Role of Ischemia in Acute Pancreatitis
Hemorrhagic Shock Converts Edematous Pancreatitis to Hemorrhagic Pancreatitis in Rats

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Ischemia has been considered to play a role in the development of acute pancreatitis. The aim of this study was to investigate the effect of ischemia, caused by hemorrhagic shock, on cerulein-induced acute pancreatitis in rats. Acute pancreatitis was induced by the intravenous infusion of a supramaximally stimulating dose of cerulein (10 μg/kg/hr) for 6 hr. Hemorrhagic shock was induced by the removal of blood until the mean arterial blood pressure reached 35 mm Hg. This level was maintained for 30 min, after which time all the blood was reinfused. Hemorrhagic shock alone induced no morphological change in the pancreas. However, after the induction of hemorrhagic shock in animals treated with cerulein, hemorrhage and parenchymal necrosis were frequently observed in the pancreas. Seven of 20 rats (35%) receiving cerulein plus hemorrhagic shock had died by 48 hr after the start of cerulein infusion, whereas none of the rats in the cerulein or shock group died during this experiment. Cathepsin B activity in the pancreas of the cerulein plus shock group was significantly higher than in the other groups at 48 hr. These results suggest that ischemia may be a contributing factor in the pathogenesis of acute pancreatitis.

KEY WORDS: experimental pancreatitis; cerulein; hemorrhagic shock; cathepsin B; rat.

The pathogenesis of acute pancreatitis remains an unsolved problem, and many different theories have been reported for many years. Recently, circulatory disturbance or ischemia has been reported to be one of the pathogenetic factors in acute pancreatitis. Clinical studies have documented the occurrence of acute pancreatitis after cardiac surgery, which may be due to pancreatic ischemia following secondary obstruction of blood vessels and hypoperfusion (1). In addition, autopsies of patients dying after shock have demonstrated a high incidence of acute pancreatitis or pancreatic necrosis that could be correlated with the severity and duration of shock (2–6). In experimental studies, pancreatic edema caused by duct ligation and hyperstimulation has been considered to lead to parenchymal necrosis in the presence of temporary arterial obstruction (7, 8). Pfeffer et al (9) and Redha et al (10) produced hemorrhagic–necrotizing pancreatitis by the intraarterial injection of microspheres in dogs and rats. Broe et al (11) showed that 2 hr of total ischemia can produce significant injury in the isolated ex vivo perfused canine pancreas. Printz et al (12) demonstrated that hemorrhagic shock worsened the microscopic evidence of pancreatitis induced by supramaximal secretagogue stimulation in rats. Klar et al (13) demonstrated that a pharmacological vasoconstrictor, phenylephrine, increased severity of cerulein-induced pancreatitis. More-
over, many authors have shown histological vascular alterations in acute hemorrhagic pancreatitis (14, 15) and impairment of pancreatic microcirculation and reduction of pancreatic perfusion in acute pancreatitis (16).

Acute edematous pancreatitis was induced in rats by the infusion of a supramaximally stimulating dose of cerulein (17). However, this pancreatitis is reversible and not fatal. In clinical cases, treatment of severe fatal hemorrhagic-necrotizing pancreatitis is more important. However, the factors that convert mild edematous pancreatitis to severe hemorrhagic-necrotizing pancreatitis have not yet been clarified. In the present study, our aim was to determine whether hemorrhagic shock had morphologic and enzymatic influences on cerulein-induced acute pancreatitis in rats and to clarify the role of ischemia and microcirculatory disturbance in the development of severe hemorrhagic pancreatitis. Particular interest was focused on morphologic changes of the pancreatic microvasculature.

**MATERIALS AND METHODS**

**Experimental Protocol.** Male Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) weighing 170–240 g were given free access to standard rat chow and water before the experiments. After an overnight fast, they were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg body weight), and PE-50 cannulas (Clay Adams, Parsippany, New Jersey) were placed in both femoral arteries and veins and tunneled subcutaneously to exit at the base of the tail. The catheter placed in the right femoral vein allowed continuous administration of physiologic saline containing heparin (5 units/ml) at a rate of 0.40 ml/hr. Arterial blood pressure was monitored via the catheter placed in the right femoral artery, and the left femoral artery was used for the removal and reinfusion of blood. The animals were housed in individual cages without restraints.

