

Modafinil ameliorated pancreatic injury and inflammation through upregulating SNIP1

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Abstract. Acute pancreatitis (AP) is the inflammatory response of the exocrine pancreas to various causes. Modafinil has significant anti-inflammation and anti-oxidation effects. No experiment has assessed the effects of modafinil on AP. Thus, the study aims to study the effects of modafinil on AP and its potential mechanism *in vivo* and *in vitro*. 5% sodium taurocholate was retrograde injected into pancreatic duct to establish AP rat model. The severity of AP was detected by H&E staining, serum amylase and lipase levels. The inflammation, oxidative stress and apoptosis were detected separately by ELISA, MDA and SOD kits, tunnel staining and Western blotting in rats. Besides, SNIP1 expression was analyzed by qPCR and Western blotting. *In vivo*, AR42J cells were stimulated by cerulein and lipopolysaccharide to establish AP cell model. Flow cytometry examined cell apoptosis. After the plasmids silencing SNIP1 were transfected into AP cells, the inhibitory effects of modafinil on inflammation, oxidative stress and apoptosis were significantly reversed. The results indicated that modafinil showed significant curative and therapeutic effects by regulating SNIP1 levels.

Key words: Acute pancreatitis — Modafinil — SNIP1 — Inflammation

Introduction

Acute pancreatitis (AP) is an inflammatory response in which pancreatic enzymes (such as trypsin, pancrelipase) are activated in the pancreas owing to various causes, leading to pancreatic tissue autodigestion, edema, hemorrhage and necrosis. The acute inflammation and autodigestion of pancreas usually contribute to the release of amylase and lipase into blood. Serum amylase and lipase are used as the diagnose standards in AP (Steinberg et al. 2017). Furthermore, C-reactive protein (CRP) can be applied as an important indicator for detecting pancreatic necrosis in AP patients (Komolafe et al. 2017).

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Modafinil is a wakefulness enhancer used in the treatment of narcolepsy, which includes shift work sleep disorders and obstructive sleep apnea (Lillicrap et al. 2016). In addition to these indications, modafinil is also widely used in other diseases. Animal studies have shown that modafinil can inhibit inflammation *via* decreasing pro-inflammatory factors and increasing anti-inflammatory factors (Han et al. 2018; Yousefi-Manesh et al. 2019b). In addition, it affects oxidative stress partly by decreasing malondialdehyde (MDA), one of products of membrane lipid peroxidation (Yousefi-Manesh et al. 2019b). And modafinil regulates Akt/NF- κ B pathway to ameliorate inflammation in atherosclerosis mouse with apoE deficiency (Han et al. 2018). Besides, modafinil derivatives exert anti-inflammatory effects much better than aspirin *via* decreasing iNOS and COX-2 in LPS-treated BV2 microglia cells. Modafinil can also inhibit the apoptosis and autophagy in rapamycin-induced hippocampal neuronal cells (Jung et al. 2012). Furthermore, the systematic review indicates the procognitive effects of modafinil on major depressive disorder (Cao et al. 2019).

Smad nuclear interacting protein1 (SNIP1) is mainly located in the nucleus. SNIP1 interacted with TGF- β signal transduction factor Smad4, thus inhibiting the recruitment

of Smad4 to co-activator CBP/p300 and the activation of TGF- β downstream genes (Vaccarino et al. 2019). SNIP1 is proved to be modified at its lysine site by small ubiquitin-like modifier (SUMO) *in vitro* and *in vivo*. The SUMOylation of SNIP1 decreased its inhibitory effects on TGF- β downstream genes (Liu et al. 2016). TGF- β is involved in limiting the development of inflammation process (Schmitz-Winnenthal et al. 2010). Besides, TGF- β signal pathway is involved in many cellular processes including cell apoptosis, cell growth, dynamic equilibrium and other functions (Chen et al. 2019; Schlegel et al. 2019; Yang et al. 2019).

It is observed that SNIP1 levels are down-regulated in patients with pancreatitis through searching Gene Expression Omnibus (GEO) database (Chu et al. 2019), implying that SNIP1 may play a vital role in AP. There have been several studies show that SNIP1 modulates epithelial barrier function in intestinal epithelial cells to suppress inflammation and its overexpression decreases cardiac hypertrophy induced by pressure overload *via* inhibiting nuclear factor- κ B signaling (Lu et al. 2016; Shi et al. 2018). Furthermore, SNIP1 mediates DNA damage-induced apoptosis through TET2-SNIP1-c-MYC pathway and its knockdown in pituitary adenoma cells suppresses proliferation and induces apoptosis (Chen et al. 2018). Thus, the study mainly focused on the effects of modafinil on AP rats and the potential mechanism in AP.

