



Overexpression of aquaporin 2 in renal tubular epithelial cells alleviates pyroptosis

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Background: Severe renal ischemia-reperfusion injury results in worse outcomes of kidney transplantation. Compared to the collecting duct, the proximal tubule is more likely to exhibit severe pyroptosis and damage during renal ischemia-reperfusion. Aquaporins were reported of having regulatory roles in pyroptosis. We explored whether aquaporin 2 overexpression in proximal tubular cells could alleviate ischemia-reperfusion injury related pyroptosis.

Methods: A renal ischemia-reperfusion model of mice was established, and human kidney 2 cells were treated with hypoxia-reoxygenation. Aquaporin 2 overexpression was achieved in human kidney 2 cells transfected with lentivirus, which were then cultured with murine cells. Renal tissues and serum of the mice, and human kidney 2 cells were subjected to histological, molecular, and biochemical examinations.

Results: Compared with the sham group, the renal function of the ischemia-reperfusion group was significantly decreased, and the tissue injury was severe and accompanied by more nuclear dissolved and necrosis. Besides, the expression of aquaporin 1-5 decreased significantly, while the expression of Töll-like receptor 4, caspase-1, kim-1 and interleukin 1 β and 18 increased significantly in ischemia-reperfusion group. Similar results were observed in the human kidney 2 cells test. Overexpression of aquaporin 2 partially reversed the cell damage, pyroptosis, and molecular expression changes of human kidney 2 cells induced by hypoxia-reoxygenation.

Conclusions: Our findings suggest that aquaporin 2 overexpression can potentially reduce pyroptosis in proximal tubular cells, and thus might be a novel target for relieving pyroptosis and injury in renal ischemia-reperfusion injury.

Keywords: Aquaporin; ischemia-reperfusion injury; kidney transplantation; pyroptosis

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Introduction

Kidney transplantation is the optimal treatment for end-stage renal disease and is far superior to hemodialysis in terms of long-term survival rate, quality of life and treatment cost (1). In most cases, allografts undergo warm

and cold ischemia and subsequent ischemia-reperfusion injury (IRI). Allografts with longer ischemic time (14 hours) were reported to be more likely to demonstrate delayed graft function, and the risk of graft loss increased by 1% for per hour increase in the duration of total ischemia in

comparison with allografts with shorter ischemic time (<14 hours) (2). As a result of necrosis of renal tubular epithelial cells, renal IRI not only delays the recovery of graft function, but also promotes rejection. Because of specific immunity that results from antigen exposure caused by injury, renal IRI affects the long-term survival rate of renal allografts and recipients (3). Evidence indicating that allografts with early resistance to IRI have significantly enhanced function 5 years after transplantation in recipients and may prolong life is robust (4). Although good results have been achieved, the alleviation of IRI and the improvement in prognosis are still not ideal (5).

During IRI, in comparison with the collecting duct, the renal proximal tubule is more susceptible to tissue damage that is more likely to be severe. Phenotypic differences between the collecting duct and the proximal tubule might account for the difference in susceptibility. Aquaporin 2 (AQP2), one member of aquaporin (AQP) family, is considered to be expressed mostly in the collecting duct, and its regulating action is responsible for fine-tuning the final urine volume to meet the current demands of the body (6). Besides being involved in fluid transport, research has revealed the modulatory role of AQPs in interleukin (IL)-1 β -related inflammation (including pyroptosis), in pathophysiological processes in tissue and organ inflammation, in oxidative stress and in IRI (7). AQP deletion is linked to worse prognosis of patients with renal IRI (8). Moreover, the regulating activities of AQPs can affect the survival of allografts (7). However, there is no evidence clarifying the effect of AQPs on pyroptosis in renal IRI.

Because of the differences between the collecting duct and the proximal tubule in response to IRI and their differential expressions of AQPs, we evaluated the potential effects of AQPs on renal IRI, with a particular focus on the AQP2 and the process of pyroptosis.

We present the following article in accordance with the ARRIVE reporting checklist (available at <http://dx.doi.org/10.21037/tau-21-71>).

Methods

The renal IRI model in mice

Adult C57BL/6 male mice (eight weeks old; Chengdu Dossy Experimental Animals, Co., Ltd., Chengdu, China) were raised under standard guidelines. The Animal Ethics Committee of West China Hospital, Sichuan University, Chengdu, China (No. 2019141A) approved this study. All experimental

procedures were followed by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Fifteen mice were fed adaptively for 7 days and then randomly divided into two groups. To establish a renal IRI model, the bilateral renal pedicles were clamped simultaneously with a non-invasive artery clamp for 30 minutes (Figure S1, n=8); for the sham model, the operation lasted for 30 minutes without clamping (n=7). All mice were sacrificed after 24 hours, and their blood and renal tissues were collected.

The human kidney 2 (HK-2) cell hypoxia-reoxygenation (H/R) model

Both HK-2 and 293T cells were obtained from the Institute of Urology, West China Hospital of Sichuan University, Chengdu, China. HK-2 cells were cultured according to the recommended methods of the American Type Culture Collection (9). Some cells were cultured in conditions of hypoxia for 24 hours and then in normal oxygen conditions for 12 hours to achieve H/R, as previously described (10).

A lentiviral expression vector (pLVX-AcGFP1-N1) was utilized to construct plasmids containing the target gene AQP2 (NCBI Reference Sequence: NM_000486.5). The lentivirus was packaged and concentrated through 293T cell transfection. The virus titre was measured under a fluorescence microscope (Figure S2). HK-2 cells were transfected to obtain cells that overexpressed AQP2 (AQP2hi cells) and control cells created through the use of non-related blank vectors (NR cells).

HK-2 cells were added to 6-(3×10^5 cells per well), 24-(2.5×10^4 cells per well), or 96-well plates (4×10^3 cells per well) and cultured with or without H/R. Thereafter, cells were divided into the following six groups: negative control (NC, cells cultured without H/R and transfection), H/R (cells cultured with H/R, but without transfection), NR (NR cells cultured without H/R), AQP2hi (AQP2hi cells cultured without H/R), NR + H/R (NR cells cultured with H/R), and AQP2hi + H/R (AQP2hi cells cultured with H/R).

