Effect of Complement C1q Expression on Hepatic Ischemia-Reperfusion Injury in Rats*

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Summary: The effect of the complement C1q expression on total hepatic ischemia-reperfusion (I/R) injury in rats was investigated. Sixty healthy male Sprague Dawley (SD) rats weighing 180–200 g were randomly divided into 5 groups: sham-operation group (S group, \( n = 12 \)); group of I/R for 1 h (I/R 1 h group, \( n = 12 \)); group of I/R for 3 h (I/R 3 h group, \( n = 12 \)); group of I/R for 6 h (I/R 6 h group, \( n = 12 \)); group of I/R for 24 h (I/R 24 h group, \( n = 12 \)). The hepatic I/R model of rats was established, and liver tissues were obtained 1 h, 3 h, 6 h and 24 h after hepatic I/R, respectively. Furthermore, the tissues were stained using hematoxylin-eosin, and the liver injuries of rats were observed using a microscope. The malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in liver tissue were determined.

1 MATERIALS AND METHODS

1.1 Experimental Animals and Grouping

Sixty healthy male SD rats (180–200 g) were provided by the Experimental Animal Center, School of Medicine, Wuhan University (China), and these rats were randomly divided into 5 groups: sham-operation group (S group, \( n = 12 \)), group of total hepatic I/R for 1 h (I/R 1 h group, \( n = 12 \)), group of total hepatic I/R for 3 h (I/R 3 h group, \( n = 12 \)), group of total hepatic I/R for 6 h (I/R 6 h group, \( n = 12 \)), group of total hepatic I/R for 24 h (I/R 24 h group, \( n = 12 \)).
group, \(n=12\), and group of total hepatic I/R for 24 h (I/R 24 h group, \(n=12\)). This study was approved by constituted ethical committee of Zhongnan Hospital, Wuhan University, China.

1.2 Establishment of Total Hepatic I/R Model

The total hepatic I/R model was established using the methods of Parasrapumiria et al\cite{5}. Rats were anesthetized using 3% sodium pentobarbital at a dose of 50 mg/kg. Supine position was taken and the limbs and head were fixed. Abdominal median incision was made, and non-invasive arterial clip was used to close proper hepatic artery, portal vein, common bile duct, suprahepatic vena cava and hepatic inferior vena cava. Major hepatic blood vessels were blocked to simulate total hepatic ischemia stage. Non-invasive arterial clip was released 30 min later, and the reperfusion phase was simulated by restoring the blood supply. Next, abdominal cavity was sutured, and the rats were put back into the cages. Rats were sacrificed by taking femoral arterial blood after 1 h, 3 h, 6 h, and 24 h of reperfusion, respectively. The specimens were used for testing. The operation in the S group was the same as hepatic I/R groups except that arterial clip was not given.

1.3 Specimen Collection

Rats were sacrificed by taking femoral arterial blood after 1 h, 3 h, 6 h and 24 h of reperfusion, respectively. The liver tissue was washed using cold saline, and there was no residual blood. Then the tissue was wiped by clean filter paper. Tissues were prepared for fluorescence real-time quantitative PCR, Western blotting, and determination of superoxide dismutase (SOD) and malondialdehyde (MDA). The remaining specimens were immediately fixed in 4% paraformaldehyde, and further used for histopathological examination.

1.4 Liver Histopathological Examination

Liver tissue was fixed in 4% paraformaldehyde for 24 h, and further dehydrated in gradient alcohol. Tissues were vitrified using dimethylbenzene and anhydrous ethanol, and embedded using paraffin. Sections were stained using hematoxylin-eosin. Finally, the pathological changes of the liver were observed under a microscope.

