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Laser speckle contrast imaging for assessment of liver microcirculation

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ABSTRACT

Objective: Laser speckle contrast imaging (LSCI) is a novel technique for microcirculation imaging not previously used in the liver. The aim of the present experimental study was to evaluate the use of LSCI for assessing liver microcirculation.
Materials and methods: In six male Wistar rats, the median liver lobe was exposed through a midline laparotomy. Liver blood perfusion was measured simultaneously with LSCI and sidestream dark-field (SDF) imaging at baseline and during sequential temporary occlusions of the portal vein, hepatic artery, and total blood inflow occlusion. Both the inter-individual variability associated with perfusion sampling area and comparisons in perfusion measurements between both imaging techniques were investigated and validated for the application of LSCI in the liver.
Results: Occlusion of the hepatic artery, portal vein, and total inflow occlusion resulted in a significant decrease in LSCI signal to 74.7 ± 6.4%, 15.0 ± 2.3%, and 10.4 ± 0.5% respectively ($p<0.005$ vs. baseline). The LSCI perfusion units correlated with sinusoidal blood flow velocity as measured with SDF imaging (Pearson's $r=0.94$, $p<0.001$). In a 10 mm diameter region of interest, as measured with LSCI, baseline inter-individual variability measured by the coefficient of variability was 13%.
Conclusion: Alterations in LSCI signal during sequential inflow occlusions were in accordance with previously published results on hepatic hemodynamics in the rat and correlated well with our SDF imaging-derived sinusoidal blood flow velocity measurements. We found that LSCI was able to produce reproducible real-time blood perfusion measurements of hepatic microcirculation. Compared to established techniques for liver blood perfusion measurements LSCI holds the advantages of non-contact measurements over large surfaces with a high speed of data acquisition.

Introduction

Intraoperative measurements of hepatic microcirculation are of interest in liver transplantation and liver resection, where damage is inflicted to the liver by, e.g., ischemia/reperfusion (Puhl et al., 2005) and preoperative chemotherapy (Soubrane et al., 2010), which may compromise (micro)vascularity. In the experimental setting, liver microcirculation measurements have been used to investigate, e.g., the impact of portal vein occlusion on liver regeneration and microcirculatory response (Gock et al., 2011). Portal vein occlusion is frequently used clinically to increase the volume of the future liver remnant prior to liver resection to prevent postoperative liver failure (Sturesson et al., 2010).

Different techniques for intraoperative liver microcirculation determination exist, including laser Doppler flowmetry (LDF), intravital fluorescence microscopy and video microscopic techniques such as sidestream dark-field (SDF) imaging. LDF typically measures relative blood perfusion at a single point (Sturesson et al., 2011), but suffers from large inter-site and inter-individual variability (Richter et al., 2010; Wheatley et al., 1993). Therefore, LDF appears more suitable for relative perfusion assessment as change from baseline. The SDF imaging technique has the advantage over the laser-based techniques with the capability to quantitatively measure microcirculatory parameters like sinusoidal blood flow velocity and functional sinusoidal density (Langer et al., 2001; Puhl et al., 2003). Additionally, SDF imaging does not require toxic fluorescent dyes for contrast enhancement, as used for intravital fluorescence microscopy. The SDF imaging technique comprises a handheld microscope with green light emitting diodes concentrically placed around the tip of a central light guide (Goedhart et al., 2007). The green light illuminates the tissue by scattering and is absorbed by hemoglobin in red blood cells (RBCs).

Abbreviations: CV, coefficient of variability; LDF, laser Doppler flowmetry; LSCI, laser speckle contrast imaging; LSPU, laser speckle perfusion units; MAP, mean arterial blood pressure; RBC, red blood cell; ROI, region of interest; SDF, sidestream dark-field; SEM, standard error of the mean.

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In the resulting image conveyed by the central light guide, the RBCs are displayed as dark moving globules contrasted against a light gray background. By analyzing the recorded video sequences offline, microcirculatory parameters can be quantified. Orthogonal polarization spectral imaging, from which SDF imaging technique has evolved, has been validated for liver microcirculatory measurements (Langer et al., 2001). However, much like LDF, both orthogonal polarization spectral imaging and SDF imaging evaluate perfusion in a small area and additionally demand time consuming offline analysis.

Application of a full-field (micro)vascular measurement, as provided by the relatively novel laser speckle contrast imaging (LSCI) technique (Mahé et al., 2012; Roustit and Cracowski, 2012), may reduce inter-site and inter-individual variability (Rousseau et al., 2011; Tew et al., 2011). The technique relies on measuring fluctuations in the interference pattern (speckle), created by back-scattered light from a biological tissue surface that is laser-illuminated (Boas and Dunn, 2010; Fercher and Briers, 1981). Analyzing these fluctuations provides information on the motion of moving scattering particles in the tissue, i.e. RBCs. Furthermore, LSCI provides non-contact real-time full-field imaging with a high spatial and temporal resolution without the need for surface scanning. Applications for LSCI include skin, brain, retinal and kidney perfusion and are usually interpreted in laser speckle perfusion units (LSPU) (Bezemer et al., 2010b; Boas and Dunn, 2010). To our knowledge, no previous reports on evaluating liver perfusion using LSCI have been published. The aim of the present study was to evaluate the use of LSCI for assessing liver microcirculation during different blood inflow occlusions and to compare the technique to blood flow measurements obtained simultaneously with SDF imaging.

