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Effect and mechanism of propofol in hepatic ischemia/reperfusion injury of rat

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Abstract. – OBJECTIVE: Hepatic ischemia/reperfusion (I/R) injury remains to be one of the most common clinical diseases. This study aimed to explore the potential effect and mechanism of propofol in protecting rat liver from I/R injury.

MATERIALS AND METHODS: The hepatic I/R model was established in Sprague-Dawley (SD) rats by perfusing the liver with heparinized cold saline through the portal vein for 20 min. The rats were then received a 100 mg/kg/d propofol administration for the continuously 10 days. The hepatic function indexes of ALT, AST and GGT were detected by ELISA. The apoptosis of hepatic cells was assessed by TUNEL assay, and Bax and Bcl-2 expression changes were detected by qRT-PCR and Western blotting. In addition, serum pro-inflammatory factors and PI3K/AKT signaling pathway-related protein expression were detected.

RESULTS: Propofol markedly attenuated the increases of ALT, AST, and GGT induced by I/R. Propofol reduced I/R-induced apoptosis and pro-inflammatory factors release. Furthermore, propofol could inhibit the expression of phosphorylated PI3K, AKT and mTOR, and inhibited the expression of p-mTOR.

CONCLUSIONS: Propofol protects hepatic I/R injury partly by reducing apoptosis and reducing the release of pro-inflammatory cytokines, which is possibly involved in the modulation of the PI3K/AKT/mTOR signaling pathway. All these data suggest that propofol may play certain positive roles in protecting the liver from I/R injury.

Key Words:
Propofol, Hepatic ischemia/reperfusion (I/R) injury, Apoptosis, Inflammatory cytokines, PI3K/AKT/mTOR.

Introduction

Hepatic ischemia-reperfusion (I/R) injury refers to liver tissue ischemia and hypoxia because of liver blood flow interruption or inadequate perfusion in consequence of various diseases, which is recognized as a type of uncontrolled inflammatory cascade[1-3]. The reperfusion not only can't be able to restore functions of ischemic tissues, but aggravate dysfunction of the liver cell metabolic disorder and structural damage[4-6]. Thus, hepatic I/R leads to organs dysfunction and failure, and other complications after liver resection and liver transplantation surgery directly affecting the achievement of surgery and postoperative survival rate[7-11]. With the deepening research of the hepatic I/R injury mechanism, the researchers found that pharmacological preconditioning (PPC) could effectively protect hepatic I/R injury[12-14].

Regarding the present work, the hepatic I/R injury protective drugs are mainly oxygen-free radical scavenger, calcium antagonist, Kupffer cell activation inhibitors and drugs that could improve microcirculation or cell energy metabolism[15-18]. In the report of Li et al[18], animal experiments were constructed and shown the role of the calcium channel blockade on hepatic I/R injury protection and found it assist the recovery of secretory function in hepatocytes. Moreover, the research of Chen et al[19] illustrated that gadolinium chloride (GdCl3) significantly weaken I/R-induced myocardial apoptosis in rats by inhibiting activation of both death receptor and mitochondria-mediated pathway. However, the toxic and side effects of these drugs affect its clinical application. Propofol is a new type of clinical commonly used and named as a kind of safety anesthetics, but the role of propofol in hepatic I/R injury research is still insufficient[20,21].

This research works on the new uses of anesthetic, aiming to explore the function and mechanism of propofol in hepatic I/R. The hepatic function was detected by ELISA, and propofol was found remarkably restores the liver function. In addition, propofol significantly ameliorated

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apoptosis by increasing the Bcl-2/Bax ratio. Additionally, we found that propofol reduced the release of pro-inflammatory cytokines in hepatic I/R injury. qRT-PCR and Western blot results showed that propofol is possibly involved with the modulation of the PI3K/AKT/mTOR signaling pathway in hepatic I/R protection. In summary, propofol protects against hepatic I/R partly by reducing apoptosis and reducing the release of pro-inflammatory cytokines, which is possibly involved with the modulation of the PI3K/AKT/mTOR signaling pathway. All these findings suggest that propofol may be a new therapeutic target for hepatic I/R injury.

