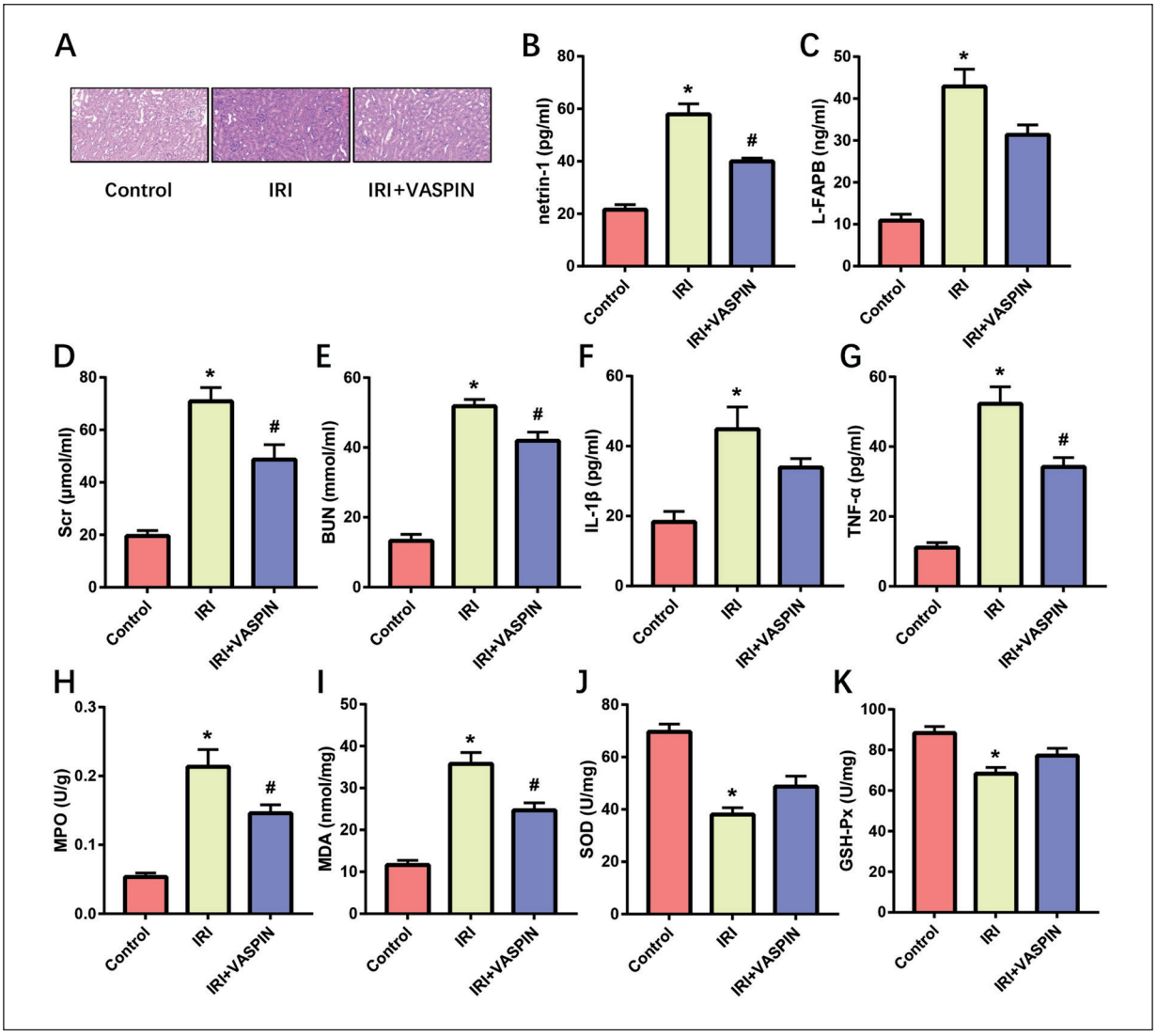

Figure 1. Exogenous VASPIN reduces mouse kidney IRI. **A,** HE staining of mice kidney (magnification: 40×). **B, C,** ELISA results of netrin-1 and L-FAPB, **D-G,** ELISA results of Scr, BUN, IL-1β, and TNF-α. **H-K,** Content of MPO, MDA, SOD, and GSH-Px in mice kidney tissue. ("*" means $p<0.05$ *vs.* the control group and "#" means $p<0.05$ *vs.* the IRI group).

age and increased inflammation in mice, while VASPIN can reduce their expression (Figure 1D-1G). In addition, the expressions of oxidative stress related indicators (MPO, MDA, SOD, and GSH-Px) in mouse kidney tissues were examined (Figure 1H-1K). The results showed that VASPIN could reduce MPO and MDA in kidney tissue and increased SOD and GSH-Px, showing good anti-oxidant capacity.