Histopathological staining

The collected renal tissues of the mice were formalin-fixed, paraffin-embedded and then sectioned for subsequent haematoxylin-eosin (HE) staining. The following procedures were performed as previously described.

Immunofluorescence staining was performed to assess the expression of AQP2 and caspase-1. For frozen-section immunofluorescence staining, sections were fixed, blocked

and then stained with the primary antibodies AQP2 (1:500, PAA580Hu01; Cloud-Clone Corp., Wuhan, China) and caspase-1 (1:100, PAB592Hu01; Cloud-Clone Corp.). The fluorescent secondary antibodies were fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G (1:500, A0562; Beyotime Biotechnology Co., Ltd., Haimen, Jiangsu, China) and 4',6-diamidino-2-phenylindole (C1002; Beyotime Biotechnology Co., Ltd.). Sections were then mounted with anti-fade mounting medium (Abcam Co., Ltd, Cambridge, MA, USA).

FAM-FLICA kits (ICT098; ImmunoChemistry Technologies LLC., Bloomington, MN, USA) were utilized to conduct FAM-YVAD-FMK/Hoechst/propidium iodide (PI) staining according to the manufacturer's instructions. Images were collected using a fluorescence microscope.

Cell proliferation assay

Cell proliferation was assessed with the Cell Counting Kit-8 (CCK8; C0037; Beyotime Biotechnology Co., Ltd.). Cells were added to 96-well plate (5×10^3 cells per well) and cultured as described above. Then, reagent containing 10% CCK8 solution was added, and plates were incubated for 4 hours. Optical density (OD) values for calculating cell proliferation were measured at 450 nm with using a microplate reader (EnVision, PerkinElmer Co., Ltd., Shanghai, China).

The concentration lactate dehydrogenase (LDH), IL-1 β , IL-18, serum creatinine (Scr), blood urea nitrogen (BUN), and caspase-1 activity

The release rates of LDH (C0016; Beyotime Biotechnology Co., Ltd.), Scr (C011-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) and BUN (C013-2; Nanjing Jiancheng Bioengineering Institute) were measured with corresponding test kits; IL-1 β (SEA563Mu; Wuhan USCN Business Co., Ltd., Wuhan, China) and IL-18 (SEA064Mu; Wuhan USCN Business Co., Ltd.) were measured with enzyme-linked immunosorbent assay kits; and caspase-1 activity was measured with the Caspase 1 Activity Assay kit (C1101; Beyotime Biotechnology Co., Ltd.). All tests were performed according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNAs of tissues/cells were extracted with TRIzol;

complementary DNAs were synthesized with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). After reverse transcription and dilution, primers (see [Appendix 1](#) for details) were applied to PCR reactions.

Western blotting

Radioimmunoprecipitation assay lysate solution was used to prepare cell/tissue lysates. Protein concentrations were measured with the BCA Protein Assay Kit (Beyotime Biotechnology Co. Ltd, Haimen, Jiangsu, China). After membrane transfer and blocking, primary antibodies, including caspase-1, IL-1 β , AQP2, kidney injury molecule 1 (kim-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were incubated. Then, the corresponding secondary antibodies were incubated, and gels were detected with chemiluminescence.

Statistical analysis

The data were calculated as means \pm standard deviations. All analyses were performed with SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA, USA). Among multiple groups, the one-way NOVA analysis, followed with a Tukey test was used to compare the differences of all column pairs. For difference between two groups, Student's *t*-test was applied. $P < 0.05$ was considered to be significant.

Results

Renal IRI resulted in pyroptosis and downregulated the expression of aquaporins

In renal tissues of mice with IRI ($n=8$), severe exfoliation of renal tubular epithelial cells was observed, the nuclei of which were dissolved and necrotic. Some renal tubules were dilated and ruptured (*Figure 1*). These renal tissues had significantly less relative messenger RNA (mRNA) expressions of AQP1-AQP5 (all $P < 0.05$; *Figure 1*), and less protein expression of AQP2 than did the renal tissues from the sham group ($P=0.019$; *Figure 1*; $n=7$), and on the immunofluorescence test ($P=0.031$; *Figure 2*). However, tissues from the IRI group ($n=8$), compared with those from the sham group ($n=7$), exhibited significantly more mRNA expression of Toll-like receptor 4 (*Figure 1*); higher activity of caspase-1 ($P < 0.001$; *Figure 1*); and more protein expression of kim-1 ($P=0.010$; *Figure 1*), IL-1 β ($P=0.015$;

