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Original Research Article

Bilateral vagotomy attenuates the severity of secretagogue-induced acute pancreatitis in the rat

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ABSTRACT

Purpose: We assessed the effect of bilateral vagotomy (BV) on the course of acute caerulein-induced pancreatitis (AP) in the rat.
Material/methods: The study was performed on Wistar rats surgically prepared by subdiaphragmatic BV. Control group underwent sham operation. Four days later, AP was induced by subcutaneous injection of caerulein (25 μg/kg/5 h) to the conscious animals with or without BV. After administration of caerulein the blood samples were taken for determination of serum lipase activity and interleukin-10 (IL-10) concentration. Pancreatic tissue samples were subjected to histological examinations and to the measurement of lipid peroxidation products (MDA + 4-HNE) concentration and the activity of an antioxidant enzyme – glutathione peroxidase (GPx). After application of caerulein pancreatic blood flow was measured by laser Doppler flowmetry.
Results: AP was manifested by oedema and neutrophil infiltration of the pancreatic tissue and accompanied by significant increases of serum lipase activity, serum concentration of IL-10 and pancreatic concentration of MDA + 4HNE (*ca.* 50×, 2× and 4× respectively $p \geq 0.05$). Pancreatic activity of GPx and pancreatic blood flow were decreased (both by 60%). In vagotomised rats with AP serum lipase activity and pancreatic concentration of MDA + 4-HNE were lower whereas Il-10 concentration and pancreatic activity of GPx, as well as pancreatic blood flow were significantly higher as compared to AP rats with intact vagal nerves. In AP rats with vagotomy all histological signs of pancreatitis were significantly reduced.
Conclusions: Bilateral vagotomy resulted in the significant attenuation of caerulein-induced pancreatitis in the rat.

1. Introduction

Pancreas, both in its exocrine and endocrine parts is richly innervated by cholinergic fibres of the vagal complex. Cholinergic activity of vagal fibres innervating the pancreas originates from the dorsal vagal complex (DVC) neurons of the brainstem [1–4].

It is well known that vagal nerves are involved in the stimulation of pancreatic exocrine secretion, but implication of these nerves in the pathogenesis of acute pancreatitis (AP) and in the intestinal inflammation has not been clarified [2–7]. Previous studies show, that the tonic activity of vagal nerves is responsible for pancreatic secretion under basal conditions [8–10]. Stimulation of vagal nerves increases pancreatic blood flow and activates gastrointestinal motility [9]. Vagotomy reduces the secretion of pancreatic enzymes, diminished pancreatic blood flow and decreased gastric acid secretion [3,10–13].

Acute pancreatitis (AP) is a serious disease with varied etiology [14]. Caerulein-induced acute pancreatitis is an experimental model of oedematous (mild) form of this disease. Pathomechanism of acute pancreatitis is related to the process of premature activation of digestive proenzymes (mainly trypsinogen) in the pancreatic tissue. It is accompanied by significant reduction of pancreatic blood flow and activation of coagulation and complement systems [7]. Impairment of pancreatic microcirculation leads to the accumulation in the pancreatic tissue of proinflammatory mediators such as oxygen and nitrogen species (ROS and RNS),

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platelets activating factor (PAF), nitric oxide (NO), and cytokines [5,7,15,16]. Above substances could get into the peritoneal cavity and could produce the irritation of the tissues as well as the increase of capillary permeability [5,17,18].

Among the cytokines which are released in the course of acute pancreatitis are proinflammatory cytokines: tumour necrosis factor α (TNFα), proinflammatory interleukins 1β, 6 and 8 (IL-1β, IL-6 and IL-8), as well as an anti-inflammatory interleukin 10 (IL-10). These mediators have been mostly studied as markers of severity of acute pancreatitis [15].

IL-1β and TNFα are both produced in the inflamed pancreas and they are responsible for detrimental effects of the disease, but they do not appear to play a pivotal role in the development of AP [15,18–20]. The importance of IL-6 in the acute phase response has been confirmed by the observation that this interleukin stimulates the synthesis of acute phase proteins, including C reactive protein (CRP), from hepatocytes *in vitro* and *in vivo* [15].

Interleukin 10 (IL-10) is known as an anti-inflammatory cytokine that inhibits the release of proinflammatory cytokines from macrophages. It has been proved that treatment with IL-10 decreases the severity of pancreatitis *in vitro*, mainly by inhibiting cellular necrosis [19,21].

Oxidative stress in AP is manifested by the reduction of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). Malonyldialdehyde and 4-hydroxynonenal (MDA + 4-HNE) are lipid peroxidation products and level of MDA + 4-HNE correlates with the severity of AP, while GPx is a useful tool for estimating the antioxidant activity in clinical settings [7,22,23].