Methods

Animals

The adult Sprague Dawley (SD) rats (weight 230 ± 30 g, age 7 ± 1 weeks) were purchased by Kay Biological Technology (Shanghai, China) ($n = 40$). All rats were raised at 23–25°C, and at 50–60% humidity. The rats were divided into AP group ($n = 30$) and Control (Sham) group ($n = 10$). The rats in the AP group were used to establish AP model. The rats were anesthetized by intraperitoneal injection with 3% pentobarbital sodium (Chemrrt, Beijing, China). After incision in the middle of the abdomen of rats, duodenum was exposed, and pancreatic and hilar bile ducts were differentiated. Biliopancreatic duct was temporarily blocked using an artery clamp. The catheter was used to enter biliopancreatic duct transduodenally. 5% sodium taurocholate (Sangon Biotech, Shanghai, China) was retrograde injected into pancreatic duct (1 mg/kg). For Control group, the rats were injected into the same amount of normal saline under same laparotomy treatment. After surgery, the rats were intraperitoneally administered with modafinil (10 mg/kg, Sigma-Aldrich, Merck KGaA, Germany) dissolved in dimethyl sulfoxide (50% DMSO) once daily for 3 days

(AP+modafinil group). The dose of modafinil could exert protective roles partly through reducing inflammation (Yousefi-Manesh et al. 2019b). In addition, the preliminary study showed that the dose of modafinil could ameliorate pancreatic injury.

The Control group was administered with equal amount of DMSO. After final modafinil treatment, the rats were anesthetized by pentobarbital sodium and fixed on the operating table. The ascites were extracted with a 5 ml syringe and the result was recorded. Blood was collected from the abdominal aorta, placed in the sterilized EP tube and centrifuged at 3000 rpm at 4°C for 10 min. After 40 min, the supernatant was collected and stored at -70°C. The intact pancreatic tissue was also taken out, the section of which was fixed with 4% paraformaldehyde. The rats were sacrificed by pentobarbital sodium. The rest of the pancreas was stored in liquid nitrogen for further experiment.

All experiments were performed according to Ethics Committee of Suzhou Municipal Hospital (KL901099).

The detection of amylase and lipase

The amylase and lipase content in serum was separately detected by amylase or lipase assay kit according to the manufacturer's protocol (amylase kit: C016-1-1, lipase kit: A054-1-1, Nanjing JianCheng, Nanjing, China).

Western blot

Pancreas tissue was taken and cut into pieces, which were cleaved by protein lysate. Pancreas tissue lysate was centrifuged at 12000 rpm for 10 min. The supernatant was collected and used to detect total protein concentration. Subsequently, the target proteins were separated by using SDS-PAGE electrophoresis and then transferred into PVDF membrane. Skim milk was used to block the protein bands, followed by routine washing. Afterwards, diluted primary antibody was added and incubated with target protein at 4°C overnight (Bcl-2: #3498, caspase-3: #9662, cleaved caspase-3: #9664, Bax: #14796, GADPH: #5174, CST, USA. SNIP1, ab126194, England). The bands were washed and incubated at 37°C for 2 h. After ECL developed color, Image J software was used to analyze gray value. The relative expression of target protein was calculated by the formula: target protein band gray value/GADPH gray value.

Hematoxylin and eosin (H&E) staining

Paraffin sections of pancreatic tissue were prepared for H&E staining (Liu et al. 2018). Schmidt pathology score method was used to assess damage of pancreatic tissue (Liu et al. 2017) (Table 1). The scoring process was as following: 5 fields were randomly selected from each section, which were indi-

Table 1. Histopathological scoring criteria of rat pancreas (Schmidt method)

	0	1	2	3	4
Edema	No	regional edema of lobular septa	diffused edema of lobular septa	acini swelling, diffused expansion of acinar septa	pulmonary lobules clearly separated
Inflammation	No	2–10 (white blood cell)/HPF	11–20/HPF	21–30/HPF	>30/HPF
Bleeding	No	0–25%	25–50%	50–70%	>75%
Necrosis	No	The surrounding of the gland	<20%	20–50%	>50%

HPF, high-power field.

vidually graded through the four aspects of edema, inflammation, bleeding and necrosis. Then, the average scores of the 5 fields were taken as the final score. The higher score stood for more severe damage of pancreatic tissue.

