



Impact of propofol on renal ischemia/reperfusion endoplasmic reticulum stress¹

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Abstract

Purpose: To investigate the protective mechanisms of propofol (Pro) on renal ischemia/reperfusion (I/R) injury by studying its impact on renal I/R endoplasmic reticulum stress.

Methods: Eighteen male Sprague-Dawley rats (SD rats) were randomly divided into three groups: the I/R group, the Pro pretreatment group, and the control group, and corresponding treatments were performed. The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) of each group were detected. The expression levels of CCAAT-enhancer-binding protein (C/EBP) homology protein (CHOP) and caspase-12 protein within renal tissue samples were detected by western blot.

Results: The periodic acid-Schiff (PAS) staining was performed to observe the morphological changes within the renal tissues, and the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay was performed to detect the presence of renal apoptosis. The Pro pretreatment significantly reduced the serum Cr and BUN levels, as well as the expressions levels of CHOP and caspase-12 protein inside the kidney of I/R rats, improving renal pathological injury and reducing the I/R-induced renal apoptosis.

Conclusion: Propofol could downregulate the expression of stress-apoptotic proteins CHOP and caspase-12 in the endoplasmic reticulum, thus reducing renal I/R injury.

Key words: Endoplasmic Reticulum Stress. Reperfusion Injury. Propofol. Kidney. Rats.

■ Introduction

Renal ischemia/reperfusion (I/R) injury is the main cause of acute renal failure. It is common in clinical patients with hypovolemic shock, kidney transplantation, cardiovascular surgery, etc., and can be life threatening¹. Renal I/R is known to promote the upregulation of CCAAT-enhancer-binding protein (C/EBP) homology protein (CHOP), therefore increasing apoptosis². CHOP and caspase-12 are key proteins in the endoplasmic reticulum stress apoptosis pathway. Hence, it is particularly important to have measures that can promptly prevent this type of injury. Studies have found that intravenous anesthetic propofol (Pro) shows properties such as antioxidation, anti-inflammation, and anti-apoptosis^{3,4}, and could reduce I/R injury in organs including the liver, kidney, and brain⁵⁻⁷; however, its effects on the signals of the endoplasmic reticulum stress apoptosis pathway, during renal I/R injury, are yet to be reported. In this study, we observed the effects of Pro on the expressions of CHOP and caspase-12 in renal I/R injury, aiming to explore the molecular mechanisms related to the actions of Pro in renal I/R injury prevention.

■ Methods

The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Zhengzhou University. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

The male SD rats (SPF grade, purchased from the Experimental Animal Center of the Henan Province), weighed 250-300 g, and had unrestricted access to food and water. All the animal-related operations were in line with

the regulations of the experimental animal management.

Preparation of the renal I/R model and animal groupings

To produce the renal I/R injury model in rats, anesthesia was induced by intraperitoneal injection of 60 mg/kg sodium pentobarbital. Then the rat was placed on an operation plate equipped with a thermostat system. The rat's rectal temperature was maintained at 37°C. The abdominal cavity was opened along the ventral abdominal midline and the right kidney was resected. The left renal pedicle was freed and an intraperitoneal injection of 50 U/kg heparin was administered; after 5 min, the left renal pedicle was occluded with a non-injury artery clamp. The renal pedicle was reopened 60 min later. The abdominal cavity was then closed and after 24 h, perfusion was performed and the blood serum and renal specimens were collected. The 18 male SD rats were randomly divided into three groups. The I/R group comprised rats that had undergone the procedure described above. The Pro pretreatment group comprised rats in which 25 mg/kg/h Pro (Libang Pharmaceutical Co., Ltd., Xi'an, China) was pumped via the tail vein for two consecutive hours after anesthesia, and the renal I/R injury model was prepared during this time. Finally, the control group comprised rats that had been treated in the same manner as the I/R group except occlusion of the left renal artery pedicle. The serum and renal specimens of each group were sampled.

Detection of serological indicators

The blood was sampled from the inferior vena cava, and serum creatinine (Cr) and blood urea nitrogen (BUN) were detected using a standard clinical automatic biochemical analyzer (Hitachi7600-020).

