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Protective effects of tropisetron on cerulein-induced acute pancreatitis in mice

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ABSTRACT

Acute pancreatitis (AP) causes morbidity and mortality. The aim of the present study was to investigate the protective effect of tropisetron against AP induced by cerulein.

Cerulein (50 μg/kg, 5 doses) was used to induce AP in mice. Six hours after final cerulein injection, animals were decapitated. Hepatic/pancreatic enzymes in the serum, pancreatic content of malondialdehyde (MDA), pro-inflammatory cytokines and myeloperoxidase (MPO) activity were measured.

Tropisetron significantly attenuated pancreatic injury markers and decreased the amount of elevated serum amylase, lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), MPO activities and pro-inflammatory cytokines levels caused by AP in mice. Tropisetron didn't affect the pancreatic levels of MDA.

Our results suggest that tropisetron could attenuate cerulein-induced AP by combating inflammatory signaling. Further clinical studies are needed to confirm its efficacy in patients with AP.

1. Introduction

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas and the leading cause of hospitalization among different types of gastrointestinal disorders worldwide [1]. Epidemiologic analysis revealed that hospital admissions for AP increased from 40 per 100,000 in 1998 to 70 per 100,000 in 2002 [2]. The molecular mechanisms underlying AP are not fully understood yet, but it has been shown that inflammatory mediators play an important role in the progression and severity of AP [3]. Acinar cell injury caused by factors like gallstones in the distal common bile duct, alcohol abuse and endoscopic retrograde cholangiopancreatography (ERCP) led to release of digestive enzymes [4,5]. Subsequent to acinar cell injury, inflammatory cytokines including interleukin 6 (IL-6), interleukin 8 (IL-8), Tumor necrosis factor alpha (TNF-α) are

Abbreviations: ALT, alanine aminotransferase; AP, acute pancreatitis; AST, aspartate aminotransferase; CLP, cecal ligation and puncture; DM, diabetes mellitus; ELISA, enzyme linked immunosorbent assay; ERCP, endoscopic retrograde cholangiopancreatography; IL-1β, Interleukin 1 beta; IL-6, interleukin 6; IL-8, interleukin 8; IQR, interquartile range; IκBα, Inhibitor kappa B-alpha; MAPK, mitogen-activated protein kinase; m-CPBG, meta chlorophenylbiguanide; MDA, malondialdehyde; NAFT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PPARγ, peroxisome proliferator activated receptor gamma; SDS, sodium dodecyl sulfate; TBA, thiobarbituric acid; TNF-α, Tumor necrosis factor alpha; α7 nAChR, alpha7 nicotinic acetylcholine receptor.

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produced within pancreas [6]. These cytokines can induce apoptosis in acinar cells and stimulate neutrophils to be recruited at the site of inflammation [7,8]. Furthermore, pancreatic enzymes could reach distant organs like lung and live through systemic circulation and cause injury [6]. For example, pancreatic elastase induced liver inflammation and upregulation of hepatic TNF predominantly within Kupffer cells [9,10]. Also, it was shown that interleukin-1 and TNF can increase the expression of other cytokines and related genes, which finally resulted in systemic inflammatory response to acute pancreatitis and the subsequent organ failure [6,11].

Current therapies including antisecretory agents, protease inhibitors, antioxidants, immunomodulators and etc., are insufficient for treatment of this disease. So, there is a pressing need for more effective therapies [12].

Tropisetron, a 5-HT_3 receptor antagonist, is widely used to treat chemotherapy associated emesis [13]. Anti-inflammatory and antioxidant aspects of tropisetron have been shown in various peripheral and central inflammatory settings [14], including experimental colitis[15], cisplatin-induced nephrotoxicity [16], ischemic stroke [17], beta-amyloid-induced neuroinflammation [18] and animal model of multiple sclerosis[19]. Furthermore, in a rat model of cecal ligation and puncture (CLP)- induced sepsis tropisetron administration significantly inhibited TNF-α and IL-6 overproduction in serum [20]. Although the exact mechanisms underlying anti-inflammatory effects of tropiseton have not been identified yet, different canonical pathways involved in inflammation such as Nuclear factor-κB (NF-κB), calcineurin, mitogen-activated protein kinase (MAPK), peroxisome proliferator-activated receptor gamma (PPARγ) and Alpha7 nicotinic acetylcholine receptor (α7 nAChR) have been shown as targets of tropisetron [14].

