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Laser speckle contrast imaging for assessment of abdominal visceral microcirculation in acute peritonitis: does sequential impairments exist?

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ABSTRACT

Objective: It is believed that the microcirculation of multiple organs is impaired during acute peritonitis, however whether distinct susceptibilities of visceral microvasculature exist is still unknown. The present study aims to verify whether the microcirculatory alterations occur sequentially among multiple abdominal viscera during acute peritonitis.

Materials and methods: Acute peritonitis was achieved on 29 Sprague–Dawley rats through colon ascendens stent peritonitis (CASP) model. With laser speckle contrast imaging (LSCI), the microcirculation of the liver, ileum and renal cortex was monitored in each rat at baseline before CASP sepsis and continued monitoring at 4 h, 8 h, or 12 h after the surgery. Another 9 rats served for sham operation. One-way analysis of variance with a post hoc Dunnett's test was used for analysis.

Results: The ileum microcirculation was impaired earliest from 342.1 ± 61.0 laser speckle perfusion unit (LSPU) at baseline to 271.7 ± 74.0 LSPU at 4 h ($P < 0.05$), while the decline of renal microcirculation was not obvious until 8 h after peritonitis (289.1 ± 111.2 vs 376.2 ± 53.4, $P < 0.05$). However hepatic microcirculation was not significantly changed during 12 h of observation period.

Conclusion: The microcirculation of various viscera has shown distinct susceptibilities to acute peritonitis: the ileum is more susceptible than the kidney, while the hepatic microcirculation seems to be the most resistant to peritonitis.

Introduction

As recommended in the "surviving sepsis campaign" Dellinger et al., 2013, systemic hemodynamic variables (MAP, CVP, $Scvo_2$) have been widely used to guide quantitative fluid resuscitation. However, though the systemic variables could be ameliorated due to the resuscitation, considerable quantities of patients exhibit tissue hypoxia and impaired microvascular perfusion. Microcirculatory dysfunction plays an essential role in the pathogenesis of sepsis induced organ failure Vincent and De Backer, 2005; Sakr et al., 2004. A recent clinical study De Backer et al., 2013 has demonstrated that microcirculatory variables are stronger predictors of outcome than global ones. Persistent microcirculatory dysfunction predicts a higher mortality in critically ill patients. The correction of systemic hemodynamic variables cannot indicate the recovery of microcirculation De Backer et al., 2013; Tachon et al., 2014.

Several apparatus have been developed and introduced to monitor the microcirculatory alterations both in animals and human beings. The most commonly used techniques include intravital microscopy, orthogonal polarization spectral (OPS) imaging, sidestream dark-field (SDF) imaging, and laser doppler flowmetry (LDF). Intravital microscopy Sharawy et al., 2011 requires injections of toxic fluorescent dyes while OPS Bracht et al., 2008 and SDF Pranskunas et al., 2012 can investigate microvasculature directly without the dyes. Clear microvascular networks can be seen through these techniques, but only a small area is detected at a time, several times should be monitored to depict the microcirculation of a whole organ surface. The process and the offline analysis are time-consuming. Similarly, LDF can only detect the perfusion of the spot to which the probe is attached. In addition, the physical attachment of the probes may obstruct the microcirculation of the pressed region. Until now, most clinical studies focused on the sublingual microcirculation due to the limitation of available areas/organs. Though sublingual microcirculatory variables were reported to be useful in predicting the outcome of critically ill patients, it's still unclear whether it could reflect the microcirculation of other organs Pranskunas et al., 2012; Boerma et al., 2007; Verdant et al., 2009.

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Unlike the conventional techniques mentioned above, the relatively novel laser speckle contrast imaging (LSCI) technique allows for a full-field perfusion measurement. Through increasing the "region of interest" and "time of interest" measured under LSCI Rousseau et al., 2011, the variability of blood flow measurements could be reduced. Furthermore, LSCI does not require physical contact with the measured region, which could minimize the interference and possible injury to the tissue. A recent study has demonstrated the feasibility and accuracy of LSCI in detecting liver microcirculation compared to SDF Sturesson et al., 2013.

Since structures and distributions of visceral microvascular beds varied, the vascular beds of different organs may have distinct tolerance to endotoxin or other bacterial products during sepsis. The capillary in the liver, unlike that in the gut, is composed of hepatic sinusoidal endothelium and the Kupffer cells. The sinusoidal endothelial cells (ECs) are featured by relatively large and aggregated fenestration (approximately 100 nm in diameter). That structure allows sufficient passage of small particles between the hepatocyte and blood within the space of Disse. Endotoxin tolerance of sinusoidal EC contributes to reduced leukocyte adhesion and improved hepatic microcirculation Uhrig et al., 2005. Thus our hypothesis in the present study is that the microvascular perfusion alterations occur in different time intervals during acute peritonitis. To the best of our knowledge, abdominal visceral organ microcirculation monitored using LSCI in polymicrobial abdominal sepsis has not been reported. We carried out the present study with LSCI to assess the microcirculatory alterations of multiple abdominal organs including the liver, kidney and intestine in acute peritonitis.