Materials and methods

The study protocol was reviewed and approved by the institutional Animal Experimentation Committee of the Academic Medical Center of the University of Amsterdam. Care and handling of the animals were in accordance with the Dutch legislation guidelines and the European Institutional Animal Care and Use Committee Guidelines.

Animals

Six male Wistar rats with a mean body weight of 400 g (range 380–410 g) were allowed to acclimatize for 10 days on standard food chow and water ad libitum prior to the experiments. Rats were anesthetized by an intraperitoneal injection of a mixture of ketamine (Nimatek, Eurovet Animal Health BV, Bladel, The Netherlands; 90 mg/kg body weight), Dexmedetomidine (Dexdomitor, Pfizer Animal Health BV, Madison, NJ; 0.5 mg/kg body weight) and atropine sulfate (Pharmachemie, Haarlem, The Netherlands, 0.05 mg/kg body weight). Maintenance anesthesia was obtained by intravenous administration of ketamine (50 mg/kg body weight/h) via a tail vein cannula. All animals were intubated orotracheally and mechanically ventilated at a frequency of 40/min with 40% oxygen in air. The right carotid artery was cannulated for measurement of mean arterial blood pressure (MAP).

Surgical preparation

The liver was accessed via a midline incision and the peritoneal attachments were divided. Hereafter, the portal vein was carefully dissected from the hepatic artery. To perform individual or combined controlled intermittent vascular occlusions, a rubber sling was passed around each vessel (i.e. the portal vein and hepatic artery). The ends of each rubber sling were threaded through a 5 cm long silicon tube and affixed using a clamp. Tightening the rubber slings resulted in blood vessel occlusions of either the venous or arterial flow sequentially or simultaneously. The median liver lobe was exteriorized and gently positioned on a warmed metal arch (supporting platform) to minimize movement artifacts but without compromising hepatic perfusion. The liver surface was kept moist throughout the procedure by irrigation of warm (37 °C) sterile NaCl 0.9% (saline) solution at regular intervals.

Laser speckle contrast imaging settings

Hepatic blood perfusion measurements were obtained using a commercially available LSCI instrument (moorFLPI Speckle Contrast Imager, Moor Instruments, Axminster, UK). The LSCI instrument was mounted on a fully adjustable tabletop stand and positioned 30 cm above the exposed liver surface. A low-resolution/high-speed imaging setting was used with a 25 Hz display rate, 1 s time constant and 4 ms camera exposure time. Image resolution was 152×113 pixels and light was collected through a zoom lens, which provided adjustable magnification. All LSCI blood perfusion measurements are presented in LSPU.

Sidestream dark-field imaging settings

The SDF imaging microscope (MicroScan Video Microscope System, MicroScan BV, Amsterdam, The Netherlands) was mounted on a modified mechanical micromanipulator stand for stable reproducible positioning. The microscope probe was covered with a sterile, 10 mm diameter, disposable cap (MicroScan Lens, MicroVision Medical, Amsterdam, The Netherlands), and positioned at the right caudal region of the medial lobe. The mechanical micromanipulator enabled fine control of the SDF microscope onto the liver surface without inducing pressure artifacts and obstruction of hepatic (microvascular) perfusion. Imaging was performed using a ×5 objective lens system (onscreen magnification of 380×), which was captured by a charge couple device video camera with a 720×576 pixel resolution, resulting in a 1.0×0.75 mm imaged tissue segment. All measurements were captured on digital video interface tapes and recorded on a Sony DSR-11 DVCAM video recorder (Sony, Shinagawa-ku, Tokyo, Japan), and viewed on a 19-inch Samsung SyncMaster 932MV liquid crystal display monitor (Samsung, Seoul, South Korea) with a 1440×900 screen resolution.

LSCI and SDF imaging procedures

Image acquisition was initiated after a 15 min stabilization period across seven different time points as shown in Fig. 1. Three different sequential hepatic blood inflow restrictions performed were portal venous occlusion, hepatic artery occlusion, and simultaneous total inflow occlusion by the portal vein and the hepatic artery, respectively. To eliminate the possibility of motion-induced artifacts and to measure proper blood perfusion dynamics, data acquisition was performed during a 10 s period of apnea, representing the time of interest. The periods of vessel occlusions were followed by a 5 min period of full blood inflow. Continuous LSCI and SDF imaging was obtained across all time points in all animals. At the end of the experiments the animals were sacrificed by an intravenous overdose of sodium pentobarbital.

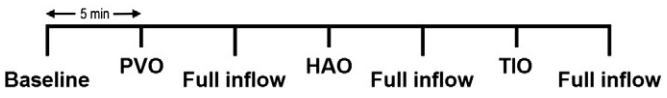

Fig. 1. Experimental design. Vascular occlusions were performed after 5 min of full inflow stabilization, except for baseline measurement, which was preceded by a 15 min stabilization period. Perfusion measurements were obtained before occlusion (i.e. during continuous full inflow) and 3 min after occlusion for 10 s during apnea. Total inflow occlusion (TIO) was defined as simultaneous occlusion of both the portal vein and hepatic artery. PVO portal vein occlusion; HAO hepatic artery occlusion.