Regarding the remarkable anti-inflammatory properties of tropisetron and its wide therapeutic window, it would be plausible to investigate the protective aspects of tropisetron in an experimental model of AP. In this context the possible protective actions of tropisetron was studied in cerulein-induced AP in mice.

2. Materials and methods

2.1. Animals

Male NMR mice weighing 25–30 g were randomly assigned to 3 experimental groups (each group contains 6 animals). The animals were housed for one week before the experiments to acclimatize to new conditions. The experimental procedures were approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran in accordance with the Standards for the Care and Use of Laboratory Animals. Animals were kept in a room with 12-h light/12-h dark cycle and 22–24 °C.

2.2. Induction of pancreatitis

Acute pancreatitis was induced by cerulein (50 μg/kg, ip,5 doses at hourly intervals) [21]. In treatment group, tropisetron (2 mg/kg, ip) was administered twice at the intervals of one and six hours after the first cerulein administration. Control group received normal saline in the same manner.

Six hours after the last cerulein injection, animals were euthanized, blood samples were collected and pancreases removed and snap frozen in liquid nitrogen and then kept at −80 °C for later biochemical measurements. Blood samples were used to determine the serum amylase and lipase activities. A portion of pancreases was fixed in 10% formalin for histopathological evaluation.

2.3. Biochemical analysis

The serum amylase, lipase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using the commercially available kits (Pars-Azmun Co. Tehran, Iran) [22].

2.4. Determination of lipid peroxidation

Malondialdehyde (MDA) level was measurement as an indicator of lipid peroxidation with thiobarbituric acid (TBA) based reaction [23]. Briefly, the pancreas tissue was homogenized in 1.15% KCl solution. Then, 0.1 ml of the homogenate was added to a reaction solution containing 0.2 ml of sodium dodecyl sulfate (SDS) (8.1%), 1.5 ml of acetic acid (20%), 1.5 ml of TBA (0.8%) and 0.7 ml of distilled water. Samples were boiled for 30 min at 95 °C. TBA-treated samples were mixed with *n*-butanol and centrifuged at 3000 g for 10 min. The absorbance of the supernatant was read at 532 nm with a spectrophotometer. MDA level was expressed as μM/100 g tissue.

2.5. Determination of total myeloperoxidase activity (MPO) activity

MPO activity, an indicator of neutrophil sequestration in the pancreas, was determined as previously decribed [24]. Concisely, pancreatic tissues were homogenized in 10 mM potassium phosphate buffer (pH 7.4) containing 0.5% (w/v) hexadecyl trimethyl ammonium bromide (50 mg tissue/mL). The homogenates were centrifuged for 30 min at 20,000 *g* at 4 °C. After that, the supernatant was allowed to react with a solution containing tetramethyl benzidine (1.6 mM) and hydrogen peroxide (0.1 mM). The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 mmol peroxide/min at 37 °C. Values were expressed as MPO in units/g wet tissue.

2.6. Histopathological examination and scoring

After decapitating of mice, a portion of pancreases was fixed in 10% formalin for 24–48 h. Then, pancreases were embedded in paraffin to be cut into 5 μm-thick sections and dying with hematoxylin and eosin. Finally, a pathologist observed the tissue sections under light microscope blindly and scored them (1–4) through evaluating the following criteria: edema, pro-inflammatory cell infiltration, acinar vacuolization and necrosis (one is normal and 4 is severe) [25].

2.7. Determination of inflammatory mediators

To determine the inflammatory cytokine levels, the pancreatic tissues were homogenized as described by previously [26]. Briefly, a lysis buffer was prepared containing 200 mM NaCl, 5 mM EDTA, 10 mM tris, 10% glycerin and 1 mM PMSF. Next, one tablet of protease inhibitor cocktail was added for 10 ml of lysis buffer. Finally, the pH was adjusted to 7.4. After that, we added 200 μl lysis buffer to 10 mg tissue before homogenization. Samples were centrifuged twice (1500 g at 4 °C for 15 min) to avoid contamination of cell debris. The supernatant was used for measurement of cytokines. Enzyme-linked immunosorbent assay (ELISA) kits were used for measurement of TNF-α, Interleukin 1 beta (IL-1β) in the pancreas samples (duplicate experiments).

2.8. Statistical analysis

Histologic damage score is presented as median ± interquartile range (IQR). For analysis of this factor, Kruskal-Wallis test followed