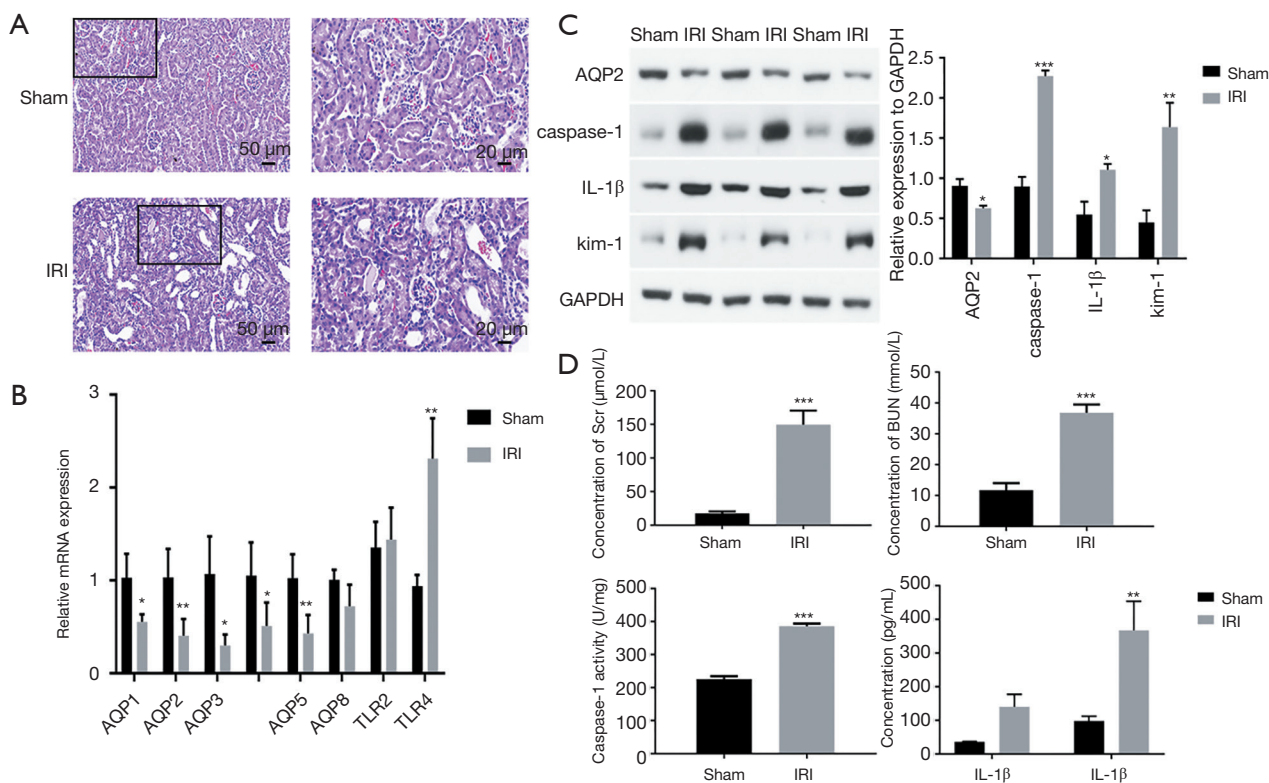


Figure 1 Renal IRI resulted in pyroptosis and downregulation of AQPs *in vivo*. (A) HE staining. In the renal tissues of the IRI group (n=8), severe exfoliation of renal tubular epithelial cells was observed, of which the nuclei were dissolved and necrotic. Some renal tubules were dilated and ruptured. (B) qRT-PCR analysis. IRI renal tissues had significantly less mRNA expressions of AQP1-AQP5 and numerically less mRNA expression of AQP8 but significantly more relative mRNA expression of Toll-like receptor 4. (C) Western blot analysis. IRI renal tissues had significantly less expression of AQP2 but significantly more expression of kim-1, IL-1 β and caspase-1 than renal tissues of the sham group (n=7). (D) Measurement of serum concentration of IL-1 β , IL-18, Scr and BUN, and renal activity of caspase-1 test. IRI group had higher serum concentrations of Scr, BUN, IL-1 β and IL-18, and higher activity of caspase-1 than the sham group. Compared with the sham group: *means $0.01 \leq P \leq 0.05$; ** means $0.001 \leq P \leq 0.01$; ***means $P < 0.001$. AQP, aquaporin; BUN, blood urea nitrogen; HE, haematoxylin-eosin; IL, interleukin; IRI, ischemia-reperfusion injury; kim-1, kidney injury molecule 1; mRNA, messenger RNA; Scr, serum creatinine.

Figure 1) and caspase-1 (on Western blot test, $P < 0.001$, Figure 1; and on immunofluorescence test, $P < 0.001$, Figure 2). In addition, the IRI group, in comparison with the sham group, exhibited higher serum concentrations of Scr ($P < 0.001$), BUN ($P < 0.001$), IL-1 β ($P = 0.003$) and IL-18 ($P = 0.002$; Figure 1).

H/R promoted pyroptosis and downregulated AQP2 in HK-2 cells

In the CCK8 test (Figure 3), OD values were all significantly lower in the H/R group than that in the NC group after 24-, 36-, 48-, and 60-hour cultures (all $P < 0.01$) at the same time

point. The H/R group, in comparison with the NC group, demonstrated significantly less expression of AQP2 by mRNA ($P = 0.019$, Figure 3) and protein ($P = 0.008$, Figure 3), while the H/R group had significantly higher activity of caspase-1 ($P < 0.001$, Figure 3) and more expression of kim-1 ($P < 0.001$, Figure 3), IL-1 β ($P < 0.001$, Figure 3) and caspase-1 ($P = 0.012$, Figure 3). In the supernatant of the H/R group, in comparison with the control group, concentrations of IL-1 β ($P = 0.023$) and IL-18 ($P = 0.012$) were higher, as well as the release rate of LDH (Figure 3). According to FAM-YVAD-FMK/Hoechst/PI staining, the fluorescence intensity of both FAM-YVAD-FMK and PI in the H/R group was higher than that in the control group (Figure 4).

