Role of Wnt/β-Catenin pathway agonist SKL2001 in Caerulein-induced acute pancreatitis

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Abstract

Objective: To clarify the protective role of Wnt/β-Catenin pathway agonist SKL2001 in Caerulein-induced acute pancreatitis (AP).

Methods: AR42J cells and rats were divided into Control, Caerulein, SKL2001 + Caerulein and SKL2001 + Control groups respectively. Cell apoptosis was examined by flow cytometry. Hematoxylin-eosin (HE) staining was performed to observe pathological changes of pancreas and small intestinal tissues. Inflammatory cytokines were detected by enzyme linked immunosorbent assay (ELISA) while Wnt/β-Catenin pathway-related genes by quantitative real-time PCR (qRT-PCR).

Results: In-vitro results showed Caerulein promoted cell necrosis with inhibited Wnt/β-Catenin pathway and increased inflammatory cytokines. However, SKL2001 reduced cell necrosis and inflammatory cytokines with activation of Wnt/β-Catenin. Additionally, in-vivo results demonstrated 6 h after final Caerulein induction, accumulation of fluid or edema, hemorrhage, inflammation and necrosis of the pancreatic acini occurred and reached to maximal level at 12 h after final Caerulein induction; meanwhile, Wnt/β-Catenin pathway was evidently inhibited with enhanced inflammatory cytokines, and the above damages were further aggravated 12 h later. Nevertheless, the pancreatic and small intestinal tissue damages were alleviated in Caerulein-induced rats treated with SKL2001.

Conclusion: Activation of Wnt/β-Catenin pathway could inhibit Caerulein-induced cell apoptosis and inflammatory cytokines release, thus improving pancreatic and intestinal damages of AP rats.

Key words: Acute pancreatitis, Wnt/β-Catenin pathway, Caerulein, SKL2001, AR42J cells
**Introduction**

As a common acute gastrointestinal disease requiring hospitalization, acute pancreatitis (AP) presented an increasing trend in global incidence during the past decades (Sun et al. 2017). Although AP is generally considered as a mild self-limiting disease with good prognosis and low mortality in most patients, about 20% patients would turn to severe acute pancreatitis (SAP) (Ioannidis et al. 2008). Recently, multiple evidences indicated that the activated leucocyte and its released inflammatory cytokines during the occurrence of systemic inflammatory response syndrome caused by SAP, would not only lead to local pathological damages (pancreas and intestine), but also multiple organ dysfunction syndrome (MODS) with the mortality rate of approximately 30% (Jacob et al. 2016; Sun et al. 2017). Hence, domestic and foreign scholars are still exploring the pathogenesis of AP for an ideal treatment.

Currently, several lines of studies have put forward a close relation of Wnt signaling pathway and pancreas-related diseases, for example, the dysregulation of Wnt pathway was found to be related to the presence and progression of pancreatic mucinous cystic neoplasm (MCN) (Svala et al. 2014), and the Wnt signaling pathway was also noticed to be activated during the process of acinar-to-ductal metaplasia after chronic pancreatitis or AP (Zechner et al. 2016). To the best of our knowledge, the Wnt signaling pathway with high evolutionary conservation are ubiquitously present in organisms, and exerts important effects on embryonic development, cell division, apoptosis and necrosis (Kawakami et al. 2013). As a classic Wnt signaling pathway, Wnt/β-catenin becomes the research hotpot due to its broad effects, and Wnt/β-catenin mediates the stabilization of β-catenin in cytoplasm through their bindings with the Frizzled receptors to increase β-catenin nuclear translocation, thereby regulating cell process (Caspi et al. 2014). There was evidence stating that the activation of Wnt/β-catenin could facilitate the development of pancreatic cancer and enhance its malignant potential (Sano et al. 2016). More importantly, its activation could mediate the activation and proliferation of pancreatic stellate
cells (PSC), which resulted in the fibrosis of pancreas islet, consequently promoting the occurrence of chronic pancreatitis (Hu et al. 2014). However, no research focuses on investigating the role of Wnt/β-catenin pathway in AP. Therefore, this study was undertaken to construct AP model in vivo and in vitro by Caerulein induction together with pre-treatment of Wnt/β-Catenin pathway agonist SKL2001 to explore the protective effects of inhibiting Wnt/β-Catenin pathway on AP.

