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P-Selectin Inhibition Reduces Severity of Acute Experimental Pancreatitis

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Key Words
Acute pancreatitis · P-selectin · Antibody therapy

Abstract
Background: Acute pancreatitis (AP) is characterized by disturbed pancreatic microcirculation with tissue necrosis. Platelet and leukocyte activation contributes to microcirculatory disorders and inflammatory tissue infiltration. P-selectin mediates the adhesion of both activated platelets and leukocytes to the vessel wall. The aim of this study was to investigate the effect of P-selectin inhibition by monoclonal antibodies (AB) on experimental AP. ***Methods:*** AP was induced in rats by glycodeoxycholic acid (GDOC) intraductally and by cerulein infusion. Animals were divided into 4 groups: (1) severe AP (GDOC); (2) severe AP + platelet inhibition (GDOC + selectin AB); (3) control (Ringer); (4) control + platelet inhibition (Ringer + selectin AB). 24 h after AP induction, histology and serum (amylase, thromboxane A2) were evaluated (6 animals per group). In additional 12 animals of each group, platelet and leukocyte activation as well as erythrocyte flow patterns were evaluated by intravital microscopy 12 h after AP induction. ***Results:*** AP induction caused significant tissue inflammation and necrosis with increased amylase and thromboxane levels. Prophylactic inhibition of P-selectin reduced tissue inflammation and necrosis significantly. Severe AP led to significantly more adherent platelets and leukocytes in capillaries and venules. In contrast, antibody-treated animals showed significantly reduced platelet-endothelium interaction compared with untreated AP animals. Antibody application in control animals without AP did not induce any changes compared with healthy control animals. ***Conclusion:*** Inhibition of P-selectin reduces tissue damage in experimental AP. This is associated with a reduction in platelet- and leukocyte-endothelium interaction and an improvement in pancreatic microcirculation.

Introduction

Acute pancreatitis (AP) is characterized by an inflammatory affection of the exocrine pancreatic tissue and disturbance of pancreatic microcirculation [1]. Depending on the severity, irreversible perfusion failure with consecutive tissue hypoxia and necrosis complicates the course of the disease and triggers systemic inflammatory and septic complications [2]. The pathophysiology of AP has been investigated with regard to microcirculatory changes in several studies [1–5]. Attention was paid especially to erythrocyte flow patterns, leukocyte-endothelium interaction and rheological approaches to reconstitute perfusion and inhibit irreversible tissue damage [1, 3–5]. Leukocyte-endothelium interaction as an early step of the inflammatory response has been characterized as a key step in the pathophysiology of AP [6]. In addition to this cellular factor, activation of the humoral coagulation cascade has also been shown to play an important role in the development of microcirculatory disorders in AP [7, 8]. Platelets as cellular elements of hemostasis can

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functionally link inflammatory cells, and humoral coagulation factors have recently been characterized to play a decisive role in the pathogenesis of AP [9, 10].

P-selectin mediates the adhesion of activated platelets to the vessel wall [11]. In addition, P-selectin acts on activated leukocytes during the inflammatory process with a consequent margination and adhesion of these cells to the endothelium [12]. With these two functions, P-selectin is involved as a central element in the pathophysiological processes of inflammation and coagulation.

The aim of this study was to investigate the effect of P-selectin inhibition by monoclonal antibodies (AB) on the course and severity of experimental AP.

Methods

Animals

The experiments were performed in 72 male Wistar rats weighing 250–320 g. Animals were fasted overnight with free access to water before the experiments. Care was provided in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, 1985). Surgical anesthesia was induced with i.p. injection of pentobarbital (25 mg/kg) and i.m. injection of ketamin (40 mg/kg) for the procedures of catheter placement and induction of pancreatitis. Anesthesia during intravital microscopy was induced by i.v. injection of pentobarbital (10 mg/kg). Polyethylene catheters (inner diameter 0.5 mm) were placed in the right jugular vein and left carotid artery, tunneled subcutaneously to the suprascapular area and brought out through a steel tether that allowed the animals' free movement and access to water during the experiments.

