Early Microcirculatory Derangement in Mild and Severe Pancreatitis Models in Mice

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Introduction

It has been reported that microcirculatory derangements are important features of experimental acute pancreatitis in animals. The leukocyte–endothelial cell interaction which produces effects such as adhesion and migration is presumed to be involved in the progression of acute inflammation following endothelial injury and tissue damage. Moreover, the generation of inflammatory mediators further complicates the interaction between leukocytes and endothelial cells. Changes in vascular permeability always accompany endothelial damage, and hyperpermeability to large molecules, such as albumin, is a hallmark of the inflammatory response of the endothelial lining. It remains unelucidated to what extent and at what stage the leukocyte–endothelium interaction contributes to changes in permeability during the course of acute pancreatitis.

Abstract

An in vivo microscopic technique was used to clarify the increase in microvascular permeability and enhanced leukocyte–endothelium interaction of pancreatic microcirculation in experimental pancreatitis of differing severity. Using bovine albumin fluorescein isothiocyanate (FITC) and carboxyfluorescein diacetate succinimidyl ester (CFDASE) as tracers, the change in permeability and the behavior of leukocytes in the acinar microcirculation were quantified during the initial 1, 2, 6, and 12 h after the induction of caerulein pancreatitis in mice. Cold stress was added to produce the severe model. It was revealed that the early microcirculatory changes in the pancreas of caerulein pancreatitis included the increased permeability of endothelial lining and an accumulation of extravasated fluid in the perilobular space, which were more severe if cold stress was added. A decrease in flow velocity was also noted 2 h after the onset of severe pancreatitis. Leukocyte adherence to the endothelial cells was not observed during the first 12 h in either model of severity. In contrast, observation of the hepatic microcirculation revealed a significant number of adherent leukocytes 2 h after the induction of severe pancreatitis. These results suggest that during the early course of acute pancreatitis, leukocyte adherence in the pancreatic microcirculation is a secondary event following the increase in pancreatic vascular permeability.

Key words

Microcirculation · Acute pancreatitis · In vivo microscope · Leukocyte adherence · Vascular permeability

Materials and Methods

Induction of Acute Pancreatitis

Male ICR mice aged 10–14 weeks and weighing 25–35 g were purchased from CLEA Japan (Tokyo) and maintained in the Institute for Experimental Animals at Tohoku University School of Medicine. We followed...
the NIH Guidelines for the Care and Use of Research Animals. To induce mild pancreatitis, a 5% solution of caerulein (ceruletide diethylamine, Pharmacia, Milan, Italy) was injected intraperitoneally at 50 mg/kg. To induce severe pancreatitis, the mice were exposed to 15 min of cold stress, 15 min after the intraperitoneal injection of the same dose of caerulein. The cold stress was produced by immersing the mice into iced water up to the level of the xyphoid process. The in vivo microscopic observation was done 1, 2, 6, or 12 h after the initial injection of caerulein. Mice given an intraperitoneal injection of 1 ml normal saline (control) and mice not subjected to any intervention (baseline) were employed as normal groups. Control mice were observed 1 h after the normal saline injection. There were five mice in each group at all observation times. A total of 50 mice were used in the in vivo microscopic experiment and two functional fluorochromes with different emission profiles were employed; carboxyfluorescein diacetate succinimidyl ester (CFDASE) to label circulating leukocytes, and fluorescein isothiocyanate (FITC) to load the plasma component.

In the second experiment, the same animal models and in vivo microscopic technique were used to qualify the initial events in acute pancreatitis. Animals were observed 15, 30, or 45 min after the intraperitoneal injection of caerulein at same dosage. Only mild pancreatitis was produced in this experiment. The same protocol was followed as in the first experiment for performing the in vivo microscopic technique. There were five mice in each group observed.

**Dual-Color Digital Microfluorography Using FITC and CFDASE**

Mice were anesthetized with intraperitoneal pentobarbital, given at a dose of 50 mg/kg diluted in 5 mg/ml solution. The abdomen was opened through a midline laparotomy. The inferior vena cava was punctured with a 27-F needle, then injected with 0.05 ml 4% bovine albumin FITC (Sigma, St. Louis, MO, USA) and 0.05 ml CFDASE (CFSE, Molecular Probes, Eugene, OR, USA). Local compression was carried out for 2 min for hemostasis. The pancreatic body and tail, together with the spleen, were then mobilized out of the peritoneal cavity, with the mice placed in the left decubitus position. The left lateral lobe of the liver was observed under the microscope. Warm saline-soaked cotton was placed around the pancreas and on the surface of the exposed abdominal contents. After preparation, the animals were placed on the stage of the microscope, and put in a closed chamber connected to an incubation warmer (Nikon, Tokyo, Japan) adjusted to keep moisture and temperature. The in vivo microscopic examination was performed 15 min after the injection of FITC and CFSE tracers. No mouse was observed for any longer than 1 min at a time to avoid damage to the microcirculation from the epi-illumination itself.

**In vivo Microscope System**

The organ was placed above the objective lens of a inverted type fluorescence microscope (TMD-300, Nikon) with an epi-illumination unit, and the light of the mercury vapor lamp passed through an excitation filter set (450–490 nm). The microcirculation was studied with the ×10 and ×20 objective lens (Nikon), resulting in a magnification on the video monitor of ×160 and ×320. A video camera (C2400, Hamamatsu, Hamamatsu, Japan) was attached to the microscope, and the experiments were recorded on video tape by a VHS video recorder (BR-S382, JVC, Tokyo, Japan). Using video analyzing software (NIH Image 1.55, NIH, Bethesda, MD, USA) every image was digitalized in 320 × 240 pixels. Each pixel had 8 bits, with a final resolution of 256 gray-scale values, from 256 of white to 0 of black, being the inverted value of the original gray scale in the software. With a standard microscope scale (Nikon) we calibrated the software to determine 100 μm as 38.2 pixels.

**Analysis of Velocity and Wall Shear Rate**

The velocity of intravascular fluorescent dye was assessed by playing back the video tapes frame by frame, using image software: Avid Video Shop (Avid Technology, Cambridge, MA, USA). The observation areas were chosen at the collecting venules, 20–25 μm in diameter, of the acinar capillaries. Centerline red blood cell velocity ($V_{max}$, mm/s) was measured using the distance from a red blood cell between two subsequent frames in a fixed time interval of 0.03 s. Venular blood flow was calculated from the value of mean red blood cell velocity ($V_{mean} = V_{max}/1.6$) and the cross-sectional area assuming a cylindrical geometry of vessels. The venular wall shear rate ($g$) was calculated as: $g = 8 (V_{mean}/D_v)$, $D_v$ being the diameter of the examined venule (mm).

**Data Analysis of Microvascular Permeability**

Extravasation of the FITC-bound albumin was noted after the injection of the fluorescein tracer, resulting in an increase of gray-scale values in the digitized images (Fig. 1). The gray-scale values of intravascular (IV) space, perivascular space (PV), and perilobular space (PL) in the areas of interest were determined by an analysis using NIH 1.55 software. Permeability changes were accessed with PV/IV ratios and PL/IV ratios.