Kidney & Blood Pressure Research

Kidney Blood Press Res 2018;43:1607-1622
DOI: 10.1159/000494450
Published online: 22 October 2018
Accepted: 12 October 2018

Original Paper

The Protective Effect of Propofol Against Ischemia–Reperfusion Injury in the Interlobar Arteries: Reduction of Abnormal Cx43 Expression as a Possible Mechanism

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Key Words
Propofol • Ischemia reperfusion injury • Connexin 43 • Gap junction • Renal vasoconstrictor response

Abstract
Background/Aims: This experimental study aims to observe whether the protective effect of propofol against renal ischemia–reperfusion injury (IRI) in the rat interlobar artery occurs through altered expression of the gap junction protein connexin 43 (Cx43). ***Methods:*** This study randomly divided male Sprague Dawley (SD) rats into an untreated control group, a sham-operated control group (sham group), an ischemia–reperfusion group (IR group), a propofol group (propofol+IR group) and a fat emulsion group (Intralipid group). The ischemia/reperfusion model was prepared through resection of the right kidney and noninvasive arterial occlusion of the left kidney. Forty-five minutes after renal ischemia–reperfusion, an automatic biochemical analyzer was employed to measure blood urea nitrogen (BUN) and serum creatinine (SCr); changes in renal tissue pathology were observed using hematoxylin and eosin (HE) staining, and the vasomotor activity of the interlobar artery was detected using a pressure mechanogram technique. The protein expression of Cx43 in renal artery cross-sections was determined through western blotting. ***Results:*** The experimental study confirmed that the BUN and SCr of rats markedly increased after ischemia–reperfusion injury; additionally, we observed some coagulation necrosis and shedding of cells, some solidification of nuclear chromatin, degeneration of cytoplasmic vacuoles, high renal interstitial vascular congestion

Y.-C. Chang, W.-J. Xue and W. Ji contributed equally to this work.

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and obvious inflammatory cell infiltration, characterized by focal hemorrhages. Furthermore, the contraction activity of the renal interlobar artery greatly decreased, and the tension of the arteries in the renal lobe increased remarkably. After the gap junction blocking agents 2-APB and Gap27 were applied, the systolic velocity of blood vessels and the vascular contraction rate both decreased. In addition, the expression of Cx43 in kidney tissues increased markedly. The damage was more severe after 24 h of ischemic reperfusion than after only 4 h. However, after pretreatment with propofol, regardless of whether ischemia–reperfusion was applied for 4 h or 24 h, the previously increased expression of Cx43 decreased obviously, and all forms of renal damage were reversed. ***Conclusion:*** Our research suggests new ways for propofol to relieve ischemia–reperfusion injury by decreasing the abnormal expression of the gap junction protein Cx43. This study reveals a novel mechanism for the action of propofol against IRI, and we hope this finding will lead to new treatments for IRI.

Introduction

Reperfusion after ischemia can restore the function of the tissue or organ, repair the structural damage and allow the patient to recover in most cases. Sometimes, however, reperfusion not only fails to restore the function of tissues and organs but also aggravates their dysfunction and structural damage after ischemia. This phenomenon of irreversible damage is known as ischemia–reperfusion injury (abbreviated as IRI) [1]. Increased free radicals, intracellular calcium overload, the opening of the mitochondrial permeability transition pore, endothelial dysfunction, the presence of a prothrombogenic phenotype, the development of capillary no-reflow, and pronounced inflammatory responses also play major roles in the development of reperfusion injury [2-4]. Renal IRI is a very sophisticated pathophysiological phenomenon, and its mechanism remains somewhat unclear.

A gap junction (GJ) is a type of special connection channel generally existing in cells. These junctions are critical for the transmission of electrical and biochemical signals between cells [5]. Studies have suggested that GJs exist not only in renal vascular endothelial cells and smooth muscle cells but also in other renal tissue cells. In addition, GJs are critical for renal circulation [6, 7]. However, there has been little research into whether the expression of gap junction proteins is altered in renal IRI. In particular, gap junction 43 (Cx43) is present in almost all tissue. Intact endothelial cells are essential for the maintenance of normal physiological function in normal vascular tissue, and Cx43 is critical for the maintenance of endothelial continuity and integrity [8].

Propofol is commonly used as an anesthetic. In recent years, studies have demonstrated the protective effects of propofol against ischemic injury in the kidneys, liver and heart [9-11]. It has been suggested in early studies that propofol protects against opioid-induced hyperresponsiveness of airway smooth muscle during target-controlled infusion anesthesia [12]. Propofol relieves renal oxidative injury and contributes to repair when administered during IRI, and the anesthetic may exert a protective effect by regulating bone morphogenetic protein 2 expression in renal IRI [13]. Some studies have identified that IRI increases the production of the lipid peroxidation by-product malondialdehyde (MDA) in whole-kidney homogenates. In contrast, propofol pretreatment decreases MDA levels, increases superoxide dismutase (SOD) levels and improves renal dysfunction when administered during IRI. For example, rats pretreated with propofol are protected from kidney dysfunction and histological damage. Protection is associated with a decrease in proinflammatory cytokine generation and a concomitant increase in BMP2 expression [13]. Additionally, propofol pretreatment can relieve IR damage in LLC PKl cells by activating K_{ATP} channels [14]. Another study suggests that the microvascular injury occurs during renal ischemia–reperfusion injury and that propofol can protect against oxidation, improve the inhibition of intestinal injury and inflammation, and protect the permeability of capillaries [15]. It is believed that the protective effect of propofol on the kidney may be due to preconditioning

of cell or tissue protective mechanisms such as HO-1 or heat shock protein [16] or may act on the K_{ATP} ion pathway in the kidney [14]. The latter played a role in fighting oxygen free radicals, inflammation and apoptosis pathway and played a role in alleviating renal cell and tissue damage. The study suggested that propofol pretreatment might alleviate hypoxia reoxygenation injury by stimulating p38/MAPK [17]. Propofol can not only reduce renal ischemia–reperfusion injury but also adjust GJ function to reduce acute renal injury in orthotopic liver transplantation [13, 18]. However, it is not clear what part propofol plays in protection against renal IRI. It is necessary to further study whether propofol is associated with expressional changes and functional regulation of gap junction proteins.

This study began by generating a kidney ischemia–reperfusion model in rats and comparing the changes in contraction in the renal interlobar artery before and after ischemia–reperfusion. Additionally, the regulatory effects of propofol on arterial contraction and gap junction (Cx43) expression during renal IRI were examined. The aim of this study was to explore the protective effect of propofol against kidney IRI and the mechanism of the effect, providing new insights for the future clinical treatment of IRI.