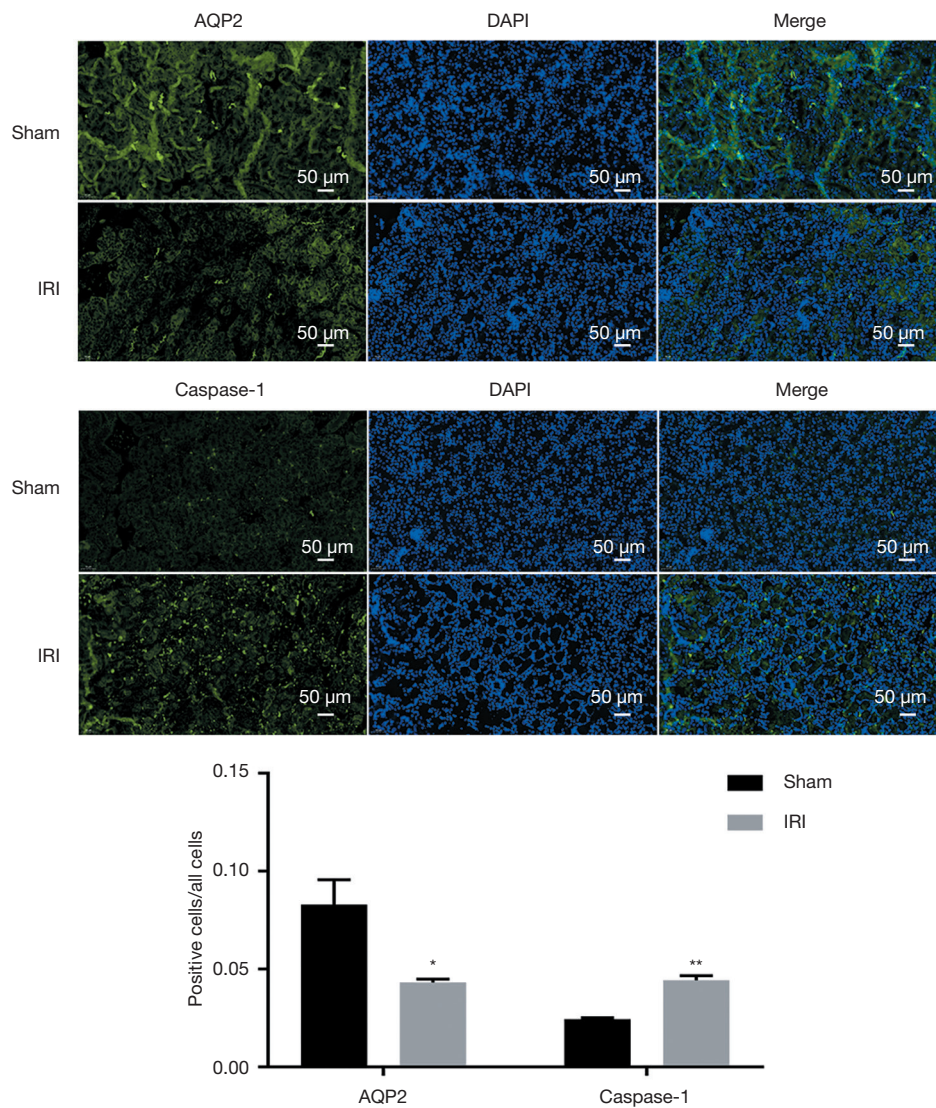


Figure 2 Immunofluorescence staining of renal tissues. Tissues from mice with IRI (n=8) demonstrated significantly lower fluorescence intensity of AQP2 but higher fluorescence intensity of caspase-1 than those from the mice that underwent sham surgery (n=7). AQP, aquaporin; IRI, ischemia-reperfusion injury. *means $0.01 \leq P \leq 0.05$; **means $0.001 \leq P \leq 0.01$.

Upregulation of AQP 2 alleviated H/R induced pyroptosis of HK-2 cells

The AQP2 vector achieved acceptable overexpression efficiency (Figure 5; supplementary materials showed details). In the CCK8 test (Figure 5), OD values were all significantly lower in the NR + H/R group than in the NR and AQP2hi + H/R group after 24-, 36-, 48-, and 60-hour culture (all $P < 0.05$) at the same time point. In contrast, after 24-, 36-, 48-, and 60-hour culture, OD values were higher

in the AQP2hi group than in the AQP2hi + H/R group (all $P < 0.05$) and were comparable with those in the NR group (all $P > 0.05$). In the Western blot assay (Figure 5), the NR + H/R group, in comparison with the NR group, exhibited significantly less expression of AQP2 ($P = 0.014$) but more expression of kim-1 ($P < 0.001$), IL-1 β ($P = 0.010$) and caspase-1 ($P = 0.003$). Similarly, the AQP2hi + H/R group, in comparison with the AQP2hi group, expressed less AQP2 ($P = 0.009$) but more kim-1 ($P = 0.012$), IL-1 β ($P = 0.006$) and