ELISA

TNF- α (tumor necrosis factor α), IL-6 (interleukin 6) and CRP levels in serum were separately detected using corresponding kits by the manufacturer's protocol (TNF- α : RAB0479, IL-6: RAB0311, or CRP kit: RAB0097. Sigma-Aldrich, Merck KGaA, Germany).

The detection of super oxide dismutase (SOD) and malondialdehyde (MDA)

The pancreatic tissue was homogenized and centrifuged to collect the supernatant. Oxidative stress parameters were evaluated by oxidative stress parameters (SOD and MDA). SOD levels were analyzed by total superoxide dismutase assay kit with nitro blue tetrazolium (NBT; S0109, Beyotime, Shanghai, China). MDA was detected by thiobarbituric acid method according to the manufacturer's protocol (S0131).

Tunnel staining

Paraffin section of pancreatic tissue was prepared for TUNNEL staining (MK1027, Boster, Wuhan, China). The paraffin section was deparaffinized using xylene I for 15 min and then using xylene II for 15 min. Afterwards, the section was in turn soaked into absolute alcohol for 20 min, 95% ethanol for 5 min, 90% ethanol for 5 min, 85% ethanol for 3 min and washed with distilled water for 2 min. DNase-free proteinase K (20 μ g/ml) was added to the sections and incubated with sections for 20 min at 20°C. Subsequently, the sections were washed with PBS for three times. The apoptotic cells in pancreatic tissue were stained using TUNNEL kit according to the manufacturer's protocol. The end of DNA 3-OH in apoptotic nucleus of pancreatic tissue was labeled by d UTP notch terminal labeling mediated by terminal deoxynucleotide

transferase. Furthermore, the apoptotic cells were labeled and displayed by *in situ* fluorescence. The apoptotic cells presented green fluorescence and all nucleated cells showed blue fluorescence after DAPI staining.

Cell line

Acinar cell necrosis was closely correlated with the development of AP. The rat pancreatic acinar AR42J cells were purchased from American type culture collection (YS-ATCC030, Manassas, VA, USA). Cells were cultured at RPMI-1640 medium containing 10% fetal bovine serum at 37°C with 5% CO₂. Cerulein (CR; 0.5 nM) and lipopolysaccharide (LPS; 1.0 μ g/ml) were used to culture AR42J cells for 12 h and establish AP cells model. The Control group was treated using equal DMSO. Modafinil was dissolved into DMSO. AR42J cells were pretreated with modafinil for 48 h at different concentrations (0.1, 1, 3 and 10 mM) separately.

Plasmid transfection

SNIP1 knockdown plasmids (shRNA-SNIP1-1, shRNA-SNIP1-2) and its negative control (shRNA-SNIP1-NC) were separately transfected into AR42J cells using Lipofectamine 2000 (Invitrogen, England) through the manufacturer's protocol (plasmid: GenePharma, Shanghai, China). Afterwards, the cells were cultured at 37°C, at 5% CO₂ for 24 h for further experiment.

CCK8 assay

Cells were seeded into 96-well plates with 1×10^4 cells in each. 10 μ l CCK8 solution was added into each well and incubated with cells for 1 h (Cusabio Biotech, Wuhan, China). The absorbance in 450 nm was detected using a Microplate Reader.

RT-qPCR

The total RNA was extracted using TRIzol lysate (Invitrogen, CA, USA). Then, we used PrimeScript RT Reagent Kit

Table 2. The score of pancreatic histopathological changes

Group	Time (h)	
	3	72
Sham	0.52 ± 0.19	0.18 ± 0.14
Modafinil	0.55 ± 0.17	0.16 ± 0.15
AP	4.41 ± 1.05**	11.65 ± 1.60**
AP+modafinil	3.42 ± 0.90**,#	7.12 ± 1.19**,#

Data are means ± SD. ** $p < 0.01$ vs. Sham group; # $p < 0.05$, ## $p < 0.01$ vs. AP group.

(RR047A, Takara, Japan) to reverse transcript total RNA into cDNA. PCR reaction system containing cDNA, SYBR Green mix and primers was performed using the 7500 Real-Time PCR Detection System. GAPDH was used as internal parameter. The relative expression of SNIP1 was calculated using $2^{-\Delta\Delta CT}$ method (Chen et al. 2013).

Flow cytometry

The cells were washed two times with PBS and then incubated with 5 μ l Annexin-V-FITC at room temperature in dark for 15 min. 2.5 μ l propidium iodide (PI) was added before detection. The flow cytometry was used to cell apoptosis levels. The fourth quadrant (Q4) of the scatter plot stood for health (FITC⁻/PI⁻) cells. The third quadrant (Q3) is viable apoptotic cell (FITC⁺/PI⁻) and the second quadrant (Q2) is non-viable apoptotic cell (FITC⁺/PI⁺). The cell apoptotic rate was calculated by the formula: cell apoptotic rate = Q3 + Q2 (Liao et al. 2019).