Materials and Methods

Drugs and reagents

The materials purchased were as follows: anti-Cx43 monoclonal antibody from Abcam (UK), national drug approval No. ab170190; peroxidase-Conjugated AffiniPure goat anti-rabbit IgG from ZSGB-BIO (China), national drug approval No. ZB-2301; anti-β-actin monoclonal antibody from ZSGB-BIO (China), national drug approval No. TA-09; peroxidase-conjugated AffiniPure goat anti-mouse IgG from ZSGB-BIO (China), national drug approval No. ZB-2305; propofol, fat emulsion, phenylephrine and acetylcholine from Sigma, USA; 2-aminoethyl diphenylborinate (2-APB) from Sigma, USA, approval No. 047K1192; ketamine from Gutian Fujian Pharmaceutical Co., Ltd. (Fujian, China), national drug approval No. H35020148; VITROS Chemistry Products CREA Slides from Ortho-Clinical Diagnostics, Inc. (China), national drug approval No. YZB/USA 0073-2015; VITROS Chemistry Products BUN/UREA Slides from Ortho-Clinical Diagnostics, Inc. (China), national drug approval No. 20152402807; acetylcholine chloride from Sigma, USA, approval No. A2661; phenylephrine hydrochloride from Sigma, USA, national drug approval No. PHR1017.

Animals

One hundred adult male SD rats (body weight, 240-260 g) were provided by the Animal Center of Xinjiang Medical University (Urumqi, China). The use of animals in the present study was approved by the Committee of Animal Experimental Ethics of The First Affiliated Hospital of Medical College, Shihezi University (Shihezi, China) (animal use certificate No. SCXK Xin 2003-0001). The animals were singly housed in cages in a specific-pathogen-free environment at 22±1°C with a relative humidity of 40-70% under a 12 h/12 h light/dark cycle and provided with food and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the Medical College of Shihezi University and complied with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health [19].

The establishment of a rat renal IRI model

All rats were fasted for 12 h and anesthetized with 10% chloral hydrate (0.04 g/kg). The left renal artery was isolated and occluded with a noninvasive microvascular clamp. The vascular clamp was released after reperfusion. In the sham operation group, silk sutures were passed under the left renal artery but not tied. In the renal ischemia–reperfusion (IR) group, the left kidney was subjected to ischemia for 45 min, followed by reperfusion for 4 h or 24 h. In the propofol+IR group, the rats received propofol (50 mg/kg) by intraperitoneal injection once daily for three days before being subjected to 45 min of kidney ischemia and 4 h of reperfusion. The drug doses of propofol and the administration method were derived from the literature [20-22]. The fat emulsion group was treated in the same manner as the propofol group, except that the fat emulsion Intralipid was injected instead of propofol.

Experimental groups and protocol

One hundred male SD rats were randomly divided into ten groups as follows: propofol intervention (Pro 50 mg/kg) 4 h and 24 h; fat emulsion (Intralipid 50 mg/kg) 4 h and 24 h; renal ischemia–reperfusion (IR) 4 h and 24 h; sham operation (Sham) 4 h and 24 h; and blank control (Control) 4 h and 24 h. Each group contained 10 rats. After 4 h or 24 h of reperfusion, the rats were reanesthetized with the same dose of 10% chloral hydrate; their abdomens were opened, and 5 ml of blood was drawn from the abdominal aorta. Plasma samples were immediately transferred to a biochemistry laboratory and stored in a refrigerator (DW-86L626; Haier Special Electric Appliance Co., Ltd, Qingdao, China) at -80˚C to measure BUN and SCr. Then, the rats' left kidneys were quickly removed and placed in a 4˚C oxygen-saturated (95% O_2 and 5% CO_2) saline solution. The kidneys were cut in the longitudinal direction and fixed on a silica gel petri dish. The interlobar arteries were exposed, and the perivascular connective tissue was removed under a dissecting microscope. A 2 to 3 mm segment of each renal interlobar artery was cut for a stress electromyogram detection. The other part of the blood vessel was used for the measurement of protein expression.

Measurement of BUN and SCr levels

BUN and SCr levels were measured using a Roche automatic biochemical analyzer (Modular DPP-H7600; First Affiliated Hospital of Shihezi University Laboratory, China). The results of both measurements were recorded in mg/dl.

Histopathological examination

The blood vessels from the different groups of rats were collected and immediately washed twice with phosphate-buffered saline. Then, the vessels were modified and fixed in 10% neutral formalin. The samples were successively dehydrated and embedded in paraffin. Tissue sections (4 μm) were fixed in ethanol at ambient temperature, stained with hematoxylin and eosin (HE), and photographed under a light microscopy (automatic biochemical analyzer, Olympus).

Preparation of renal interlobar arterial segments in rats of each group

The literature [13] was referenced for the preparation of interlobar arterial segments and the technique of Stress electromyogram. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.04 g/kg). The kidneys were quickly removed and placed in a 4˚C oxygen-saturated (95% O_2 and 5% CO_2) saline solution (physiological salt solution, PSS). The kidneys were cut longitudinally and fixed on a silica gel petri dish. The interlobar arteries were exposed, and the perivascular connective tissue was removed using a dissecting microscope. The arteries were cut into 2 to 3 mm sections for pressure-induced myotome experiments.

Detecting the change in vessel diameter using a pressure myograph system

The arterial segments were placed in a perfusion vessel containing 5 ml of PSS and were cannulated at both ends with glass micropipettes (1.2 mm; World Precision Instruments, LLC, Sarasota, FL, USA), which were stretched to a diameter of 300 to 400 μm diameter for the MRA and protected using an 11-0 nylon monofilament suture. The glass micropipettes were shaped with a P-97 puller (Sutter Instrument, Novato, CA, USA). To reinforce the vessel and prevent leaks, a syringe containing PSS liquid was used to slowly wash away the residual blood in the lumen, which was drained through the glass micropipette fixed to the other end of the vessel. The perfusion tank was moved to the stage of an inverted microscope at 100× magnification. The blood vessels were submerged in PSS liquid at 37˚C and 100% oxygen saturation. The baths were aerated with 95% O_2 and 5% CO_2 at a constant rate. Vascular segments were standardized after reaching equilibrium. KPSS solution (KCl 60 mmol/L) was used to stimulate vascular contraction. The vessels were washed with PSS and incubated for 30 min; then, PE (10^{-5} mol/L) was administered. ACh (10^{-5} mol/L) was used to relax the blood vessels when the vasoconstriction plateaued. If the diastolic rate was above 80%, the endothelium would be considered intact, suggesting that the vascular activity was suitable for the experiment. To continuously record the outer diameter of the isolated vascular segments, we transferred the vessel and the perfusion chamber to an inverted trinocular microscope with an analog video camera and a computer-assisted image capture system (Pressure Myograph System; Danish Myo Technology A/S,

Aarhus, Denmark) [14] once the tissues were mounted. Pressure type arterial measuring instrument (DMT, 110P, Denmark).

The experiment was initiated after 30 min of stabilization. The intra-arterial pressure was maintained at 7.98 kPa; the temperature was controlled at 37˚C; the PSS water bath was controlled at a volume of 5 ml, and 60 mmol/L KCl was added for vascular stability. Then, PSS was added until the maximum blood vessel stability was reached, followed by 60 mmol/L KCl, and the process was repeated for three cycles; changes in diameter were observed when the blood vessels were in the stable state. The variable of interest was the vessel diameter; changes in this variable were calculated as D(m)=Dp-Dk (Dp: diameter of vascular segment stabilized in PSS; Dk: diameter of vessel after stabilization in 60 mmol/L KCl); to reduce the differential error caused by different vessel diameters, we calculated the vascular shrinkage as a percentage of diameter: (%)=(Dp-Dk)/Dp)×100%[23, 24].