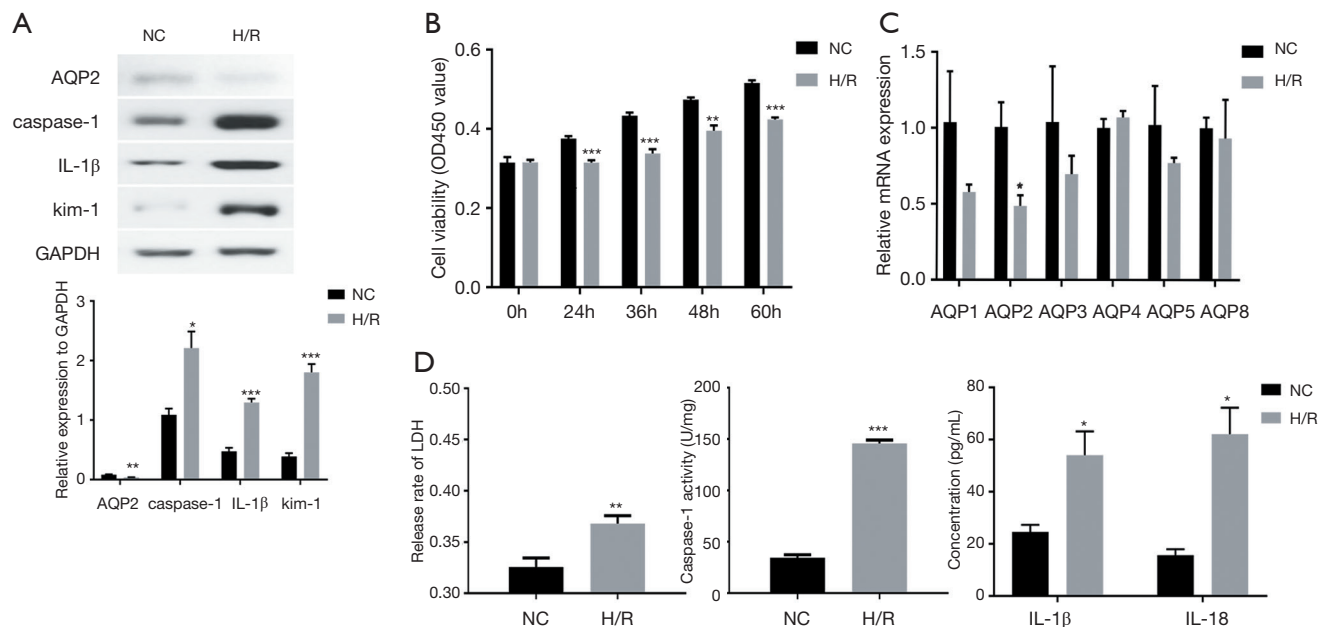


Figure 3 H/R resulted in pyroptosis and downregulation of AQP2 in HK-2 cells. (A) Western blot analysis. Tissues from mice that received H/R (the H/R group) expressed significantly less AQP2 but significantly more kim-1, IL-1β and caspase-1 than did those of the NC group. (B) Testing with the Cell Counting Kit-8. The OD values at the same time point were all significantly lower in the H/R group than in the NC group after 24-, 36-, 48-, and 60-hour culture. (C) qRT-PCR analysis. In the H/R group, mRNA expressed significantly less AQP2 and numerically less AQP1, APQ3 and AQP5 than did the NC group; expressions of AQP4 and AQP8 were comparable between the two groups. (D) Measurement of concentration of IL-1β and IL-18 in supernatant, and release rate of LDH and activity of caspase-1 in cells. Concentrations of IL-1β and IL-18 in the supernatant were significantly higher for the H/R group than for the NC group. The release rate of LDH and activity of caspase-1 was significantly higher in the H/R group than in the NC group. Compared with the sham group: *means $0.01 \leq P < 0.05$; **means $0.001 \leq P < 0.01$; ***means $P < 0.001$. AQP, aquaporin; HK-2, human kidney 2; H/R, hypoxia-reoxygenation; IL, interleukin; IRI, ischemia-reperfusion injury; kim-1, kidney injury molecule 1; LDH, lactate dehydrogenase; mRNA, messenger RNA; NC, negative control; OD, optical density.

caspase-1 ($P=0.002$). However, the AQP2hi + H/R group, in comparison with the NR + H/R group, expressed less kim-1 ($P=0.002$), IL-1β ($P=0.049$) and caspase-1 ($P=0.017$).

The AQP2hi + H/R group had lower caspase-1 activity than the NR + H/R group ($P=0.002$, *Figure 5*). The concentrations of IL-1β, IL-18, and the release rate of LDH were higher in the supernatant of the NR + H/R group than that of the NR group and higher in the AQP2hi + H/R group than in the AQP2hi group (all $P < 0.05$). The supernatant of the AQP2hi + H/R group had a significantly lower concentration of IL-18 ($P=0.024$) and release rate of LDH ($P=0.004$) and a non-significantly lower concentration of IL-1β ($P=0.065$) than did the NR + H/R group. In FAM-YVAD-FMK/Hoechst/PI staining, the fluorescence intensities of both FAM-YVAD-FMK and PI were higher in the NR + H/R and AQP2hi + H/R groups than in the NR

and AQP2hi groups (*Figure 4*). The AQP2hi + H/R group showed weaker FAM-YVAD-FMK and PI fluorescence intensities than did the NR + H/R group.

Discussion

Previous study has reported that the urinary exosomal release of AQP1 and AQP2 during severe acute kidney injury in mice were significant decreased (11). The expression of renal AQP1 and AQP2 were also reported to be downregulated when urine volume was decreased in recipients of kidney transplants, and AQP2 downregulation was related to a decline in the glomerular filtration rate (12). In our study, we found the mRNA expression of AQP1-AQP5 were significantly decreased and the AQP8 was numerically decreased after IRI initiation in mice kidneys.