Statistical analysis

Statistical data was analyzed using GraphPad prism software 7.0. *In vivo*, the comparison among different groups was performed through two-way analysis of variance followed by Turkey's test. *In vitro*, comparison beyond two groups was performed with one-way analysis of variance followed by Turkey's test. Data was shown as mean ± standard deviation (SD). A value $p < 0.05$ was considered as statistically significant.

Results

Modafinil significantly decreased pancreatic damage in AP rats

In Sham group, H&E staining showed that pancreatic tissue was normal and pancreatic lobule was intact. There was no inflammatory cell infiltration, hyperemia and necrotic tissue in the interstitium. After 5% sodium taurocholate was retrograde injected into pancreatic duct of rats to establish AP rat model, many pancreatic acinar cells showed necrosis with fuzzy structure and fused into one piece in AP group. The structure of pancreatic lobule was blurred, along with inflammatory cells infiltration. After modafinil treatment, histologic assessment of pancreatic damage showed that cell necrosis, inflammatory cell infiltration and interstitial edema were significantly ameliorated in rats with acute pancreatitis comparing to AP group (Fig. 1A), along with significantly decreased ascites ($p < 0.01$, Fig. 1B). Schmidt pathology

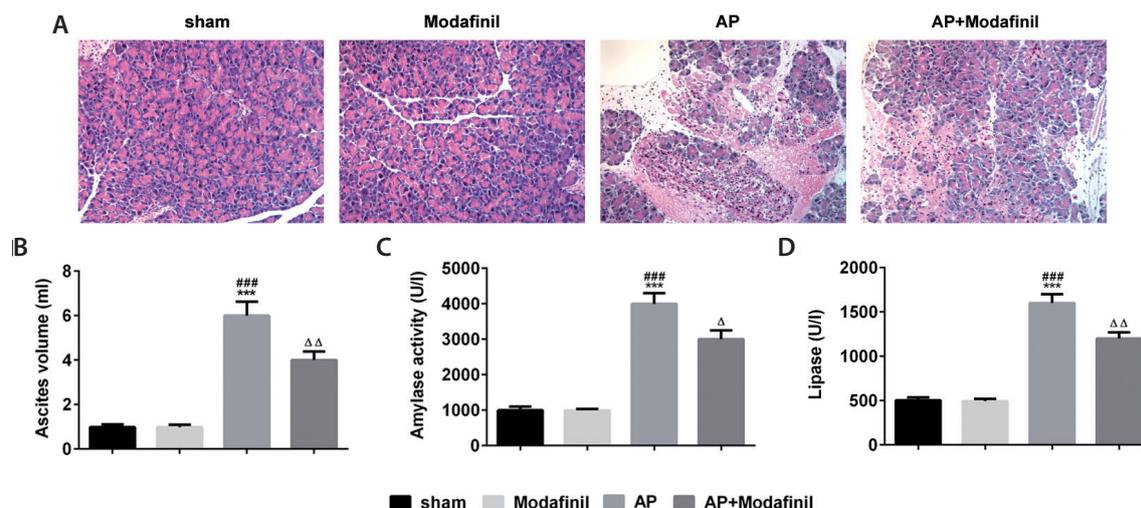


Figure 1. A. Pancreatic damage was detected by H&E staining. B. Modafinil significantly reduced ascites extracted with a 5 ml syringe in acute pancreatitis rats. C, D. Amylase and lipase levels in serum were measured through amylase and lipase assay kit in acute pancreatitis rats, respectively. All data are shown as mean ± SD, $n = 10$. *** $p < 0.001$ vs. Modafinil group; ### $p < 0.001$ vs. Sham group; Δ $p < 0.05$, $\Delta\Delta$ $p < 0.01$ vs. AP group. AP, acute pancreatitis.