Materials and methods

Ethics statement

This animal study adheres to Guide for the Care and Use of Laboratory Animals (Council 2013), and approved by Ethics Committee of The First Affiliated Hospital of Guangxi Medical University.

Cells culture and grouping

Rat pancreatic exocrine cells AR42J (American Type Culture Collection, Rockville, Maryland, USA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% Fetal bovine serum (FBS), 1 mM pyruvate, 1x penstrep and 2 mM glutamine at 37°C in 95% air and 5% CO₂. Then, 100 nM dexamethasone (Dex) from Sigma (D-8893; 1 mg vial) was added to the cells. Two to three days later, the medium was changed, and AR42J cells were sub-cultured through digesting by 0.25% trypsin and 0.01% ethylene diamine tetra acetic acid (EDTA). Then, AR42J cells were divided into 4 groups: Control group (cultured with RPMI1640 medium), Caerulein group (cultured with RPMI1640 medium supplemented with 100 nmol/L Caerulein), SKL2001 + Caerulein group (cultured with RPMI1640 medium containing 20 μM Wnt/β-Catenin agonist SKL2001 for 24 h, followed by culture in 100 nmol/L Caerulein), and SKL2001 + Control group (cultured with RPMI1640 medium containing 20 μM SKL2001 for 24 h, followed by culture in phosphate-buffered saline (PBS) at equal concentrations). SKL2001 (S8302) and Caerulein (HY-A0190) were purchased from Selleck Chemicals (Houston, TX, USA) and MedChemExpress (Shanghai, China), respectively.
Annexin V-FITC/PI staining

After cells treated for 24 h, the medium was abandoned, and the cells were collected using EDTA-free trypsin and washed by phosphate buffer solution (PBS) twice. Next, a total of 1-5×10^5 cells were centrifuged and resuspended by 500 μl binding buffer followed by adding 5 μl Annexin V-FITC and 5 μl Propidium Iodide (PI). After reaction for 5-15 min at room temperature at dark, specimens were tested in 1 h using the Epics XL flow cytometry (Beckman Instruments, Inc., CA, USA). The left inferior quadrant showed normal cells (FITC-/PI-); the right upper quadrant was the necrotic cells (FITC+/PI+); the right lower quadrant was the apoptotic cells (FITC+/PI-) (Takahashi et al. 2004). The experiment was repeated in triple.

Rats grouping and AP model preparation

A total of 40 Sprague-Dawley (SD) rats (half male and half female in weight of 250-300 g) were provided by the First Affiliated Hospital of Guangxi Medical University. All animals were kept in the laboratory for 1-week adaptive breeding maintained at 21 ± 2°C and a 12 h light- dark sequence with free food and water. The rats were randomly divided into 4 groups with 10 rats in each group, including Control group, Caerulein group, SKL2001 + Caerulein group and SKL2001 + Control group. Rats in Caerulein group adopt 6 hourly intraperitoneal injections of Caerulein (20 mg/kg) dissolved in normal saline. Rats in SKL2001 + Caerulein group and SKL2001 + Control group had the intraperitoneal injection of 25 mg/kg SKL2001 in 100% dimethyl sulfoxide (DMSO) with dilution in 50% Tween 20 to adjust the concentration of DMSO to 20%, and one hour later, rats were intraperitoneally injected with 20 mg/kg Caerulein and normal saline hourly for 6 h, respectively. The rats in Control group were injected with normal saline in the same volume.

