Protective Effects of Endothelin-1 on Acute Pancreatitis in Rats

MASAFUMI KOGIRE, MD, KAZUTOMO INOUE, MD, SHUN-ICHI HIGASHIDE, MD, KYOICHI TAKAORI, MD, YOSHIYA ECHIGO, MD, YUAN-JUN GU, MD, SHOICHIRO SUMI, MD, KOUTARO UCHIDA, MD, and MASAYUKI IMAMURA, MD

Endothelin-1, a 21-residue peptide isolated from vascular endothelial cells, has a broad spectrum of actions. To clarify the involvement of endothelin-1 in acute pancreatitis, we examined the effects of endothelin-1 and its receptor antagonist BQ-123 on cerulein-induced pancreatitis in rats. Rats were infused intravenously with heparin-saline (control), endothelin-1 (100 pmol/kg/hr), cerulein (5 µg/kg/hr), or cerulein plus endothelin-1 for 3.5 hr. In another experiment, cerulein or cerulein plus BQ-123 (3 mg/kg/hr) was infused. Infusion of cerulein caused hyperamylasemia and pancreatic edema. Endothelin-1, when infused with cerulein, decreased the extent of pancreatic edema with a significant increase in the pancreatic dry- to wet-weight ratio. Histological changes induced by cerulein were markedly attenuated when endothelin-1 was given with cerulein. In contrast, endothelin-receptor blockade with BQ-123 further augmented pancreatic edema caused by cerulein. The extent of inflammatory cell infiltration was greater when BQ-123 was given with cerulein. Endothelin-1 or BQ-123 had no influence on hyperamylasemia. This study suggests that endothelin-1 has protective effects on experimental acute pancreatitis.

KEY WORDS: endothelin-1; BQ-123; cerulein; acute pancreatitis; rat.

While intrapancreatic activation of digestive enzymes is generally believed to be an important early event in acute pancreatitis (1), factors affecting the course of the disease are not well understood. In dogs, pancreatic blood flow decreased as acute pancreatitis progressed from mild to severe (2). Alpha-adrenergic vasoconstricting drugs caused deterioration in experimental acute pancreatitis (3). These findings suggest that pancreatic ischemia plays a role in the aggravation of the disease (4). On the other hand, some agents, such as prostaglandins (5, 6) and protease inhibitors (6, 7), have shown protective effects against experimental acute pancreatitis.

Endothelin-1 is a 21-residue peptide isolated from the supernatant fraction of cultured vascular endothelial cells (8). Endothelin-1 produces potent and protracted contraction of vascular smooth muscles in various animal species, possibly contributing to the control of systemic blood pressure and/or local blood flow (8, 9). In addition to its vasopressor properties, endothelin-1 has a broad spectrum of actions, including the stimulation of the production of prostaglandins (9-11) and the suppression of increased vascular permeability (12). Because of these activities, we postulated that endothelin-1 affects the course of acute pancreatitis. The present study was designed to determine the effects of endothelin-1 on cerulein-induced acute pancreatitis in rats; to this end, we administered exogenous endothelin-1 to rats or inhibited the actions of endogenous endothelin-1 by endo-
MATERIALS AND METHODS

Male Wistar rats (150–250 g) from Japan SLC (Hamamatsu, Japan) were used in the present study, which was performed under the guidelines on animal experimentation of the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University. After an overnight fast, each rat was lightly anesthetized with ether. A PE-50 cannula (Clay Adams, Parsippany, New Jersey) was placed in the femoral vein and tunneled subcutaneously to exit at the base of the tail as described previously (14). Its patency was maintained by continuous infusion of saline containing heparin (5 units/ml) at a rate of 0.4 ml/hr. In some rats, another PE-50 cannula, which was connected to a Statham P23 pressure transducer, was placed in the femoral artery to monitor arterial pressure during the experiments. The animals were housed in individual cages and allowed at least 4 hr to recover from the effects of anesthesia.

