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Effect of propofol on the skeletal muscle insulin receptor in rats with hepatic ischemia-reperfusion injury

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Abstract

Objective: To investigate the effect of propofol on the expression and phosphorylation of the skeletal muscle insulin receptor and its substrates following hepatic ischemia-reperfusion injury (HIRI).

Methods: Sixty healthy Wistar rats were divided randomly into a propofol group (P) and an ischemia-reperfusion group (I/R). Rats in the P group received propofol infusion prior to ischemia and during a 120-minute post-reperfusion period. Plasma glucose and insulin concentrations were measured, as well as expression levels of the insulin signaling proteins insulin receptor (IR) β unit (IRβ) and IR substrate 1 (IRS-1). In addition, tyrosine phosphorylation levels of these proteins were measured in skeletal muscle.

Results: Plasma glucose levels in the two groups were higher at 2 hours after reperfusion (T2) versus exposure of the hepatic hilum (T1). Plasma glucose levels in the I/R group were higher than those in the P group, while insulin levels at T2 were lower. In addition, phosphotyrosine levels of IRβ and IRS-1 were decreased by 32.1% and 22.4%, respectively.

Conclusion: Propofol increased phosphotyrosine levels of IRβ and IRS-2, resulting in an alleviation of increased plasma glucose levels following HIRI.

Keywords

Propofol, ischemia-reperfusion injury, phosphorylation, plasma glucose, insulin signaling, phosphotyrosine

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Introduction

Hepatic ischemia-reperfusion injury (HIRI) is a common pathophysiological process that occurs during surgical procedures such as liver resection and liver transplantation[1] and can induce a series of physiological effects on the body. Previous studies have shown that HIRI can lead to a critical increase in serum glucose levels,[2] leading to hyperglycemia, which in turn can lead to liver metabolism and immune dysfunction and have a negative effect on post-surgical recovery.[3] Glucose control is directly related to the insulin signal transduction pathway.[4] Previous studies have demonstrated that both HIRI and propofol can affect the insulin signaling pathway to induce insulin resistance.[5,6] In addition, propofol has been shown to have contradictory effects on hepatic ischemia-reperfusion injury.[7] Whether propofol has an effect on the insulin signaling pathway after HIRI remains unclear. We therefore used a rat model of hepatic ischemia-reperfusion to investigate the effects of propofol on serum glucose and hepatic insulin signaling pathways.

Materials and methods

Animal model and surgical procedures

Sixty healthy male Wistar rats (200–255g) were purchased from the Animal Experimental Center of Nanjing Medical University and housed in a ventilated room with a constant temperature of 20°C. All rats were fasted for 14 hours prior to the experimental procedures and were randomly divided into a propofol group (P group) and an ischemia-reperfusion group (I/R group). Prior to surgical procedures, rats were administered L-pentobarbital sodium (40 mg/kg) by intraperitoneal injection. Under sterile conditions, the midline of the upper abdomen to the hilum was incised and the hepatic portal blood vessels were clamped for 30 minutes to induce complete hepatic ischemia. After 30 minutes, the clamp was removed to restore hepatic blood flow for 2 hours. The time point at which the hepatic hilum was exposed was designated T1. For rats in the P group, propofol was infused 20 minutes prior to hepatic occlusion (10 mg/kg/hour) and until 2 hours after reperfusion (T2). Rats in the I/R group were infused with a similar volume of normal saline at the same rate. All animal procedures were approved by the Ethics Committee of Changzhou First People's Hospital (reference no. 051968870201).

Measurement of plasma glucose levels

Blood samples (5 mL) were collected from the inferior vena cava after exposure the hepatic hilum (T1; $n = 10$ in each group) and 2 hours after reperfusion (T2; $n = 10$ in each group). Serum glucose levels were measured using the glucose oxidase method, and the insulin secretion (IS; IS = insulin/PG) and insulin resistance (HOMA-IR; HOMA-IR = insulin*PG/22.5) indices were calculated.[8]

Western blot analysis

Protein expression levels for IRβ and IRS-1 in skeletal muscle tissue were determined in each group at T2. In brief, tissues were frozen in liquid nitrogen and placed at −86°C. The frozen tissues were then cut, lysed, and centrifuged. Protein concentrations were determined from the supernatants. Proteins were denatured and then electrophoresed on a 6% polyacrylamide gel, transferred to membranes, and blocked. The membranes were incubated with anti-rat IRβ or IRS1 antibody (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Next, the membranes were washed and incubated with diluted affinity-purified secondary antibody at 37°C. Membranes

were washed and then incubated with chromogenic substrate trace AB solution. Bands were visualized using a Gel Pro-Analyzer (JEDA3.3, Jiangsu, China), and the optical density of the immunoreactive bands was calculated. The ratio of each protein band to an internal reference was then calculated.