Specimen collection

After final injection of Caerulein, at the time point of 6 h and 12 h, 5 rats at each time point from each group were anesthetized via intraperitoneal injection of 2% pentobarbital sodium (4 mg/kg). Then, the abdominal cavity was cut along the middle line to isolate the pancreas bluntly. Next, the pancreas
tissues in the same site were cut and fixed in 4% neutral polyformaldehyde and stored at room temperature. A total of 10 cm small intestinal tissues (5 cm from the ileocecum) were taken to wash its content by PBS. The small intestinal tissue of 3 cm was placed in EP tube treated by DEPC (diethyl-pyrocarbonate) and stored at -80°C, while 2 cm was fixed in 4% paraformaldehyde for 24-48 h to prepare the paraffin specimen.

**Hematoxylin-eosin (HE) staining**

The paraffin specimens were made into a series of slices in thickness of 4 μm, and then placed in a water bath. After the tissue was fully extended, the slice was dragged and placed at 60°C for 4 h for fixation. Then, the slice was dewaxed with xylene, dehydrated by gradient alcohol, and stained with hemotoxylin for 1 min. After washed by running water, slice was placed in 1% hydrochloric acid alcohol for 5 s, treated with 1% ammonia, and stained with eosin. After dehydrated by gradient alcohol, slice was transparentized by xylene, sealed in neutral gum and observed for pathological changes under light microscope. According to Schmidt score (Schmidt et al. 1992), the pancreatic tissue was graded. While the pathological grade of intestinal mucosal damage was evaluated based on Chiu’s scoring standard (Chiu et al. 1970). The experiment was repeated for three times.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

The total RNA was extracted using Trizol method (Takara Biotechnology Ltd., Dalian, China), and the extracted RNA was detected for its OD260/280 value using ultraviolet spectrophotometer and calculated for its concentration, followed by storing at -80°C. On the basis of gene sequence published in the Genbank database, Primer 5.0 software was applied to design the primers (Table 1), which were synthesized by Shanghai Biological Engineering Co., Ltd (Shanghai, China). The total RNA reverse transcriptase PCR was conducted in accordance with procedures provided by ABI PRISM cDNA Archive kit (Applied Biosystems by Life Technologies Corporation, Foster city, California, USA). Then, qRT-PCR followed procedures of ABI One-step RT-PCR Kit (Applied Biosystems by Life Technologies Corporation, Foster city, California, USA). Using ABI 7300 PCR instrument (Applied
Biosystems by Life Technologies Corporation, Foster city, California, USA), PCR amplification conditions were as follows: 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. With GAPDH as internal control, the relative expression of target gene was calculated based on \(2^{-\Delta\Delta Ct}\), and the experiment was repeatedly conducted in triple.

**Enzyme linked immunosorbent assay (ELISA)**

Cell supernatant, pancreatic and small intestinal tissues were collected from each group to determine the protein expressions of IL-1β, IL-6 and TNF-α using ELISA kit (Wuhan Boster Biological Technology Ltd., Wuhan, Hubei, China). The microplate reader (Thermo Fisher Inc., Waltham, MA, USA) was used to determine the absorbance at 450 nm wavelength, which used to get the concentration of inflammatory cytokines. Each experiment was in triple repetition.

**Statistical analysis**

All data was analyzed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA). All data was expressed as mean ± standard deviation (\(\bar{x} \pm s\)). The comparison between two groups was analyzed by independent sample \(t\)-test, and among multiple groups was analyzed by One-way ANOVA. While pairwise comparison was examined using least significant different (LSD) test. A value of \(P < 0.05\) was considered as the significant difference.

