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Research paper

Pretreatment of a matrix metalloproteases inhibitor and aprotinin attenuated the development of acute pancreatitis-induced lung injury in rat model

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ABSTRACT

Objective: Acute lung injury (ALI) is one of the most common extra-pancreatic complications of acute pancreatitis. In this study, we examined the protective effect of protease inhibitor aprotinin and a matrix metalloproteinase inhibitor (MMPi) on pulmonary inflammation in rats with severe pancreatitis-associated ALI.
Method: A rat model of acute pancreatitis (AP) was established by injecting sodium glycodeoxycholate (GDOC) into the pancreatic duct. Pharmacological interventions included pretreatment with a protease inhibitor aprotinin (10 mg/kg) and a matrix metalloproteinase inhibitor (MMPi, 100 g/kg). The extent of pancreatic and lung injury and systemic inflammation was assessed by examinations of blood, bronchoalveolar lavage (BAL), and lung tissue. Pancreatic or lung tissue edema was evaluated by tissue water content. Pulmonary arterial pressure and alveolar-capillary membrane permeability were evaluated post-injury via a catheter inserted into the pulmonary artery in an isolated, perfused lung model.
Results: Pre-treatment with aprotinin or MMPi significantly decreased amylase and lactate dehydrogenase (LDH), and the wet/dry weight ratio of the lung and pancreas in AP rats. Compared to the GDOC alone group, administration of aprotinin or MMPi prevented pancreatitis-induced IL-6 increases in the lung. Similarly, treatment with aprotinin or MMPi significantly decreased the accumulation of white blood cells, oxygen radicals, nitrite/nitrates in both blood and BAL, and markedly reduced lung permeability.
Conclusion: Pretreatment with either aprotinin or MMPi attenuated the systemic inflammation and reduced the severity of lung and pancreas injuries. In short, our study demonstrated that inhibition of protease may be therapeutic to pulmonary inflammation in this GDOC-induced AP model.

1. Introduction

Acute pancreatitis often triggers a systemic inflammatory reaction, resulting in impairment of other organ functions (Chan and Leung, 2007). Studying acute pancreatitis gives insight into the systemic inflammatory response syndrome (SIRS) and its wide-reaching consequences. Acute lung injury (ALI) is one of the often seen complications of acute pancreatitis and the systemic inflammatory response syndrome (Bhatia and Moochhala, 2004). By studying the proinflammatory mediators involved in acute pancreatitis-induced lung injury, we learn more about the factors leading to pancreatic cell damage and distant organ injury, in the hope that key processes might be identified and modified to reduce morbidity and mortality.

Many aspects of acute pancreatitis have been explored by previous researchers. The pancreas plays a key role in digestion of food particles. It releases proteases, lipase, and amylase into the intestinal lumen to aid breakdown and absorption of nutrients. Packaging of the protease precursors trypsinogen and chymotrypsinogen along with a trypsin inhibitor is one mechanism to prevent runaway autodigestion (Hirota et al., 2006). Once pancreatic proteases are in the systemic circulation, they may cause significant cell damage and induce a systemic inflammatory response. Blocking the action of pancreatic proteases in this situation may check the acute pancreatitis-associated widespread inflammation (Shi et al., 2007). A non-specific serine protease inhibitor, aprotinin, has been used for pancreatic proteases blockage, but the clinical benefit was limited (Smith et al., 2010). Aprotinin could

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possibly prevent blood loss in cardiac surgery and reduced the mortality and hospital stay after cardiac surgery (Mahdy and Webster, 2004).The application of aprotinin in major surgery was started from the 1960′s (Tice et al., 1964).Another often implicated player in inflammation is matrix metalloproteinases (MMP). MMPs are a diverse group of zinc-dependent endoproteinases with functions in cell growth, host defense, and tissue repair, among others. They have been observed and documented in many inflammatory states in human diseases (Manicone and McGuire, 2008). In the lungs in particular, MMPs have been implicated in the development of acute respiratory distress syndrome (Ricou et al., 1996) and associated increased alveolar capillary membrane permeability (Keck et al., 2002). In fact, MMP9 level in serum was found to correlate with the development of pulmonary complications in acute pancreatitis (Keck et al., 2006). Although some studies have investigated the effect of various inhibitors including protease inhibitors, MMP inhibitors (MMPi), and phospholipase A2 inhibitors on acute pancreatitis and associated lung injury, the complex cell-signaling process leading from pancreatitis to systemic inflammation is still to be elucidated.

In our study, we aimed to gain further understanding of acute pancreatitis-induced lung injury (ALI) and the effect of the pancreatic protease inhibitor, aprotinin, and MMPi on attenuating pancreatic and lung injury. We found that both aprotinin and MMPi could ameliorate acute pancreatitis-induced lung injury. This result demonstrates the vital role of proteases in pancreatic and lung injury.

2. Material and methods

2.1. Preparation of animals

Male Sprague-Dawley rats (250–300 g, pathogen-free) were purchased from the National Animal Center, Taiwan. They were housed in a controlled environment at ambient temperature of 22 ± 1 °C under a 12 h/12 h light/dark cycle. Food and water were available *ad libitum*. Care and use of the animals were in accordance with the principles of the National Animal Center guidelines. Rats fasted overnight prior to the operation with free access to water 12 h before the experiment.