Material and Methods

Experimental Animals

Male Sprague-Dawley (SD) rats, aged 10 weeks (200 ± 10 g) were obtained from the Laboratory Animal Center of Tianjin Medical University (Tianjin, China). The rats were randomly divided into three groups: 1) Sham group (n = 20 rats), in which rats received a sham oper...; 2) I/R group (n = 30 rats), in which I/R... was established; 3) I/R + propofol grou... = 30 rats), in which I/R model was first est... shed and then the cells were treated with ... mg/kg/d propofol (Shanghai Sh... n Biolog... cal Technology Co., LTD, Sh... ina) for... 10 days. Each group shou... sure t... at least 15 rats survived until th... d of th... rch. Five rats in each grou... w... k... h, and 24 h after re... usio... the animal experiments were ... roved by ... hics Committee of Huna... incial Peopl... ospital, The First Aff... ed ... ital of Huna... Normal Universit...

Anima... odel of Hepatic ...

Th... ts we... erformed a midline laparoto... nder ... % chlo... l hydrate anesthesia. After ... g the h... ic artery, portal vein, ...rahep... ena ... (SHVC), and infrahe... ena c... C), the portal vein and the IH... were can... ated with a polyethylene tube and ... liver was also perfused through the por... ... parinized cold saline (2.5 IU/mL, ... mL/min) to wash out all the blood through ...HVC. The transfixion pin was removed and t... cture site was repaired after 20 minutes of c... d perfusion. The anhepatic phase ended after all clips were unclamped[22].

Determination of Plasma Liver Enzyme and Oxidation-related Parameters

The serum was obtained by centrifug... d samples. The level of alanine amin... (ALT), gamma-glutamyl transpeptid... (GGT) and aspartate aminotransferase (AST... re respectively detected by the spectrophoto... using an automated clinical chemistr... analyze... 5400; Beckman Coulter, Brea, C... SA)[22].

TUNEL Assay

The frozen liver s... sectio... into slices of 5-mm ... knes... *in situ* ...optosis was perform... ing TU... ass... kit (Promega, Madi... WI, USA). W... ed the cells displa... bl... taining with... the nucleus as apoptot... cells. ... mber of apoptotic cells were e... ted by a per... nded to the group assig... y 3 nonoverlap... g microscopic eyes... under high-power magnification (× 400) and ...ressed as per... tage[22].

D... minatio... f TNF-α and IL-6 Lev... ...

Seru... α and IL-6 levels were determined ...sing an ELISA kit (Biosource International, Ca... CA, USA)[21].

Western Blot Analysis

Liver tissues were rapidly homogenized in 200 mL of extraction protein buffer containing 50 mM tris-HCl, pH 7.4, 2.0 mM EDTA, 2 mM Na_3VO_4, 50 mM NaF, 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), 10 µg/mL aprotinin, 10 µg/mL leupeptin, and 10 µg/mL pepstatin A[23]. After incubation for 30 min on ice, the supernatant was centrifuged at 300 g for 10 min. Protein samples were separated on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA)[3], after boiling for 5 min at 95°C. Then, the membranes were incubated with the appropriate primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight after blocking with 5% skim milk in TBS-T (powder-Tris-buffered saline with 0.1% Tween 20) for 1 h. Membranes were washed 3 times with TBS-T and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h. The final results were obtained by exposure to Kodak film (NY, USA).