The experimental model of bilateral vagotomy (BV) in rats with induced AP was previously reported by Li et al. [20]. They showed that BV could abolish the disadvantageous effect of AP induced by sodium taurocholate and observed the beneficial effect of BV on the following parameters estimated during AP: serum amylase activity, IL-6 and CRP concentrations, as well as histological changes in morphology of pancreatic tissue [20].

The aim of this study was to evaluate the effect of bilateral vagotomy on the severity of caerulein-induced pancreatitis in the rat. Serum lipase activity, IL-10 serum concentration, concentration of MDA + 4-HNE and GPx activity in the pancreatic tissue as well as histological changes and pancreatic blood flow, were evaluated in these animals as indicators of the intensity of inflammatory process in the pancreas.

2. Material and methods

2.1. Materials

We used Caerulein (Sigma–Aldrich; St. Louis, USA) to induce acute pancreatitis, Kodak Ektachem DT Slide (LIPA) to determine the serum lipase activity (Eastman Kodak Company; Rochester NY, USA), Vetbutal to anesthetise rats (Biowet; Puławy, Poland), ELISA IL-10 kit (Camarillo, CA, USA) to determine serum IL-10.

2.2. Animals and experiment's protocol

The experiments were carried out on 40 male Wistar rats (weight about 250 g). Animals were housed in a temperature-controlled environment with a 12/12 light/dark cycle, with free access to food and water. Four days before induction of acute pancreatitis bilateral vagotomy was performed. Animals underwent a total vagotomy of both right and left branches of the vagus. The surgery was done under pentobarbital anaesthesia (Vetbutal), administered intraperitoneally at a dose 0.06 g/kg body weight.

Prior to the experiments, rats were fasted for 24 h, but access to water was not limited.

Animals were allocated to four separate groups. Each experimental group constituted of 10 rats:

1. Sham-operated group – rats injected subcutaneously (s.c.) with 0.2 ml of vehicle saline every 30 min.
2. AP group – rats with repeated injection of caerulein (given every 30 min at dose 5 μg/kg/h for 5 h), dissolved in 0.2 ml of saline;
3. Bilateral vagotomy group – rats subjected to vagotomy injected with 0.2 ml vehicle saline; every 30 min.
4. Bilateral vagotomy group with AP – rats subjected to vagotomy and given subcutaneous repeated every 30 min injection of caerulein (dose 5 μg/kg/h for 5 h), dissolved in 0.2 ml of saline.

All procedures were approved by the Jagiellonian University Ethic Committee and performed in accordance with the policies regarding the human care and use of laboratory animals.

2.3. Pancreatic weight and histological examination

After 6 h of experiment, the pancreata were carefully dissected, rinsed and weighted. Samples of pancreatic tissue were collected and processed for histopathological assessment. Histological studies were carried out on pancreatic samples fixed in 10% formalin and stained with haematoxylin and eosin. The slides were examined under light microscopy (magnification 400×) by an expert who was not familiar with experimental code. The histological changes were scored according to Jaworek et al. [23] in 250 fields (5 fields × 50 slides from each group). The histological grading of oedema, perivascular neutrophil infiltration and haemorrhage changes were assessed using from 0 to 3 range as described previously (for oedema: 0 = no oedema, 1 = interlobular oedema, 2 = interlobular oedema and moderate intralobular oedema, 3 = interlobular oedema and severe intralobular oedema; for perivascular neutrophil infiltration: 0 = no infiltration, 1 = mild perivascular neutrophil infiltration, 2 = moderate perivascular and interlobular neutrophil infiltration, 3 = abundant perivascular inter- and intralobular neutrophil infiltration [23].

2.4. Biochemical parameters

After 5-h administration of caerulein rats were subjected to pentobarbital anaesthesia and blood samples were collected from the vena cava to estimate the serum lipase activity and serum concentration of IL-10. To assess lipase activity Lipa DT slides were used as was reported in the previous paper [23]. Serum concentration of IL-10 was measured by ELISA commercial kit (R&D systems, Minneapolis MN, USA). The blood samples, were left for 2 h at room temperature for clotting, and then centrifuged (at 3500 rpm for 10 min). Serum samples were immediately frozen and kept at −80 °C.

2.5. Determination of MDA + 4-HNE concentration and GPx activity in pancreatic tissue

Samples of pancreatic tissue were taken to determine lipid peroxidation products: MDA + 4-HNE and the activity of GPx. Samples were homogenized according to the procedure and measured using Bioxytech LPO-586 kit (Oxis International, Inc., Portland, USA) as reported previously [23]. MDA + 4-HNE concentration was calculated per gram of pancreatic tissue. GPx activity was calculated as international units per gram of pancreatic tissue.

2.6. Examination of pancreatic blood flow (PBF)

Following 5 h of caerulein administration rats were subjected to measure pancreatic blood flow, which was measured by laser