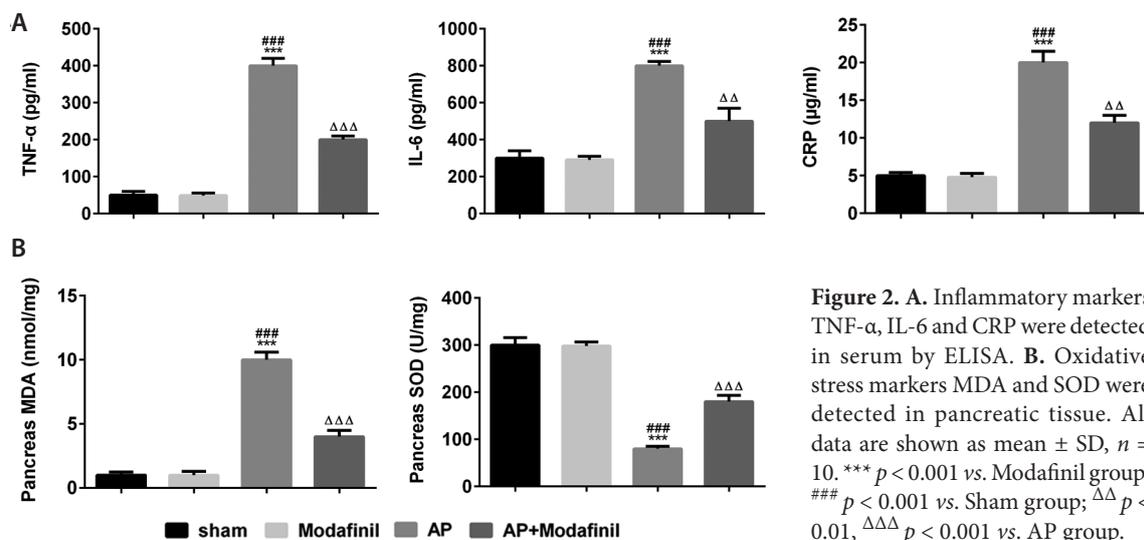


Figure 2. A. Inflammatory markers TNF- α , IL-6 and CRP were detected in serum by ELISA. B. Oxidative stress markers MDA and SOD were detected in pancreatic tissue. All data are shown as mean \pm SD, $n = 10$. *** $p < 0.001$ vs. Modafinil group; ### $p < 0.001$ vs. Sham group; $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.001$ vs. AP group.

score in AP group was markedly increased comparing to sham group. Besides, modafinil administration significantly reduced Schmidt pathology score in rats of AP+modafinil

group comparing to AP group ($p < 0.01$, Table 2). Amylase and lipase levels in serum also markedly were decreased by modafinil administration ($p < 0.05$, Fig. 1C, D).

