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Laser speckle contrast imaging for assessing microcirculatory changes in multiple splanchnic organs and the gracilis muscle during hemorrhagic shock and fluid resuscitation

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ABSTRACT

Objective: Hemorrhagic shock induces both macrocirculatory and microcirculatory impairment. Persistent microcirculatory dysfunction is associated with the dysfunction of multiple organs, especially in the splanchnic organs. However, few studies have simultaneously investigated microcirculation in multiple organs. In the present study, we used laser speckle contrast imaging to simultaneously investigate microcirculatory changes secondary to hemorrhagic shock and after fluid resuscitation among multiple splanchnic organs and the gracilis muscle.
Materials and methods: 72 male Wistar rats were subjected to sham operation, hemorrhagic shock (total blood loss of 30 mL/kg) and saline resuscitation. Macrocirculatory parameters, including the mean arterial pressure (MAP) and heart rate, and microcirculatory parameters, including microcirculatory blood flow intensity and tissue oxygen saturation in the liver, kidney, intestine (mucosa, serosal muscular layer, and Peyer's patch), and gracilis muscle were compared in a period of 3 h.
Results: Hemorrhagic shock induced a significant reduction of microcirculatory blood flow intensity in the kidney and intestine (especially the mucosa). Tissue oxygen saturation reduction secondary to hemorrhagic shock was comparable among the various splanchnic organs but lower than the gracilis muscle. Fluid resuscitation restored the MAP but not the microcirculatory blood flow in the intestine and the tissue oxygen saturation in each splanchnic organ.
Conclusion: Hemorrhagic shock induced the largest reduction in microcirculatory blood flow intensity in the intestinal mucosa. By comparison, the reduction of tissue oxygen saturation was not significantly different among the various splanchnic organs. Although fluid resuscitation restored the MAP, the intestinal microcirculation remained damaged.

Introduction

Hemorrhagic shock is one of the major causes of mortality in traumatic injury. Both macrocirculatory and microcirculatory dysfunctions have been characterized in the acute phase of hemorrhagic shock (Dubin et al., 2009; van Iterson et al., 2012). Recently, there is a paradigm shift from macrocirculatory to microcirculatory investigations because the persistence of microcirculatory dysfunction is associated with organ failure. During a hemorrhage, blood flow and tissue oxygenation of nonvital organs decrease to maintain the circulation required by vital organs. However, the acute changes of microcirculation blood flow and tissue oxygen saturation in multiple splanchnic organs secondary to hemorrhagic shock are insufficiently clear. Understanding the microcirculatory changes of splanchnic organs during a hemorrhage and resuscitation may provide crucial information for further research and treatment because splanchnic ischemia is one of the major causes of multiple organ dysfunction syndrome (Pastores et al., 1996).

Laser speckle contrast imaging (LSCI) is an increasingly prevalent technique for monitoring microcirculatory blood flow. Because it enables full-field imaging in near real time with multiple regions of interest, it is suitable for investigating microcirculatory changes among multiple organs (Boas and Dunn, 2010; Ding et al., 2014; Draijer et al., 2009). LSCI in combination with tissue oxygen saturation measurements may offer a comprehensive understanding of acute microcirculatory changes among multiple organs. In this study, we investigated the heterogeneity of microcirculatory responses to hemorrhagic shock among multiple splanchnic organs and the gracilis muscle by using LSCI and tissue oxygen saturation measurements. Additionally, we also clarified the microcirculatory effects of fluid resuscitation during the acute phase of hemorrhagic shock. Because different tissues of the intestine may have different susceptibilities to shock (Yeh et al.,

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2012b), further investigation was also conducted to compare the microcirculatory changes of the intestinal mucosa, serosal muscular layer, and lymph node.

Materials and methods

Experimental animals

A total of 72 male Wistar rats were used (body weight 250 ± 50 g; Biolasco Taiwan Co., Taipei, Taiwan). The rats were kept on a 12-h light–dark cycle and had free access to water and food. All experimental procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan University and performed in accordance with its guidelines.

Anesthesia and surgical procedure

The rats were anesthetized and prepared as described in our previous study (Yeh et al., 2012b). A tracheostomy was performed, and a 14G catheter (Surflo; Terumo Corporation, Laguna, Philippines) was inserted into the trachea to maintain spontaneous breathing. The anesthesia was maintained using 1.2% isoflurane. Subcutaneous atropine 0.05 mg/kg in 10 mL/kg of saline was injected for replacement of water evaporization from surgical open wound and to prevent airway secretion. The body temperature was continuously monitored rectally, and a warmer pad was applied to maintain the body temperature between 36 and 37 °C. Polyethylene catheters (PE-50; Intramedic 7411, Clay Adams, Parsippany, NJ, USA) were inserted into the right common carotid artery and external jugular vein. The right common carotid artery catheter was used to continuously monitor macrocirculatory hemodynamics, including the mean arterial pressure (MAP) and heart rate (HR), and to withdraw blood to establish hemorrhagic shock. The external jugular vein was used for infusion of resuscitation fluid.

