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ORIGINAL ARTICLE

# Ischemic preconditioning in hepatic ischemic–reperfusion injury

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**KEYWORDS**
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**Abstract** *Background/Introduction:* Ischemic preconditioning is a method in which brief periods of ischemia render tissues resistant to injury resulting from prolonged ischemia and reperfusion, so-called ischemia–reperfusion injury.
*Purpose:* To elucidate the possible protective role of ischemic preconditioning in rat livers with ischemia–reperfusion injury.
*Methods:* Rats were first allocated to either a sham control or an ischemic preconditioning group. On the following day, the rats from each group were administered either 30 minutes or 45 minutes hepatic ischemia. Next, rat livers were harvested for measuring proliferating cell nuclear antigen, heme oxygenase-1, inducible nitric oxide synthase, and heat shock protein 70 mRNA levels.
*Results:* Both proliferating cell nuclear antigen and hemeoxygenase-1 expression increased significantly after 45 minutes hepatic ischemia compared with those after 30 minutes hepatic ischemia, but they decreased significantly with ischemic preconditioning. However, ischemic preconditioning did not affect inducible nitric oxide synthase or heat shock protein 70 expression.
*Conclusion:* From the preliminary findings, further elucidation is warranted.


## 1. Introduction

During ischemic preconditioning, brief periods of tissue ischemia are used to render tissue resistant to injury resulting from prolonged ischemia and reperfusion, so-called ischemia–reperfusion injury. Hepatic ischemia–reperfusion injury plays a major role in hepatic resection and shock, particularly during liver transplantation.[1–4]

Hepatic vascular control is commonly used to prevent massive hemorrhage during hepatectomy. Hepatic vascular occlusion provides a relatively bloodless operative field, enabling unhurried, meticulous, and accurate intrahepatic dissection and hemostasis.[5–7]

Conflict of interest: The authors report no conflict of interest.

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Hemeoxygenases (HOs) catalyze the formation of carbon monoxide (CO), biliverdin, and $Fe^{2+}$ ions during heme degradation. HO-1 is the inducible isoform of HO and is induced for cytoprotection under various stress-related conditions including hyperemia, hypoxia, and ischemia—reperfusion.[8,9]

Nitric oxide (NO) plays a crucial role in maintaining the hepatic vascular tone.[10] NO synthase (NOS) metabolically produces NO from L-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate. In the liver, inducible NOS (iNOS) is a key molecule catalyzing NO synthesis.[11,12]

Proliferating cell nuclear antigen (PCNA), also called cyclin, is an auxiliary protein of DNA polymerase-δ[13]; its expression levels are directly correlated with cell proliferation and DNA synthesis rates.[14] PCNA is synthesized in the late G1 and S phases and remains present in the nuclei throughout the cell cycle; thus, the PCNA levels are correlated with the cell proliferative state.[15]

## 2. Methods

This study elucidated the pathophysiology of ischemia—reperfusion injury in a rat liver model and evaluated the possible protective role of ischemic preconditioning in rat livers with ischemia—reperfusion injury.

### 2.1. Samples and experimental design

We used male Sprague—Dawley rats, weighing 250—300 g, housed in stainless-steel cages under controlled temperature, humidity, and 12-hour light—dark cycles. They were allowed free access to food and water before and after surgery. All the rats received humane care, and the study protocol complied with the guidelines and animal research laws of our institution. The study protocol was reviewed and approved by the Chang Gung Memorial Hospital Animal Care and Use Committee, Kaohsiung, Taiwan. An intramuscular dose of anesthesia containing 30 mg/kg of Zoletil 50 (VIRBAC, France) and 10 mg/kg of Xylazine (Bayer Vital GmbH, Germany) was administered before surgery. After surgery, 2200 IU/100 g of penicillin (Y. F. Chemical Ltd., Taiwan) and 3 mg/kg of ketorolac tromethamine (Standard Chem & Pharm Co. Ltd., Taiwan) were administered intramuscularly for infection prophylaxis and pain relief, respectively. The rats were euthanized after the experiments by surgical removal of the heart under anesthesia.

All the rats were divided into six groups ($n = 6$ in each group): Group 1 underwent sham operation (laparotomy and closure only); Group 2 underwent laparotomy and total hepatic ischemia, performed using a small atraumatic clamp to occlude the hepatoduodenal ligament for 10 minutes (preconditioning); Group 3 underwent laparotomy and closure, and after 24 hours (Day 2) again underwent laparotomy followed by total hepatic ischemia for 30 minutes; Group 4 underwent the 10-minute preconditioning performed in the Group 2 rats, and after 24 hours (Day 2) again underwent laparotomy followed by total hepatic ischemia for 30 minutes; Group 5 underwent laparotomy and closure, and after 24 hours (Day 2) again underwent laparotomy followed by total hepatic ischemia for 45 minutes; and Group 6 underwent the 10-minute preconditioning performed in the Group 2 rats, and after 24 hours (Day 2) again underwent laparotomy followed by total hepatic ischemia for 45 minutes.

Tissue samples were harvested as follows: Groups 1 and 2, 24 hours later (Day 2); and Groups 3—6, 15 minutes (reperfusion) after removal of the temporary occlusion on Day 2. All harvested hepatic and intestinal tissue samples were stored in liquid nitrogen until analysis.

### 2.2. RNA extraction and reverse-transcription polymerase chain reaction for measuring PCNA, HO-1, iNOS, and heat shock protein 70 mRNA levels

Total tissue RNA was extracted using guanidine isothiocyanate, according to the method described by Chomczynski and Sacchi.[16] First-strand cDNA was synthesized using oligo-dT primers and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI, USA).

Hot start Taq polymerase was used for cDNA polymerase chain reaction (PCR) performed at 88°C in a Thermal Cycler (Perkin—Elmer, Norwalk, CT, USA). PCNA-specific primers were added to each tube at a final concentration of 0.2 μmol/L. The 5′—3′ sequences of the primer pairs and predicted sizes of the amplified PCR fragments have been provided previously.[17,18] After a 5-minute initial melting step at 97°C, 35—40 PCR cycles were performed as follows: denaturation at 94°C for 1 minute, followed by annealing at 50°C—60°C (52°C for lipopolysaccharide binding protein (LBP), 50°C for tumor necrosis factor-α, and 60°C for CD14) for 1 minute and then extension at 72°C for 2 minutes. The final cycle was followed by a 10-minute soak at 72°C.

We used the constitutively expressed β-actin and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) as internal controls to confirm the validity of the target gene expression observed under different experimental conditions for standardizing the PCR products. The cycle numbers of each primer pair were selected in a linear range to prevent the plateau effect. The ratios of PCNA/G3PDH, HO-1/β-actin, INSO/β-actin, and heat shock protein (HSP) 70/β-actin signals were calculated for each sample. Each experiment included a negative control (a sample RNA, not subjected to reverse transcription).

### 2.3. Statistical analysis

All data were statistically analyzed and the results are presented as means ± standard deviations. The data were compared using the Mann—Whitney *U* test. Differences with $p < 0.05$ were considered statistically significant.

## 3. Results

PCNA expression did not differ significantly between Group 3 (30-minute hepatic ischemia only) and Group 4 (30-minute hepatic ischemia with preconditioning; $p = 0.9813$). It significantly increased in Group 5 (45-minute hepatic ischemia only) compared with that in Group 3 ($p = 0.0007$), whereas it decreased significantly in Group 6 (45-minute ischemia with preconditioning) compared with that in Group 5 ($p = 0.0065$; Figure 1).