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Pretreatment with obestatin reduces the severity of ischemia/reperfusion-induced acute pancreatitis in rats

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ABSTRACT

Obestatin, as ghrelin, has been originally extracted from the stomach, which remains its major source. Previous studies have shown that administration of obestatin exhibits protective and healing-promoting effects in several organs, including the stomach and kidney. In pancreas, pretreatment with obestatin inhibits the development of cerulein-induced acute pancreatitis and promotes survival of pancreatic beta cells and human islets. The aim of the present study was to check the universality of protective effect of obestatin in the pancreas. For this reason we investigated the influence of obestatin administration on the development of ischemia/reperfusion-induced pancreatitis. Acute pancreatitis was induced by pancreatic ischemia followed by reperfusion of the gland. Obestatin (4, 8 or 16 nmol/kg/dose) was administered intraperitoneally twice: 0.5 h before exposure to ischemia, and 3 h after the first injection. The effect of obestatin on the course of necrotizing pancreatitis was assessed after 6-h reperfusion, and included histological, functional, and biochemical analyses. Treatment with obestatin reduced morphological signs of pancreatic damage including edema, vacuolization of acinar cells, hemorrhages, acinar necrosis, and leukocyte infiltration of the gland. These effects were accompanied by an improvement of pancreatic DNA synthesis and superoxide dismutase activity, and a decrease in serum level of lipase and pro-inflammatory interleukin-1β. Moreover pretreatment with obestatin reduced myeloperoxidase activity and malondialdehyde concentration in pancreatic tissue of rats with acute pancreatitis. *Conclusions*: Administration of obestatin inhibits the development of ischemia/reperfusion-induced acute pancreatitis. This observation, taken together with previous findings that obestatin protects the pancreas against cerulein-induced pancreatitis, indicates that protective effect of obestatin in the pancreas is universal and independent of the primary cause of acute pancreatitis.

1. Introduction

Despite substantial improvements in the management of acute pancreatitis (AP) over the last decade, the disease still remains associated with high morbidity and mortality rates reaching up to 30% in severe cases (Working Group IAP/APA, 2013). This is mainly due to the lack of targeted treatment for pancreatitis owning to the poor understanding of its pathogenesis. A cascade of pathophysiological mechanisms including inflammation, apoptosis, necrosis and oxidative stress have been associated with the disease and are responsible for irreversible morphological and structural changes of the gland (Hammer, 2014).

Obestatin, a circulating 23-amino-acid peptide, derived from the preproghrelin precursor produced predominantly in the stomach, exhibits numerous physiological functions involving inhibition of food intake, body weight gain, gastric emptying and regulation of jejunal motility, as well as affects paracrine and autocrine functions of the pancreas (Li et al., 2011; Plusczyk et al., 2003; Trovato et al., 2014). Accumulating evidences suggest that obestatin promotes survival and proliferation, and prevents apoptosis in both beta-cells and

Abbreviations: AP, acute pancreatitis; DNA, deoxyribonucleic acid; IL-1β, interleukin-1β; MPO, myeloperoxidase; MDA, malondialdehyde; SOD, supeoxide dismutase

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human islets (Chen et al., 2009; Granata et al., 2008; Lacquaniti et al., 2011; Li et al., 2011). In addition, obestatin-induced modulation of FGFR/Notch/Ngn3 developmental pathways, together with its expression in the fetal pancreas, implies its possible role in formation of the pancreas and organ regeneration (Baragli et al., 2013; Gesmundo et al., 2013). Several previous reports have indicated that obestatin exerts protective and healing-promoting effects in various organs including the stomach and kidney (Dembiński et al., 2011; Koç et al., 2014). Similarly, a positive effect of this peptide has also been observed in the pancreas, where obestatin exhibited protective action in cerulein-induced AP (Ceranowicz et al., 2009). Protective effects of obestatin seem to be related to the inhibition of the inflammatory process in the pancreas, manifested by the reduced liberation of interleukin-1β (IL-1β), the inhibition of leukocyte infiltration of the gland tissue, and attenuation of decreased DNA synthesis in the organ (Ceranowicz et al., 2009).

Although, protective action of obestatin in the cerulein-evoked AP has been proved, it still remains unknown whether this peptide exerts universal protective effect in AP evoked by the primary vascular mechanism. Pancreatic ischemic injury plays an important role in the pathogenesis of AP. Microvascular perfusion failure is essential for the development of AP in humans after hypovolemic shock (Warshaw and O'Hara, 1978), hypothermia (Maclean et al., 1973), cardiac (Lonardo et al., 1999) or aortic surgery (Gullo et al., 1996; Sakorafas et al., 1998), and transplantation of the pancreas (Fernandez-Cruz et al., 1993; Schaser et al., 2005). Animal studies are in agreement with the results from clinical observations. Experimental data have shown that ischemia not only may be the primary factor of AP, but also in AP induced by the primary non-vascular factors, pancreatic ischemia always increases the severity of this disease (Klar et al., 1990; Vollmar and Menger, 2003; Waldner, 1992). Moreover, a subsequent tissue reperfusion results in further tissue damage (Andican et al., 2005; Schanaider et al., 2014). Therefore, the aim of this study was to investigate whether the pretreatment with obestatin could prevent the development of severe necrotizing pancreatitis evoked by pancreatic ischemia followed by reperfusion.