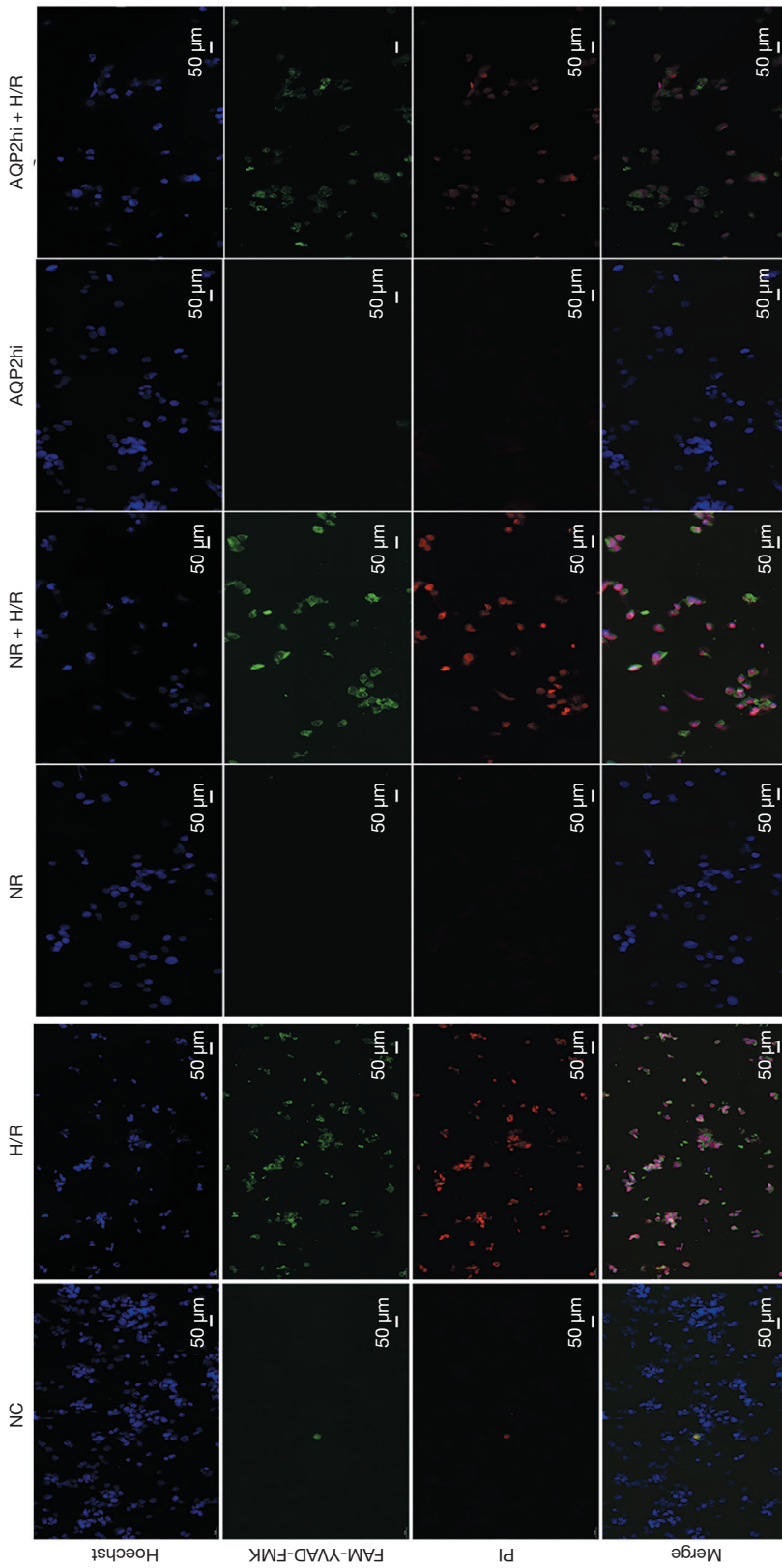


Figure 4 FAM-YVAD-FMK/Hoechst/PI staining of HIK-2 cells. Fluorescence intensity of FAM-YVAD-FMK staining and PI staining was significantly greater in the tissues of mice treated with H/R than in the NC group. The fluorescence intensities of FAM-YVAD-FMK and PI staining were greater in cells created with non-related blank vectors cultured without H/R (NR + H/R group) and in cells that overexpressed AQP2 and were cultured with H/R (AQP2hi + H/R group) than in the NR group and in cells that overexpressed aquaporin 2 but were not treated with H/R (AQP2hi group). FAM-YVAD-FMK and PI fluorescence intensities were weaker in the AQP2hi + H/R group than in the NR + H/R group. AQP, aquaporin; HK-2, human kidney 2; H/R, hypoxia-reoxygenation; NC, negative control; NR, non-related; PI, propidium iodide.

