INT-777, a bile acid receptor agonist, extenuates pancreatic acinar cells necrosis in a mouse model of acute pancreatitis

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A B S T R A C T

Bile acids receptor TGR5 and its agonist INT-777, which has been found to be involved in the NLRP3 inflammasome pathway, play an important role in inflammatory diseases. However, the role of INT-777 in acute pancreatitis (AP) has not been reported. In this present study, we found that TGR5 was expressed in pancreatic tissue and increased after AP onset induced by caerulein and further evaluated the impact of INT-777 on the severity of AP. The results showed that INT-777 could reduce the severity of AP in mice, which was manifested as decreased pancreatic tissue damage as well as the decrease of serum enzymes (amylose and lipase), pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and the expression of necrosis related proteins (RIP3 and p-MLKL). Furthermore, we found that INT-777 reduced the reactive oxygen species (ROS) production in pancreatic acinar cells and inhibited the activation of NLRP3 inflammasome pathway. In conclusion, our data showed that INT-777 could protect pancreatic acinar cell against necrosis and reduce the severity of AP, which may be mediated by inhibiting ROS/NLRP3 inflammasome pathway.

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1. Introduction

Acute pancreatitis (AP) is one of the most common gastrointestinal diseases in clinical practice. Although the treatment of AP had great progress in recent years, the reported mortality of severe AP patients was still as high as 15%–30% [1–3]. AP is a non-infectious acute inflammatory disease which develops from pancreas local inflammation to excessive systemic inflammatory response and even multiple organ dysfunction syndrome (MODS). So far, the pathogenesis of AP has not been fully understood yet. It is generally accepted that pancreatic acinar cells (PACs) injury, including necrosis, apoptosis and autophagy, is the most important early biological event after AP onset. Among them, the necrosis of PACs is considered as the trigger for the development of severe AP [4–7]. It has been a key issue in the clinical treatment of AP about how to effectively alleviate necrosis.

Bile acids, the important ingredients of the bile, are considered as the crucial metabolic regulators which play a key role in the digestion as well as absorption of diet lipids and the maintenance of cholesterol balance [8]. Nowadays, substantial studies have found that in addition to regulating metabolism, bile acids are also involved in the regulation of inflammatory responses and the maintenance of immune homeostasis [9,10]. Guo et al. found that bile acids can reduce the severity of many inflammatory diseases, such as LPS-induced sepsis model [11]. By inhibiting the macrophages producing inflammatory mediators, secondary metabolites of bile acids such as deoxycholic exert prominent anti-inflammatory effects [12].

As a member of the G protein-coupled receptor family, Takeda G-protein coupled receptor clone 5 (TGR5) is a novel bile acid receptor first discovered in 2003 [13]. TGR5 gene is widely expressed in the liver, pancreas, brown fat tissue, immune cells, bile duct
epithelium and other tissue cells of humans and animals. Many studies have suggested that TGR5 is involved in the inflammatory response and INT-777, a TGR5 agonist, has a protective effect on many inflammatory diseases, such as sepsis [11], atherosclerosis [14], diabetic nephropathy [15] and hepatic steatosis [16]. However, whether it has a protective effect on AP has not been reported yet. In the current article, INT-777 was used to investigate its effect on caerulein-induced AP model in mice.

2. Materials and methods

2.1. Animal and reagents

Male ICR mice (8 weeks old) were purchased from the Model Animal Center of Yangzhou University. Before the experiment, animals were fed alternately 12 h light/12 h dark for one week under suitable room temperature (22–25 °C) and humidity environment. All methods were performed in accordance with the Principles of Laboratory Animal Management (NIH Publication 85/23, revised in 1996). All the animal experiment procedures were approved by the Animal Ethics Committee of Nanjing University, Jinling Hospital.

Caerulein was purchased from AnaSpec (AnaSpec, Inc., Fremont, USA); INT-777 was purchased from MCE (MCE, Inc., New Jersey, USA); anti-phospho-mixed lineage kinase domain like pseudokininase (p-MLKL) antibody was purchased from Abcam (Abcam, Cambridge, UK); anti-receptor-interacting protein 3 (RIP3) antibody, anti- associated speck-like protein containing a caspase recruitment domain (ASC) antibody and anti-cysteinyl aspartate specific proteinase1 (Caspase-1) antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-IL-1β and TNF-α antibody against RIP3 and NLRP3, goat anti-rabbit and rabbit anti-mouse secondary antibodies were purchased from Abcam (Abcam, Cambridge, UK); Goat anti-rabbit and rabbit anti-mouse secondary antibodies were purchased from Abcam (Abcam, Cambridge, UK). Dihydroethidium (DHE) and 4',6-Diamidino-2-Fluorescein (DAPI) staining solution were purchased from Servicebio (Wuhan Servicebio Technology Co., Ltd., Wuhan, China.). ELISA kits including interleukin (IL)-6, IL-1β and tumor necrosis factor-α (TNF-α) were purchased from eBioscience (Affymetrix eBioscience, Santiago, USA). Lipase kits were purchased from Nanjing Jiancheng (Nanjing Jiancheng Company, Nanjing, China). Amylase kits were purchased from BioSino (BioSino Bio-Technology & Model Science Inc., Beijing, China).

