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Differential effects of endothelins on histological and ultrastructural changes and trypsinogen activation in the secretagogue-induced acute pancreatitis in rats

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ABSTRACT

The role of endothelins in acute pancreatitis remains obscure. To assess the effects of endothelins (ETs) in early (4 h) caerulein-induced acute pancreatitis (AP) in rats, ET-1, ET-2 and ET-3 (0.5 or 1.0 nmol/kg) were applied twice with *i.p.* caerulein (2×40 μg/kg) at 1 h interval. Histological and ultrastructural examinations of pancreases and the assay of trypsinogen activation in whole homogenate were performed. All ETs, especially ET-1 at the higher dose, decreased inflammatory cell infiltration despite an increase in the edema score. The vacuolization and necrosis of acinar cells were slightly increased after the lower dose of ET-1 and ET-2. Ultrastructural changes were generally improved after the higher dose of ETs. Trypsinogen activation increased from $4.8 \pm 1.3\%$ in control to $18.4 \pm 3.8\%$ in AP ($p < 0.01$). It was attenuated to $6.4 \pm 1.3\%$ ($p < 0.01$) by the higher dose of ET-1 and to $8.8 \pm 1.5\%$ ($p < 0.05$) by the lower dose of ET-3. In summary, ETs, especially ET-1 at the higher dose, were found to have some beneficial effects on morphological changes and trypsinogen activation in the pancreas in early caerulein-induced AP.

Introduction

The 2–10% mortality rate in acute pancreatitis (AP) is still appreciable and in the most severe forms approaches 20% (Imrie and McKay, 1999). Both the premature activation of trypsinogen and the impairment of pancreatic microcirculation are thought to play a pivotal role in the pathogenesis of AP (Sherwood et al., 2007; Cuthbertson and Christophi, 2006). In mild, edematous AP almost a double increase was noted in the pancreatic capillary flow (PCF) within the first 6 h, whereas in the severe, necrotic form, the PCF decreased by half within the same time (Schmidt et al., 2002). The role of circulating trypsin in promoting pancreatic microcirculatory failure in experimental AP has been reported (Keck et al., 2005). Numerous data have been collected on the role of endothelin-1 (ET-1) in microvascular deterioration in AP (Foitzik et al., 2001; Inoue et al., 2003; Plusczyk et al., 2003). ET-1 exerts its effects by binding to two different receptors: ET_A, responsible for vasoconstriction and ET_B, related to vasodilatation (Rossi et al., 2000). The protective effects of the selective ET_A or nonselective $ET_{A/B}$ antagonists in severe AP have been described (Foitzik et al., 2000; Eibl et al., 2002; Andrzejewska and Dlugosz, 2003). Nonetheless, important data on the lack of beneficial effects of such antagonists in severe models of AP have also been reported (Fiedler et al., 1999; Martignoni et al., 2004).

The caerulein (cholecystokinin analog)-induced AP is a model of mild, edematous form of the disease. The "trigger mechanism" begins in the acinar cell, with a functional blockade in the secretory pathway leading to premature trypsinogen activation inside these cells (Saluja et al., 1999). It has been found that vascular factors also play an important role in the ignition and perpetuation of caerulein-induced AP (Sunamura et al., 1998). Additional ischemia or stress leads to the transformation of a mild, edematous AP into its severe necro-hemorrhagic form (Kyogoku et al., 1992; Chen et al., 2001). ET-1 has been incriminated for progressive pancreatic ischemia, which may lead to necrotic changes in the pancreas (Plusczyk et al., 1999). Nevertheless, the effect of ET-1 on the caerulein-induced AP remains controversial. A delayed application of ET-1 in sustained caerulein AP increased the capillary permeability in the pancreas, whereas the selective ET_A antagonist exerted the opposite effect (Eibl et al., 2000). ET-1 given as intra-arterial repeated bolus aggravated morphologic changes in caerulein-induced AP, whereas the ET_A-selective antagonist ameliorated the course of such AP intensified by water immersion stress (Liu et al., 1995, 1999).

On the contrary, early infusion of ET-1 with caerulein abrogated histological changes, whereas the ET_A antagonist further augmented pancreatic edema and the inflammatory cell

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infiltration (Kogire et al., 1995). In our previous study, we did not find any appreciable positive effects of both selective ET_A and nonselective $ET_{A/B}$ antagonist in the early course of caerulein-induced AP (Andrzejewska et al., 2005). Therefore, we presume that in the early course of caerulein AP, when pancreatic blood flow is increased, ET-1 could exert some beneficial effects, which could have some clinical implications in the prevention of the so-called "post-ERCP" acute pancreatitis.

Little is known about the effects of ET-2 and ET-3 on the pancreas. ET-2 and ET-3 reduced pancreatic blood flow to a lesser degree than ET-1 (Takaori et al., 1992). A topical superfusion of the pancreas by these ETs has shown that there are essential differences between ET-1, ET-2 and ET-3 in their effect on the pancreatic microcirculation, post-capillary leukocyte accumulation and histological changes (Plusczyk et al., 2001). Only ET-1 and ET-3 expressions have been found in the isolated pancreatic acini, and ET_A and ET_B receptors for these ETs have been identified; however, their role remains obscure (Hildebrand et al., 1993).