Detection of renal CHOP and caspase-12 protein

We took 50 mg of frozen renal tissue, extracted the proteins according to the instructions provided with the RIPA clearance solution and used a BCA protein concentration detection kit to detect the protein concentration (Beyotime Biotechnology Co. Ltd, Shanghai, China). Then, SDS sample-loading buffer was added to the isolated protein, and the mixture was boiled for 5 min. A 10% SDS-polyacrylamide separation gel was prepared, to which 20 µg of protein was added per lane. Following electrophoresis and membrane transferring, 5% nonfat milk was used to block the PVDF membrane. The primary CHOP antibody (Santa Cruz Biotech., California, USA) and primary caspase-12 antibody (Santa Cruz Biotech., California, USA) (1:1000) were added and the membrane was incubated overnight at 4°C. The membrane was washed with TBST, and then, the appropriate secondary antibody (Beijing Kangwei Century Biotech. Co., Beijing, China) (1:5000) was added and the membrane was incubated for 1 h at room temperature. Following another wash with TBST, ECL luminescent was added to the membrane. After 5 min, the blots were developed by exposure in darkness. The expression of β-actin protein (antibody purchased from Santa Cruz Biotech., California, USA) was used as a control to correct the calculated expression levels of the target proteins.

Pathomorphological observations

We cut the paraffin-embedded renal tissue samples into 5-µm-thick paraffin slices, followed by dewaxing, hydration, periodic acid-Schiff (PAS) staining, hematoxylin re-staining, routine dehydration, hyalinization, and fixation of the samples. The pathomorphological changes of the renal tissues from each group were then observed under a microscope.

Determination of renal cell apoptosis

The tissue samples were cut into 5-µm-thick paraffin slices, dewaxed, and hydrated. The samples were then labeled according to the instructions of the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) apoptosis kit (Chemicon International Co., California, USA). The apoptotic nuclei appeared brown. Each slice was observed in at least three non-overlapping fields to count the number of the apoptotic cells.

Statistical analysis

SPSS17.0 statistical software was used for the statistical analysis; the quantitative data were analyzed by using a t-test, with $P < 0.05$ considered as a statistically significant difference.

■ Results

Changes in serum Cr and BUN

The serum Cr levels of the I/R group, the Pro pretreatment group, and the control group were 201.3 ± 27.5 µmol/L, 140.7 ± 25.6 µmol/L, and 37.7 ± 9.3 µmol/L, respectively. The serum BUN levels of the above three groups were 22.3 ± 4.5 mmol/L, 11.6 ± 5.3 mmol/L, and 4.4 ± 3.3 mmol/L, respectively. Compared with the I/R group, the Pro pretreatment group showed notably reduced serum Cr and BUN levels, and the differences were statistically significant ($P < 0.05$).

Expressions of CHOP and caspase-12 protein

The expression levels of CHOP and caspase-12 protein in the I/R group significantly increased, while the Pro pretreatment significantly reduced the protein levels of CHOP and caspase-12 (Figure 1).

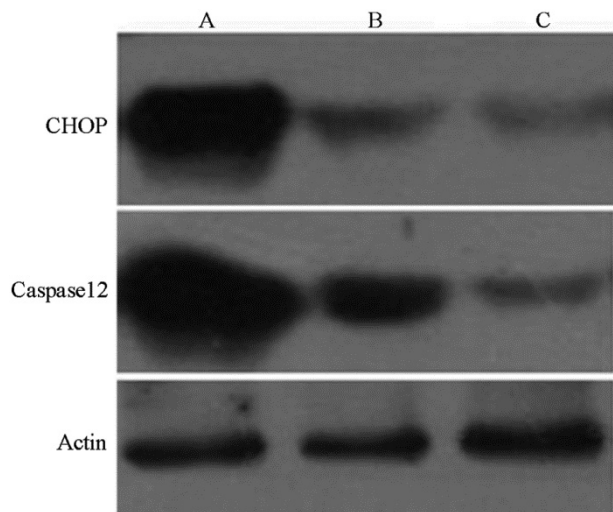


Figure 1 - Expressions of CHOP and Caspase12 protein in each group by Western blot. **A:** I/R group; **B:** Pro pretreatment group; **C:** Sham group.

