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Laser speckle contrast imaging for measurement of hepatic microcirculation during the sepsis: A novel tool for early detection of microcirculation dysfunction

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Background: Sepsis is a fatal systemic inflammatory response syndrome caused by severe infection. The aim of this study was to measure hepatic microcirculation during the sepsis with laser speckle contrast imaging (LSCI), as well as investigating the underlying mechanisms.
Methods: Sepsis was induced by cecal ligation and puncture. Rats were divided into the sham group and sepsis group. The hepatic microcirculation was monitored with LSCI. In addition, hepatic endothelial function (expression of cell adhesion molecules, coagulation and vascular permeability) and neutrophils accumulation in the liver were compared between the two groups.
Results: During the sepsis, hepatic microcirculation decreased dramatically (290.3 ± 70.1 LSPU (laser speckle perfusion units) at baseline vs. 230.4 ± 60.7 LSPU at 12 h vs. 125.2 ± 25.4 LSPU at 48 h, $P < 0.001$). The rats developed hyperbilirubinemia since 6 h. In the early phase of sepsis, the accumulation of neutrophils and formation of microthrombus increased rapidly. In the late phase, hepatic neutrophils accumulation was already at its maximum level. Meanwhile, the endothelial coagulation status shifted from procoagulation to anticoagulation. The vascular leakage was involved in the microcirculation dysfunction since 12 h after sepsis.
Conclusions: Hepatic microcirculation dysfunction occurs early during the sepsis and is associated with liver injury. This microcirculation dysfunction is due to neutrophil–endothelium interactions, microthrombus formation and vascular leakage.

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Introduction

Sepsis is the 11th leading cause of death in the United States, while liver is the second most commonly affected organ during the sepsis (Marshall, 2013). Liver injury is an early event, as reflected by hyperbilirubinemia within hours after sepsis (Recknagel et al, 2012). It has been established that patients with hyperbilirubinemia have a 3-fold increased risk of infection compared with healthy controls (Field et al, 2008). The severity of hyperbilirubinemia prompts clinicians to investigate underlying mechanisms and potential therapies.

Hepatic hypoperfusion may contribute to the liver injury. The microcirculation rather than the macrocirculation is implicated as the underlying culprit (De Backer et al, 2004). Microcirculation consists of the smallest blood vessels (<100 μm in diameter). Microcirculation is responsible for delivering oxygen to tissues, exchanging nutrients and waste products, as well as modulating inflammation and coagulation (Trzeciak et al, 2007). The discordancy of microcirculation and macrocirculation has been recognized recently (De Backer et al, 2013). It is indicated that restoring macrocirculation is not sufficient to restore microcirculation and preserve organ perfusion (Dyson et al, 2012). The microcirculatory variables are better predictors of outcomes than global ones in septic patients (De Backer et al, 2013). These data emphasize the importance of microcirculation measurement.

Several techniques have been developed, including fluorescence intravital microscopy, sidestream dark-field imaging (SDF), orthogonal polarization spectral imaging (OPS) and laser Doppler flowmetry (LDF). Intravital microscopy only records blood flow intermittently. The applications of OPS, SDF, and LDF are limited by the small measurement area (Senarathna et al, 2013). As a novel technique, laser speckle contrast imaging (LSCI) has gained extensive attention. It allows continuous and real-time recordings of microcirculation with a larger area. LSCI has been applied successfully to evaluate microcirculation in

Abbreviations: aPTT, activated partial thromboplastin time; CLP, cecal ligation and puncture; EPCR, endothelial PC receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HR, heart rates; ICAM-1, intercellular adhesion molecule; i.p., injected intraperitoneally; LPS, lipopolysaccharide; LSCI, laser speckle contrast imaging; MAP, mean arterial pressure; MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; ROI, region of interest; RT-PCR, reverse transcription-PCR; RT-PCR, reverse transcription-PCR; TF, tissue factor; TM, thrombomodulin; TUNEL, terminal deoxyribonucleotidyltransferase-mediated dUTP nick-end labeling; VCAM-1, vascular cell adhesion molecule.

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the skin, gastrointestinal mucosa, and brain cortex (Senarathna et al, 2013, (Schiessler et al, 2002, (Briers et al, 2013). LSCI has been used to monitor liver microcirculation during temporary occlusion of blood inflow (Sturesson et al., 2013a, 2013b). Notably, microcirculation is heterogeneous between the organs (Lendemans et al, 2008, (Verdant et al, 2009).

To the best of our knowledge, the hepatic microcirculation has not been evaluated with LSCI during the sepsis. In addition, the underlying mechanisms of hepatic microcirculation dysfunction remain unclear. Hence, we aimed to monitor hepatic microcirculation during the sepsis, as well as investigating the potential mechanisms.

Methods

Ethics statement

This study was approved by Science and Technology Department of Jiangsu Province [Permit Number: SYXK (Su) 2013-0003]. All experimental procedures were performed according to the guidelines set out by the animal research committee of Nanjing University.

Experimental protocols

One hundred and thirty-two 8-week-old Sprague-Dawley male rats (weight between 220 ± 25 g, obtained from Xi'an, Shanxi province, China) were used in the experiments. Prior to experiments, all rats were kept for at least 2 weeks to recover from transport. The rats were maintained in the environment with 50–60% humidity and 12-hour light/dark cycle at 25 ± 2 °C. All rats had free access to water and standard food. They were fasted for 12 h before surgery. Sixty rats were randomly divided into two groups (rats were sacrificed at 0 h, 6 h, 12 h, 24 h and 48 h after surgery for sample collections; $n = 6$ in each time point). Group 1 was the sham group; Group 2 was subjected to cecal ligation and puncture (CLP). Another set of twelve rats was randomly divided into the sham group or the sepsis group, serving for macrocirculatory and microcirculatory measurements. Another sixty rats were also divided into the two groups, serving for vascular permeability assay.