Monitoring, Blood Samples

Mean arterial pressure and heart rate were monitored during intravital microscopy by an electromechanical pressure transducer (Baxter Uniflow; Baxter Healthcare Cooperation, Deerfield, Ill., USA). Arterial blood samples for determination of serum amylase were obtained before (baseline) and 24 h after (end point) pancreatitis was induced. Serum amylase was determined by standard laboratory methods (Hitachi automatic analyzer; Boehringer Mannheim, Mannheim, Germany).

Animal Models

Animals were divided into 4 groups. In each group, pancreatic microcirculation was evaluated in 12 animals by intravital microscopy (6 animals per group for platelet microscopy, 6 animals per group for erythrocyte and leukocyte microscopy) and morphological changes assessed in additional 6 animals by histology. In the control groups, animals underwent sham operation and received either Ringer's solution or Ringer's solution and P-selectin AB. Severe AP was induced in the two other groups by infusion of bile salt (glycodeoxycholic acid, 2.5 mM) into the pancreatic duct in combination with intravenous infusion of caerulein (5 μg/kg/h) over 6 h as described in detail by Schmidt et al. [13]. Caerulein was reconstituted in saline solution and infusion volume was 4 ml/kg/h. Bile salt infusion into the pancreatic duct was performed in a volume (1.2 ml/kg)-, time (5 min)- and pressure (30 mm Hg)-controlled manner. In the therapy group, monoclonal P-selectin AB were administered as described below.

In each of the models, animals received saline solution during the observation period (0.9%, 4 ml/kg/h). Intravital microscopy was performed 12 h after induction of pancreatic injury and histological changes, tissue and blood samples were assessed 24 h after infusions were started.

P-Selectin AB

Commercially available monoclonal P-selectin AB were used in the experiments (Biocytex, Marseille, France). AB were purchased at a concentration of 1 mg/ml. Antibody dosage was 1 mg/kg body weight. AB were applied 30 min before AP induction as a bolus infusion. Application was repeated 3 h after AP induction in the same dosage.

Platelet Preparation

Prior to intravital microscopy, 1 ml of whole blood was withdrawn. Platelets were separated and stained according to the method originally described by Massberg et al. [11]. Briefly, platelets were stained by rhodamine 6G and separated by two cycles of centrifugation under addition of prostacyclin. After suspending and washing the separated platelets, blood cell count was performed to calculate the number of platelets per μl and to rule out animal-specific differences in the number of platelets. Platelets were then reinjected and intravital microscopy was performed.

Intravital Microscopy

The abdomen was reopened and the pancreas carefully exteriorized in a horizontal position through the midline incision. The duodenal loop with the head of the pancreas was carefully placed on an anatomically designed stage in a temperature controlled (37°C) Ringer's bath. Afterwards, intravital microscopy was performed as described below. The animals were sacrificed after completion of intravital microscopy by a pentobarbital overdose.

Erythrocyte and Leukocyte Assessment

Erythrocytes (hematocrit 50%) labeled with fluorescein isothiocyanate (FITC) were applied intravenously at a dose of 0.5 ml/kg as described before [14]. In addition, 1 ml/kg of rhodamine-6G solution was applied intravenously to label leukocytes in vivo [15]. Intravital microscopy was performed after an equilibration period of 15 min using a fluorescent microscope (Leitz, Wetzlar, Germany) with a 20-fold water immersion objective. An epi-illuminescent xenon lamp with an excitation filter of 450–490 nm was used for visualization of FITC-labeled erythrocytes and an excitation filter of 540–630 nm for rhodamine-labeled leukocytes.

Platelet Assessment

After platelet reinjection, intravital microscopy was performed by an epi-illuminescent xenon lamp with an excitation filter of 540–630 nm.

Off-Line Analysis

Images were transferred onto a monitor and simultaneously recorded on a videotape recorder. In each animal, 5 capillary fields of the exocrine pancreas and 5 postcapillary venules (20–40 μm) were recorded over 1 min. Off-line analysis was performed