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SRT1720 ameliorates sodium taurocholate-induced severe acute pancreatitis in rats by suppressing NF-κB signalling

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ABSTRACT

Severe acute pancreatitis (SAP) is a medical emergency that is often associated with multiple organ failure and high mortality. Although an SAP diagnosis requires prompt treatment, therapeutic options remain limited. SRT1720 is a newly formulatedSIRT1 activator that exerts multiple pharmacological activities with beneficial health effects. However, its potential as an SAP treatment has not been explored. The current study assessed the effect of SRT1720 on a rat model of sodium taurocholate-induced SAP and explored the underlying mechanism. SAP was induced in rats by retrograde injection of a 3.5% sodium taurocholate solution (1 ml/kg) in the biliopancreatic duct. SRT1720 (5 mg/kg) was administered intraperitoneally after sodium taurocholate exposure. Serum samples were analysed for inflammatory cytokine levels and select enzymatic activities using the enzyme-linked immunosorbent assay and commercial enzyme activity assay kits, respectively; protein expression levels were evaluated by western blotting; mRNA levels of biomarkers were determined by quantitative real-time PCR; histopathological changes were analysed by haematoxylin and eosin staining and immunohistochemistry.SRT1720 treatment significantly reduced serum amylase, lipase, pancreatic histological scores, proinflammatory cytokine (TNF-α and IL-6) levels, and expression of NF-κB and p65 in sodium taurocholate-induced SAP rats. Importantly, the treatment stimulated SIRT1 and IκBα levels in pancreatic tissue. Our data suggest that SRT1720 protects rats from sodium taurocholate-induced SAP by suppressing the NF-κB signalling pathway.

1. Introduction

Severe acute pancreatitis (SAP) is one of the most common acute abdominal diseases of the digestive system that is associated with a high mortality of approximately 20–30%. The death of patients with pancreatitis is typically caused by systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndromes (MODS) [1]. However, the underlying mechanism of the rapid progression of a local pancreatic inflammation into a systemic disease has not been completely identified. Some studies have indicated that the process of SAP is a complex pathophysiological process involving multiple factors, including the disturbance of microcirculation, oxidative stress, overload of calcium, and excessive release of proinflammatory mediators [2–4]. Upon pancreatic enzyme activation, tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are released [5]. Pathological manifestations of pancreatitis are inflammatory cell infiltration, oedema, adiponecrosis, necrosis of pancreatic acinar cells, and haemorrhage. Today, treatment of SAP is limited to spasmolysis, microcirculation improvement, anti-inflammatory and analgesic drugs, and other supportive therapies [6].Thus, finding new and more effective treatment options is of great importance.

SRT1720 is a novel pharmaceutical formulation developed by

Abbreviations: ANP, acute necrotizing pancreatitis; AREA, pixel area; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; HE, haematoxylin and eosin; IHC, immunohistochemistry; IL, interleukin; IOD, cumulative optical density; IP, intraperitoneal; MODS, multiple organ dysfunction syndrome; p, probability; PVDF, polyvinylidene difluoride; qRT-PCR, quantitative real-time PCR; SAP, severe acute pancreatitis; SD, Sprague-Dawley; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis; Sir2, silent information regulator 2; SIRS, systemic inflammatory response syndrome; SIRT1, sirtuin 1; STAC, sirtuin activating compound; TNF-α, tumour necrosis factor-α

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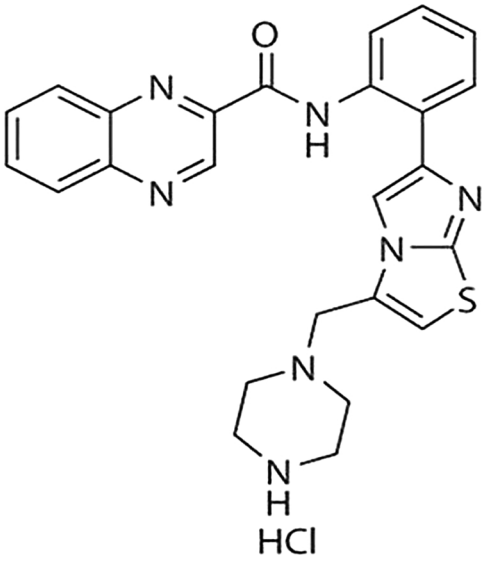

Fig. 1. Chemical structure of SRT1720.