**Results**

**Activation of Wnt/β-Catenin pathway inhibited Caerulein-induced AR42J cell necrosis and the release of inflammatory cytokines**

The qRT-PCR was used to detect expressions of Wnt/β-Catenin pathway-related genes. As shown in Figure 1A, the mRNA expressions of \(Wnt1\), \(Wnt2\), \(\beta\)-catenin, \(c\)-Myc and \(Axin-2\) were greatly lower in Caerulein-induced AR42J cells than Controls (all \(P < 0.05\)), but these expressions were evidently
increased in Caerulein-induced AR42J cells pre-treated with Wnt/β-Catenin pathway agonist SKL2001 (all \( P < 0.05 \)). Moreover, AR42J cells in SKL2001 + Control group showed the highest expressions of \( Wnt1, Wnt2, \beta\text{-catenin}, c\text{-Myc} \), and \( Axin-2 \) among the four groups (all \( P < 0.05 \)). In addition, the protein expressions of TNF-α, IL-1β, and IL-6 were significantly enhanced in Caerulein group and SKL2001 + Caerulein group when compared with Control group and SKL2001 + Control group (all \( P < 0.05 \)), as detected by ELISA analysis in Figure 1B, whereas these expressions were dramatically reduced in SKL2001 + Caerulein group, as compared with Caerulein group (all \( P < 0.05 \)). Annexin V-FITC/PI staining (Figure 1C-D) revealed that Caerulein could obviously induce AR42J cell necrosis in contrast to Controls, while SKL2001 + Caerulein group presented less AR42J cell necrosis in comparison with Caerulein group (all \( P < 0.05 \)).

**Activation of Wnt/β-Catenin pathway ameliorated the pathological changes of pancreatic tissues in Caerulein-induced AP rats**

HE staining analysis presented in Figure 2A showed that the pancreatic tissues in Control group and SKL2001 + Control group at each time point (6h and 12h) was clear with complete lobule structure, without bleeding and necrosis, as well as without inflammatory-cell infiltration in the interlobular septa. By contrast, in Caerulein group, accumulation of fluid or edema, hemorrhage, inflammation and necrosis of the pancreatic acini occurred and reached to maximal lesion at 12 after the final Caerulein induction, which was prevented when SKL2001 was administrated, and no significant differences were found between 6 h and 12 h. According to Schmidt scoring method, the Schmidt scores at the 6 h and 12 h were higher in Caerulein group and Caerulein + SKL2001 group than in Control group, but were sharply decreased in SKL2001 + Caerulein group compared to Caerulein group (all \( P < 0.05 \), Figure 2B).

**Activation of Wnt/β-Catenin pathway on the pathological changes of small intestinal tissues in**
**Caerulein-induced AP rats**

In Control group and SKL2001 + Control group, the intestinal mucosa of rats was intact and regularly arranged with integrated structure of intestinal crypt and gland. However, the rats in Caerulein group showed obvious inflammatory reaction in the intestinal mucosa, manifested by the enlargement of intestinal edema, the destroyed structure of intestinal crypt and gland, and the atrophy of small intestinal villi at 6 h after the final induction. Besides, the damage was more serious 12 h later with increased mucosal bleeding and gland injury. As shown in Figure 3A, compared with Caerulein group, the intestinal villus edema and inflammatory-cell infiltration were alleviated in SKL2001 + Caerulein group with improved structure of intestinal crypt and gland. In comparison with Control group, Chiu’s score of Caerulein group was significantly elevated, but was slightly lower in SKL2001 + Caerulein group at all time-points (all $P < 0.05$, Figure 3B).

**Expressions of Wnt/β-Catenin pathway-related genes in pancreatic and small intestinal tissues in each group**

The qRT-PCR was conducted to determine expressions of Wnt/β-Catenin pathway-related genes in pancreatic and small intestinal tissues at different time points (6h and 12h) after the final induction in each group (Figure 4). Compared with Control group, $Wnt1$, $Wnt2$, $β$-catenin, $c$-Myc and $Axin$-2 mRNA expressions in pancreatic and small intestinal tissues at 6h and 12h were evidently reduced in Caerulein group, and more specifically, these mRNAs were remarkably lower at 12 h than 6 h in Caerulein group (all $P < 0.05$). In addition, mRNA expressions of $Wnt1$, $Wnt2$, $β$-catenin, $c$-Myc and $Axin$-2 were greatly higher in SKL2001 + Caerulein group and SKL2001 + Control group than Control group and Caerulein group at 6 h (all $P < 0.05$). Nevertheless, no significant differences were observed in these mRNAs between SKL2001 + Caerulein group and Control group at 12 h (all $P > 0.05$).

