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Effect of breviscapine against hepatic ischemia reperfusion injury

Yan-zhu Lin, BD,[a,c,1] Zhi-yuan Lu, MD,[b,1] Xiao-hui Liang, MD,[c] Kang Li, MD,[d] Bo Peng, MD,[c] and Jin Gong, MD,PhD[c,*]

[a] Clinical Medicine, International School, Jinan University, Guangzhou, China
[b] Department of Stomatology, Medical College, Jinan University, Guangzhou, China
[c] Department of General Surgery, The First Affiliated Hospital, Jinan University, Guangzhou, China
[d] Department of Gastrointestinal Surgery, YueBei People'Hospital, Shaoguan, China

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ABSTRACT

Background: Breviscapine is an active ingredient extracted from traditional Chinese medicine *Erigeron breviscapus*. The purpose of this study was to investigate the effect of breviscapine injection on hepatic ischemia and/or reperfusion injury.

Methods: Forty rats were randomly divided into five groups ($n = 8$): Sham group, Ischemia reperfusion 1 (I/R1) + normal saline (NS) group, I/R1 + breviscapine (Bre), I/R2 + NS group, and I/R2 + Bre group. Group1 and group2 represent ischemia time for 10 min and 30 min, respectively. Breviscapine or normal saline was administered to rats (single dose of 10 mg/Kg, intravenously) 30 min before hepatic ischemia. Serum transaminases, histopathologic changes, malondialdehyde (MDA), and superoxide dismutase (SOD) in liver tissues were evaluated. The expression level of mitochondrial fusion 2 (Mfn2) was also investigated.

Results: After 24-h reperfusion, based on the histopathologic analysis, compared with NS control group, the liver function was improved in breviscapine group. Liver enzymes aspartate and alanine aminotransferase levels were significantly lower in the I/R + Bre group, when compared with the I/R + NS group. Pretreatment with breviscapine reduced MDA level ($P < 0.05$) and increased SOD activity significantly in I/R + Bre compared with I/R + NS group. Western blot and RT-q polymerase chain reaction showed that Mfn2 was significantly downregulated in breviscapine preconditioning group as compared to normal saline control group.

Conclusions: Breviscapine preconditioning attenuates liver ischemia reperfusion injury via inhibiting liver oxidative stress reaction. The protective mechanism probably inhibits Mfn2 protein and mRNA expression.

Introduction

Hepatic ischemia/reperfusion (I/R) injury is the common pathophysiological process associated with several clinical conditions, including liver transplantation, hepatectomy, trauma, and hypovolemic shock.[1] The exact pathogenesis of I/R injury is complex and is currently believed to be connected with energy metabolic disorder, generation of oxygen radicals, intracellular calcium overload, and activation of Kupffer cells and neutrophil granulocytes.[2,3] These events may lead to liver

* *Corresponding author*. Department of General Surgery, The First Affiliated Hospital, Jinan University, Guangzhou 510632, China. Tel.: +86 020 38688613; fax: +86 020 38688888.
E-mail address: gongjin51@gmail.com (J. Gong).
[1] Y.-z.L. and Z.-y.L. contributed equally to this work.
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damage and increase postoperative morbidity and mortality rates.[4] Therefore, it is very important to find an effective hepatic protection to minimize hepatic ischemia reperfusion injury.

Traditional Chinese medicine is one of the research hotspots in therapeutic intervention of hepatic ischemia reperfusion injury because of less side effect and more target spots.[5] Breviscapine is a flavonoid extracted from the natural plant *Erigeron breviscapus*. Scutellarin is the main active ingredient, and its structural formula is 4, 5, 6-trihydroxyflavone-7-glucuronide.[6] Clinical trials and animal studies have shown that breviscapine has medical effects including anticoagulation, improving microcirculation, as well as activating blood supply to the heart and brain.[7,8] It has been prepared into some Chinese patent medicines including injection and used to treat cardiovascular and cerebrovascular diseases in clinical practices for a long time in China.[9,10] Currently, its protective effect against myocardial and cerebral ischemia reperfusion injury is widely studied and applied. However, studies about its effect on hepatic ischemia/reperfusion injury, especially about its protective mechanism, are still limited.