VASPIN Reduces ERS Levels in HK-2 Cells

HRO treatment was performed on HK-2 cells to construct IRI model at the cellular level. CCK-8 (Figure 2A) examined the effect of 1, 3, 5, 10, 20 μM VASPIN on the viability of HK-2 cells. The results indicated that 10 μM was the optimal concentration of VASPIN to stimulate HK-2 cells. Cell experiments were divided into three groups: control group, HRO group, and HRO+VASPIN group. The results of IF staining (Figure 2B, 2C) showed that the expressions of GRP78 and ATF6 in HK-2 cells of HRO group were significantly higher than those of the control group, and VASPIN could decrease their expressions. In addition, the results of Western blot (Figure 2D) and qRT-PCR (Figure 2E-2H) also confirmed the inhibitory effect of VASPIN on ERS-related signaling pathways.

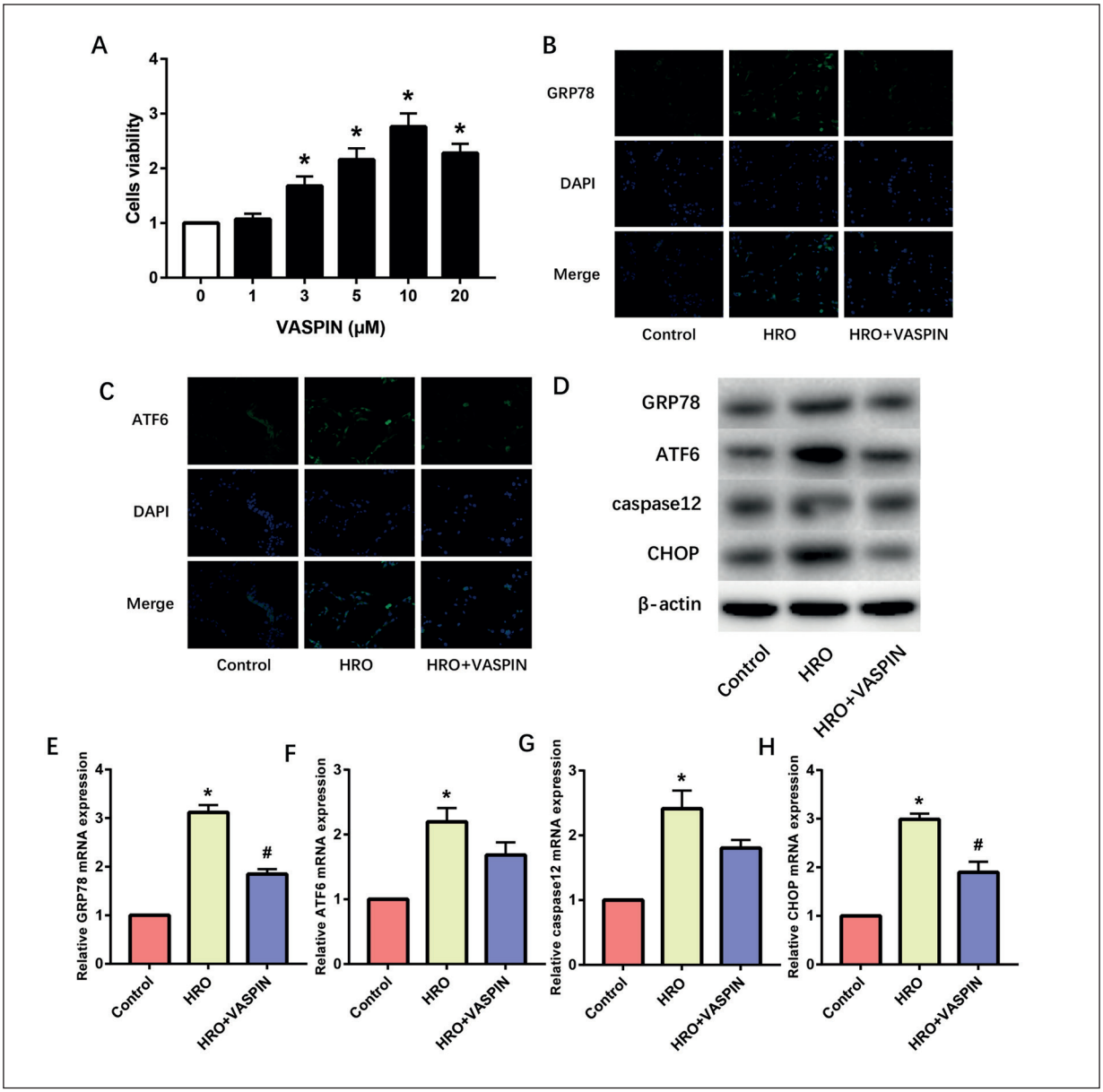

Figure 2. VASPIN reduces ERS levels in HK-2 cells. **A,** CCK8 assay of VASPIN. **B, C,** IF staining of GRP78 and ATF6 (magnification: 400×). **D,** Western blot results of GRP78, ATF6, caspase12, and CHOP, **E-H,** qRT-PCR results of GRP78, ATF6, caspase12, and CHOP. ("*" means $p<0.05$ *vs.* the control group and "#" means $p<0.05$ *vs.* the HRO group).

VASPIN Reduces the Expression of HMGB1 and Lenti-HMGB1 Attenuates the Anti-Inflammatory and Anti-Oxidative Effects of VASPIN

The expression changes of HMGB1 in HK-2 cells were firstly detected. The results of IF staining (Figure 3A), Western blot (Figure 3B), and qRT-PCR (Figure 3C) showed that HK-2 cells in the HPO group expressing more HMGB1 and VASPIN could decrease the expression of HMGB1. To determine the effect of HMGB1 on HK-2 cells, Lenti-HMGB1 was used to increase expression in HK-2 cells and detect its effect on HK-2 cells. The transfection efficiency of Lenti-HMGB1 was verified by Western blot (Figure 3D) and qRT-PCR (Figure 3E). The results of IF staining (Figure 3F-3G) indicated that HMGB1 increased the expression of GRP78 and ATF6, indicating that HMGB1 increased ERS levels in HK-2 cells. The results of the ELISA (Figure