Detection of Cx43 protein expression in the renal artery by western blotting

Total protein was extracted from the renal artery using lysis buffer, and the protein concentration was determined using the BSA method. Equal amounts of renal artery homogenates (25 mg/lane) were separated by 10% SDS-PAGE and transferred to a 0.2-mm nitrocellulose membrane. The nitrocellulose blots were blocked by incubation in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% TWEEN 20) containing 5% milk for 1 h. Next, the samples were mixed with anti-CX43 polyclonal antibody (1:1000 dilution)/β-actin polyclonal antibody (1:20000) and kept at 4˚C overnight. Afterwards, the blots were washed in TBST five times for 15 min. The blots were incubated with horseradish peroxidase-linked anti-rabbit IgG for 1 h at ambient temperature and then washed five times in TBST for 15 min. A chemiluminescent peroxidase substrate was applied in line with the manufacturer's instructions, and the membranes were exposed briefly to X-ray film. Protein expression was examined using an imaging software program (EN61000-6-1; Bio-Rad Laboratories, Inc., 1000 Alfred Nobel Drive, Hercules, California 94547, USA)[22].

Statistical analysis

Experimental data were presented as the mean±standard error of the mean. SPSS 17.0 software processing (SPSS, Inc., Chicago, IL, USA) was applied for statistical analysis. Variables that followed the normal distribution were compared among different groups by one-way analysis of variance, and those that did not follow the normal distribution were compared by the rank-sum test; enumeration data were analyzed by the chi-squared test. $P<0.05$ was considered statistically significant.

Results

Changes in BUN and SCr in each group

Renal IR can result in abnormal increases in serum BUN and SCr, and renal function is often assessed by measuring BUN and SCr [25]. Propofol markedly reduced the levels of BUN and SCr. The serum levels of BUN and SCr increased by a dramatic 4- to 6-fold after IR (n=10, $P<0.05$ or $P<0.01$) and was reduced by propofol pretreatment (n=10, $P<0.05$ or $P<0.01$) (Table 1). As the results suggest, propofol may attenuate IR-induced renal damage. There was no difference between the sham operation group and the control group, and there was no difference between the Intralipid group (receiving only the fat solvent used to dissolve propofol) and the IR group.

Pathological changes in rat kidneys under light microscopy

The glomeruli and renal tubules did not show significant morphological changes between the control group and the sham group at 4 h or 24 h (Fig. 1). However, the renal tubules were damaged 4 h after renal ischemia–reperfusion, with the proximal tubule being the most severely damaged. The clarity of the tubular structures was reduced, and the lumen was dilated. Tubular cells and exfoliated cells were observed in the tubular epithelium. Some cells that had undergone coagulation necrosis were being shed, and some of the nuclear chromatin was subject to cytoplasmic vacuolar degeneration; renal interstitial hypervascular hyperemia

with inflammatory cell infiltration was significant, showing a focal hemorrhage area. Renal IRI and the accompanying damage were more severe in the 24 h group than in the 4 h group. Both the extent and the degree of renal tubular injury were markedly lower in the propofol+IR group than in the IR group. In the former group, the nuclei were normal, and the tubules showed only a small amount of tubule-type interstitial hyperemia, with the bleeding obviously reduced. The effects of Intralipid were similar to those of renal IRI (not shown in Fig. 1, 4 h). The method of Paller et al. was employed to calculate the pathological score of the renal tissue [26], specifically, the pathological score of HE staining of the renal tissue from the rats of each group, and the data are presented as the mean±standard error (Table 2).

Table 1. Serum levels of BUN and SCr (mg/dl) (mean±SD, n=10). Compared with the sham group: *P<0.05, **P<0.01; compared with the IR group: #P<0.05, ##P<0.01; compared with the Propofol+IR group: ΔP<0.05, ΔΔP<0.01

Group (n=10)	BUN		SCr	
	4h	24h	4h	24h
Control group	5.45±0.92	5.44±1.01	26.54±6.99	27.00±7.11
Sham group	7.81±1.43	7.78±1.48	31.77±3.23	34.93±6.69
I/R group	15.99±3.31*	23.91±3.18**	68.27±13.41*	152.82±28.76**
Propofol+I/R group	10.51±1.60#	14.02±3.36**##	40.32±5.90#	103.10±23.21**##
Intralipid group	15.85±1.96*Δ	25.84±3.67**ΔΔ	68.83±9.26*Δ	144.06±24.90**ΔΔ

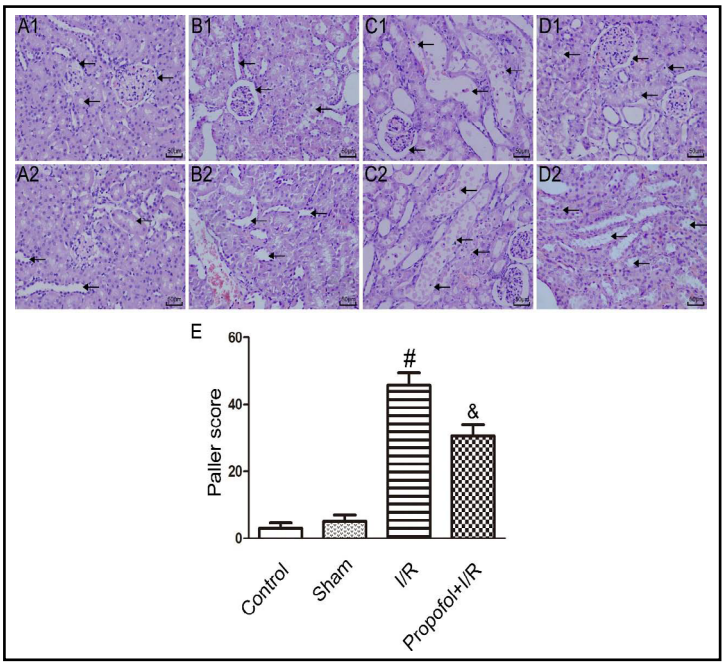

Fig. 1. HE staining and Paller scores of renal ischemia–reperfusion 24 h. The first row is the glomerulus: A1: control group; B1: sham group; C1: IR group; D1: propofol+IR group. The second row is the renal tubules: A2: control group; B2: sham group; C2: IR group; D2: propofol+IR group. E: Paller scores of renal ischemia–reperfusion 24 h. #P<0.05 versus the control group, &P<0.05 versus the IR group.