**Activation of Wnt/β-Catenin pathway on the release of inflammatory cytokines in pancreatic and small intestinal tissues of Caerulein-induced AP rats**

As shown in Figure 5, at all time-points, the protein levels of inflammatory cytokines (including
TNF-α, IL-1β and IL-6) in pancreatic and small intestinal tissues were significantly higher in Caerulein group and SKL2001 + Caerulein group than Control group and SKL2001 + Control group, whereas the protein levels of inflammatory cytokines were decreased in SKL2001 + Caerulein group, as compared to Caerulein group (all $P < 0.05$). Moreover, these inflammatory cytokines protein expressions in Caerulein group were higher at 12 h than that at 6 h (all $P < 0.05$), and there were no significant differences between Control group and SKL2001 + Caerulein group at all time-points (all $P > 0.05$).

**Discussion**

As a vital signaling pathway, once Wnt/β-catenin signaling pathway to be dysregulated, it would cause some obstacles or damages to affect body growth and development to a certain extent (Clevers and Nusse 2012). Recently, the role Wnt/β-catenin pathway has been attracted much attention in pancreatic biology, including pancreatitis (Arensman et al. 2014; Wells et al. 2007). For example, activation of PSCs, a crucial cell in the pancreatic fibrosis, could motivate Wnt/β-catenin pathway, eventually leading to the formation of chronic pancreatitis (CP) (Hu et al. 2014). In return, through inhibiting Wnt/β-Catenin pathway, retinoic acid could improve the pancreatic fibrosis of CP rats and suppress the activation of pancreatic islet cells (Xiao et al. 2015), which indirectly indicated that the activation of Wnt/β-Catenin pathway might be one of the causes to be involved in CP, possibly because Wnt/β-Catenin could increase tissue fibrosis, and notably, pancreatic fibrosis has been suggested as a typical pathological feature of CP (Tan et al. 2014). Interestingly, the Wnt/β-catenin pathway in our findings showed to be inhibited, as evident by the down-regulated $Wnt1$, $Wnt2$, $β$-catenin, $c$-Myc and $Axin-2$ in Caerulein-induced AR42J cells and pancreatic tissues of rats. As demonstrated by the previous studies, Caerulein could induce inflammatory cells releasing a large amount of pre-inflammatory factors (including TNF-α, IL-1β and IL-6) to initiate the inflammatory cascade in pancreas, leading to the occurrence and development of pancreatitis (Dabrowski et al. 1999; Sun et al.
Of note, Zhang X et al. found that the TNF-α could inhibit Wnt/β-catenin signaling pathway in osteoblasts (Zhang et al. 2017) by decreasing the expressions of DKK-1, GSK-3β, and β-catenin in a time-dependent manner (Sang et al. 2016). In addition, IL-1 signaling promoted production of IL-1β and other proinflammatory molecules, thus suppresses β-catenin signaling in neurons (Kitazawa et al. 2011). Besides, a previous study demonstrated that IL-6 stimulation decreased chondrocyte expression of the Wnt/β-catenin signaling pathway transactivator β-catenin (Svala et al. 2014). Moreover, the Wnt/β-catenin signaling pathway possessing anti-inflammatory functions could inhibit the production of proinflammatory cytokines in different cell types (Dessimoz et al. 2005). All mentioned above indicating Wnt/β-Catenin signaling pathway affected by the Caerulein treatment possibly by the feedback regulation of inflammatory cytokines. Besides, there was also stated that the inhibition of Wnt/β-catenin signaling was mediated by suppression of Notch signaling in vivo, consequently resulted in damages in the regeneration of exocrine pancreas (Siveke et al. 2008). Of note, β-catenin has pointed out to facilitate the proliferation of human acinar cells after damage, thereby contributing to the resolution of AP (Keefe et al. 2012), and its inactivation causes acute edematous pancreatitis (AEP), characterized by infiltrated fluid and vacuolated tissue (Dessimoz et al. 2005), which provided the possibility that activation of Wnt/β-Catenin signaling could be the new target to treat AP.