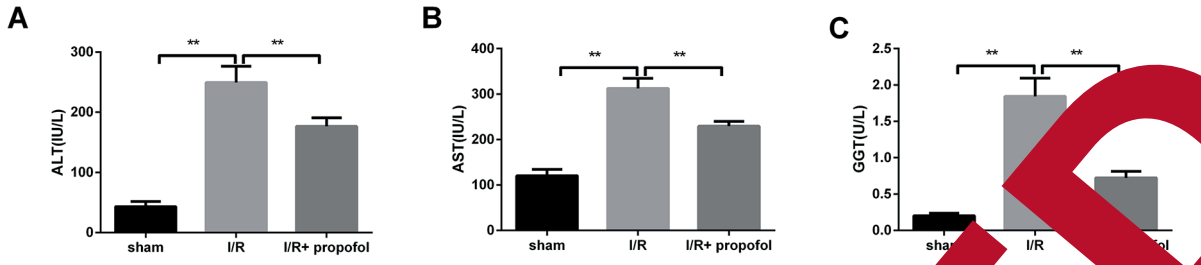

Figure 1. Effect of propofol treatment on hepatic ischemia-reperfusion (I/R) injury. Serum samples were collected from the rats in sham, I/R and I/R + propofol groups. ***(A)*** Alanine aminotransferase (ALT), ***(B)*** aspartate aminotransferase (AST), and ***(C)*** gamma-glutamyl transpeptidase (GGT) indexes were tested. Data presented as mean ± SD (n = 6 per group). ** $p < 0.01$ (Student *t*-test).

qRT-PCR

Total RNA was isolated from the liver tissue sample using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). qPCR was performed using a Light-Cycler® 480Real-Time PCR System (Roche, Basel, Switzerland) and the SYBR Green qPCR Master Mix (2X) (Fermentas, Waltham, MA, USA).

Statistical Analysis

The data are presented as the mean ± SD. Statistical analyses were performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Differences between two groups were analyzed by Student *t*-test. $p < 0.05$ was considered to be statistically significant.

Results

Propofol Restored the Liver Function in I/R

I/R mode was established in rats and the rats were received a 10 mg/kg/d propofol administration for the consecutive 3 days. Then, the serum of the rats was collected and the hepatic index was detected using ELISA. As shown in Figure 1, ALT, AST, GGT indexes in I/R group were all significantly increased, while the liver function improved significantly after propofol administration. These suggested protective role of propofol in I/R injured rats.

Propofol Ameliorates Cell Apoptosis in I/R

TUNEL assay was performed to assess the number of apoptotic cells in the liver of the three groups. As results show in Figure 2A, the hepatic cell apoptosis induced by the I/R was decreased by propofol intervention. Next, we extracted the total mRNA and protein in the liver of the three groups and detected the expression levels of Bax and Bcl-2. Bax is one of the apoptosis promoting gene, while Bcl-2 is an anti-apoptotic gene. As shown in Figure 2 B and 2C, Bax was highly expressed in I/R group and the Bcl-2 was lowly expressed. More important, propofol intervention significantly alleviated I/R induced Bax up-regulation and Bcl-2 down-regulation.

Propofol Reduces Release of Pro-inflammatory Cytokines in I/R

HIRI always accompanied by inflammation, thus we tested the influence of propofol on the release of pro-inflammatory cytokines in I/R. The results given in Figure 3 showed that propofol reduces pro-inflammatory factors release of IL-6, TNF-α, and MIP2 in I/R.

Propofol Promotes AKT Phosphorylation and Inhibits p-mTOR

Previous studies[24,25] have reported the hepatic I/R injury may involve AKT/mTOR signaling pathway. So we performed qRT-PCR and Western blot experiments to analysis RNA and protein expression of AKT and mTOR in the liver of these three groups. As shown in Figure 4, the phosphorylated forms of AKT and mTOR were both up-regulated after I/R injury, while total AKT and mTOR was both down-regulated. Propofol administration could enhance I/R induced abnormal expression of p-AKT and AKT, and alleviate I/R induced abnormal expression of p-mTOR and mTOR.

Discussion

Hepatic I/R injury is confirmed an unavoidable consequence during hepatic resection, liver transplantation, and hypovolemic shock[7]. It has been implicated in the pathophysiology of many clinical entities following hepatic surgery and transplanta-