Intergroup differences in the diameter of the renal interlobar artery

By fixing the renal interlobar artery of rats in a pressure myograph system, we measured the initial average diameter of the blood vessels. The mean diameter of all blood vessels was 448.76±61.43 μm (n=60), while the group means for the sham, IR and propofol+IR groups were 468.45±23.24 μm (n=20), 396.37±16.75 μm (n=20), and 457.45±24.76 μm (n=20), respectively. When 60 mmol/L KCl was used to induce renal interlobar artery vasoconstriction, the blood vessels contracted, decreasing in average diameter. The vascular diameter decreased to an average of 120.13±9.67 μm (n=30), and the mean diameters of the blood vessels of the sham group, IR group and propofol+IR group decreased to 73.51±9.28 μm (n=10), 175.47±13.32

Table 2. Pathological score of HE staining of renal tissue from rats of each group (mean±SD, n=10). Compared with sham group: *P<0.05, **P<0.01, compared with I/R group: #P<0.05, ##P<0.01, compared with Pro group: ΔP<0.05, ΔΔP<0.01

Group	Pathological score	
	4 h	24 h
Control group	7.18±1.68	7.11±1.24
Sham group	6.96±1.65	7.02±1.17
I/R group	20.90±10.31*	49.75±16.37**
Propofol+I/R group	11.62±2.56*#	29.83±12.15*##
Intralipid group	21.58±9.6*Δ	41.63±13.65**ΔΔ

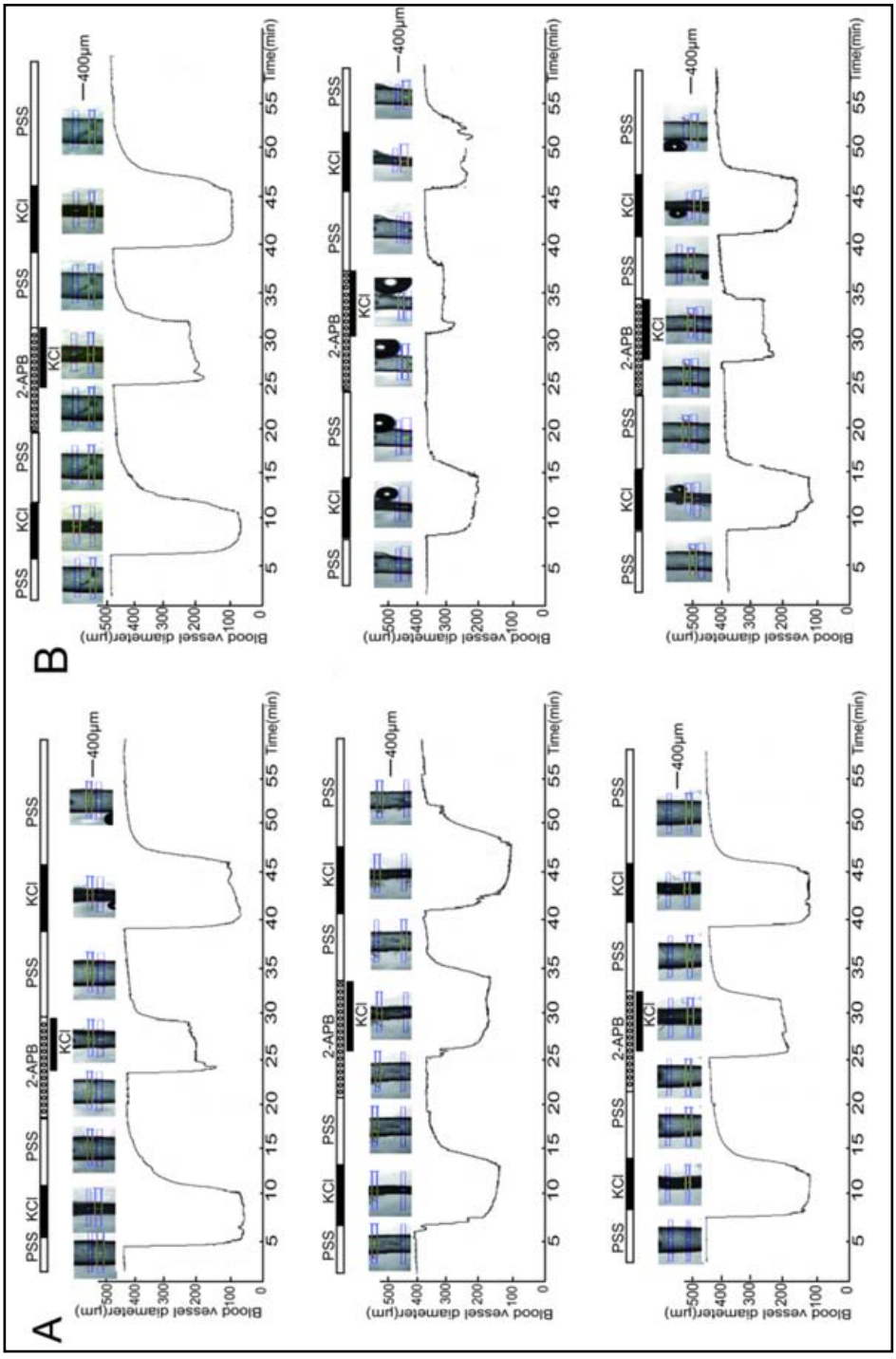

Fig. 2. The contractile condition of the interlobar arteries after ischemia followed by different durations of reperfusion. *A*: Ischemia followed by 4 h of reperfusion; B: Ischemia followed by 24 h of reperfusion. The top row represents the sham group, the middle row represents IR, and the bottom row represents the propofol+IR group.

μm (n=10) and 116.37±11.36 μm (n=10), respectively, when reperfusion lasted 4 h. After 4 h of reperfusion, the vascular diameter decreased to an average of 136.33±10.21 μm (n=30), and the mean diameters of the blood vessels of the sham group, IR group and propofol+IR group decreased to 69.13±10.34 μm (n=10), 208.65±11.46 μm (n=10) and 132.43±9.91 μm (n=10), respectively (Fig. 2).

The interlobar arteries of the IR group were markedly smaller in initial diameter than those of the sham group (n=20, $p<0.05$), and propofol+IR noticeably restored the initial diameter of the arteries, as shown in Fig. 3. The effect of high KCl (60 mmol/L) on vasoconstriction

was obviously weaker than that of the sham treatment, and 24 h of reperfusion (n=10, p<0.01) was obviously weaker than 4 h (n=10, p<0.05). However, whether reperfusion lasted 4 h or 24 h, the arterial vasoconstriction induced by 60 mmol/L KCl propofol+IR group slightly restored constriction capability.

Changes in the amplitude and velocity of renal interlobar artery constriction induced by high KCl in each group

The rate of change of vascular contraction amplitude in response to high potassium, (%)=((Dp-Dk)/Dp)×100%, was calculated for each experimental group. For the sham, IR and propofol+IR groups after 4 h of reperfusion, the contraction rate values were 86.37±11.12% (n=10), 65.21±13.43% (n=10) and 74.56±10.14% (n=10), respectively. The rates of shrinkage after 24 h of reperfusion were 85.67±9.84% (n=10), 47.36±8.97% (n=10) and 71.05±11.33% (n=10), respectively, for the sham, IR and propofol+IR groups, as calculated with the abovementioned formula.

The rate of change of vasoconstriction caused by high potassium was markedly lower after ischemia and 4 h or 24 h of reperfusion, with or without propofol pretreatment, than in the sham operation group, as shown in Fig. 4. In addition, the decrease in the rate of change of vasoconstriction was more obvious after 24 h of reperfusion than after only 4 h. The results also suggested that propofol could, to some extent, correct the decrease in the rate of vasoconstriction induced by ischemia–reperfusion.