Currently, evidence have confirmed that pancreatic cells predominantly die via necrosis in AP, and the severity of necrosis was not only positively correlated with acinar cell necrosis, but also was the key factor to determine the occurrence, development and prognosis of AP (Kitazawa et al. 2011). In this study, the obviously induced AR42J cell necrosis by Caerulein indicated the successful construction of AP model in vitro. Then, the cells pretreated with (Wnt/β-Catenin pathway agonist) SKL2001 to further investigate the role of Wnt/β-Catenin pathway in AP, and we found a significant decrease of Caerulein-induced cell necrosis. As we know, reactive oxygen species (ROS)-mediated oxidative stress could inhibit secretion of pancreatic acinar cells, activate cell protease and promote stress factor release, which causes inflammation and cell necrosis via various ways, thereby promoting...
AP progression (Dabrowski et al. 1999; Pereda et al. 2006). Thus, it is reasonably to suspect that the activation of Wnt/β-catenin signaling pathway may play a protective role in AP by eliminating ROS in AP and reducing the acinar cells AR42J necrosis (Musil et al. 2003). Similarly, Wu et al. also found that activation of Wnt/β-Catenin pathway could inhibit macrophages necrosis via blocking ROS-mediated PARP-1/AIF pathway, exerting a protective role in infectious tuberculosis (Wu et al. 2015).

Besides, necrosis is classically accepted as a passive cell death process, which can directly damage nearby cells and tissues, resulted in inflammation and further promoted the release of more inflammatory factors, ultimately accelerating AP development (Franco-Pons et al. 2013; Franco-Pons et al. 2010). IL-1β, as a key inflammatory cytokine produced by neutrophils and monocytes that migrate to the pancreas, would stimulate the secretion of pancreatic enzymes by acinar cells and promote the release of other inflammatory mediators (Fink and Norman 1997). In terms of IL-6 and TNF, they have been independently used to identify mild AP from early severe AP (Pooran et al. 2003).

In our study, expressions of TNF-α, IL-1β and IL-6 were significantly up-regulated in Caerulein-induced AR42J cell supernatant and pancreatic tissues, but sharply decreased with the pre-treatment of SKL2001, further indicating that activation of Wnt/β-Catenin signaling pathway could lower the release of inflammatory cytokines. Several lines of evidence identified the anti-inflammatory and pro-inflammatory functions of Wnt/β-catenin in organisms (Keerthivasan et al. 2014; Tian et al. 2013). But so far, most of literatures supported the opinion that the Wnt/β-catenin signaling pathway in different cell types can inhibit the release of pro-inflammatory cytokines (including IL1β, IL-6, IL-8 and TNF-α), to be implicated in the development of diseases (Ma and Hottiger 2016). In agreement, NF-κB activation was found to be enhanced in monocytes/macrophages in AP, which could negatively regulate Wnt/β-catenin pathway to facilitate the inflammation (Liu et al. 2003).

Furthermore, the inflammatory mediator cascade is triggered and induces damages to the pancreas, as well as other vital organs, like intestinal injury (Sun et al. 2017). Meanwhile, a growing number of
evidences exhibited that SAP is the reason for impaired intestinal mucosal barrier function, since the intestinal mucous membrane permeability was increased to cause the intestinal flora displacement and intestinal endotoxemia, thus leading to release a large amount of cytokines and inflammatory mediators, further stimulating the systemic inflammatory response syndrome (SIRS) and MODS (Li et al. 2016; Zhongkai et al. 2012). That’s why intestinal mucosal barrier is so important for SAP recovery.