2.2. Establishing acute pancreatitis with sodium glycodeoxycholate (GDOC)

Acute pancreatitis was induced by injecting GDOC into the pancreatic duct. The rats were anesthetized with pentobarbital (50 mg/kg i.p.) until non-responsive to pain and then secured onto the operating table. A 5 cm midline incision was made in the abdomen. The duodenum and pancreatic duct were identified. A PE10 tube was inserted into the pancreatic duct and secured in place. A PE50 tube was then inserted into the femoral artery for blood draws. Glycodeoxycholate (GDOC, 10 mM, 0.4 mL/kg in saline) was infused into the pancreatic duct with a syringe infusion pump (Kd Scientific, USA) at 20 μL/min. After infusion the PE10 tube was removed and the pancreatic duct ligated. The abdominal wound was closed. Rats in the sham group underwent operation with nothing infused. 48 h later we observed and documented the degree of inflammation and injury in the pancreas and lung. We analyzed the inflammatory responses by measuring the changes in white blood cell count (WBC), oxygen radicals, nitric oxide (NO), lactate dehydrogenase (LDH), IL-6, and TNF-α.

2.3. Experimental design

Sham experimental operation (N = 7) was performed without actual cannulation of the pancreatic duct to mimic the process of inducing pancreatitis. All other procedures were identical to GDOC alone group (see below). For aprotinin alone control, a PE50 tube was cannulated into the femoral vein for aprotinin (3 mg/ml) pre-treatment (10 mg/kg at 0.2 mL/min for 5 min) 30 min before laparotomy. MMPi alone control (N = 4): Animals were pretreated with MMPi II (Calbiochem, Cat no. 444247, N-Hydroxy-1,3-di-(4-methoxybenzenesulphonyl)-5,5-dimethyl-[1,3]-piperazine-2-carboxamide, 1 mg dissolved in 1 mL of DMSO) 100 μg/kg via intraperitoneal injection 1 day prior to experimentation. GDOC alone (N = 7): The hemodynamic data were recorded and blood drawn from the femoral artery before and 48 h after GDOC infusion. The blood was analyzed quantitatively for WBC, amylase, LDH, NO, H_2O_2, IL-6, and TNF-α. The animal was sacrificed by pentobarbital overdose 48 h after the experiment. The lungs and the pancreas were harvested to determine their wet/dry weight ratio, nitrite/nitrate, oxygen radical, and cytokines in the bronchoalveolar lavage fluid, as well as PCR and protein analyses for MMP, iNOS, NFkB and TNFα. For GDOC plus aprotinin (N = 7): Thirty min after aprotinin infusion, acute pancreatitis was induced as in GDOC alone group. Hemodynamic monitoring and blood draws before and after GDOC as well as organ harvesting 48 h after experimentation were performed as in GDOC alone group. For GDOC plus MMPi (N = 7): Animals were pretreated with MMPi 100 μg/kg i.p. 1 day prior to experimentation. Acute pancreatitis induction, hemodynamic monitoring, blood draws, and specimen processing were performed as in GDOC alone group. For apillary membrane permeability (N = 24): A total of 24 rats from sham and GDOC-treated groups underwent the additional isolated perfused lung venous challenge protocol to determine the capillary membrane permeability.

2.4. Hemodynamic monitoring

Blood pressure was recorded with a photoplethysmographic monitoring device (MK-2000ST NP-NIBP, Japan) before acute pancreatitis was induced and again before sacrificing the animal.

2.5. WBC count, amylase and LDH

Ten μL of whole blood was withdrawn from the femoral artery catheter before and at the end of the experiment to be analyzed by cell counter. Blood and lavage samples were kept at 4 ° C and centrifuged (3000 x g, 5 min). Fifty μL of the supernatant was withdrawn and analyzed for amylase and LDH (Fuji Dri-Chem 3000, Japan).

2.6. Free oxygen radicals

After anesthesia and at the end of the experiment, 20 μL of whole blood withdrawn from the femoral artery was added to hydrogen peroxide R2 reagent (pH 5.0, diluted) and R1 reagent ($CrNH_2$) for free oxygen radical testing. Samples were centrifuged for 1 min (2000 x g) and analyzed for hydrogen peroxide (H_2O_2, wavelength 505 nm, Cataellani, FORM OX, Italy).

2.7. Methyl guanidine measured by spectrofluorometer

As the formation of methyl guanidine (MG) is an index of hydroxyl radical production, we measured MG in the bronchoalveolar lavage fluid to reflect AP-induced hydroxyl radical production in the lung. A spectrofluorometer (Jusco 821-FP, Hachioji, Japan) was used and the fluorescence spectra measured for emission maximum at 500 nm and excitation maximum at 395 nm. The assay was calibrated with authentic MG (Sigma M0377, St. Louis, MO, U.S.A.).

2.8. Measurement of Nitrite/Nitrate by HPLC

Levels of nitrite/nitrate, the metabolites of NO, in lung lavage and blood samples were determined by high-performance liquid chromatography (HPLC, ENO-20, Eicom Nox Analyzer, Kyoto, Japan). The samples were diluted, and serum samples deproteinized by ultrafiltration. The samples were separated on a strong anion-exchange column (Spherisorb SAX, 250 × 4.6 mm I.D., 5 μm) followed by two on-line post-column reactions. The first involved nitrate reduction to nitrite on

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