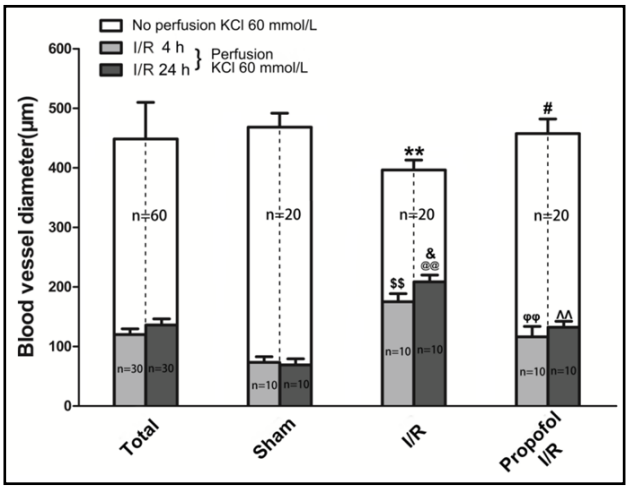

Fig. 3. Differences in arterial diameter under different conditions. **P<0.01 vs sham group (no perfusion with 60 mmol/L KCl); #P<0.05 vs IR group (no perfusion with 60 mmol/L KCl); $$P<0.01 vs sham group, 4 h (perfusion with 60 mmol/L KCl); @@P<0.01 vs sham group, 24 h (perfusion with 60 mmol/L KCl); ψψP<0.01 vs IR group, 4 h (perfusion with 60 mmol/L KCl); ^^P<0.01 vs IR group, 24 h (perfusion with 60 mmol/L KCl); &P<0.05 vs sham group, 24 h (perfusion with 60 mmol/L KCl).

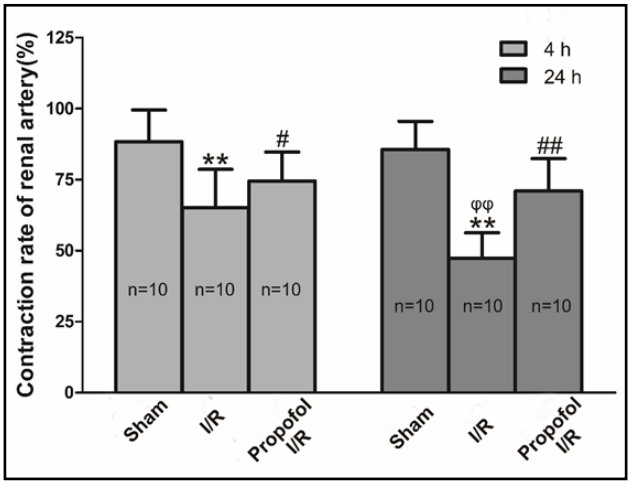

Fig. 4. The rate of change of vascular contraction amplitude of the interlobar artery in response to high KCl in different groups. **P<0.01 vs sham group; #P<0.05 or ##P<0.01 vs IR group; ψψP<0.01 vs IR group for 4 h.

The systolic velocity of each experimental group was also examined to measure the change in blood vessel constriction rate in the interlobar arteries after ischemia–reperfusion. The systolic velocities of blood vessels in the sham, IR and propofol+IR groups at 4 h were 3.82±0.44 μm/s (n=10), 2.17±0.54 μm/s (n=10) and 3.21±0.41 μm/s (n=10), respectively. The values for the same groups at 24 h were 3.67±0.51 μm/s (n=10), 1.25±0.42 μm/s (n=10) and 2.34±0.63 μm/s (n=10), respectively.

The systolic velocity of the renal interlobar artery caused by high potassium after 4 h or 24 h of reperfusion was obviously slower than that of the sham operation group, as shown in Fig. 5. In addition, the decrease in the systolic velocity of the renal interlobar artery was more obvious after 24 h of reperfusion than after only 4 h. The results also suggested that propofol could correct the slowing of the systolic velocity of the renal interlobar artery induced by ischemia–reperfusion.

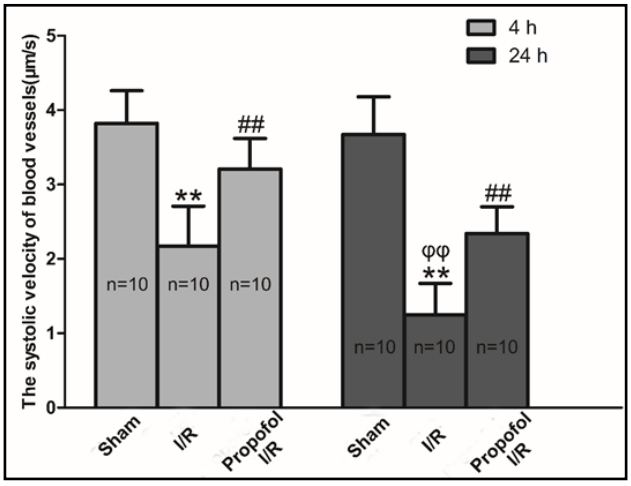

Fig. 5. Changes in the vascular systolic velocity of the renal interlobar artery in different groups in response to high KCl. **P<0.01 vs sham group; ##P<0.01 vs IR group; ψψP<0.01 vs IR group for 4 h.

The effect of 2-APB on the amplitude and velocity of the renal interlobar artery constriction induced by high KCl in each group

2-APB has several pharmacological functions, including the nonspecific blocking of gap junction communication. Without application of 2-APB, the ischemia–reperfusion 4 h groups (sham, IR and propofol+IR groups) had contraction rate values of 86.37±11.12% (n=10), 65.21±13.43% (n=10) and 74.56±10.14% (n=10), respectively, after the rat renal interlobar artery was treated with KCl (60 mmol/L). After 24 h of reperfusion, the sham, IR and propofol+IR group had contraction rates of 85.67±9.84% (n=10), 47.36±8.97% (n=10) and 71.05±11.33% (n=10), respectively. With application of 2-APB (100 mmol/L), the shrinking rates of the sham 4 h group, the IR 4 h group and the propofol 4 h group were 66.67±12.21% (n=10), 52.78±13.37% (n=10) and 63.04±1179% (n=10), respectively. The shrinking rates of the sham 24 h group, the IR 24 h group and the propofol 24 h group were 64.14±10.16% (n=10), 30.56±9.87% (n=10) and 42.5±10.58% (n=10), respectively.