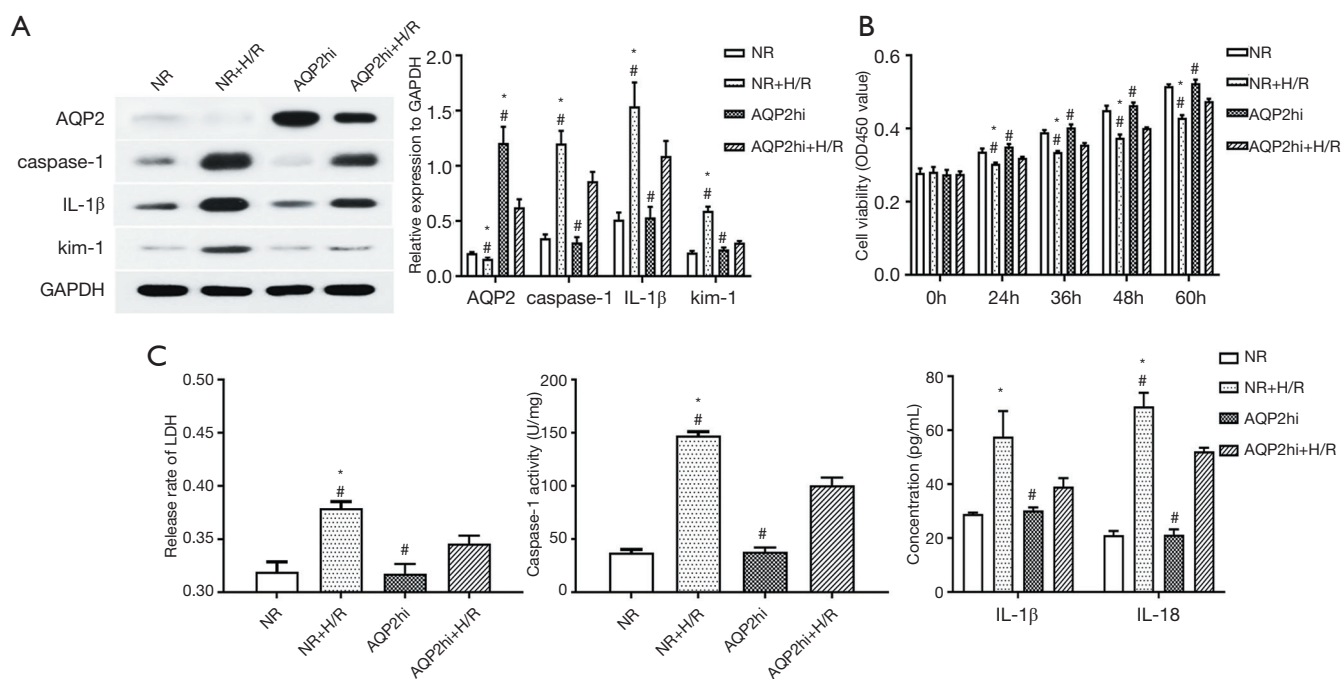


Figure 5 AQP2 overexpression alleviates the pyroptosis of HK-2 cells. (A) Western blot analysis. The cells that overexpressed AQP2, whether treated with H/R (the AQP2hi + H/R group) or not (the AQP2hi group) showed significantly higher AQP2 expression than did the cells created with non-related blank vectors, whether treated with H/R (the NR + H/R group) or not (the NR group), respectively. The NR + H/R and AQP2hi + H/R groups expressed significantly less AQP2 but more kim-1, IL-1 β and caspase-1 than did the NR and AQP2hi groups, respectively. The AQP2hi + H/R group expressed less kim-1, IL-1 β and caspase-1 than the NR + H/R group. (B) Testing with the Cell Counting Kit-8. The OD values at the same time point were all significantly lower in the NR + H/R group than in either the NR or AQP2hi + H/R group after 24-, 36-, 48-, and 60-hour culture. After 24-, 36-, 48-, and 60-hour culture, the OD values of the AQP2hi group were higher than those of the AQP2hi + H/R group but comparable with those of the NR group. (C) Measurement of concentration of IL-1 β and IL-18 in supernatant, and release rate of LDH and activity of caspase-1 in cells. The NR + H/R and AQP2hi + H/R groups had significantly higher caspase-1 activity, release rates of LDH and supernatant concentrations of IL-1 β and IL-18 than did the NR and AQP2hi groups, respectively. The AQP2hi + H/R group had lower caspase-1 activity, release rates of LDH and supernatant concentrations of IL-1 β ($P=0.065$) and IL-18 than did the NR + H/R group. * means $P<0.05$ (compared with the NR group); # means $P<0.05$ (compared with the AQP2hi + H/R group). AQP, aquaporin; HK-2, human kidney 2; H/R, hypoxia-reoxygenation; IL, interleukin; IRI, ischemia-reperfusion injury; kim-1, kidney injury molecule 1; LDH, lactate dehydrogenase; NC, negative control; NR, non-related; OD, optical density.

Current research has confirmed that during IRI, amounts of radical oxygens were generated, accompanied by pro-inflammatory response, which increased renal cell apoptosis, autophagy and irreversible cell death (13). While, increasing expression of AQPs could reduce cell injury by reducing oxidative stress, promoting metabolism, and inhibiting the signals involving apoptosis and inflammation. We also confirmed that the IRI group *in vivo* had higher expression of Toll-like receptor 4, caspase-1, kim1, and IL-1 β ; higher caspase-1 activity; and higher concentrations of IL-1 β and IL-18 in blood; while less AQP2 expression were observed. Similar results were also observed in HK-2 cells

in vitro experiments, and the H/R group cells were found to have more pyroptosis by FAM-YVAD-FMK/Hoechst/PI staining. The overexpression of AQP2 partially reversed the above changes of HK-2 cells. These results revealed the potential modulatory role of AQP2 in pyroptosis in the proximal tubule, and overexpression of AQP2 might reduce injury from renal IRI and improve renal outcome.

Pyroptosis is a type of cell death with different mechanism, characteristics, and outcome from those of apoptosis or necrosis (14). According to reported studies, the process of tissue IRI accompanies the occurrence of pyroptosis and decrease of AQPs levels (11,15), and these

were consistent with the results we observed in the mice kidneys of IRI model. Caspase-1 is capable of cleaving Gasdermin D, which is essential to promote pyroptosis, to the formation of large pores in the plasma membrane (16). Furthermore, caspase-1 promote IL-1 β and IL-18 from inactive precursors to maturation through these formed pores or upon cell lysis, then leading to pyroptosis (14). Pyroptosis plays an important role in renal IRI (17). In this study, the upregulation of caspase-1, IL-1 β , and IL-18 were observed in IRI group, suggesting that caspase-1-mediated pyroptosis may be involved in renal IRI. The suppression of pyroptosis potentially prevented the process of organ IRI. Chun et al. found that, in rats, hydrogen sulfide protected against lung injury after limb ischemia-reperfusion by alleviating inflammation and AQP1/AQP5 expression abnormalities (18). Besides, AQP3 deletion in murine kidneys resulted in worse IRI outcomes (8). However, to our knowledge, no other study has focused on the relationship between AQPs and pyroptosis in renal IRI. In this study, the expression of AQP2 in HK-2 cells in the H/R group was significantly down-regulated, accompanied by lower cell proliferation rate, more release of IL-1 β and IL-18, and more pyroptosis. However, the overexpression of AQP2 in HK-2 cells exhibited milder pyroptosis and less cell injury than that of the control cells after H/R. The results of this study indicate that increased AQP2 expression in HK-2 cells may reduce pyroptosis during H/R.

The lower expression of AQP2 was observed in IRI groups, accompanied by an increase in pyroptosis, which was consistent with other studies (19,20). Besides, this study showed that the exogenous overexpression of AQP2 attenuated the HK-2 cell damage and pyroptosis caused by H/R, which may be related to the down-regulation of caspase-1 expression and activity. However, this study still had some limitations. On the one hand, we only studied the effects of overexpression of AQP2 on the proliferation and pyroptosis of HK-2 cells, the exact mechanism of AQP2 in modulating pyroptosis and the changes in renal physiological function after AQP2 overexpression remained unknown. On the other hand, it is thought that AQP2 expression is mainly expressed in the distal tubules and collecting ducts. However, some studies suggested that the expression of AQP2 could also be detected in HK-2 cells, suggesting that the weakly expressed AQP2 in renal proximal tubular cells may be a potential research target (21,22). Furthermore, the pathophysiological mechanism of kidney IRI is complex. Increasing the expression of AQP2 alone may not completely reverse the injury of

renal tubular cells induced by ischemia-reperfusion, but a potential therapeutic target. Currently, it is reported that calcium and cAMP/PKA signaling activators can induce AQP2 activation. Prostaglandin E2 (PGE2) receptors EP2 and EP4, can selectively increase the transcription and membrane accumulation of AQP2 (23). Moreover, EP4 has been confirmed to be able to attenuates renal ischemia-reperfusion (I/R) injury by against I/R-induced oxidative stress, necrosis, apoptosis and autophagy (24,25). In further studies, more *in vivo* animal experiments will be carried out to explore the effect of overexpression of AQP2 on the kidneys of IRI and its potential mechanism. Due to the large physiological differences between humans and mice, the application of the results of this study to the clinic still needs more researches.