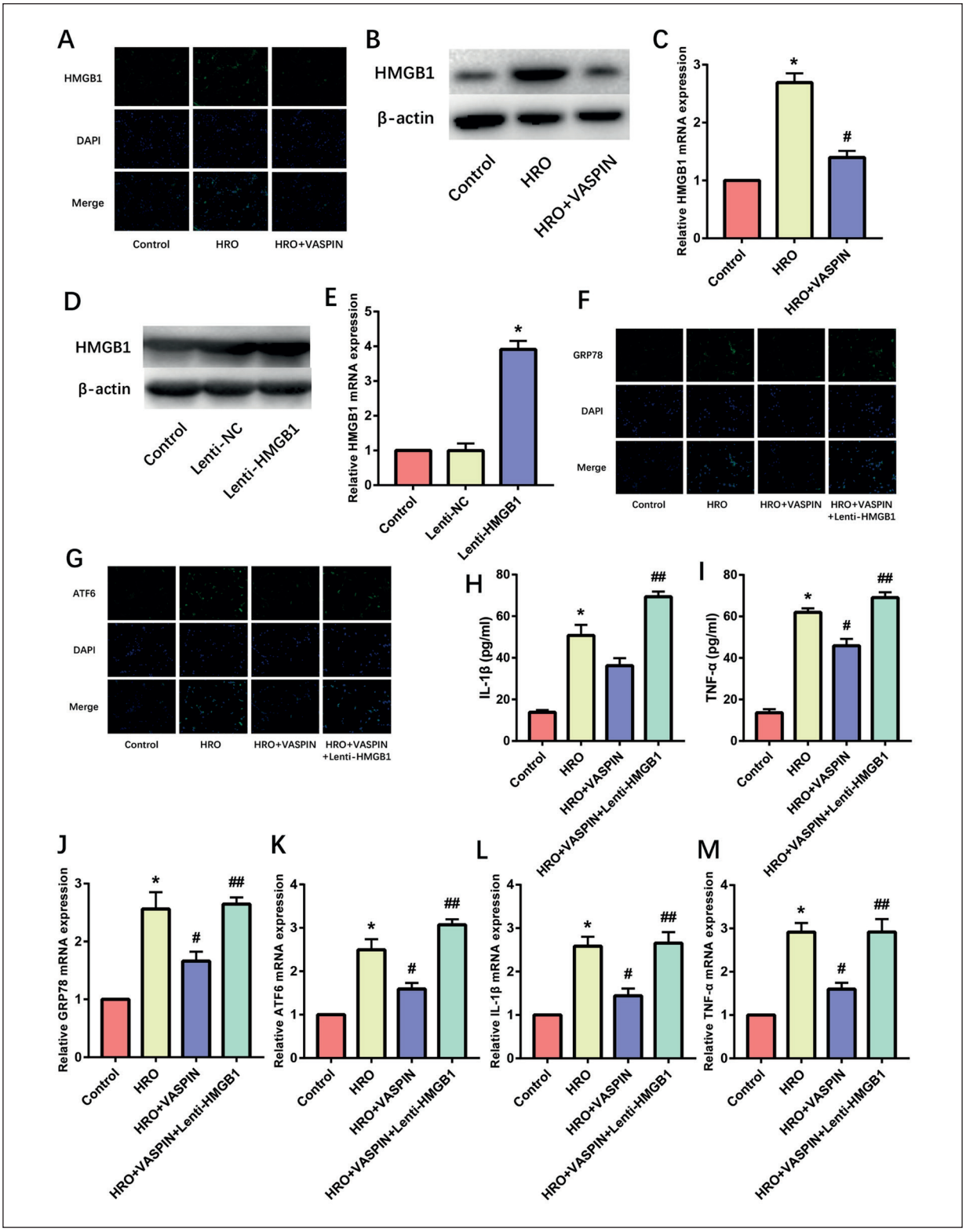

Figure 3. VASPIN reduces the expression of HMGB1 and Lenti-HMGB1 attenuates the anti-inflammatory and anti-oxidative effects of VASPIN. **A-C,** IF staining (magnification: 400×), Western blot and qRT-PCR results of HMGB1. **D, E,** Western blot and qRT-PCR results of HMGB1. **F, G,** IF staining of GRP78 and ATF6 (magnification: 400×). **H, I,** ELISA results of IL-1β and TNF-α; **J-M,** qRT-PCR results of GRP-78, ATF6, IL-1β, and TNF-α. ("*" means $p<0.05$ *vs.* the control group, "#" means $p<0.05$ *vs.* the HRO group, "##" means $p<0.05$ *vs.* the HRO+VASPIN group).

3H, 3I) also showed a pro-inflammatory effect of HMGB1 on HK-2 cells. The results of qRT-PCR (Figure 3J-3M) were similar to those of IF staining and Western blot.

VASPIN Increases the Activity of the Nrf2/ARE/HO-1 Signaling Pathway in HK-2 Cells and Inhibits the NF-κB Signaling Pathway

HMGB1 is a classical pro-inflammatory factor, so the NF-κB signaling pathway downstream of HMGB1 and the activity of the oxidative stress-related Nrf2/ARE/HO-1 signaling pathway were examined. The results of IF staining (Figure 4A, 4B) indicated that VASPIN could increase the expression of Nrf2 and decrease the expression of NF-κB p65. The results of Western blot (Figure 4C) and qRT-PCR (Figure 4D-4G) also showed that VASPIN could increase the expressions of Nrf2, ARE, and HO-1 and decrease the expression of NF-κB p65. These results indicate that VASPIN activates Nrf2/ARE/HO-1 signaling pathway and inhibits NF-κB signaling pathway.