A long midline laparotomy was performed to exteriorize splanchnic organs including the liver, left kidney, and a segment of the terminal ileum (about 6 to 10 cm proximal to the ileocecal valve). A 2-cm section was performed on the antimesenteric aspect of the intestinal lumen by using a high-frequency desiccator (Aaron 900; Bovie Aaron Medical, St. Petersburg, FL, USA) to carefully expose the opposing mucosa for examining the microcirculation. Nearby intestinal serosal muscular layer (at the midline of antimesenteric aspect) and the central Peyer's patch (identified by visualize the lymph nodes) were also identified for examining the microcirculation. Moreover, the right gracilis muscle was exposed for measuring microcirculatory changes relative to those of the splanchnic organs. The exposed viscera and tissue were kept moist hourly with saline (0.5 mL of aerosolized) prewarmed to 37 °C.

Hemorrhagic shock and fluid resuscitation protocol

After completion of the surgery, the animals were allowed to stabilize for 30 min before the baseline measurements were performed (baseline condition was considered stable when all measurement values remained at 10% for 15 min; T_0). After the baseline measurements were collected, the concentration of inhaled isoflurane was decreased to 0.7% to prevent over anesthesia for hemorrhaging animal without further surgical stimulation, and the rats were randomly assigned to either a sham operation (S) group, hemorrhagic shock (H) or fluid resuscitation with 0.9% saline (R) group. In the H group, hemorrhagic shock was initiated through controlled blood withdrawal via the right carotid arterial cannula (3 times of 10 mL/kg per 5 min; total blood loss of 30 mL/kg during 15 min). Further macrocirculatory and microcirculatory monitoring were measured according to the time points shown in Fig. 1. In the S group, the rats received the same surgical preparation but did not undergo blood withdrawal. The R group was resuscitated by a total of 30 mL/kg of 0.9% saline after hemorrhagic shock for 30 min.

Evaluation of splanchnic microcirculatory blood flow and oxygen saturation changes secondary to hemorrhagic shock

A full-field laser perfusion imager (MoorFLPI; Moor Instruments, Ltd., Devon, UK) was used from the baseline (T_0) to continuously quantify microcirculatory blood flow intensity in the splanchnic organs (Yeh et al., 2012a). The detail of choices of interested region of tissue for monitoring is mentioned in the Supplementary Material. The imager uses LSCI, which exploits the random speckle pattern that is generated when tissue is illuminated by a laser light. The random speckle pattern changes when blood cells move within the region of interest (ROI). When the level of movement is high (high flow), the changing pattern becomes more blurred, and the contrast in that region decreases accordingly. Therefore, low contrast is related to high flow and high contrast to low flow. The contrast image is processed to produce a 16-color coded image that correlates with blood flow in the tissue (e.g., blue is defined as low flow and red as high flow). The microcirculatory blood flow

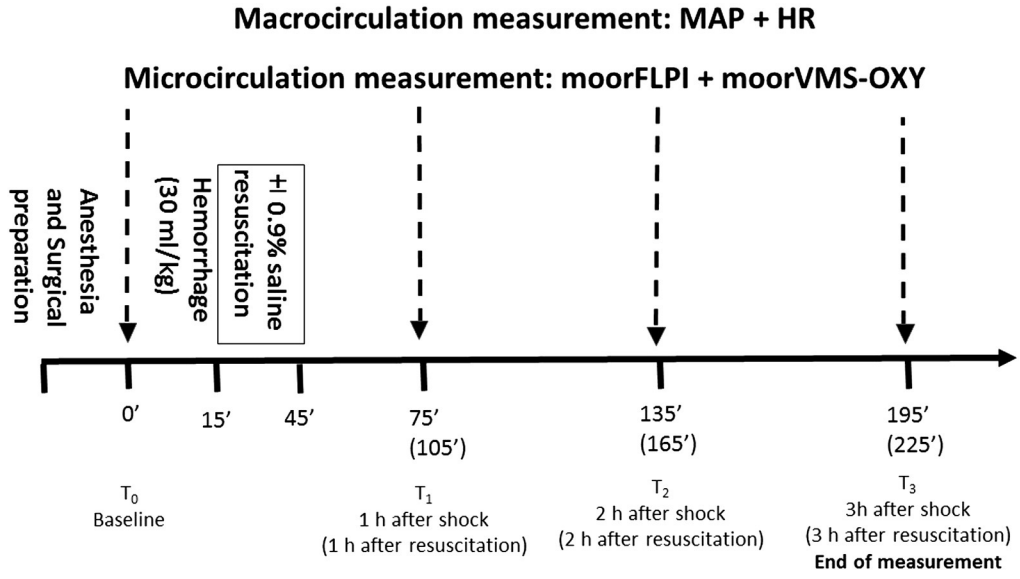

Fig. 1. Timeline of protocol.