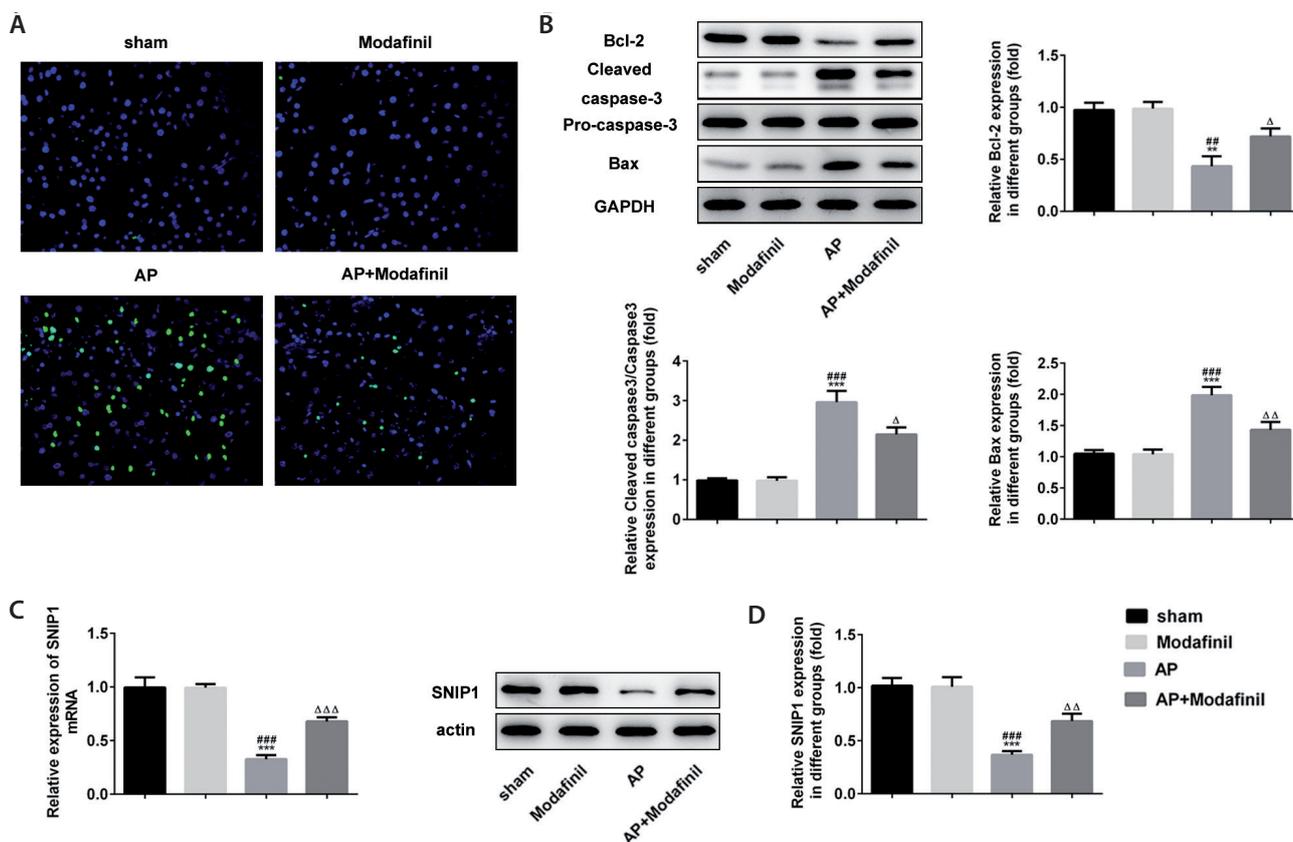


Figure 3. A. Apoptotic cells were detected in pancreatic tissue by tunnel staining. B. Apoptosis-related proteins were detected by Western blotting. SNIP1 expression was analyzed by qPCR (C) and Western blotting (D). All data are shown as mean \pm SD, $n = 10$. ** $p < 0.01$, *** $p < 0.001$ vs. Modafinil group; # $p < 0.01$, ### $p < 0.001$ vs. Sham group; Δ $p < 0.05$, $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.001$ vs. AP group.

Modafinil significantly reduced inflammation and oxidative stress in AP rats

To assess the inflammatory levels and oxidative stress in AP rats, inflammatory markers (TNF- α , IL-6, CRP) in serum and oxidative stress markers (MDA, SOD) were detected via specific kits in pancreatic tissue. The results revealed that AP-increased levels of TNF- α , IL-6 and CRP were markedly reduced when modafinil was administered; MDA was decreased and SOD was increased after administration of modafinil to AP rats ($p < 0.01$, Fig. 2A, B).

Modafinil significantly decreased apoptosis and increased SNIP1 levels

The cell apoptosis levels were evaluated by TUNNEL staining and apoptosis-related proteins levels in pancreatic tissue. Modafinil significantly reduced the number of apoptotic cells (showed green fluorescence) in acute pancreatitis rats (Fig. 3A). Simultaneously, anti-apoptosis protein (Bcl-2) levels were significantly increased, and pro-apoptosis proteins (Bax and cleaved caspase-3) levels were markedly decreased under modafinil treatment of AP rats ($p < 0.05$, Fig. 3B). The SNIP1 levels were also measured through qPCR and Western blotting. The results illustrated that SNIP1 levels were upregulated in rats of AP group. However, modafinil could distinctly down-regulated SNIP1 expression in AP rats ($p < 0.001$, Fig. 3C).

Modafinil dramatically increased cell viability and SNIP1 levels in CR and LPS-induced AR42J cells

Next step, whether modafinil had direct effects on AR42J cells *in vitro* were investigated. Modafinil treatment didn't affect the cell vitality of normal AR42J cells (Fig. 4A). Differently, modafinil obviously increased cell viability of AP cells in a dose-dependent manner ($p < 0.01$, Fig. 4B). Here, 10 nM of modafinil was used to perform further experiments. We found that SNIP1 expression was significantly decreased in AP cells, which could be significantly increased upon modafinil treatment ($p < 0.05$, Fig. 4C, D).

Modafinil lessened inflammation and oxidative stress through SNIP1 in CR and LPS-induced AR42J cells

SNIP1 knockdown plasmids (shRNA-SNIP1-1, shRNA-SNIP1-2) were separately transfected into different groups of cells. The results exhibited that the suppressive effect of shRNA-SNIP1-1 on SNIP1 expression was better than that of shRNA-SNIP1-2 ($p < 0.01$, Fig. 5A). Thus, shRNA-SNIP1-1 was selected for later experiments. Then, we analyzed the effects of modafinil on amylase, lipase, inflammation and oxidative stress in AP cells and explored its mechanism. SNIP1 knockdown significantly reversed the inhibitory effects of modafinil on amylase, lipase, inflammation response and oxidative stress in AP cells ($p < 0.05$, Fig. 5B, D).