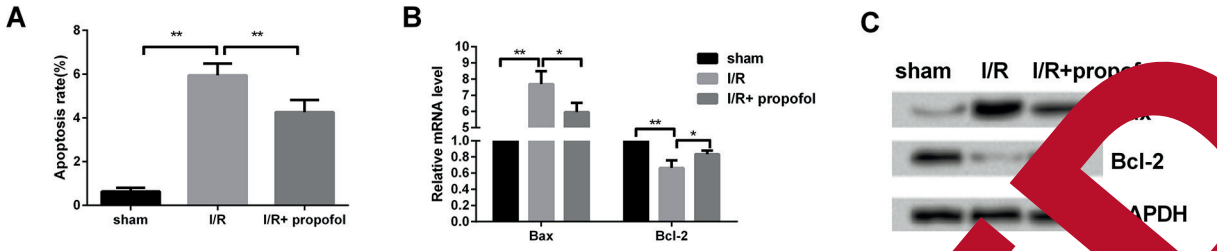

Figure 2. Effects of propofol on apoptosis in the liver after hepatic ischemia-reperfusion (I/R) injury. ***(A)*** TUNEL assay was performed to detect the apoptotic cells rate of sham, I/R, and I/R + propofol groups. ***(B)*** The mRNA and protein levels of Bax and Bcl-2 were examined by qRT-PCR and Western blotting respectively. Data presented as mean ± SD (n = 5/group). *, $p < 0.05$; **, $p < 0.01$ (Student *t*-test).

tion, as well as the dysfunction and injury of other organs[12]. Although the research on hepatic I/R injury has made great progress, it is still a major cause of morbidity and mortality following liver surgery[26]. In the present work, we found that propofol could partially recover I/R induced an increase in ALT, AST, and GGT levels. Propofol alleviated I/R induced apoptosis and the release of pro-inflammatory cytokines. Besides, propofol aggravated I/R induced p-AKT up-regulation and AKT down-regulation, as well as alleviated I/R induced p-mTOR up-regulation and mTOR down-regulation.

As a new type of anesthetics commonly used in clinical, the use of propofol has been reported to be safe for atrial fibrillation ablation, catheter ablation, internal cardioverter defibrillator implantation and many other ways. Furthermore, several investigations have revealed a protective role of propofol in I/R injury liver. For instance, propofol can protect the liver from I/R injury by reducing I/R induced increase of plasma ALT and AST[29,30]. In accordance with the reported literatures, our data also confirmed the protective effects of propofol on hepatic I/R, that it could dramatically reduce ALT, AST and GGT levels in plasma.

Apoptosis is a major mechanism of cell death after hepatic I/R injury, we detected the apoptotic cells rate and the expression of apoptosis-related proteins, *i.e.*, Bcl-2 and Bax, to further understand the role of propofol in hepatic I/R injury. The results clearly showed that propofol significantly ameliorated apoptosis by increasing the Bcl-2/Bax ratio. Also, the inflammatory response plays an important role in liver dysfunction after liver injury[21]. We tested the release of pro-inflammatory cytokines IL-6, TNF-α and MIP2 in hepatic I/R injury and finally found that propofol can decrease the release of pro-inflammatory cytokines in hepatic I/R injury. These findings were all in line with the previous research that, propofol can protect the liver from I/R injury by modulating the inflammatory responses and liver apoptosis[32].

Evidence has strongly suggested that the PI3K/AKT/mTOR signaling pathway played an important role in hepatic I/R injury[33]. To demonstrate the mechanism of propofol on hepatic I/R injury progress, we constructed qRT-PCR and Western blot experiments and tested AKT and mTOR expressions at both the mRNA and protein levels. Propofol was reported to activate