2. Materials and methods

2.1. Animals and treatment

All studies followed an experimental protocol approved by the Committee for Research and Animal Ethics of the Jagiellonian University and the Local Commission of Ethics for the Care and Use of Laboratory Animals.

The experiments were carried out in adult male Wistar rats, weighting 160–180 g, which were housed separately in cages with wire-mesh bottoms in a windowless colony room. The room was maintained at 22 ± 1 °C with relative humidity of 50 ± 10%, and 12 h:12 h light:dark photoperiod. Animals were fasted for 24 h with free access to water before induction of acute pancreatitis, food and tap water were available ad libitum later on.

Following a one-week period of acclimation to their new environment, the animals were randomly assigned to 8 equal experimental groups, as follows: (1) sham-operated normal saline-treated control group; (2–4) animals treated intraperitoneally with obestatin given twice at the dose of 4, 8, or 16 nmol/kg/dose, respectively, with 3-h interval between doses; (5) normal saline-treated rats with acute ischemia/reperfusion-induced pancreatitis; (6–8) rats with acute ischemia/reperfusion-induced pancreatitis pretreated intraperitoneally with obestatin given at the dose of 4, 8, or 16 nmol/kg/dose, respectively, the first dose of obestatin was administered 0.5 h before exposure to ischemia, the second 3 h later. Studies were repeated in three series aiming to obtain 10 observations in each experimental group. Rat obestatin was obtained from Yanaihara Institute (Shizuoka, Japan).

Surgical procedure for induction of ischemia/reperfusion-induced pancreatitis was performed according to the method described previously (Dembinski et al., 1996). Briefly, following a 24-h period of fasting with free access to water, rats were placed under general anesthesia, which was achieved by intraperitoneal administration of ketamine (50 mg/kg, Bioketan, Vetoquinol Biowet, Gorzów Wielkopolski, Poland). Using sterile technique a longitudinal laparotomy was performed and ischemia in the splenic region of the pancreas was induced by clamping the inferior splenic artery for 30 min applying microvascular clips, which were then removed for reperfusion, and the abdominal cavity was closed. In sham-operated-control animals longitudinal laparotomy and mobilization of the pancreas without artery clamping were carried out instead.

The severity of acute ischemia/reperfusion-induced pancreatitis was assessed after 6-h reperfusion.

2.2. Determination of pancreatic blood flow

At the end of study, the animals were anesthetized with ketamine again, and the abdominal cavity was opened. Pancreatic blood flow was measured by a laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (Dembiński et al., 2001). The data was presented as percent change from control value obtained in sham-operated saline-treated rats without induction of acute pancreatitis.

2.3. Determination of serum lipase activity and interleukin-1β concentration

Immediately following measurement of pancreatic blood flow, blood samples were taken from the aorta and serum was collected, and frozen at −60 °C. Serum lipase activity was determined with Kodak Ectachem DT II System analyzer (Estman Kodak Company, Rochester, NY, USA) using a commercially available Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical diagnostics, Inc., Rochester, NY, USA). Serum concentration of interleukin-1β (IL-1β) was measured using the commercial BioSource Cytoscreen rat IL-1β kit (BioSource International, Camarillo, California, USA) based on ELISA.

2.4. Determination of pancreatic myeloperoxidase activity

As an additional assessment of severity of the inflammatory response, accumulation of neutrophils within the pancreatic tissue was determined by quantifying tissue myeloperoxidase activity (MPO) (Hartwig et al., 1999). Briefly, after aortic blood samples collection, the pancreas was carefully dissected out from its attachments to the stomach, duodenum, and the spleen. Fat and peripancreatic tissues were trimmed away. Samples of pancreatic tissue were taken for determination of pancreatic MPO and superoxide dismutase activity, pancreatic DNA synthesis and malondialdehyde concentration, as well as the morphological studies. Pancreatic tissue was weighted and homogenized in 0.1 M sodium phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (Sigma-Aldrich, St. Louis, USA) and 5% soybean trypsin inhibitor (Sigma-Aldrich, St. Louis, USA), and then directly frozen on dry ice. The specimens were freeze-thawed three times, and sonicated after each cycle. Suspensions were then centrifuged at 20,000*g* for 15 min. MPO activity in supernatant was measured with a spectrophotometer at 470 nm by mixing an aliquot (25 μl) of the supernatant with 1.0 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.0016 ml of guaiacol (Sigma-Aldrich, St. Louis, USA) and 0.0005% hydrogen peroxide (Sigma-Aldrich, St. Louis, USA) as substrates. MPO activity was expressed as the percentage of MPO activity obtained in control animals of each series of studies.