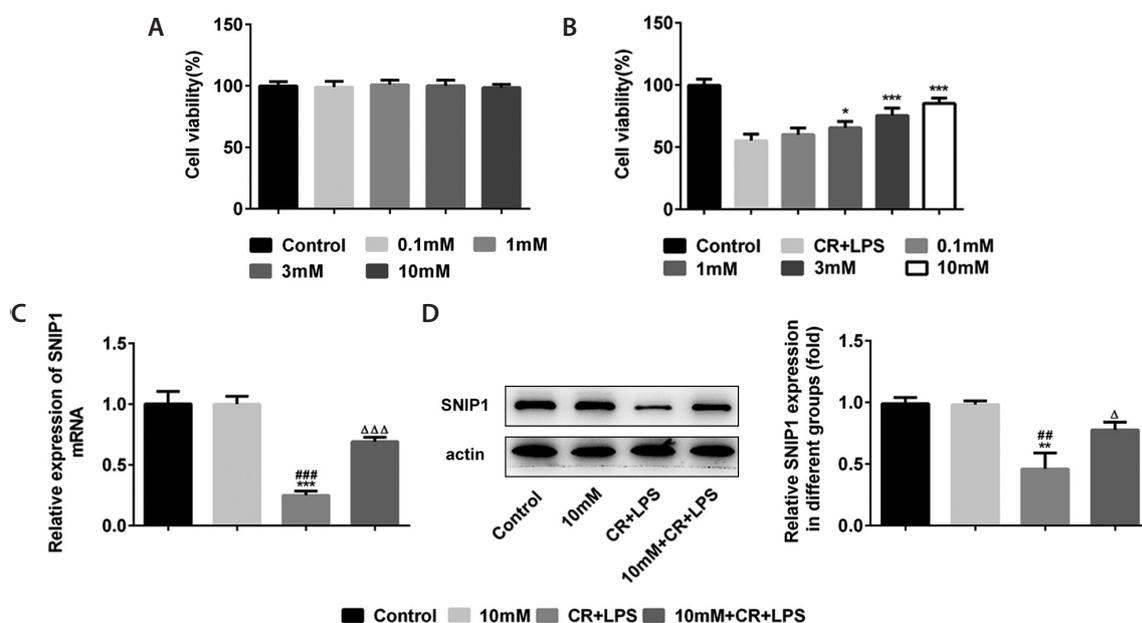


Figure 4. A. AR42J cells vitality was detected by CCK8 assay after modafinil (0.1, 1, 3 and 10 mM) treatment. B. Modafinil pretreatment (0.1, 1, 3, 10 mM) markedly increased cells vitality induced by cerulein (0.5 nM) and lipopolysaccharide (1.0 μ g/ml) (CR+LPS). * $p < 0.05$, *** $p < 0.001$ vs. CR+LPS group. C, D. Modafinil significantly recovered SNIP1 levels that were decreased by CR+LPS treatment. 10 mM, Control group treated using 10 mM modafinil. All data are shown as mean \pm SD, $n = 10$. ** $p < 0.01$, *** $p < 0.001$ vs. Control group; ## $p < 0.01$, ### $p < 0.001$ vs. 10 mM group; Δ $p < 0.05$, $\Delta\Delta\Delta$ $p < 0.001$ vs. CR+LPS group.