Discussion

There are three N-terminal transmembrane proteins in the endoplasmic reticulum, including inositol-Requiring Enzyme 1a (IRE1α), protein kinase-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6)[8]. Under normal circumstances, the above three transmembrane proteins are inactivated by binding to GRP78/BiP. When the endoplasmic reticulum homeostasis is disturbed, ERS, BiP, and transmembrane proteins are separated, resulting in three major signaling pathways PERK/eIF2α, IRElα/XBP1s and ATF6/ERSE that initiate unfolded protein response (UPR). Among them, the up-regulation of GRP78 expression is a marker protein that confirms the occurrence of UPR. UPR is the mechanism that promotes the survival of damaged cells during ERS. However, when the UPR is unable to adapt to ERS, an excessively long UPR can cause tissue injury and organ dysfunction[9]. Therefore, GRP78, PERK, IRE1α, and ATF6 are important members of the UPR transition from pro-survival to pro-apop-

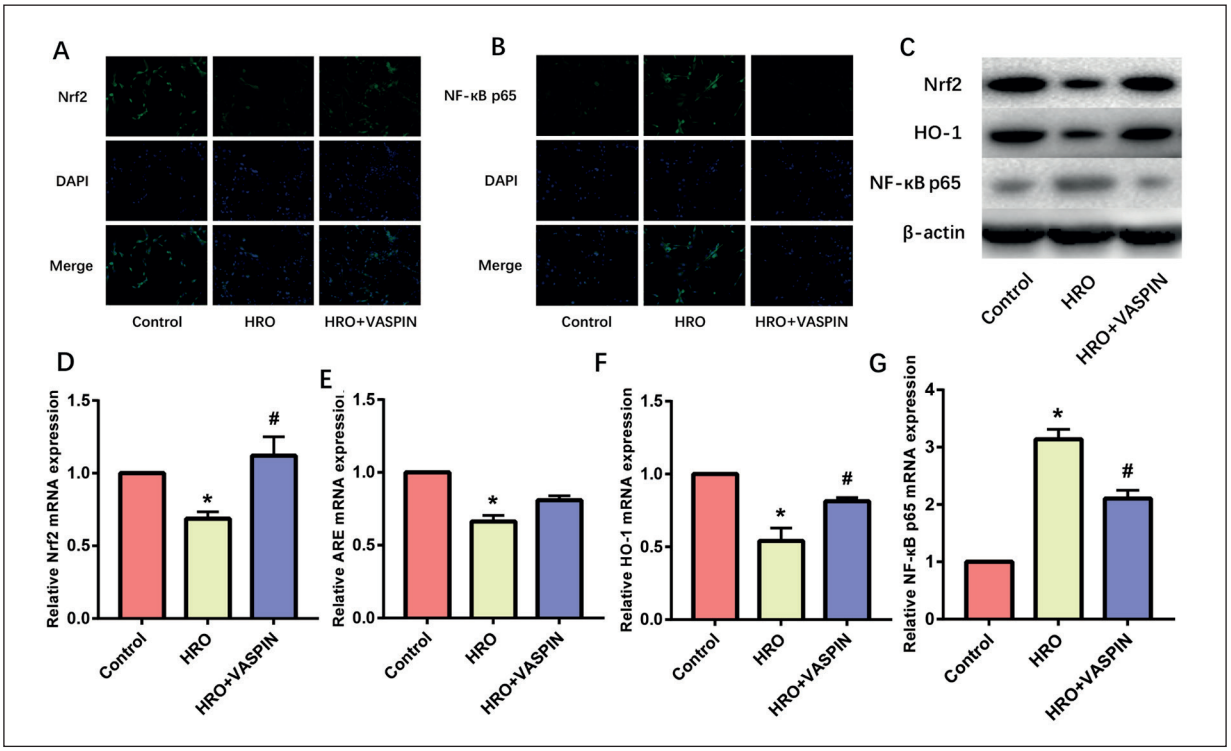

Figure 4. VASPIN increases the activity of the Nrf2/ARE/HO-1 signaling pathway in HK-2 cells and inhibits the NF-κB signaling pathway. **A, B,** IF staining results of Nrf2 and NF-κ*B* p65 (magnification: 400×). **C-G,** Western blot and qRT-PCR results of Nrf2, ARE, HO-1 and NF-κB p65. ("*" means $p<0.05$ *vs.* the control group and "#" means $p<0.05$ *vs.* the HRO group).