Currently, the role of change of mitochondrial structure in hepatic I/R has received increasing attention of domestic and foreign scholars.[11,12] Under the ischemic condition, disturbance in the cellular energy metabolism and enzyme function suggests that protection of mitochondria may play an important role in the maintenance of cellular integrity during I/R injury.[11] Mitofusion 2 (Mfn2) is embedded in the outer membrane of the mitochondria.[13] It regulates mitochondrial metabolism through regulating mitochondrial membrane potential and substrate oxidation and oxidative phosphorylation. It plays an important role in the regulation of mitochondrial morphology and function and is essential for mitochondrial fusion.[14] Disorder of mitochondrial fusion plays a role in I/R disease but the underlying mechanism is still unclear.[15] Therefore, studying Mfn2 is essential for identification of the role of mitochondria in hepatic I/R injury.

In the present study, a rat model of hepatic ischemia reperfusion was developed to investigate the effect of breviscapine on hepatic ischemia reperfusion injury after different durations of ischemia, as well as its potential mechanism. This study provides a research foundation and theoretical basis for the clinical application of hepatic surgery.

Materials and methods

Development of animal model

A total of 40 specific pathogen-free male Sprague–Dawley rats, with body weights ranging from 250 to 280 g, were provided by the Experimental Animal Center of Southern Medical University. They were housed in cages and maintained at room temperature and 12/12 h light-dark cycle and free access of water and food. The experimental protocol was approved by the local ethics committee for animal experimentation.

Before the operation, the rats were fasted (food but not water) for 12 hours and then were weighed and anesthetized with an intraperitoneal injection of 10% chloral hydrate at a dose of 3.0 mL/kg of body weight. Then, the rats were placed in a supine position, and a midline abdominal incision was made on each of them. Noninvasive vascular clamps were used to occlude the portal veins and hepatic artery branches to the median and left hepatic lobes, leading to an ischemia of almost 70% of the liver. The clamps were removed at corresponding time according to the durations of ischemia to recover the blood supply to the liver, and the rat model of hepatic ischemia reperfusion injury was developed. Narcotic injection was performed at 24 hours after reperfusion, and blood samples from the inferior vena cava, and the left lobe of the liver were collected.

Experimental grouping

The rats were randomized into five groups using a random number table. Each group contained eight rats. These groups were

(1) Sham surgery group (Sham group), which underwent abdominal surgery without occlusion to produce an ischemia;
(2) 10-min ischemia and normal saline group (I/R1+NS), in which equivalent normal saline was intravenously injected via the tail 30 min before the surgery, and the abdomen was closed after 10 minutes of ischemia and reperfusion;
(3) 10-min ischemia and breviscapine preconditioning group (I/R1+Bre), in which 10 mg/Kg of breviscapine injection was intravenously injected via the tail 30 min before the surgery, and the abdomen was closed after 10 minutes of ischemia and reperfusion;
(4) 30-min ischemia and normal saline group (I/R2+NS), in which equivalent normal saline was intravenously injected via the tail 30 min before the surgery, and the abdomen was closed after 10 minutes of ischemia and reperfusion;
(5) 30-min ischemia and breviscapine preconditioning group (I/R2+Bre), in which 10 mg/Kg of breviscapine injection was intravenously injected via the tail 30 min before the surgery, and the abdomen was closed after 10 min of ischemia and reperfusion.

The histopathology observation

Immediately after the 24 h reperfusion, the liver tissue samples were removed and placed in 10% neutral-buffered formalin. For histopathology, liver samples were routinely processed and embedded in paraffin, and 3-μm sections were stained with hematoxylin and eosin. The microscopy examination of stained sections was done with an Olympus PROVIS AX-70 microscope (Olympus Corporation, Japan). Results were interpreted by a pathologist who was blinded to the treatment groups. The lesion was graded as follows: grade 0: no injury; grade 1: mild injury in the form of cytoplasmic vacuolation to focal nuclear pyknosis; grade 2: moderate to severe injury in the form of extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; and grade 3: severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration.[16]