Acute pancreatitis was induced by intravenous infusion of a supramaximally stimulating dose of cerulin (Ceosunin; Kyowa Hakko, Tokyo, Japan) for 3.5 hr (14). In the first set of experiments, rats were divided into the following groups: (1) control—infused only with heparin-saline solution at a rate of 0.8 ml/hr; (2) endothelin-1—infused with heparin-saline solution as above but with endothelin-1 (Peptide Institute, Osaka, Japan) added to the infusate such that each animal received 100 pmol/kg/hr; (3) cerulein—infused with heparin-saline solution as above but with cerulein added to the infusate such that each animal received 5 ~g/kg/hr; (4) cerulein plus endothelin-1—as for cerulein above but with endothelin-1 (100 pmol/kg/hr) infused throughout the 3.5 hr of cerulein infusion. In the second set of experiments, rats were infused intravenously for 3.5 hr with either cerulein (5 ~g/kg/hr) alone or cerulein plus BO-123 (3 mg/kg/hr), which was a kind gift from Dr. Yano (Tsukuba Research Institute, Banyu Pharmaceutical Co., Tsukuba, Japan). This dose of BO-123 was based on a report indicating that intravenous infusion of BO-123 at rates of 1.2 and 30 mg/kg/hr produced a significant decrease in blood pressure in stroke-prone spontaneously hypertensive rats with high plasma endothelin-1 levels (15). The heparin-saline solution used as a vehicle for the agents contained 0.1% bovine serum albumin to minimize the adherence of the agents to the surfaces of tubing and syringes (16). In both experiments, rats were killed by decapitation for the collection of trunk blood and pancreatic tissue samples immediately after the infusion of the agents was completed.

Serum was separated by centrifugation, and serum amylase levels were measured using blue-dyed starch polymer (Shionogi, Osaka, Japan). The development of pancreatic edema was quantitated by comparing the pancreatic weight obtained immediately after killing the animals (wet weight) to that of the same sample after incubation at 150°C for 48 hr (dry weight) (7). For histological examination, samples of pancreatic tissue were 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. Inflammatory cell infiltration was scored on a scale from 0 (absent) and 1 (minimal) to 4 (maximal). The grading of acinar cell vacuolization was based on the percentage of acinar cells with cytoplasmic vacuoles in the examined field: 0, less than 2%; 1, 2–5%; 2, 5–15%; 3, more than 15%.

In a separate experiment, we determined plasma endothelin-1 levels in rats infused with the heparin–saline solution, cerulein (5 ~g/kg/hr), or cerulein plus endothelin-1 (100 pmol/kg/hr). After 3.5 hr of infusion of the agents, the rats were killed by decapitation. Mixed arteriovenous trunk blood was collected in iced, heparinized tubes containing 100 KIU of aprotenin (Bayer, Leverkusen, Germany) per milliliter of whole blood. Plasma was separated by centrifugation and stored at -60°C for subsequent radioimmunoassay for endothelin-1.

Plasma endothelin-1 concentration was measured by radioimmunoassay after extraction of endothelin-1 on octadecylsilisica particles (Preparative C18; Waters Associates, Milford, Massachusetts). Plasma samples (2 ml) were mixed with particles, which were then washed with distilled water, 40% methanol, and acetone. Immunoreactive endothelin-1 was eluted twice with 1.0 ml of 60% methanol, and the extracts were dried and reconstructed for the radioimmunoassay, in which the antibody to endothelin-1 (Peninsula Laboratories, Belmont, California) was used. The recovery rate for the extraction procedure was 72.1 ± 3.9%. The sensitivity of the radioimmunoassay was 0.82 pg/tube. Intra- and interassay variances were 9.8% and 13.3%, respectively.

The results were expressed as the mean ± SEM. Data from the measurements of serum amylase levels, pancreatic dry- to wet-weight ratio, arterial pressure, and plasma endothelin-1 levels were analyzed by a one-way classification analysis of variance; the separation between means was done by the least significant difference procedure. P < 0.05 was considered significant.

RESULTS

Infusion of cerulein for 3.5 hr caused an increase in serum amylase levels and edema of the pancreas. Serum amylase levels in rats given cerulein plus endothelin-1 did not differ significantly from the values in rats given cerulein alone (Figure 1). However, pancreatic edema of rats given cerulein plus endothelin-1 was less pronounced macroscopically compared with that of rats given cerulein alone, which was objectively quantitated by a significant increase in the pancreatic dry- to wet-weight ratio from 16.3 ± 1.1% in rats given cerulein alone to 19.6 ± 0.9% in rats given cerulein plus endothelin-1 (Figure 2). On microscopic examination, inflammatory cell infiltration into the pancreatic parenchyma and acinar cell vacuolization were noted in rats given cerulein. These histological changes were markedly attenuated when endothelin-1 was infused with cerulein (Table 1 and