Material and methods

Experimental animals

The pilot and main studies were carried out on 7- to 8-week-old Sprague–Dawley male rats (weight between 270 ± 25 g). The animals were kept in the environment with 50–60% humidity, 12-hour light/dark cycle at 25 ± 2 °C, all the animals had free access to water and standard food. All experimental procedures were performed in accordance with the guidance of Animal Investigation Ethics Committee of Jinling Hospital.

Surgical procedures

In the pilot study, three rats were anesthetized by ketamine (80 mg/Kg i.p.) and xylazine (5 mg/Kg i.p.), and kept anesthetized by inhaling 2% fluothane in O_2. The right carotid artery was dissected, then cannulated by a 2F catheter connected to a pressure transducer. Thereafter, acute peritonitis was achieved by colon ascendens stent peritonitis (CASP) procedure which was described in detail previously Lustig et al., 2007. Briefly, a 4-cm-long midline incision was performed and a 14 G venous indwelling cannula was inserted through the colon ascendens to the ileocecal valve at the antimesenteric site (about 1.5 cm) and fixed to the wall of the colon with two sutures (4-0 Ethicon). The cecum was squeezed carefully until the stent was filled with feces and a small drop of stool appeared in the orifice. Then the colon with the cannula was repositioned into the abdominal cavity properly and abdominal layers (muscular, skin) were sutured (2-0 Ethicon). A baseline measurement of systemic circulatory parameters and visceral microcirculation was made on all the subjects before the stent insertion. After 10-minute resting from CASP surgery, the arterial pressure and heart rate were monitored continuously. The microcirculatory perfusion of the liver, ileum and renal cortex was monitored 6, 12 and 24 h after CASP surgery. The rats were kept anesthetized during the whole observation and a 10 ml/kg/h normal saline solution resuscitation was carried out subcutaneously.

In the main study, thirty-six animals of the CASP group were divided into three subgroups in which abdominal organ microcirculation was detected at 4, 8, and 12 h after CASP surgery (12 rats in each subgroup). After surgical procedures or the microcirculation monitoring, the animals were taken back to living environment with free access to food and water, and the fluid resuscitation was identical to the pilot study.

Sham operation. For the sham group (n = 9), the animals underwent the same laparotomy except that the stent was only fixated at the antimesenteric site of the ascending colon instead of inserting into the bowel lumen. They were also assigned to three groups and got LSCI monitor at 4, 8, 12 h after the surgery (n = 3 for each subgroup).

With respect to the severity of sepsis in our experiments, we monitored the survival rate during a 48-hour period. Animals that survived for 48 hours were sacrificed through cervical dislocation under general anesthesia.

Laser speckle contrast imaging settings

The multiple visceral microcirculation was determined by a commercially available LSCI instrument (PeriCam PSI System, Perimed, Sweden). At predetermined time points, experimental animals were anesthetized again and laid supine on a warming pad. The left lateral lobe of the liver was carefully exteriorized, injury to the surface of the organ should be avoided. A 1 cm × 1 cm square region was set as the region of interest (ROI), then the microcirculation was monitored for 1 min by the scan head 12 cm higher above the measurement site. Subsequently, the liver was repositioned gently and followed by monitoring of the terminal ileum and left kidney one by one, 1 min for each measurement. All the measurements began when the subjects kept sedative and breathed evenly. ROIs on the kidney and ileum were determined in accordance with the shape of the organ (areas within white line, see Fig. 2). We used the same monitoring range (0–500 LSPU) through all our study. The images and data were recorded and analyzed in real time by the software PimSoft 1.4 version (Perimed, Sweden).

Statistical analysis

The results are presented as mean ± SD. One-way analysis of variance (ANOVA) was used with Dunnett's test as a post test for analysis. A P value smaller than 0.05 was considered statistically significant.

Results

Pilot study

Rat 1 kept alive during the 24-hour observation with a stable mean arterial pressure (MAP). Rats 2 and 3 died 9 h and 18 h after CASP respectively. The MAP did not change obviously till approximately 2 h before death (Fig. 1A). During the last 2 hours, MAP declined rapidly until death. Heart rate had a similar trend (not shown in the figure). Unlike systemic circulatory parameters, the microcirculation of ileum and renal cortex was dropped gradually during the observation, while hepatic perfusion did not change greatly (Fig. 1B).

Main study

In the CASP group, three rats in the 4-hour subgroup and two rats in 12-hour subgroup died of an anesthetic complication during first or second anesthesia. They were excluded from the study. Another 2 rats in the 12-hour subgroup died within 12 h after peritonitis, thus the data at 12 h after CASP were unavailable. The final rats available for analysis in the CASP group were 9, 12 and 8 in each subgroup. The proportion of rats that died as early as 12 h after CASP was nearly 20%. During the first 24 h, 55% of the animals died. Mortality within 48 h reached almost 90%. In contrast, all the animals in the sham group survived during the observation period (Fig. 2).

Detailed data of the microcirculation in the septic and sham group are presented in Table 1. Fig. 3 shows representative images of the three