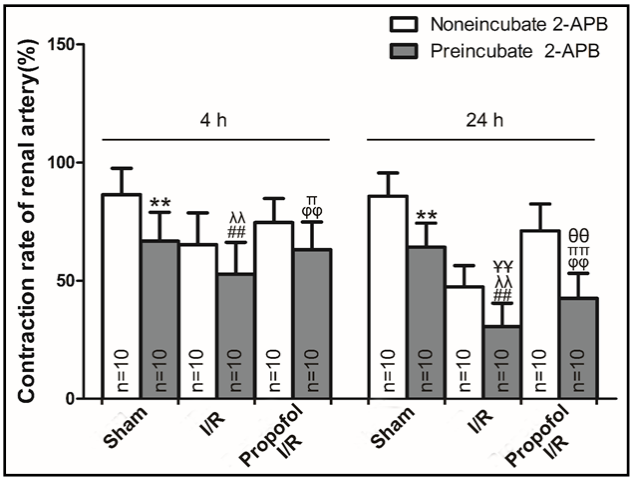

Fig. 6. The rate of change of the vascular contraction amplitude of the renal interlobar artery in different groups in response to high KCl without and with 2-APB. **P<0.01 vs sham group; ##P<0.01 vs IR group; ψψP<0.01 vs IR group; λP<0.05 or λλP<0.01 vs sham group of the same reperfusion time with 2-APB; πP<0.05 or ππP<0.01 vs IR group of the same reperfusion time with 2-APB; ¥¥P<0.01 vs IR group with 4 h of reperfusion and 2-APB; θθP<0.01 vs propofol+IR group with 4 h of reperfusion 2-APB.

Each group had a significantly lower shrinkage rate after incubation with 2-APB (100 mmol/L) than without 2-APB treatment (n=10, p<0.01) (Fig. 6). After incubation with 2-APB (100 mmol/L), the renal interlobar artery shrinkage rate of the IR group treated with high KCl decreased in comparison with that of the sham group, and the difference was statistically significant at 4 h (n=10, P<0.05) and 24 h (n=10, P<0.01); the renal interlobar artery

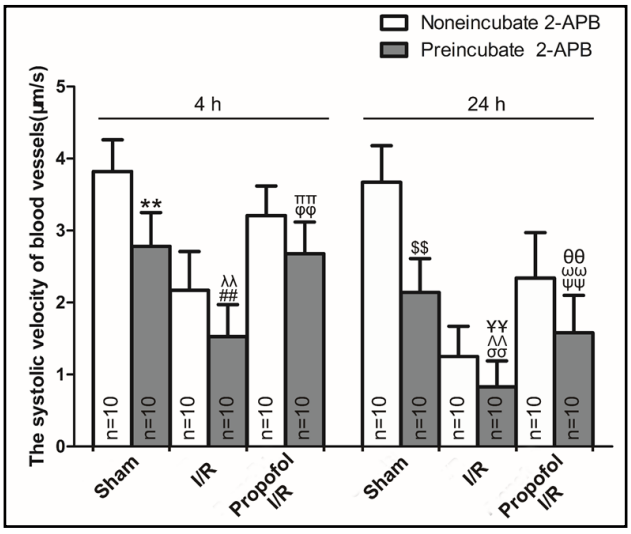

Fig. 7. Changes in the vascular systolic velocity of the renal interlobar artery in different groups induced by high KCl without and with 2-APB. **P<0.01 vs sham group; ##P<0.01 vs IR group; ψψP<0.01 vs IR group; λλP<0.01 vs sham group of the same ischemia–reperfusion time with 2-APB; ττP<0.01 vs IR group of the same ischemia–reperfusion time with 2-APB; ¥¥P<0.01 vs IR group with 4 h of reperfusion followed by 2-APB; θθP<0.01 vs propofol+IR group with 4 h of reperfusion followed by 2-APB.

shrinkage rate of the propofol+IR group treated with high KCl increased in comparison with that of the IR group, and the difference was statistically significant at 4 h (n=10, P<0.05) and 24 h (n=10, P<0.01); the renal interlobar artery shrinkage rates of the IR and propofol+IR groups were lower after 24 h of reperfusion than after 4 h, and the difference was statistically significant (n=10, P<0.01) (Fig. 2).

Without 2-APB application, the systolic velocities of blood vessels in the sham, IR and propofol+IR groups after 4 h of reperfusion were 3.82±0.44 μm/s (n=10), 2.17±0.54 μm/s (n=10) and 3.21±0.41 μm/s (n=10), respectively, after the rat renal interlobar artery was treated with KCl (60 mmol/L). The systolic velocities of blood vessels in the experimental groups (sham, IR and Pro groups) after 24 h of reperfusion were 3.67±0.51 μm/s (n=10), 1.25±0.42 μm/s (n=10) and 2.34±0.63 μm/s (n=10), respectively. The systolic velocity of blood vessels in the sham, IR and Pro groups at 4 h of reperfusion were 2.14±0.47 μm/s (n=10), 0.83±0.36 μm/s (n=10) and 1.58±0.52 μm/s (n=10), respectively, with application of 2-APB (100 mmol/L),. Finally, the systolic velocities of blood vessels in the experimental groups (sham, IR and Pro group) for 24 h of reperfusion were 2.78±0.47 μm/s (n=10), 1.53±0.44 μm/s (n=10) and 2.68±0.43 μm/s (n=10), respectively.

The systolic velocity of blood vessels of each group was lower after incubation with 2-APB (100 mmol/L) than without 2-APB incubation, and the results were statistically significant (n=10, P<0.01) (Fig. 7). After incubation with 2-APB (100 mmol/L), the systolic velocity of blood vessels of the IR group treated with high KCl decreased in comparison with that of the sham group blood vessels, and the difference was statistically significant at 4 h (n=10, P<0.01) and 24 h (n=10, P<0.01); the systolic velocity of blood vessels of the propofol+IR group treated with high KCl increased compared with the vessels of the IR group, and the difference was statistically significant at 4 h (n=10, P<0.01) and 24 h (n=10, P<0.01); the systolic velocities of blood vessels of the IR and propofol+IR groups at 24 h treated with high KCl decreased compared with those of the IR and propofol+IR groups at 4 h (n=10, P<0.01) (Fig. 7).

The effect of Gap27 on the amplitude and velocity of renal interlobar artery constriction induced by high KCl in each group

2-APB is a compound that interferes with many pathways (through IP_3 receptors, TRP channels); thus, Gap27 was also selected as a gap junction 43 blocker. The shrinkage rate of each group was lower after incubation with Gap27 (100 mmol/L), than without Gap27 treatment, as shown in Fig. 8E, and the results were statistically significant (n=6, p<0.01). After incubation with Gap27 (100 mmol/L), the renal interlobar artery shrinkage rate of the IR group treated with high KCl decreased compared with that of the sham group, and the difference was statistically

