Microvascular Research 94 (2014) 28–35

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

The monitoring of microvascular liver blood flow changes during ischemia and reperfusion using laser speckle contrast imaging

Chong Hui Li, Hong Dong Wang, Jian Jun Hu, Xin Lan Ge, Ke Pan, Ai Qun Zhang, Jia Hong Dong *

Department of Hepatobiliary Surgery, Chinese PLA General Hospital, Chinese PLA Medical College, Beijing, China
Institute of Hepatobiliary Surgery, Chinese PLA General Hospital, Chinese PLA Medical College, Beijing, China

ARTICLE INFO

Article history:
Accepted 24 April 2014
Available online 2 May 2014

ABSTRACT

Objective: The recovery of microvascular liver blood flow (LBF) after ischemia is an important determinant of the degree of hepatocellular injury. Laser speckle contrast imaging (LSCI) was recently suggested to be a suitable instrument for monitoring the LBF. This study was designed to evaluate LSCI in monitoring the LBF changes during liver ischemia and reperfusion (IR).

Methods: A rat model with 120-min ischemia and 60-min reperfusion to 90% of the liver (entire liver except the caudate lobe, which was kept as portal blood bypass) was used. The LBF of the sham operation (SO) group and the IR group was measured with LSCI at the following time points: before ischemia (Baseline), 5 min after the start of ischemia (I-5 min), 5 min before the end of ischemia (I-115 min) and 5 and 60 min after the start of reperfusion (R-5 min and R-60 min). The reproducibility among different rats or repeated measurements, the liver histopathology, the liver biological zero (BZ) and the influence of liver movement on the LSCI measurements were investigated.

Results: The entire exposed liver surface after laparotomy was suitable for full-view LSCI imaging. Establishing many circular or oval regions of interest (ROIs) on the LSCI flux image was a simple and convenient method for calculating and comparing the LBF of different ROIs and different liver lobes. There was good-to-moderate intra-individual and inter-individual reproducibility for the LSCI measurements of the LBF in the rats of the SO group. In the IR group, the total blood inflow occlusion resulted in a notable drop of the LBF from the baseline ($P < 0.05$) that remained for the 120 min of ischemia. The LBF decreased further after the reperfusion ($P < 0.05$), reflecting the IR-induced liver microcirculation dysfunction. The histopathological examination revealed severe hepatic sinus congestion and damaged hepatocytes in the IR group. The no flow BZ and liver movement contributed to the LBF values.

Conclusions: LSCI technology is a simple, convenient and accurate method for the real-time monitoring of microvascular LBF changes during ischemia and reperfusion, regardless of the contribution of biological zero and liver movement. This finding suggests the possible application of LSCI for monitoring the microvascular LBF changes intraoperatively.

Introduction

The Pringle maneuver (temporary occlusion of the hepatoduodenal ligament) is widely employed during hepatectomy to reduce intraoperative blood loss (Dixon et al., 2005). This maneuver inevitably results in ischemia and subsequent reperfusion injury, which could cause significant postoperative complications (Lesurtel et al., 2009). The ischemic time and restoration of liver perfusion are important determinants of the degree of hepatocellular injury because microcirculatory collapse corresponds to a profound reduction in tissue oxygenation (Vollmar et al., 1994). It would be helpful to monitor the microvascular liver blood flow (LBF) changes during ischemia and reperfusion (IR) intraoperatively with an appropriate and convenient instrument in a surgical environment.

Laser speckle contrast imaging (LSCI) is a recently marketed technique that is based on speckle contrast analysis (Basak et al., 2012). High frame rate LSCI provides non-contact full-field imaging over wide areas with excellent spatial and temporal resolutions and theoretically combines the advantages of laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) (Puissant et al., 2013; Roustit et al., 2010). Applications of LSCI include pre-clinical studies of neurological disorders and clinical applications, including dermatological (Kernick and Shore, 2000), neurosurgical and endoscopic studies (Boas and Dunn, 2010; Dunn, 2012).

Abbreviations: LSCI, laser speckle contrast imaging; IR, ischemia and reperfusion; LBF, liver blood flow; BZ, biological zero; ROI, regions of interest; TOI, time of interest; LSPU, laser speckle perfusion unit; AU, arbitrary unit.

* Corresponding author at: Department of Hepatobiliary Surgery, Chinese PLA General Hospital, 28 Fuxing Road, Beijing 100853, China.
E-mail address: Dongjh301@163.com (J.H. Dong).

http://dx.doi.org/10.1016/j.mvr.2014.04.010
0026-2862/

LSCI was recently used to assess the LBF during sequential liver inflow occlusions, and the method was able to produce reproducible real-time blood perfusion measurements of hepatic microcirculation that correlated well with sidestream dark field imaging-derived sinusoidal blood flow velocity measurements (Sturesson et al., 2013). Compared with the established techniques for LBF measurements, LSCI has the advantage of non-contact measurement over a large surface with high-speed data acquisition (Richter et al., 2010). In Sturesson et al. (2013) study, the liver blood inflow was occluded for only 3 min, and there was no obvious IR injury to the liver. The LBF measurements were obtained during a period of apnea to minimize movement artifacts. Apnea affects blood pressure and circulation and is not regularly used in small animal experiments. It is well known that the laser signal never reaches zero in skin recordings under a situation of arrested flow (tourniquet ischemia) because of spontaneous Brownian motion of macromolecules and the remaining red blood cells in venules. The remaining non-zero signal is called biological zero (BZ) (Kernick et al., 1999). The liver BZ in LSCI measurement has not been addressed.