Conclusions

This study provided a novel insight into the modulatory role of AQP2 in pyroptosis of HK-2 cells. These results indicated that increasing AQP2 expression in proximal tubular cells could potentially reduce pyroptosis during renal IRI. Drugs targeting AQPs may have broad potential clinical applications (26). The development of reagents that increase AQP2 expression for the treatment of renal IRI is expected in the foreseeable future.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at <http://dx.doi.org/10.21037/tau-21-71>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Animal Ethics Committee of West China Hospital, Sichuan University, Chengdu, China (No. 2019141A) approved this study. All experimental procedures were followed by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Primers included (from 5' to 3'):

h-AQP1 (194 bp): F- ATTTTCTGGGTGGGGCCATT, R- GGGCCAGACCCCTTCTATTT;
m-AQP1 (124 bp): F- AGGCTTCAATTACCCACTGGA, R- GTGAGCACCGCTGATGTGA;

h-AQP2 (137 bp): F- CAGTTCCTGGCATCTCTTGT, R- CCTTTGTCCTTCCCTAACCTATC;
m-AQP2 (137 bp): F- ATGTGGGAACTCCGGTCCATA, R- ACGGCAATCTGGAGCACAG;

h-AQP3 (107 bp): F- ACCCCTCTGGACACTTGGAT, R- GGGGTTGTTGTAGGGGTCAA;
m-AQP3 (128 bp): F- GCTTTTGGCTTCGCTGTAC, R- TAGATGGGCAGCTTGATCCAG;

h-AQP4 (124bp): F- TGGTGCCAGCATGAATCCC, R- CTCATAAAGGCCACCAGCGA;
m-AQP4 (199 bp): F- AGTCACCACGGTTCATGGAAA, R- CATGCTGGCTCCAGTATAATTGA;

h-AQP5 (224 bp): F- CACGTATGAGCCTGACGAGG, R- CCCCTCTCTAACTGTGCAGC;
m-AQP5 (220 bp): F- AGAAGGAGGTGTGTTTCAGTTGC, R- GCCAGAGTAATGGCCGGAT;

h-AQP8 (126 bp): F- TGCCTGTTCGGTCATTGAGAA, R- CGCAGGGTTGAAGTGTCCA;
m-AQP8 (145 bp): F- TGTGTAGTATGGACCTACCTGAG, R- ACCGATAGACATCCGATGAAGAT;

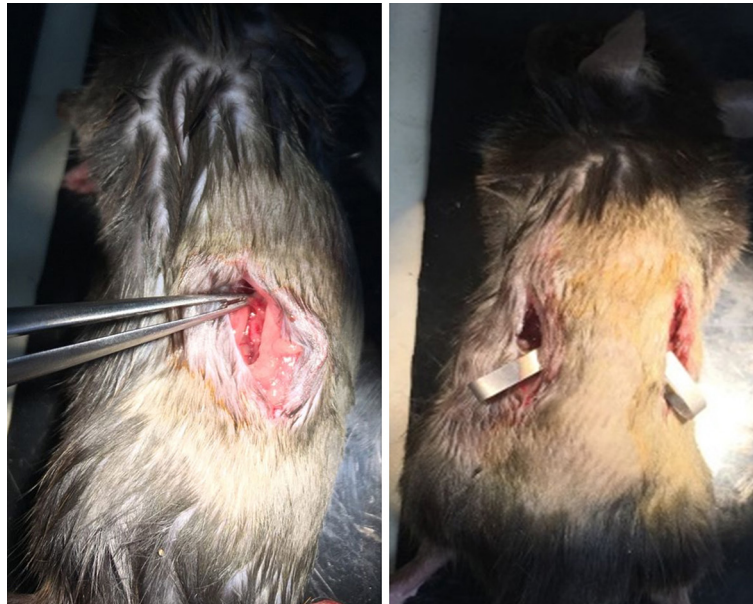


Figure S1 Establishment of renal IRI model. Proper anesthesia was achieved by intra-peritoneal injection of pentobarbital into mice. Both kidneys were exposed along the dorsal incision. After being dissociated, both renal pedicles were clamped with clips simultaneously for 30 minutes. The clips were then removed, and the wound was sutured. IRI, ischemia-reperfusion injury.

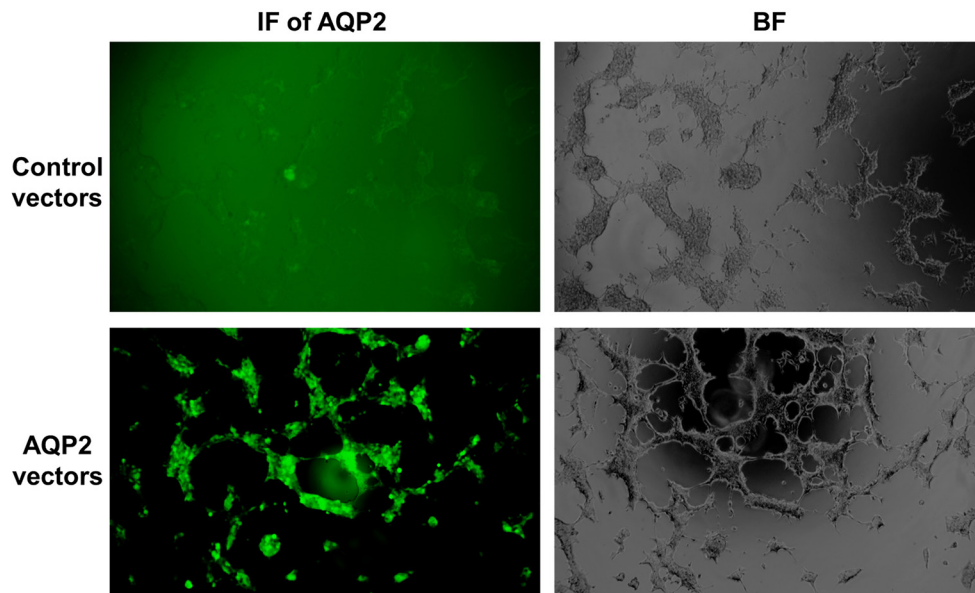


Figure S2 Measurement of AQP2 vectors. The titres of the AQP2 and control vectors were 2.2×10^5 tu/mL and 2×10^5 tu/mL, respectively. AQP, aquaporin.