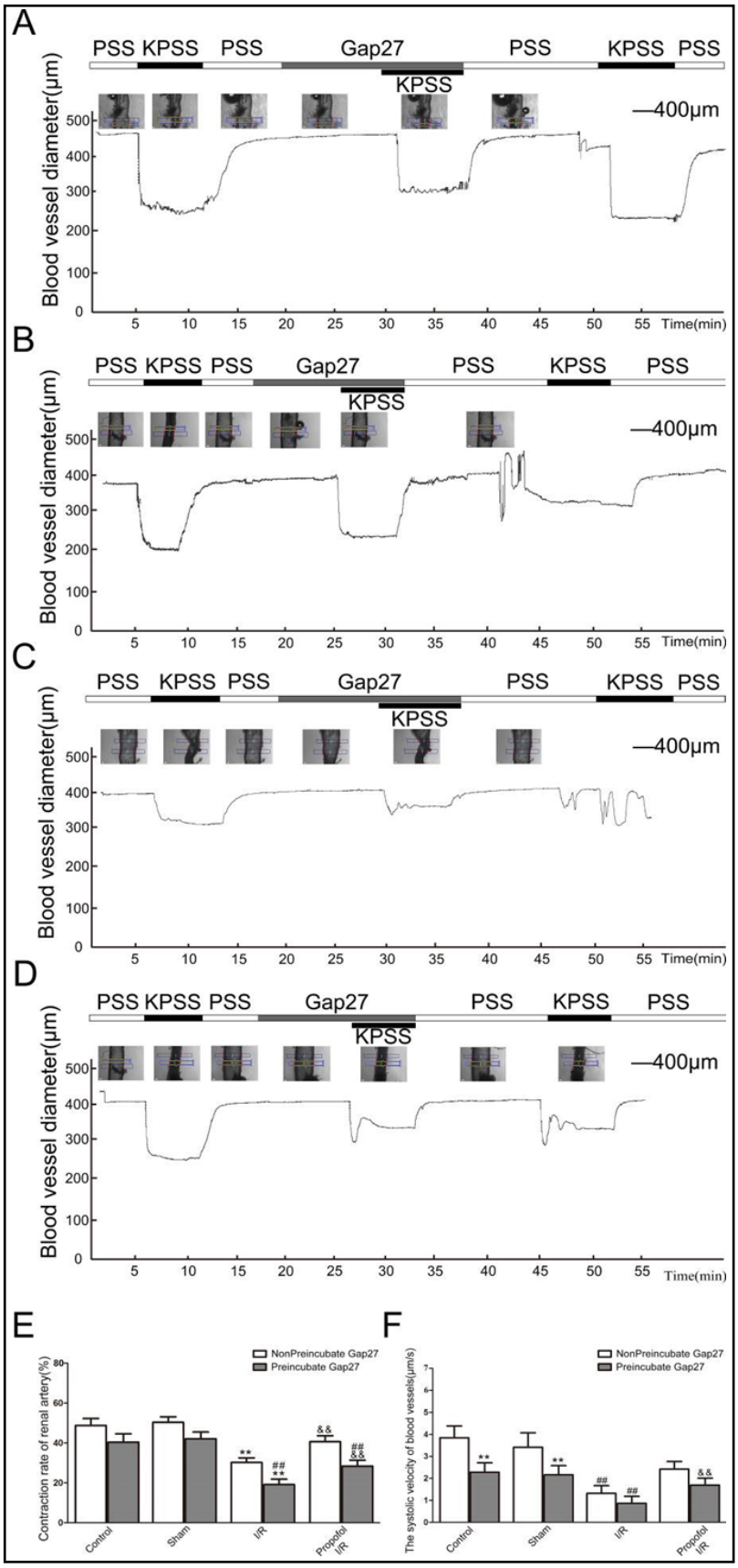

Fig. 8. The rate of change of vascular contraction amplitude and the vascular systolic velocity of the renal interlobar artery in different groups exposed to high KCl without and with Gap27. A: control group; B: sham group; C: IR group; D: propofol+IR group. E: vascular contraction rate. $^{**}p<0.01$ versus Sham group. $^{\#\#}p<0.01$ preincubated with Gap27 versus not preincubated with Gap27. $^{\&\&}p<0.01$ propofol+IR group versus IR group. F: vessel contraction rate. $^{**}p<0.05$ preincubated with Gap27 versus not preincubated with Gap27. $^{\#\#}p<0.05$ IR group versus sham group. $^{\&\&}p<0.05$ propofol+IR group versus IR group.

significant at 24 h (n=6, P<0.01); the renal interlobar artery shrinkage rate of the propofol+IR group treated with high KCl increased compared with that of the IR group, and the difference was statistically significant at 24 h (n=6, P<0.01); the renal interlobar artery shrinkage rates of the IR and propofol+IR 24 h groups treated with high KCl decreased, and the difference was statistically significant (n=10, P<0.01) (Fig. 8E).

The systolic velocity of blood vessels from each

group after incubation with Gap27 (100 mmol/L), was lower than that of the vessels without Gap27, as shown in Fig. 8F, and the results were statistically significant (n=6, p<0.01). After incubation with Gap27 (100 mmol/L), the systolic velocity of blood vessels of the IR group treated with high KCl decreased compared with that of the sham group vessels, and the difference was statistically significant at 24 h (n=6, P<0.01); the systolic velocity of blood vessels of the propofol+IR group treated with high KCl increased compared with that of the IR group, and the difference was statistically significant at 24 h (n=6, P<0.01); the systolic velocity of blood vessels of the IR and propofol+IR 24 h groups treated with high KCl decreased, and the difference was statistically significant (n=6, P<0.01)

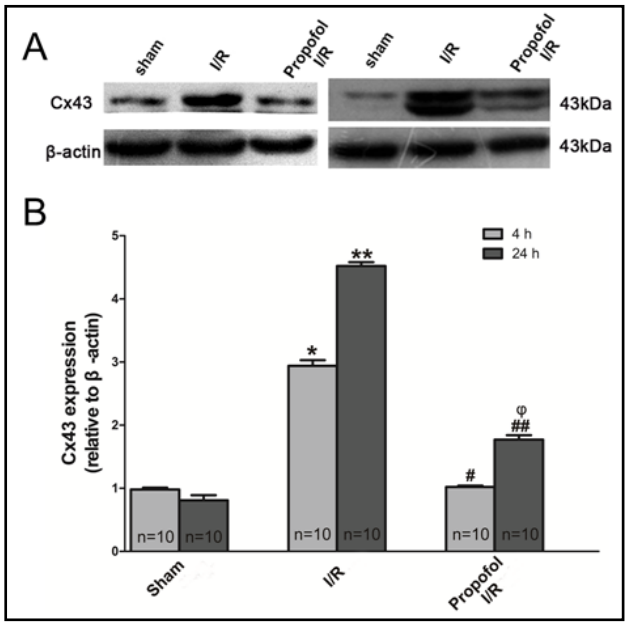

Fig. 9. The change in the expression of Cx43 in the renal interlobar artery of each group. A: The expression of Cx43 protein in the renal interlobar artery in each group after 4 h and 24 h of reperfusion. B: The differences in the expression of Cx43 protein in the renal interlobar artery of each group. *P<0.05 or **P<0.01 vs sham group of the same ischemia–reperfusion time; ##P<0.01 or ##P<0.01 vs IR group of the same ischemia–reperfusion time; ψP<0.05 vs IR group of the same ischemia–reperfusion time.

The expression of Cx43 protein in the renal interlobar artery of each group

As the western blot results suggested, the expression of Cx43 protein in the IR 4 h group increased obviously (P<0.05) compared with that of the sham group (Fig. 9A). No significant difference was found in Cx43 protein expression in the renal artery between the sham 4 h group and the propofol 4 h group (P>0.05) (Fig. 9A). The expression of Cx43 protein was obviously lower in the propofol 4 h group (P<0.05) than in the IR 4 h group (Fig. 9A). The expression of Cx43 protein increased markedly (P<0.05) in the IR 24 h group and the propofol 24 h group compared with the sham group (Fig. 9B). The expression of Cx43 protein was obviously lower in the propofol 24 h group (P<0.05) than in the IR 24 h group (Fig. 9B).