Furthermore, we found Caerulein could lead to the disorder of intestinal tissue structure in rats, increased infiltration of interstitial neutrophils and enhanced expressions of inflammatory cytokines, whose severity was increased as time went on, which is in accordance with plenty of researches.

However, upon treatment by SKL2001, those symptoms were ameliorated, suggesting that Wnt/β-Catenin pathway agonist can relieve Caerulein-induced damage to the small intestine. As reported by Yamamoto et al., the combination of intestinal epithelial cells (IEC) and Wnt could further activate the Wnt/β-catenin signaling pathway, contributing to the repair of intestinal mucosal epithelial cells. (Yamamoto et al. 2013). Mechanically, classic Wnt signaling pathways might be able to induce the activation of intestinal stem cells (a kind of adult stem cells mainly distributed in the intestinal crypt), to maintain the complete intestinal structure and function, as well as repair after injury (Suh et al. 2017).

In sum, Caerulein can inhibit Wnt/β-catenin pathway and promote AR42J cell necrosis and inflammatory cytokines release, resulting in pancreatic and intestinal tissues damages in rats. On the contrary, the reversible effects of the Wnt/β-Catenin pathway agonist SKL2001 could effectively reverse the damage induced by Caerulein, which provided a potential clue for the clinical treatment of AP.
Abbreviations list

AP, acute pancreatitis
CP, chronic pancreatitis
MODS, multiple organ dysfunction syndrome
MCN, mucinous cystic neoplasm
PSCs, pancreatic stellate cells
DMEM, Dulbecco’s modified Eagle’s medium
FBS, Fetal bovine serum
EDTA, ethylene diamine tetra acetic acid
PI, Propidium Iodide
SD, Sprague-Dawley
HE, Hematoxylin-eosin
qRT-PCR, Quantitative real-time polymerase chain reaction
ELISA, enzyme linked immunosorbent assay
ROS, reactive oxygen species
SIRS, systemic inflammatory response syndrome
IEC, intestinal epithelial cells

Acknowledgements

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Conflicts of interest
No potential conflicts of interest were disclosed.
References


**Figure Captions**

**Figure 1** Activation of Wnt/β-Catenin pathway significantly inhibited Caerulein-induced AR42J cell necrosis and the release of inflammatory cytokines

Notes: A, Relative mRNA expressions of Wnt/β-Catenin pathway-related genes in AR42J cells detected by qRT-PCR; B, The expressions of inflammatory cytokines like TNF-α, IL-1β and IL-6 in supernatant detected by ELISA method; C-D, The AR42J necrosis in each group detected by Annexin V-FITC/PI staining; *P < 0.05, compare with Control group, #P < 0.05, compare with Caerulein group, and &P < 0.05, compare with SKL2001 + Caerulein group.

**Figure 2** Observation of the pathological changes of pancreatic tissues in Caerulein-induced rats by HE staining

Notes: A, The pathological changes of pancreatic tissues at 6h and 12h after the final induction of Caerulein were observed under inverted microscope (× 200, scale bar = 50 μm); B, The pancreatic pathology at different time points (6h and 12h) after the final induction of Caerulein were evaluated by Schmidt scoring method; *P < 0.05, compare with Control group; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.

**Figure 3** Observation of the pathological changes of the small intestinal tissues in Caerulein-induced rats by HE staining

Notes: A, The pathological changes of the small intestinal tissues at different time points were observed under inverted microscope (× 400, scale bar = 50 μm); B, Chiu’s scores of the small intestinal tissues at different time points; *P < 0.05, compare with Control group; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.