Therefore, the purpose of the present study was to assess and to compare the effects of three endothelins: ET-1, ET-2 and ET-3 on the histological and ultrastructural changes in the pancreas in relation to trypsinogen activation in the early course of caerulein-induced acute pancreatitis in rats.

Materials and methods

Animals

The experiments were carried out on 52 male Wistar rats, 240–300 g of body weight (b.w.), housed individually in wire bottomed cages, at a room temperature of 21 ± 1 °C, using a 12 h light–dark cycle. They were fed with a laboratory chow diet and fasted overnight before the experiment, with free access to water. Care was provided in accordance with the current procedures for the care and use of laboratory animals. The protocol has been approved by the local Bioethical Committee.

Induction of acute pancreatitis (AP)

Acute caerulein pancreatitis was induced according to the method of Yamaguchi et al. (1989) by the injection of caerulein (Sigma Chemical Co., St. Louis, MO, USA) at a dose of 40 μg/kg b.w. *i.p.* twice, at 1 h interval. In control rats, only 0.9% NaCl was given *i.p.* In the treated rats, the solution of respective endothelin: ET-1, ET-2 or ET-3 (Sigma Chemicals Co., St. Louis, MO, USA) in 0.9% NaCl was given *i.p.* twice, simultaneously with caerulein.

Experimental design

Rats were subdivided into 8 groups as follows:

Group 1: Control group (C), received only 0.9% NaCl *i.p.* at 0 and 1 h ($n=6$).

Group 2: Rats with untreated caerulein-induced AP received only saline solution *i.p.* as in the control group ($n=10$).

Group 3: Rats with caerulein-induced AP treated with ET-1, at a dose of 0.5 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

Group 4: Rats with caerulein-induced AP treated with ET-1, at a dose of 1.0 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

Group 5: Rats with caerulein-induced AP treated with ET-2, at a dose of 0.5 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

Group 6: Rats with caerulein-induced AP treated with ET-2, at a dose of 1.0 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

Group 7: Rats with caerulein-induced AP treated with ET-3, at a dose of 0.5 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

Group 8: Rats with caerulein-induced AP treated with ET-3, at a dose of 1.0 nmol/kg b.w. twice at 1 h interval, simultaneously with caerulein ($n=6$).

The volume of 0.9% NaCl as a solvent was equilibrated in all groups to 2×2 ml/kg b.w.

Preparation of pancreatic homogenate and the plasma.

Four hours after the first caerulein injection (or saline in group C) general anesthesia was induced with *i.p.* ketamine at a dose of 40 mg/kg b.w., supported by pentabarbital at a dose of 20 mg/kg b.w. Blood samples were collected by cardiac puncture using a heparinized syringe and the rats were sacrificed by decapitation. The pancreases were quickly excised, freed from the peripancreatic tissues and weighed. The wet weight of the pancreas as % of the body weight was calculated as a measure of the gland edema (DiMagno et al., 2004). For light and electron microscopy, the representative specimens of pancreas were fixed and processed as described later. The remaining portion of the pancreas was processed for biochemical assays.

The samples of heparinized blood were centrifuged at 4000 rpm with cooling to 4 °C, the resulting plasma was collected and frozen at −80 °C until the assay of α-amylase activity as in our previous study (Andrzejewska et al., 2005; Dlugosz et al., 1997).

Histological examination

The representative specimen of the pancreatic tissue from each rat was fixed in 10% neutral-buffered formalin. Sections of the samples were stained with H&E and examined under a light microscope at ×200 magnification – intermediate power field (IPF) by a blinded observer in a hundred fields from each group. Histological changes were scored and evaluated according to Kyogoku et al. (1992).

Ultrastructural examination

Small specimens (about 1 mm^3) of pancreatic tissue (3 from each animal) were immediately fixed in 3.6% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) for 3 h and after washing in the buffer, postfixed in 2% osmium tetroxide for 1 h. The samples were dehydrated in alcohol and propylene oxide and then embedded in Epon 812. The ultrathin sections were cut from each block on a Reichert ultramicrotome, stained with lead citrate and uranyl acetate, and studied under an Opton 900 PC transmission electron microscope field by field. Fifty to 60 electron micrographs of the most characteristic changes from each group were made. The determination of pathology was made blind (Andrzejewska et al., 2005).

Biochemical assays

The remaining pancreatic tissue was homogenized in ice-cold four volumes of 50 mmol/l Tris–HCl buffer (pH 8.0), containing nonorganic detergent Triton X-100, 0.5% v/v for 1 min by 3 full up and down strokes using a motor-driven glass-Teflon homogenizer (Thomas Scientific, Swedesboro, NJ, USA) cooled with ice. The resulting homogenate was sonified for 20 s in an ice bath using