Phosphorylation levels of IRS-1 in the two groups were determined at time T2. Rats were injected with saline (0.5 mL, containing 10^{-5} mol/L insulin) through the portal vein. Skeletal muscle tissue was harvested after 30 seconds and the tissues were cut, lysed, and centrifuged. Supernatants were immunoprecipitated using anti-IRβ or anti-IRS-1 antibodies for 2 hours and the immune complexes were incubated with protein G agarose beads. After washing, the immune complexes were electrophoresed, transferred to membranes, and immunoblotted with primary and secondary antibodies as described above. Tyrosine phosphorylation levels of IRβ and IRS1 (Tyr-IRβ and Tyr-IRS1) were measured as described previously.[9]

Statistical analysis

SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Normal variables were presented as mean + standard deviation (+ s). A mean t-test or analysis of variance was used for comparison of measurement data. A Chi-square test, Fisher exact test, or rank sum test was used for counting data. Values of $p < 0.05$ were considered statistically significant.

Results

Plasma glucose and insulin levels

The plasma glucose (PG) levels, IS, and HOMA-IR were significantly increased in both groups at T2 compared with T1 ($P < 0.01$). T2 serum glucose levels in the I/R group were significantly than those in the P group ($P < 0.05$), while insulin levels and IS at T2 were reduced ($P < 0.05$) (Table 1).

Changes in the hepatic insulin signal transduction pathway

The results from western blot assays showed that there were no significant differences in IRβ and IRS-1 skeletal muscle expression levels between the two groups. However, immunoprecipitation and western blot analysis showed that the expression levels for Tyr-IRβ and Tyr-IRS1 in the I/R group were decreased by 32.1% ($P < 0.01$) and 22.4% ($P < 0.01$), respectively, compared with the P group (Figure 1).

Table 1. Changes in PG, insulin, IS, and HOMA-IR at T1 and T2 in the I/R and P groups.

Group	Point	I/R	P
PG (mmol/L)	T1	4.91 ± 0.73	4.57 ± 0.54
	T2	$10.92 \pm 0.94^{\diamond\blacklozenge}$	$9.79 \pm 0.81^{\diamond}$
Insulin (μIU/L)	T1	39.11 ± 15.48	38.38 ± 7.08
	T2	$34.93 \pm 4.75^{\blacklozenge}$	38.99 ± 4.91
IS	T1	7.75 ± 2.26	8.40 ± 1.13
	T2	$3.08 \pm 0.16^{\diamond\blacklozenge}$	$3.98 \pm 0.39^{\diamond}$
HOMA-IR	T1	9.02 ± 4.87	7.89 ± 2.18
	T2	$16.51 \pm 3.27^{\diamond}$	$17.07 \pm 3.29^{\diamond}$

$^{\diamond}p < 0.01$ Compared with T1; $^{\blacklozenge}p < 0.05$ Compared with the P group.
PG, plasma glucose; IS, insulin/PG; HOMA-IR, insulin*PG/22.5; I/R, ischemia-reperfusion.