**Figure 4** Expressions of Wnt/β-Catenin pathway-related genes in pancreatic and small intestinal tissues
in each group

Notes: Relative mRNA expressions of Wnt1 (A, F), Wnt2 (B, G), β-catenin (C, H), c-Myc (D, I) and Axin-2 (E, J) in pancreatic and small intestinal tissues in each group detected by qRT-PCR; *$P < 0.05$, compare with Control group; #$P < 0.05$, compare with Caerulein group; &$P < 0.05$, compare with SKL2001 + Caerulein group; @$P < 0.05$, compare with the corresponding group at 6 h.

**Figure 5** Activation of Wnt/β-Catenin pathway on the release of inflammatory cytokines in pancreatic and small intestinal tissues of Caerulein-induced AP rats

Notes: The expressions of IL-1β (A, D), TNF-α (B, E) and IL-6 (C, F) in pancreatic and small intestinal tissues in each group detected by ELISA method; #$P < 0.05$, compare with Caerulein group; &$P < 0.05$, compare with SKL2001 + Caerulein group; @$P < 0.05$, compare with the corresponding group at 6 h.
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<th>Reverse primer</th>
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<td>5’-CGGCGGAGGTGATTTGCGAAGATAAA-3’</td>
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<td>5’-GGGTGTTTGCAGTCCAGCGATG-3’</td>
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<td>5’-CGAGTCATTGCATACTGTCC-3’</td>
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<td>Axin-2</td>
<td>5’-TCAAGTGCACACCTTGGCAACC-3’</td>
<td>5’-TAGCCAGAACCCTATGTGATAAGG-3’</td>
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Figure 1 Activation of Wnt/β-Catenin pathway significantly inhibited Caerulein-induced AR42J cell necrosis and the release of inflammatory cytokines

Notes: A, Relative mRNA expressions of Wnt/β-Catenin pathway-related genes in AR42J cells detected by qRT-PCR; B, The expressions of inflammatory cytokines like TNF-α, IL-1β and IL-6 in supernatant detected by ELISA method; C-D, The AR42J necrosis in each group detected by Annexin V-FITC/PI staining; *P < 0.05, compare with Control group, #P < 0.05, compare with Caerulein group, and &P < 0.05, compare with SKL2001 + Caerulein group.
Figure 2 Observation of the pathological changes of pancreatic tissues in Caerulein-induced rats by HE staining

Notes: A, The pathological changes of pancreatic tissues at 6h and 12h after the final induction of Caerulein were observed under inverted microscope (× 200, scale bar = 50 μm); B, The pancreatic pathology at different time points (6h and 12h) after the final induction of Caerulein were evaluated by Schmidt scoring method; *P < 0.05, compare with Control group; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.
Figure 3 Observation of the pathological changes of the small intestinal tissues in Caerulein-induced rats by HE staining

Notes: A, The pathological changes of the small intestinal tissues at different time points were observed under inverted microscope (× 400, scale bar = 50 μm); B, Chiu’s scores of the small intestinal tissues at different time points; *P < 0.05, compare with Control group; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.
Figure 4 Expressions of Wnt/β-Catenin pathway-related genes in pancreatic and small intestinal tissues in each group

Notes: Relative mRNA expressions of Wnt1 (A, F), Wnt2 (B, G), β-catenin (C, H), c-Myc (D, I) and Axin-2 (E, J) in pancreatic and small intestinal tissues in each group detected by qRT-PCR; *P < 0.05, compare with Control group; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.
Figure 5 Activation of Wnt/β-Catenin pathway on the release of inflammatory cytokines in pancreatic and small intestinal tissues of Caerulein-induced AP rats

Notes: The expressions of IL-1β (A, D), TNF-α (B, E) and IL-6 (C, F) in pancreatic and small intestinal tissues in each group detected by ELISA method; #P < 0.05, compare with Caerulein group; &P < 0.05, compare with SKL2001 + Caerulein group; @P < 0.05, compare with the corresponding group at 6 h.