2.2. Mouse model preparation and administration of INT-777

Before induction of the animal model, mice fasted for 12 h and were free to drink water. Mice were divided equally into 3 groups (8–10 in each group): control group, AP group and AP + INT-777 group. AP was induced by intraperitoneal injection of caerulein (100 μg/kg, 1-h intervals, 10 times) as described before [17]. Mice in control group were given PBS instead of caerulein. INT-777 was dissolved in 0.5% of carboxyl methyl cellulose sodium (CMC-Na) to prepare a suspension. Vehicle (0.5% CMC-Na) or INT-777 (1mg/mice) was administered 1 h before the first injection of caerulein intraperitoneally.

Mice were anesthetized with sodium pentobarbital and sacrificed 12 h after the first injection of caerulein. Part of pancreatic tissues were fixed in phosphate-buffered 4% paraformaldehyde and the other part of the pancreas and blood samples (obtained from the tail veins) were stored at −80°C refrigerator for further analysis.

2.3. Pancreatic severity assessment

Pancreatic tissues in 4% paraformaldehyde were fixed for 12 h, and then embedded in paraffin block.Slides from each paraffin block were stained with hematoxylin and eosin. The morphological changes of the pancreas were observed under a tissue optical microscope, and the severity of the pancreatic tissue damage was scored according to previous literature reports [17,18]. Blood samples were collected for serum enzymology (amylase and lipase) and proinflammatory cytokine (IL-1β, TNF-α and IL-6) detection, all procedures were performed according to the kit instructions.

2.4. Oxidative stress injury detection

The content of reactive oxygen species (ROS) was quantified on pancreatic tissue by DHE fluorescent probe, as described previously [19]. Briefly, frozen fresh section of pancreatic tissue was incubated in the dark with DHE solution for 30 min at 37 °C. After washing with PBS, tissues were incubated by DAPI solution at room temperature for 10 min. Finally, the slides were observed under the fluorescence microscope.

2.5. Western-blot detection

Pancreatic tissue protein was extracted using Cocktail and 1% PSMF. 70 μg protein was used for 10% SDS-polyacrylamide gel electrophoresis (PAGE), transferred to PVDF membrane, blocked with 5% BCA for 2 h at room temperature and incubated with primary antibody against RIP3 (1:1000 dilution), p-MLKL (1:1000 dilution), NLRP3 (1:1000 dilution), Caspase-1 (1:1000 dilution), ASC (1:1000 dilution), IL-1β (1:1000 dilution) and β-actin (1:2000 dilution) at 4 °C overnight. Then the membranes were incubated with the corresponding secondary antibody for 1 h at room temperature in the next day. Strips were visualized by using an enhanced chemiluminescence method. And the Image J software was used to analyze the western blots band intensity.

2.6. Immunohistochemical staining

Briefly, for immunohistochemical staining of RIP3 and NLRP3, slides were incubated overnight at 4°C in a humid chamber with an antibody against RIP3 (1:200 dilution) and NLRP3 (1:200 dilution), then incubated by biotinylated secondary antibody (1:500) for 1 h. Finally, sections were counterstained with hematoxylin.

2.7. Statistical analysis

Statistical analysis was performed by SPSS 22.0 software. The data was presented as mean ± SD. The statistical difference between the two groups was analyzed by the t-test, and the one-way ANOVA test was used to evaluate the difference more than two groups. P < 0.05 was considered statistically significant (two-tailed).

3. Results

3.1. INT-777 relieved the severity of AP induced by caerulein in mice

TGR5 is a novel bile acid receptor and expressed in pancreatic tissue. Western-blot analysis showed that TGR5 was mildly expressed in normal pancreatic tissue and increased significantly after caerulein-induced pancreatitis (Fig. 1A).

Effect of INT-777 treatment on AP in caerulein induced models was evaluated after induction of the disease. The levels of serum amylase and lipase, serum pro-inflammatory factors and pancreatic

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