Renal pathomorphological changes

The I/R group exhibited widespread necrosis of tubular epithelial cells, with a large number of tubular and necrotic cells found inside the tubular cavity. In this group, the renal interstitium showed vascular dilatation and congestion, with focal hemorrhage and inflammatory cell infiltration. The Pro pretreatment group only showed focal tubular necrosis; there were fewer tubular and necrotic cells, interstitial congestion and edema significantly reduced, and inflammatory cell infiltration was lessened. The control group showed normal renal tissue structures (Figure 2).

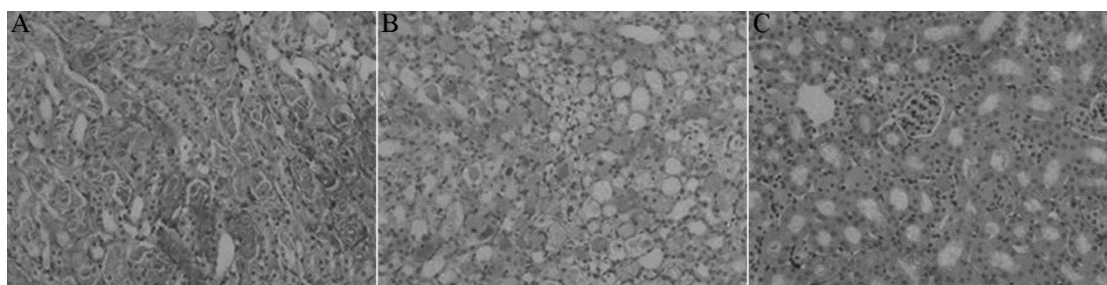


Figure 2 - Renal pathomorphological changes in each group (PAS, $\times 400$). **A:** I/R group; **B:** Pro pretreatment group; **C:** Sham group.

Renal cell apoptosis

The number of apoptotic cells in the I/R group, the Pro pretreatment group, and the control group was 171 ± 36 , 80 ± 29 , and 5 ± 3

cells respectively per field of vision. Compared with the I/R group, the Pro pretreatment group showed significantly reduced number of apoptotic cells ($P < 0.05$) (Figure 3).

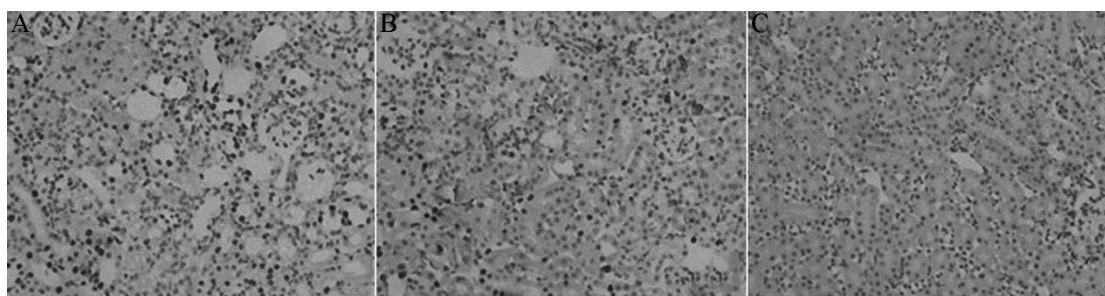


Figure 3 - Renal cell apoptosis in each group (TUNEL, $\times 400$). **A:** I/R group; **B:** Pro pretreatment group; **C:** Sham group.

■ Discussion

Renal acute I/R injury is the main cause of clinical acute renal failure, and its mortality rate is high⁸. The pathophysiological mechanism of I/R injury involves the generation of a large amount of free radicals, Ca²⁺ overload, and inflammatory cell infiltration⁹. When ischemia occurs, ATP is rapidly depleted, consequently the mitochondrial endoplasmic reticulum swells, and the change in membrane permeability causes the release of Ca²⁺ from the mitochondria and endoplasmic reticulum. During the period of reperfusion, the ATP pump has yet to be restored, so a large number of extracellular Ca²⁺ flows in, resulting in the rapid increase in intracellular Ca²⁺. The overload of Ca²⁺ impedes oxidative phosphorylation within the mitochondria, hinders ATP synthesis, and promotes the destruction of proteins and membrane structures. Consequently, a large number of oxygen free radicals are generated, and the cells exhibit apoptosis and even necrosis¹⁰. The kidney is rich in blood supply, so it is much more sensitive to ischemia; I/R causes an overload in renal Ca²⁺. Meanwhile, the rate of renal cell apoptosis increases, suggesting that Ca²⁺ overload is an important cause of renal dysfunction¹¹. Certain interventions could be implemented at the initial stages of apoptosis to reverse it, which could reduce injury to some extent.