The present study was designed to evaluate the application of LSCI in monitoring the microvascular LBF changes during ischemia and reperfusion in a condition of normal anesthesia and spontaneous breathing in rats. The experimental factors influencing the accuracy of the LSCI measurement of LBF were analyzed.

Methods

Animals

Male Wistar rats weighing 240 to 270 g were obtained from the Experimental Animal Center of the Academy of Military Medical Science (Beijing, China). The rats were maintained at 24 °C under pathogen-free conditions with a 12/12-hour dark/light cycle and allowed food and water ad libitum. All the experiments performed in this study were approved by the Animal Research Committee of Chinese PLA General Hospital.

Research design

The rats were divided into three experimental groups: the sham operation group (SO, n = 10), the ischemia and reperfusion group (IR, n = 10) and the liver biological zero group (BZ, n = 5). Ischemia and reperfusion were performed in the SO and IR groups, and the LBF was measured with LSCI at 5 time points (Fig. 1): 5 min after laparotomy, which was taken as the baseline of LBF (Baseline); 5 min after the start of ischemia (I-5 min); 5 min before the end of 120 min of ischemia (i.e., the start of reperfusion) (I-115 min); and 5 min and 60 min after the start of reperfusion (R-5 min, R-60 min). The study of liver biological zero signals and the effect of liver movement on LSCI measurements were performed in the BZ group. The left liver lobe was ligated at the base (Fig. 1B) to occlude the blood inflow and outflow. After the LSCI measurement, the rat was assigned to the induction of apnea under continuous LSCI monitoring.

The surgical procedures

A rat model with IR to 90% of the liver was used (Dong et al., 2002) (Fig. 1A). In this rat model, the caudal lobe, which represented 10% of the liver, was not occluded but was kept as a passage of the portal blood to reduce intestinal congestion during liver ischemia (also see the supplemental material). After overnight fasting but with free access to water, the rats were anesthetized with 1.5% isoflurane inhalation and placed on a thermostatically controlled heating pad that maintained the body temperature at 37 °C throughout the operation and monitoring periods.

After a midline laparotomy, the common hepatic pedicles of the left and median lobes and the right lobe were gently isolated and temporally clamped with two lengths of "0" surgical suture tied in bowknots. In the SO group, the hepatic pedicles were isolated but not clamped. A continuous suture was used to close the peritoneal skin, and the rat was returned to a cage. After 120 min of ischemia, the peritoneal skin was reopened, and reperfusion of the liver was achieved by untying the bowknots, followed by re-closure of the peritoneal skin.

In LDF and LSCI research, the BZ of a specific organ or tissue is defined as the signal obtained in the absence of vascular flow. When the hepatic BZ for LSCI was measured in the BZ group, the pedicle of the left lobe of the liver was first ligated with a 3-0 silk suture to occlude the blood inflow and outflow of the left lobe (Fig. 1B). The LBF of the left lobe was then measured with LSCI and taken as the BZ of the rat liver. Three of the BZ rats were given deep ether anesthesia to induce apnea. After approximately 20 s of apnea, the ether was removed, and the rats were given fresh air to promote recovery. This procedure was used to evaluate the influence of respiration-induced liver movement

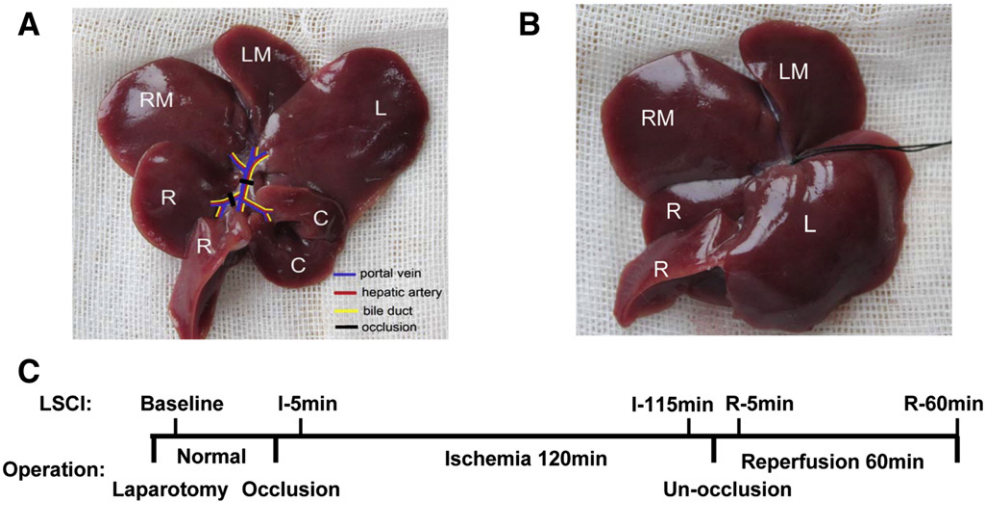

Fig. 1. Graphical illustration of the surgical operations on the rat livers and the protocol in the study. A: Schematic drawing representing the ischemic model of 90% of the rat liver by blood inflow occlusion on the pedicles of the left lobe (L), left median lobe (LM), right median lobe (RM) and right lobe (R), with no occlusion of the caudate lobe (C), which accounts for 8–10% of the liver weight and is kept as a passage of the portal blood. B: The left liver lobe is ligated to occlude the blood inflow and outflow used in liver biological zero measurements. C: The experimental procedures for LSCI measurements of ischemia and reperfusion induced liver blood flow changes at 5 min after laparotomy (Baseline), 5 min after the start of ischemia (I-5 min), 5 min before the end of ischemia (I-115 min), 5 and 60 min after the start of reperfusion (R-5 min, R-60 min).