Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.01.004

● *Original Contribution*

QUANTITATIVE EVALUATION OF HEPATIC MICROVASCULAR PERFUSION AFTER ISCHEMIA–REPERFUSION INJURY IN RABBITS BY CONTRAST-ENHANCED ULTRASOUND PERFUSION IMAGING

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(*Received* 19 *June* 2017; *revised* 11 *January* 2018; *in final form* 13 *January* 2018)

Abstract—The aim of this study was to evaluate microvascular perfusion after liver ischemia–reperfusion injury (IRI) in rabbits using the "flash-replenishment" method of contrast-enhanced ultrasound (CEUS) perfusion imaging. Twenty-eight rabbits underwent either 30, 60 or 90 min of ischemia and 120 min of reperfusion. CEUS perfusion imaging was performed using the "flash-replenishment" model, and hepatic microvascular perfusion parameters, including peak intensity (PI), area under the curve (AUC), and hepatic artery-to-vein transit time (HA-HVTT), were calculated. Prolonged ischemia upregulated intracellular adhesion molecule-1 (ICAM-1), alanine transaminase (ALT) and aspartate transaminase (AST) levels. Longer ischemia decreased PI and AUC, but increased HA-HVTT. The perfusion parameters were significantly correlated with Suzuki's pathology scores and ALT and AST levels. The "flash-replenishment" method of CEUS perfusion imaging is an accurate and non-invasive method for evaluating hepatic microvascular perfusion and provides a valuable experimental basis for early prediction of liver IRI damage after liver transplantation or liver resection. (E-mail: lyk301@163.com)

Key Words: **Hepatic microvascular perfusion, Ischemia–reperfusion injury, Contrast-enhanced ultrasound.**

INTRODUCTION

Complete or partial blocking of hepatic blood flow is required for liver transplantation, hepatectomy, and traumatic liver surgery. This inevitably results in ischemia–reperfusion injury (IRI) and significantly increases the risk of post-operative complications. Insufficient perfusion can cause metabolic disturbances and liver failure, which greatly threaten graft survival and post-operative liver function. Although the molecular mechanism underlying IRI has been studied extensively, it is difficult to deal with the complications after liver transplantation, mainly because of the lack of an effective and precise method for early monitoring and testing of post-operative live IRI (Kochhar et al. 2013).

IRI is a complex pathophysiological process involving multiple cells and multiple mediators (Tazaki et al. 2010). Because of the specific anatomic location and physiologic characteristics, liver sinusoidal endothelial cells (LSECs) become the direct target cells of IRI after blood flow is interrupted (Menger et al. 1999). The LSECs can secrete or express a variety of vasoactive substances and aggravate liver cell damage, leading to liver microcirculation dysfunction. After reperfusion, the blood flow and oxygen supply are restored, and a variety of immune inflammatory mediators are secreted, which triggers progressive aggravation of organ damage, microcirculation dysfunction, and insufficient perfusion.

Scoazec et al. (1994) detected the expression of intracellular adhesion molecule-1 (ICAM-1) in the biopsy samples of human liver grafts in IRI. ICAM-1 is expressed on the surfaces of LSECs and adjacent liver cells in the space of Disse (Ivanov et al. 2014) and is involved in a series of important pathophysiological processes, such as cell signal transduction, activation, inflammation, thrombosis, and wound healing (Lawson and Wolf 2009). Hypoxia upregulates ICAM-1 expression, promotes leukocyte–endothelial cell adhesion and induces inflammation and microvascular injury, resulting in hepatic microcirculation dysfunction.

Contrast-enhanced ultrasound (CEUS) perfusion imaging is a non-invasive functional imaging modality used

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Conflict of Interest: None declared.

for assessing blood flow perfusion. After intravenous injection of the contrast agent, the microbubbles, as an indicator, flow through the tissues and organs of the body with blood flow. A time–concentration curve (*i.e.,* time–intensity curve) of the microbubbles, with time as the horizontal axis and concentration as the vertical axis, can be obtained by observing the changes in concentration of the microbubbles at different time points in the same location. I To obtain the parameters related to tissue blood perfusion, such as time to peak of the contrast agent, peak intensity, area under the curve, blood volume and blood flow, a specific mathematical model must be used to fit the original time–intensity curve.