Surgical procedures

Rats were anesthetized with ketamine (80 mg/kg injected intraperitoneally (i.p.)) and xylazine (5 mg/kg i.p.). Subsequently, they were placed in the supine position and a midline laparotomy incision was made. In the sham group, the cecum was not ligated or punctured. In the sepsis group, the cecum was exposed, ligated about 15 mm proximal to the ileocecal valve, and perforated twice with an 18-gauge needle. The cecum was squeezed to extrude fecal contents that were spread around the cecum (Tao et al, 2004). Rats received fluid resuscitation (3 ml/100 g, 0.9% NaCl, i.p.) immediately and every 12 h thereafter. All procedures were performed by the same investigators. At indicated time points, rats were sacrificed through cervical dislocation and liver tissues (0.3 g) were harvested and stored in a −80 °C freezer.

Macrocirculatory measurement

The right carotid artery was cannulated with a 24-gauge catheter (Becton Dickinson, Belgium) for monitoring mean arterial pressure (MAP) and heart rates (HR). The data were recorded on a multichannel recorder (Powerlab, ADInstruments, Spechbach, Germany).

Laser speckle contrast imaging for measurement of hepatic microcirculation

The hepatic microcirculation was measured by a commercially available LSCI instrument (PeriCam PSI System, Perimed, Sweden) at stable room temperature (25 °C). The median liver lobe was carefully exposed through laparotomy. A 1 cm × 1 cm square region was set as the region of interest (ROI). The microcirculation was monitored for 1 min, with the scan head 12 cm high above the exposed liver. The imaging setting was used with a 25 Hz display rate, 1 s time constant, and 0.2 mm/pixel spatial resolution (Sturesson et al., 2013a, 2013b). The liver surface was kept moist throughout the experiment by irrigation of warm (37 °C) saline (0.9%) solution at regular intervals. All measurements were presented in LSPU. The data were analyzed by the PimSoft 1.4 software (Perimed, Sweden).

Histological analysis

The livers were harvested, fixed in 10% formaldehyde, embedded in paraffin, and serially cut into 5 μm-thick sections. The hematoxylin and eosin (H&E)-stained sections were evaluated using an optical microscopy (Olympus Optical, Tokyo, Japan) at ×100 and ×400 magnification. Histological damage was semi-quantified based on the methodology from previous studies (Camargo et al, 1997). The slides were analyzed in a blind fashion by two specialists to review the severity of following parameters: (1) edema, cytoplasmic vacuolation or cellular degeneration; (2) inflammatory cells infiltration; (3) necrotic zones or tissue disorganization; and (4) congestion or hemorrhage. Each parameter was scored from 0 (absent) to 3 (intense). Scoring of each sample represented the mean score of five different high microscopic power fields.

Assessment of liver function

Blood samples were obtained from the inferior vena cava. Blood was immediately centrifuged for 15 min at 4200 rpm (Laborzentrifugen SIGMA 3 K10, Germany). Liver function parameters were analyzed using an autoanalyzer (Roche Diagnostic Modular Systems, Tokyo, Japan). Coagulation parameters were measured by routine chemistry.

Reverse transcription-PCR (RT-PCR) analysis

Total RNA was isolated from the liver tissues (30–50 mg) by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), treated with RNase free-DNase (Promega, Madison, WI, USA) and reverse transcribed to cDNA. The cDNA was then amplified by RT-PCR. The primers were described in Table 1. PCR amplifications were performed for 3 min at 94 °C, followed by 35 cycles with 1 min at 94 °C, 30 s at 60 °C (for ICAM-1)/1 min at 55 °C(for VCAM-1)/30 s at 60 °C(for E-selectin)/30 s at 60 °C(for P-selectin)/1 min at 62 °C (for TF)/2 min at 72 °C (for PAI-1)/30 s at 58 °C (for TM)/30 s at 53.3 °C (for EPCR), 1 min at 72 °C, and a final 10 min at 72 °C. Following amplifications, the PCR products were analyzed on 2% agarose gels and band intensities were quantified by densitometry (Syngene gel-documentation system). The mRNA level for each gene was normalized to GAPDH mRNA.

Liver myeloperoxidase (MPO) content assay

Tissue MPO level has been identified as a marker of neutrophils sequestration (Zakaria el et al, 2007). The liver was homogenized and MPO level was assessed using spectrophotometry (655 nm). The MPO level (U/g) was defined as the quantity of enzyme degrading 1 μmol peroxide per minute per gram of tissue at 25 °C.

Immunofluorescent labeling for neutrophils in the liver

The livers were fixed in Z-Fix (Anatech Ltd., MI) for 24 h and embedded in paraffin, cut at 3 μm. For immunofluorescence staining, samples were incubated in blocking buffer (1% bovine serum albumin, 0.01% saponin, PBS) for 1 h. Sections were incubated with primary antibody: anti-MPO (1:200 dilution, Santa Cruz Biotechnology, USA), overnight at 4 °C. After washing with PBS, Alexa Fluor-conjugated secondary

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