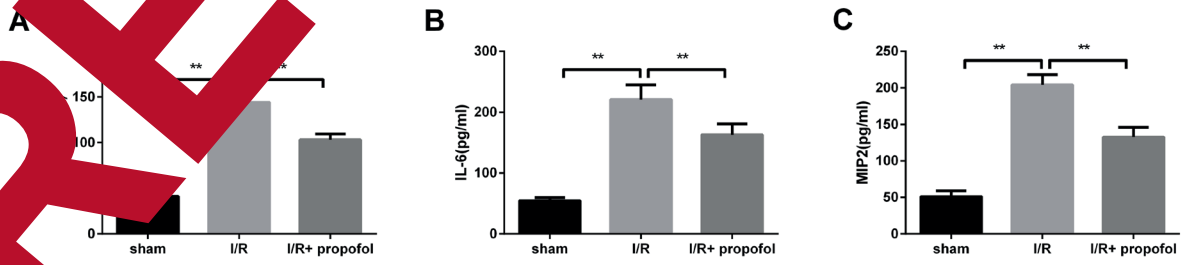

Figure 3. Effects of propofol on the pro-inflammatory factor expressions in the rats after hepatic ischemia-reperfusion (I/R). Serum in rats from the sham, I/R and I/R + propofol groups were collected. The release of ***(A)*** TNF-α, ***(B)*** IL-6 and ***(C)*** MIP2 were assessed by using ELISA kits. Data presented as mean ± SD (n = 5/group). **, $p < 0.01$ (Student *t*-test).

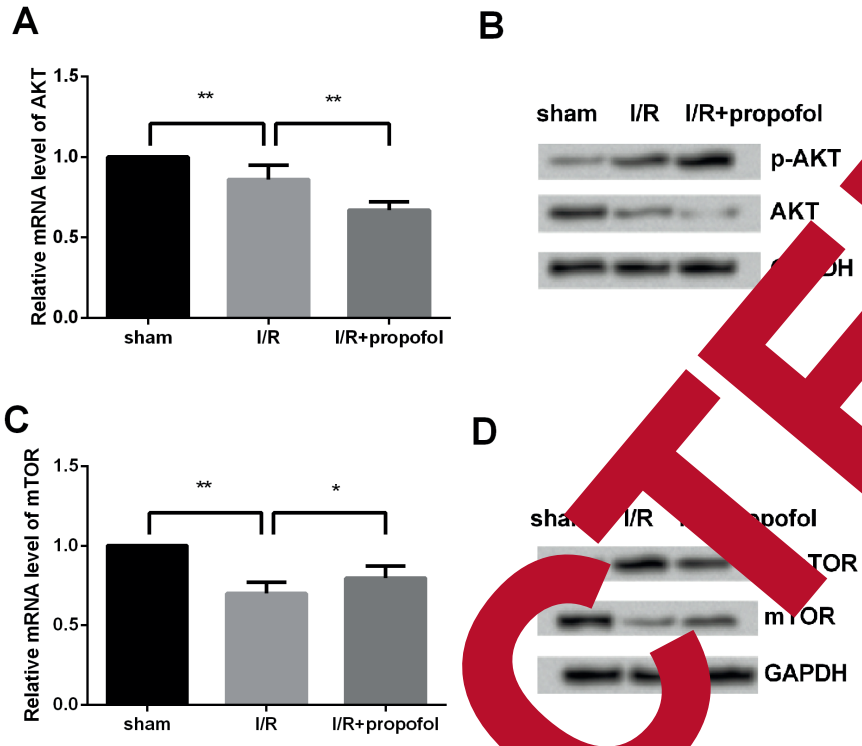

Figure 4. Effects of propofol on AKT and mTOR expression after hepatic ischemia-reperfusion (I/R). The ***(A)*** mRNA level of AKT, and the ***(B)*** protein levels of p-AKT and AKT were respectively detected by qRT-PCR and Western blotting. The ***(C)*** mRNA level of mTOR, and the ***(D)*** protein levels of mTOR were respectively detected by qRT-PCR and Western blotting. Data presented as mean ± SD (n = 5/group). $P < 0.05$ (Student *t*-test).

AKT expression in hepatic [32]; this was also confirmed in this study. Propofol significantly up-regulated p-AKT while significantly inhibited AKT. However, this study provided the first evidence that propofol also could regulate mTOR expression in hepatic I/R. In the present paper, p-mTOR was down-regulated by propofol, while mTOR was up-regulated. Similarly, propofol was reported to decrease p-mTOR/mTOR level in cerebral I/R injury[34].

Conclusions

We demonstrate that propofol preconditioning protects against hepatic I/R partly by reducing apoptosis and reducing the release of pro-inflammatory cytokines, which is possibly involved in the modulation of the PI3K/AKT/mTOR signaling pathway. Our study may provide a new method for clinical treatment of hepatic I/R injury and supply a molecular basis for the new use of anesthetic.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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