GlaxoSmithKline (Fig. 1). It is a derivative of resveratrol, a multifunctional, natural product found in red wine. SRT1720 acts as a regulator of sirtuin1 (SIRT1) [7]. In mammals, there are seven silent information regulator 2 (Sir2) homologous genes, named SIRT1-7. Mammalian SIRT1 has the highest homology to yeast Sir2 [8]. Previous studies reported that SIRT1activationinducesanti-oxidative and anti-aging effects and improves insulin sensitization and heart diseases [9–11].In mice; it improves the metabolism of various organs. These biological effects demonstrated that SRT1720 can be potentially used for treating various diseases, such as acute kidney injury caused by of ischemia|[sol]|reperfusion-induced-reperfusion injury [12], renal tubule damage caused by oxidative stress, and sepsis [13,14]. In addition, SIRT1 also controls inflammation. As reported previously, SIRT1 affects the activity of nuclear factor kappa B (NF-κB), a dominating transcription factor that regulates the production of proinflammatory cytokines [15]. To examine the effect of SIRT1 on pancreatitis, some sirtuin activating compounds (STACs), such as resveratrol and picroside II, have been developed and tested in a rat model of SAP. Several studies demonstratedthatSIRT1 agonist resveratrol can suppress inflammation in rat models of L-arginine-induced acute necrotizing pancreatitis (ANP) and sodium taurocholate-induced SAP. It can also reduce distant organ complications [16]. Considering the protective effect of SIRT1 activation in SAP rat, we hypothesized that its agonist SRT1720 may possess protective activity against sodium taurocholate-induced SAP in rats. The objective of our study was to examine the effect of SRT1720 on SAP in rats and investigate the potential involvement of the NF-κB signalling pathway in this process.

2. Materials and methods

2.1. Animals

Male Sprague Dawley (SD) rats (body weight, 200–250 g) were purchased from the Centre of Experimental Animals at the Nanjing Medicine University. All rats were kept on a 12hlight/dark cycle and given *ad libitum* access to food and water. The animal experiment and all associated procedures were approved by the Animal Ethical and Welfare Committee at the Nanjing Medical University.

2.2. Experimental design and SRT1720 dose

Thirty SD rats were randomly divided into three groups: a control group (Con, n = 10); an SAP-induced group (SAP, n = 10); and an SAP-induced group treated with SRT1720 (SAP + SRT720, n = 10). Sodium taurocholate solution (Sigma-Aldrich, Steinheim, Germany) was diluted in normal saline (0.9% NaCl) before use. SRT1720 was dissolved in dimethylsulfoxide (DMSO) and diluted with normal saline ensure a final DMSO concentration of less than 1%, which is considered to have no effect on the experiment. Before initiating the surgical procedure, the rats were not given access to food for more than12hbut had *ad libitum* access to water. Intraperitoneal (IP) injection of 10% chloraldurat (3 ml/kg) was used to anesthetize the rats prior to surgery. SAP was induced in rats according to the method published by Laukkarinenet al. [17].After opening the abdominal cavity; we intubated through the duodenum to the biliopancreatic duct and performed retrograde infusion of 3.5% sodium taurocholate solution (1 ml/kg) at a constant rate of 0.1 ml/min into the biliopancreatic duct. After completing the infusion, the part of the biliopancreatic duct that enters the duodenum was clipped for 5 min. Then, the vascular clip was removed and the incision was closed using 4-0-silk suture. The Con group was subjected to the same procedure except that the sodium taurocholate solution was replaced by an equal volume of normal saline. In the SAP + SRT1720 group, 1 h after the sodium taurocholate treatment, the rats received a dose of 5 mg/kg SRT1720 (Selleck, Houston, TX, USA) by IP injection. The SRT1720 dose has been previously reported in a study conducted by Funk et al. [18].In addition, SRT1720, a newly developed synthetic allosteric SIRT1 activator, was subjected to a drug safety test according to the method described previously [19]. According to the safety test result, a dose of 5 mg/kg could be safely used in our experiments.

All rats were killed after 12 h, and blood and pancreatic tissue samples were collected for further analysis. Blood samples were tested for serum amylase, lipase, and proinflammatory factors. A section of each pancreatic tissue sample was immediately fixed with 4% paraformaldehyde for HE staining and immunohistochemistry (IHC) analysis; the other part was stored at −80 °C for quantitative real-time PCR (qRT-PCR) and western blotting.

2.3. Histological analysis

The tissue was fixed in 4% paraformaldehyde for 12 h, embedded in paraffin, cut into 4 mm thick sections, and, finally, stained with HE. The sections were blinded for analysis by two pathologists, who randomly evaluated10 fields per section under a microscope (magnification, 200×). Pancreatic histopathology scoring was performed according to Schmidt's scoring criteria [20]: inflammatory cell infiltration, oedema, glandular cell necrosis, and haemorrhage. Each item has a score ranging from 0 to 3. The average score of 10 fields of each tissue section was used as the final pathological score.

2.4. Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 10 min to recover the serum, which wasstoredat − 80 °C until analysis. Serum amylase and lipase levels were measured using commercial assay kits (Nanjing Jiancheng Corporation, Nanjing, China).Serum tumour necrosis factor (TNF)-α (NeoBioscience, Beijing, China) and interleukin (IL)-6(NeoBioscience, Beijing, China) were detected using the enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions.

2.5. Analysis by qRT-PCR

Total RNA was extracted from pancreatic tissueusing the TRIzol reagent (Life Technologies, Carlsbad, CA, USA).Total RNA samples were analysed for concentration and purity and reverse transcribed into cDNA using the Prime Script RT Master Mix (Takara, Kyoto, Japan) according to the manufacturer's instructions. To measure the expression of the mRNAs of TNF-α, IL-6, SIRT1, IκBα and p65, qRT-PCR was