Discussion

The experimental study confirmed that, after ischemia–reperfusion injury, 1) the BUN and SCr of rats markedly increased; 2) effects at the cellular level included coagulation necrosis and shedding of some cells; solidification of some nuclear chromatin; degeneration of cytoplasmic vacuoles; high renal interstitial vascular congestion; and obvious inflammatory cell infiltration, characterized by focal hemorrhages; 3) the contractile activity of the renal interlobar artery was obviously weakened; 4) after ischemia–reperfusion, the tension of the arteries in the renal lobe increased obviously; and 5) the expression of Cx43 in kidney tissues increased obviously. In addition, the damage was more severe at 24 h of postischemic reperfusion than at 4 h. However, after pretreatment with propofol, whether reperfusion lasted for 4 h or 24 h, the previously increased expression of Cx43 decreased obviously, and renal damage (morphological and functional) was clearly reduced at each time point.

Some studies have found that renal vascular endothelial cells undergo leukocyte adhesion and the accumulation of red blood cells and platelets in the event of renal ischemia–reperfusion, which causes vascular obstruction [27, 28]. The renal vascular endothelial cell injury was coupled with increased levels of vasoconstrictor substances, along with reduced levels of vasodilator substances and many other factors, affecting blood vessel luminal obstruction, blood flow velocity, and blood vessel elasticity decreased vasoconstriction [24]. Thus, our study found that ischemia–reperfusion resulted in increased tension in the interlobar arteries, and the initial diameter of the IR vessels was obviously smaller than that of the control group vessels (Fig. 2, 4). The contraction amplitude (Fig. 2, 5) and contraction rate (Fig. 2, 6) of the IR-subjected interlobar arteries were lower than those of the control group at 4 h and 24 h (Fig. 2, 5, 6). The results suggested that the renal artery was damaged in the process of renal ischemia and reperfusion, which decreased the contraction and damaged the microcirculation. Renal function (Table 1) and corresponding morphological changes (Fig. 1 and Table 2) were reduced by microcirculation disorder in renal ischemia–reperfusion. These findings are consistent with those of previous studies.

It is widely known that GJs transmit electrical and chemical signals to synchronize the electrical and mechanical activity of the vessel wall in the vertical and horizontal directions, allowing the blood vessel to function in a unified manner. In addition, GJs are critical for regulating the coordination and synchronization of vascular motion [29]. The present study applies the nonspecific gap junction blocker 2-APB and the specific blocker Gap27, and the results suggest that renal artery contraction amplitude and contraction speed can be reduced by using 100 mmol/L 2-APB or Gap27 (including 4 h and 24 h) (Fig. 2, 7,8), and GJs may play a role in vasoconstriction induced by high KCl. Increased free radicals, intracellular calcium overload, the opening of the mitochondrial permeability transition pores, endothelial dysfunction, the appearance of a prothrombogenic phenotype, development of capillary no-reflow, and pronounced inflammatory responses are also predominant in the development of reperfusion injury [2, 3, 30]. Many of the substances produced after ischemia–reperfusion, including free radicals and Ca^{2+}, can be freely diffused through gaps between cells [1, 29]. The abnormal increase in the expression of the gap junction protein Cx43 (Fig. 9) in renal tissue (including the interlobar arteries) after ischemia–reperfusion may exacerbate the proliferation of harmful substances in the cell, and it may also exacerbate damage to the reperfused tissues. Obviously, abnormal expression of the gap junction protein Cx43 is a double-edged sword. On the one hand, GJs contribute to the synchronicity of kidney vasomotor activity, but on the other hand, they also increase the spread of harmful substances from cell to cell. As the results of our experiment suggested, a compensatory increase in Cx43 expression occurred after ischemia–reperfusion but was not able to restore the vasoconstrictive activity of the renal arteries (Fig. 2, 5, 6, 8).

Propofol is a sedative drug commonly applied in anesthesiology and intensive care medicine. Recent studies have found that propofol, in addition to its routine use in anesthesia, can fulfill many other roles, such as regulating the mesenteric microcirculation [31], which improves the ventricular arrhythmia induced by ischemia [32]. It has also been found by multiple research teams that propofol has protective effects against IRI in multiple organs [33, 34]. Propofol infusion has also been suggested to have protective effects against renal IRI in rats, but the underlying mechanism is not fully understood [10, 13, 35]. In the present study, HE staining of IRI-affected rat renal tissue (Fig. 1) and biochemical tests of intravenous blood (Table 1) have confirmed that propofol has a protective effect against renal IRI, which is consistent with what other researchers have found [35]. Although the protective mechanism of propofol against renal ischemia–reperfusion is not fully clear, there have been studies suggesting that the gap junction protein Cx43 may be involved in the role of propofol [13, 29, 32, 36]. Our research also suggests that propofol can reduce abnormal increases in kidney Cx43 protein expression caused by ischemia–reperfusion (Fig. 9). On the one hand, the state of renal vascular function state may be restored, and our findings confirm that propofol improved the amplitude and velocity of renal artery contraction after ischemia–reperfusion

(Fig. 5, 6). More importantly, the expression of propofol decreased Cx43, which also reduced the increase in renal arterial artery tension caused by ischemia–reperfusion (Fig. 2, 4). On the other hand, the reduction of Cx43 expression by propofol can also decrease the spread of harmful substances generated by ischemia–reperfusion. Although we have revealed evidence that the reduction of Cx43 gap junction protein expression in the renal arteries may explain the protective effect of propofol against kidney IRI, many questions remain unanswered. What is the mechanism by which propofol reduces CX43 expression? Are the reduced levels of Cx43 expression driven by a decrease in unphosphorylated or phosphorylated Cx43? These problems will gradually be solved in our future research.

Conclusion

Based on the above analysis, we hypothesized that the mechanism by which propofol reduces renal IRI in rats may be associated with the inhibition of abnormal Cx43 protein expression in the renal artery. We also found that in the propofol 4 h group, propofol had a protective effect against renal IRI and could mitigate the damage to the level found in the sham-operated group (Fig. 2, 4, 5, 6, 8). However, in the propofol 24 h group, the results suggested that the propofol did not protect the tissue to the same degree (Fig. 2, 4, 5, 6, 8). We showed that the damage was more severe after 24 h of postischemic reperfusion than after only 4 h, such that the use of protective drugs such as propofol could not restore it to the normal state. The results suggest that it is best to treat renal IRI as soon as possible. At the same time, our research may also reveal a novel mechanism by which propofol reduces ischemia–reperfusion injury, i.e., by inhibiting the abnormal expression of gap junctions. This study clarifies a novel mechanism for the action of propofol against IRI and may lead to new treatments for IRI.

Acknowledgements

This study was supported by grants from the National Basic Research Program of China (no. 2012CB52660000), the National Natural Science Foundation of China (nos. 31100829 and 81000411) and the High Level Talent Research Project of Shihezi University (grant no. RCSX201705). This research was supported by the Laboratory of Xinjiang Endemic and Ethnic Diseases. We are grateful to Professor Jun-Qiang Si and Professor Li Li for their expertise in statistical analysis.

Disclosure Statement

The authors declare no conflict of interests.

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