Acute pancreatitis was induced by the intravenous infusion of supramaximally stimulating doses of 10 μg/kg/hr of cerulein (Ceosunin, Kyowa Hakko, Ltd., Tokyo, Japan) dissolved in physiologic saline over a period of 6 hr. Seventy-five minutes after the start of cerulein infusion, blood was removed from the femoral artery into a syringe containing 300 units of heparin until the mean arterial blood pressure (MABP) reached 35 mm Hg (in about 10 min). This level was carefully maintained after 30 min by the removal or reinfusion of small amounts of blood. After 30 min of hypotension, all the blood was reinfused (in about 5 min). After cerulein infusion for 6 hr, the animals were given standard chow ad libitum and infused with heparin-treated saline at a rate of 0.20 ml/hr.

The animals were divided into the following four groups: (A) control—infused with physiologic saline without the induction of hemorrhagic shock; (B) shock—infused with physiologic saline with the induction of hemorrhagic shock; (C) cerulein—infused with a supramaximal dose of cerulein without the induction of hemorrhagic shock; (D) cerulein plus shock—infused with a supramaximal dose of cerulein with the induction of hemorrhagic shock.

At 6 and 24-hr after the start of the infusion of saline or cerulein, blood was drawn through the catheter in the femoral artery and rapidly centrifuged at 2000g for 15 min. Serum was collected and stored at -40º C for the measurement of serum amylase and lipase activities. Survival rates were recorded for each group (N = 20) 24 and 48 hr after the start of the infusion. Other rats in each group were killed by decapitation 6, 24, and 48 hr after the start of the infusion. The pancreas was removed rapidly, trimmed of fat and lymph nodes, and weighed. The pancreas was examined histologically, and the deoxyribonucleic acid (DNA), amylase, and cathepsin B contents were measured.

**Assays.** A portion of pancreatic tissue was homogenized in ice-cold 50 mM phosphate buffer (pH 7.4) for 1 min in a Polytron (Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 10,000g for 30 min at 4º C, and the supernatant was used for DNA, amylase, and cathepsin B assay. DNA was measured fluorometrically with Bisbenzimide H 33258 Fluorochrome (Calbiochem-Behring Corp., La Jolla, California) by the method of LaBarca and Paigen (18) with calf thymus DNA (type I, Sigma Chemical Co., St. Louis, Missouri) as the standard. Amylase activity was determined by a chromogenic method with the Phadebas amylase test (Pharmacia Diagnostics AB, Uppsala, Sweden) (19). Lipase activity was determined by the BALB-DTNB method with Lipase Kit S (Dainippon Pharmaceutical Co., Osaka, Japan) (20). Cathepsin B activity was measured with the substrate CBZ-arginy1-arginine-2-naphthylamide (Bachem Feinchemikalien AG, Bubendorf, Switzerland), as described by McDonald and Ellis (21). The degree of pancreatic edema was evaluated by a comparison of the weight of one part of the pancreas obtained immediately after killing the animal (wet weight) with that of the same sample after incubation at 70º C for 48 hr (dry weight).

**Histologic Examination.** A portion of the pancreatic tissue was fixed in 10% formalin solution, embedded in paraffin and cut into sections. The sections were stained with hematoxylin and eosin and examined under a light microscope by a blinded observer. Interstitial edema was scored as follows: 0 = absent, 1 = interlobular septa were expanded, 2 = intralobular septa were expanded, and 3 = individual acini were separated. Inflammatory infiltration was scored as follows: 0 = absent, 1 = less than 20 neutrophils per intermediate power field (IPF), 2 = 20–50 neutrophils per IPF, 3 = more than 50 neutrophils per IPF. Parenchymal hemorrhage was scored as follows: 0 = absent, 1 = 1–2 foci per slide, 2 = 3–5 foci per slide, 3 = more than 5 foci per slide. Parenchymal necrosis was graded according to the approximate percentage of the involved area: 0 = absent, 1 = less than 5%, 2 = 5–20%, 3 = more than 20%. The grading of vacuolization was based on the percentage of acinar cells with cytoplasmic vacuoles in the examined field: 0 = absent, 1 = less than 20%, 2 = 20–50%, 3 = more than 50%.