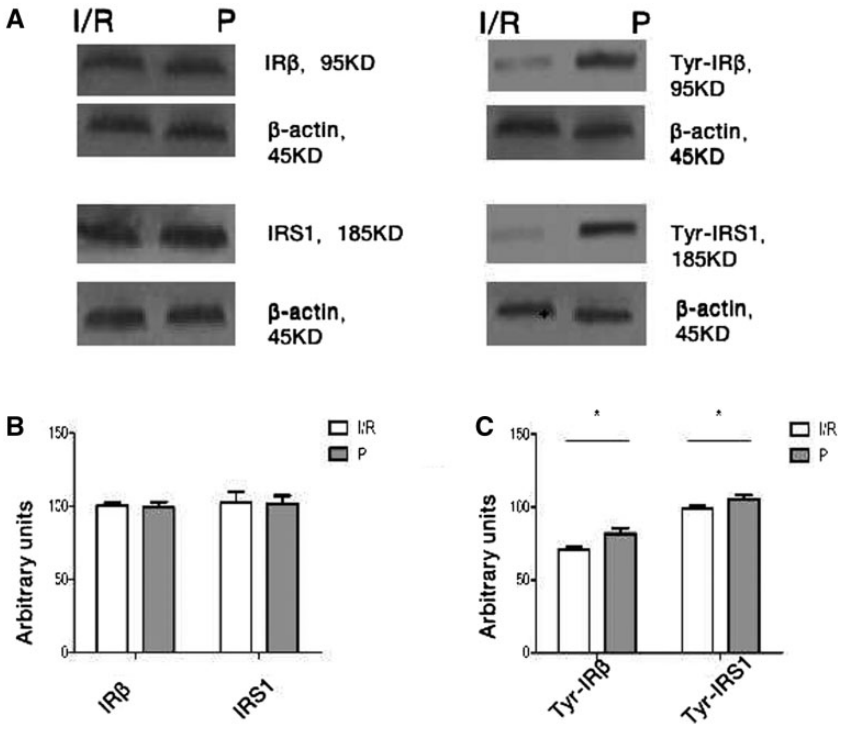

Figure 1. Change in the expression of proteins involved in the hepatic insulin signal transduction pathway. After I/R treatment, western blotting was performed to determine the expression of IRβ, IRS1, Tyr-IRβ, and Tyr-IRS1 (1A). Arbitrary units are counted in 1B and 1C.

Discussion

During liver surgery, the hepatic portal vein is occluded to reduce bleeding.[10] However, ischemia-reperfusion injury induced by hepatic blockage and its subsequent release can lead to liver damage.[11] Our study showed that rats in the I/R group had significantly higher levels of serum glucose, which may be attributable not only to neurological and endocrine changes after surgery but may also be associated with inflammatory factors such as TNF-α and IL-6, which are implicated in the insulin signaling pathway.[12,14]

Reducing high serum glucose levels after HIRI had attracted clinical attention. The present study demonstrated that propofol administration significantly attenuated the increase in serum glucose levels observed following HIRI. Compared with rats in the I/R group, propofol treatment improved Tyr-IRβ and Tyr-IRS1 expression levels, which may have led to the reduction in serum glucose observed in the P group. IRβ and IRS-1 are the main insulin signaling proteins in skeletal muscles. Studies have shown that insulin-mediated citrate metabolism is through the P13K pathway. Any signaling dysfunction in this pathway may reduce the biological effects of insulin and hence result in insulin resistance.[14,15]

Activated Kupffer cells (KCs) are the primary source of free radicals and cytokines, and previous studies have shown that rat hepatic KCs are activated during the first hour of hypoxia.[16,17] Propofol pretreatment may attenuate KC activation

during reoxygenation.[18] Reactive oxygen species (ROS) are increased during HIRI and studies have demonstrated that ROS may induce various forms of insulin resistance.[19] The use of antioxidants is believed to represent an effective treatment for insulin resistance.[20,21] As a commonly used intravenous anesthetic, propofol has a strong antioxidant capacity, and may improve the antioxidant capacity of red blood cells and tissues in rats.[22,23] Our study demonstrated that tyrosine phosphorylation levels of Tyr-IRβ and Tyr-IRS1 in the P group were higher than those the I/R group, indicating that an improvement in insulin signaling may result in reduced hyperglycemia.

The neurological and endocrine changes induced by surgical stress were responsible for the increase in blood glucose levels in rats in the two groups. Propofol reduced the increase in blood glucose levels induced by HIRI. We hypothesize that propofol improves blood glucose levels by modulating IRβ and IRS phosphorylation levels. Our study demonstrates that propofol may represent an attractive therapeutic option for the prevention and treatment of hyperglycemia induced by HIRI.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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