totic. Moreover, ERS-induced inflammation and oxidative stress exacerbate glomerular and tubular damage in AKI patients. Based on the above studies, regulation of ERS has become a new therapeutic strategy to protect the kidneys from injury. 4μ8C and MKC-3946 can inhibit IRE1α to reduce ERS and protect against organ damage. Pretreatment with tunicamycin and thapsigargin can improve UPR, reduce ERS, and improve kidney injury. The chemical chaperone 4-Phenylbutyric Acid (4-PBA) inhibits ERS-induced apoptosis, reduces renal interstitial damage, and reduces mortality in mice with proteinuria-induced progression of chronic kidney disease[10]. In this study, renal IRI in exogenous VASPIN-treated mice was significantly improved, manifested by improved morphology of renal tubular epithelial cells and decreased markers of renal injury. In addition, the expressions of CHOP, GRP78, and ATF6 in VASPIN-stimulated HK-2 cells were significantly reduced, indicating that VASPIN can attenuate ERS caused by HRO.

This research found that VASPIN could increase the expression of HMGB1 in HK-2 cells. The physiological functions of HMGB1 in cells and extracellular are vastly different. In the nucleus, HMGB1 is composed of 215 amino acids and is highly conserved. It contains two homologous DNA binding regions, BoxA and BoxB, which are the main molecular structural basis for the regulation of HMGB1 in the nucleus[11]. It can regulate the transcriptional activity of transcription factors by non-specific binding to DNA to alter the DNA double helix structure. The function of HMGB1 is not limited to the nucleus. When HMGB1 is exposed to some external stimuli, it can also be secreted from the nucleus to the extracellular space and regulate inflammation development through the high affinity with eukaryotic cell membrane receptor (such as receptor for advanced glycation end products, RAGE and Toll-like receptor, TLR)[12]. TLR4 acts as a receptor for extracellular HMGB1 and plays an extremely important role in mediating the biological effects of HMGB1. Studies have shown that inhibition of HMGB1 function in mice can effectively improve AKI caused by IRI. In TLR4 knockout transgenic mice, it can be found that knockdown of TLR4 has protective effect against AKI caused by renal IRI. Michel et al[13] found that after 4 h of AKI occurred in IRI, TLR4 expression in renal endothelial cells near the outer medulla began to increase significantly. This is the main site of the initial inflammatory response of AKI, and the inflammatory cells are abundant in the early stage. However, the infiltration of inflammatory cells into renal parenchyma requires the binding of related adhesion molecules such as ICAM-1 and VCAM-1 on renal parenchyma cells. In this case, the expression of TLR4 is increased, and the renal interstitial cells are damaged to release a large amount of HMGB1[14]. The binding of HMGB1 to TLR4 significantly increased the expression of intercellular adhesion molecules. In turn, it promotes the development of early inflammation of AKI. In this study, the expression of inflammatory factors and ERS-related molecules in HK-2 cells overexpressing HMGB1 was significantly increased, which attenuated the protective effect of VASPIN on HK-2 cells. In addition, the activity of the NF-κB signaling pathway downstream of HMGB1 was also reduced by VASPIN and VASPIN also increased the activity of the anti-oxidant signaling pathway Nrf/ARE/HO-1 signaling pathway, which also confirmed the anti-inflammatory and anti-oxidant effect of VASPIN on renal tubular epithelial cells.

To sum up, VASPIN exhibits good anti-inflammatory and anti-oxidant effects and relieves ERS in renal IRI, which is beneficial to the protection of the kidneys. Therefore, VASPIN has the potential to help clinically treat renal IRI.

Conclusions

In this study we showed that exogenous VASPIN improved renal IRI in mice, manifested by improved morphology of renal tubular epithelial cells and decreased levels of renal inflammation and oxidative stress. In addition, in HRO-treated HK-2 cells, ERS levels increased and HMGB1 activity also increased. After stimulation of HK-2 cells with VASPIN, ERS levels and HMGB1 expression were significantly reduced. In addition, VASPIN can promote the Nrf2/ARE/HO-1 signaling pathway and reduce the activity of the NF-κB signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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