The contrast agent is injected by either bolus injection or continuous infusion. In the case of bolus injection, the contrast agent is quickly injected into blood vessels in the form of boluses; and in the case of continuous infusion, the diluted contrast agent is evenly and slowly added into the blood vessels. Different mathematical models were used for fitting depending on the manner of injection to obtain the parameters related to blood perfusion of tissues and organs. The commonly used mathematical models include the bolus injection model, flash-replenishment model and contrast agent depletion model (Fujima et al. 2017; Lassau et al. 2017; Lindsey et al. 2017).

CEUS perfusion imaging was initially used for the evaluation of myocardial perfusion (Bulte et al. 2012), and subsequently has been utilized to examine blood perfusion in brain (Bilotta et al. 2016) and kidney (Fischer et al. 2016; Schwenger et al. 2014; Wang and Mohan 2016), tumor angiogenesis (Lassau et al. 2017), and liver cirrhosis grading (Berzigotti et al. 2011). The liver has a double blood supply; 25% to 30% of the blood supply is from the hepatic artery, and 70% to 75% from the portal vein, which increases the complexity of quantitative assessment of hepatic perfusion.

After a bolus injection, the arterial blood supply is responsible for the initial enhancement of the hepatic parenchyma, while the microbubbles arriving through the portal blood supply are delayed by 10 to 20 s. However, with the continuous infusion technique, hepatic replenishment reflects a combination of hepatic artery and portal vein inflow. In this study, after perfusion of the contrast agent in the liver reached a certain steady state by continuous infusion, the reperfusion process of the contrast agent was observed through flash-replenishment kinetics, to quantitatively evaluate the reperfusion capability of liver microcirculation after IRI and its correlation with ICAM-1 molecular phenotype, pathology and changes in liver function, which is the innovation of this study.

The evaluation of post-IRI hepatic microvascular perfusion is useful for the early monitoring of hepatic microcirculation and changes in molecular pathology at different stages of the disease. It is also helpful for the early diagnosis of complications and evaluation of treatment after liver transplantation and hepatectomy. Our method improved the sensitivity of ultrasound examination and might become an important imaging modality for the postoperative prediction and evaluation of hepatic microvascular perfusion.

METHODS

Animals

Thirty healthy New Zealand rabbits (weighing 3.0–3.5 kg) were provided by Chinese PLA General Hospital Experimental Animal Center (Experimental Animal License: SCXK [Beijing] 2010–0001). All rabbits were allowed to acclimate for 2 wk, with free access to food and water under 12-h light/12-h dark cycles. The experiment protocol was approved by the Experimental Animal Welfare Ethical Review Committee of Chinese PLA General Hospital (2015-x10-11). Animals with abnormal liver structures or vessels were examined and excluded using conventional ultrasonography before the experiments.

The animals were randomly assigned to 30-, 60- and 90-min ischemia groups, with 10 animals in each group. Two rabbits in the 90-min ischemia group died within 30 min of reperfusion and were excluded, leaving 8 animals in this group. No deaths or other events occurred in the other two groups.

Animal model

During the experiments, rabbits were anesthetized using 3% pentobarbital sodium (30 mg/kg) injected *via* a 24G indwelling needle inserted into the ear vein. The liver IRI model was established using Pringle's method (Sugiyama et al. 2010). Briefly, the hepatic artery, portal vein and common bile duct were blocked with a microvascular clamp for 30, 60 or 90 min, followed by 120 min reperfusion (Fig. 1). Successful establishment of the IRI model was indicated if the liver color changed from dark red to bright red within 2–5 min after releasing the clamp. Then the abdomen was closed.

CEUS perfusion imaging

The contrast agent (SonoVue, Bracco, Milan, Italy) has a mean diameter of 2.5 μm. The membrane material of SonoVue is composed of polyethylene glycol and phospholipids and is filled with stable sulfur hexafluoride (SF_6) gas. Contrast agent was diluted with 5 mL of normal saline and shaken before use, which turned it into a white microbubble suspension. The suspension contained 8 μL/mL SF_6 with a density of $1.0–5.0 \times 10^8$ bubbles/mL (mean: 3.0×10^8 bubbles/mL).

CEUS imaging was performed after reperfusion for 120 min using the Philips iU Elite ultrasound system (Philips Medical Systems, Bothell, WA, USA) with a C5-1