1.5 Determination of SOD and MDA in Liver Tissue

Rats were sacrificed by taking femoral arterial blood after 1 h, 3 h, 6 h and 24 h of reperfusion, respectively. The liver tissue was taken and frozen at \(-20^\circ\text{C}\) in a low temperature refrigerator. The tissue was thawed in a mixture of ice and water for 30 min, and the supernatant was obtained, and 0.8-fold volume of isopropanol was added and mixed. After resting for 15 min at \(-20^\circ\text{C}\), the mixture was centrifuged at \(4^\circ\text{C}\) for 10 min at 13 000 g. The supernatant liquid was discarded and 1.5 mL of 75% ethanol was added to wash the precipitate. Then the solution was centrifuged at 13 000 g for 5 min at \(4^\circ\text{C}\). Finally, the tube was placed in a clean bench and blown for 3 min, and RNA was dissolved in 20 \(\mu\text{L}\) of RNA enzyme-free water. Furthermore, 2 \(\mu\text{g}\) of RNA and 1 \(\mu\text{L}\) of Oligo (dT) 15 were added in a PCR tube, and RNase-free deionized water complemented to totally 12 \(\mu\text{L}\). Then the tube was incubated for 5 min at 70°C and quickly placed on ice for cooling. Subsequently, 4 \(\mu\text{L}\) of 5×buffer, 2 \(\mu\text{L}\) of 10 mmol/L dNTPs, 1 \(\mu\text{L}\) of RNA inhibitor and 1 \(\mu\text{L}\) of reverse transcriptase were added into the PCR tube and mixed. Then the mixture was kept at \(42^\circ\text{C}\) for 30 min and \(80^\circ\text{C}\) for 5 min, and the reverse transcriptase was inactivated. The protocol for real-time PCR was as follows: 12.5 \(\mu\text{L}\) of 2×qPCR Mix, 2.5 \(\mu\text{mol/L}\) C1q gene primers (upstream: 5'-TCACCTCA-ACTGGTTTGCTCC-3'; downstream: 5'-CAAGATT-GCCTCATTCTCATTAC-3', 174 bp) and 2.0 \(\mu\text{L}\) of 2.5 \(\mu\text{mol/L}\) internal primer, 2.0 \(\mu\text{L}\) of reverse transcription products and 8.5 \(\mu\text{L}\) of ddH2O. Each reverse transcription product was prepared in three tubes. The PCR process was as follows: 95°C for 1 min and 40 cycles with a final extension at 72°C for 5 min. Data were handled with \(\Delta\Delta^C\text{t}\) method\cite{6}. \(A=\text{CT (target gene, experimental samples)}-\text{CT (internal gene, experimental samples)}\), \(B=\text{CT (target gene, control samples)}-\text{CT (internal gene, control samples)}\), \(K=A-B\), and the expression fold was equal to \(2^AB\).

1.7 Western Blotting

Liver tissue was cut into small pieces, and cocktail, PMSF and phospho-proteasome inhibitor were added. Moreover, 10 times volume of extraction reagent was added to homogenize the tissue on ice. After 30 min of ice bath, the mixture was centrifuged at 12 000 g for 5 min, and the supernatant was collected. Protein concentration was determined using Bradford assay (GAPDH protein was used as an internal control). The proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was further blocked for 1 h at room temperature using 5% skimmed milk (prepared with 0.5% TBST). The membrane was further incubated with diluted primary-antibodies [Tris-Buffered Saline Tween-20 (TBST) dissolved in 5% skim milk] at 4°C overnight. The membrane was washed with TBST, and incubated with secondary antibodies at room temperature for 30 min. The membrane was washed again with TBST, and the target proteins were detected using an enhanced chemiluminescence (ECL) kit. Furthermore, the membrane was exposed and developed. The film was scanned and preserved. The absorbance (\(A\)) value of the target band was analyzed using Alpha software processing system. The primary-antibodies, secondary antibodies, PVDF membrane and fluorescent dye (SYBR Green) were purchased from Miao Bo Bio-Technology Co., Ltd., China.

1.8 Statistical Analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) version 17.0. Data were represented as \(\bar{x}\pm s\). Comparison between and within groups was done using single-factor analysis of variance (ANOVA). \(P<0.05\) was the level of significance.