The endoplasmic reticulum stress apoptosis pathway is one of the important endogenous apoptosis pathways, and CHOP and caspase-12 are important apoptotic proteins involved in this pathway¹². Endoplasmic reticulum stress is the progress indicator of renal injury¹³. I/R causes CHOP upregulation in the renal endothelial and epithelial cells, with an increase in apoptosis. Knocking out the CHOP gene was shown to significantly reduce renal I/R injury in mice, and improve renal microcirculation after I/R². Furthermore,

post-ischemic preconditioning was shown to reduce the I/R-induced renal endoplasmic reticulum stress by downregulating the protein expression of glucose regulating protein 78 (GRP78), activating transcriptional factor 4 (ATF4), double-stranded RNA-dependent protein kinase-like ER kinase (PERK) protein, X box binding protein 1 (XBP-1), and caspase-12, thus alleviating renal I/R injury¹⁴. Therefore, if endoplasmic reticulum stress could be subdued, renal I/R injury could in turn be reduced. Certain studies found that Pro might regulate the unfolded protein response during endoplasmic reticulum stress, as to reduce apoptotic injury in neurons¹⁵. Another study found that Pro could reduce endoplasmic reticulum stress-mediated apoptosis in the neuroblastoma cell line, SH-SY5Y¹⁶. The present study demonstrated that Pro reduces the expression levels of CHOP and caspase-12 protein in kidneys that have undergone I/R, as well as reduce renal cell apoptosis, thus reducing renal I/R injury.

Pro is an intravenous anesthetic and sedative commonly used in clinical practice; it has a phenolic hydroxyl structure similar to that of vitamin E and butylhydroxytoluene, and can act as a hydrogen donor *in vivo*, reacting with free radicals and generating phenoxy. Studies found that Pro could reduce LPS-induced macrophage inflammatory responses and reduce the generation of tumor necrosis factor α (TNF- α), interleukin (IL)-6, and IL-10 by inhibiting the reactive oxygen species/Akt/inhibitor kappa B kinase β /nuclear factor κ B (ROS/Akt/IKK β /NF- κ B) signaling pathway¹⁷. Pro has been shown to play an anti-inflammatory role in cardiopulmonary bypass, reducing the production of IL-6¹⁸, and to exert antioxidant effects in the H9c2 cells via FoxO1, thus protecting myocardial cells¹⁹. Pro was reported to reduce renal I/R injury in rats with hyperglycemia, increase the level of superoxide dismutase, and reduce the levels

of myeloperoxidase, IL-1 β , and TNF- α ⁶. Pro can reduce post-I/R liver cell apoptosis by inhibiting the activation of glycogen synthase kinase 3 β (GSK-3 β) in mitochondria, and maintaining the opening of mitochondrial permeability-transition pores, thus preventing mitochondrial swelling and potential membrane downfall⁵. Furthermore, Pro was reported to also activate the Nrf2 pathway to alleviate liver oxidative stress²⁰, while in human alveolar epithelial cells, Pro reduced LPS-induced ROS production, activated Nrf2/GSH and inhibited nicotinamide adenine dinucleotide phosphate (NADPH) oxidase^{e3}. Through inhibiting the NADPH oxidase-mediated mastocyte activation, Pro is thought to alleviate intestinal I/R injury²¹. Ultimately, Pro has anti-apoptosis, anti-inflammation, and antioxidation properties.

■ Conclusions

Propofol was shown to inhibit the expression of key apoptotic proteins in the endoplasmic reticulum apoptotic signaling pathway, thus providing an anti-apoptotic protective effect against renal I/R injury. This broadens the theoretical foundation for the Pro-mediated protection of renal functions. Nonetheless, research related to the protective effects of Pro against renal I/R injury still requires further investigation.

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