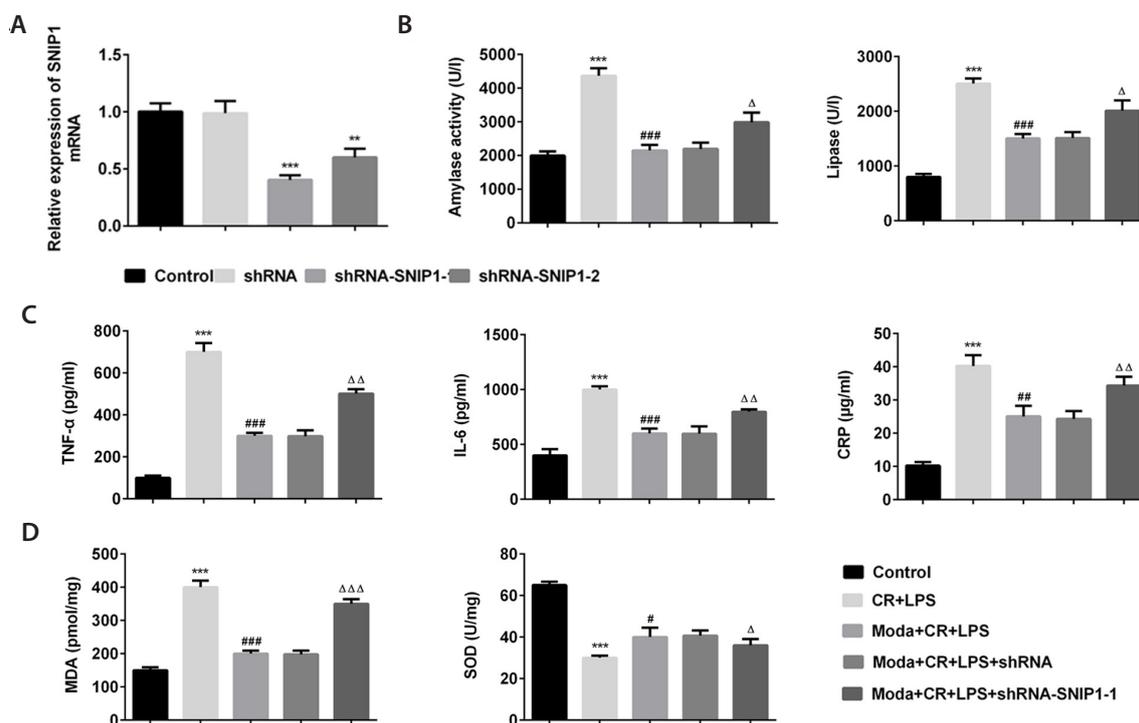


Figure 5. A. SNIP1 mRNA levels were detected by qPCR. ** $p < 0.01$ compared with shRNA-SNIP1-1, *** $p < 0.001$ vs. shRNA (expressing scrambled SNIP1). B. SNIP1 significantly affected the effects of modafinyl on amylase and lipase. C, D. SNIP1 significantly affected the effects of modafinyl on inflammation and oxidative stress. All data shown as mean \pm SD, $n = 10$. *** $p < 0.001$ vs. Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. CR+LPS group; Δ $p < 0.5$, $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.001$ vs. Moda+CR+LPS+shRNA group. CR+LPS, cerulein (0.5 nM) and lipopolysaccharide (1.0 μ g/ml); Moda, modafinyl. (For more details see Methods).

Modafinyl mitigated apoptosis through SNIP1 in CR and LPS-induced AR42J cells

The AP-induced apoptotic levels were significantly decreased by modafinyl treatment and these phenomena were significantly reversed after SNIP1 was silenced ($p < 0.01$, Fig. 6A). Besides, modafinyl decreased pro-apoptosis proteins, cleaved caspase-3 and Bax levels in AP cells, accompanied by increased anti-apoptosis protein Bcl-2. Simultaneously, the effects of modafinyl in AP cells could be reversed by decreasing endogenous expression of SNIP1 ($p < 0.05$, Fig. 6B, C). The result indicated that modafinyl might regulate mitochondrial-mediated apoptosis pathway through SNIP1.

Discussion

AP has been widely regarded as inflammation disorder and presents excess release of pro-inflammatory factors including IL-6, IL-8 and TNF- α (Pooran et al. 2003). In this study, modafinyl treatment significantly reduces IL-6, TNF- α and CRP levels in the serum of AP rats, replying that modafinyl might repress pro-inflammatory process to some extent. The

studies have demonstrated that inflammation response in AP could be regulated by p38 MAPK pathway or microRNA, which implied that modafinyl might also reduce inflammation through affecting aforementioned substance (Gu et al. 2019; Wan et al. 2019).

A recent study has shown that modafinyl can inhibit T helper 1 cells to mediate anti-inflammatory response in experimental autoimmune encephalomyelitis (Brandao et al. 2019). We discovered that the pathological damage was significantly improved by modafinyl in the present study. SNIP1 is demonstrated to mediate the inhibition of TGF- β downstream target genes, which can further ameliorate the pathological injury of pancreas and decrease amylase levels in AP (Tasci et al. 2019).

The primary substrate of caspase-3 is the poly (ADP-ribose) polymerase (PARP), which participates in DNA repair and gene integrity monitoring. The cell apoptosis induced by caspase-3 can be blocked by Bcl-2. Our study uncovered that modafinyl significantly inhibited pro-apoptotic proteins containing Bax and cleaved caspase-3 and enhanced anti-apoptotic protein Bcl-2. Furthermore, results of TUNNEL staining showed reduced apoptotic cells after modafinyl treatment. These data replied that apoptosis was

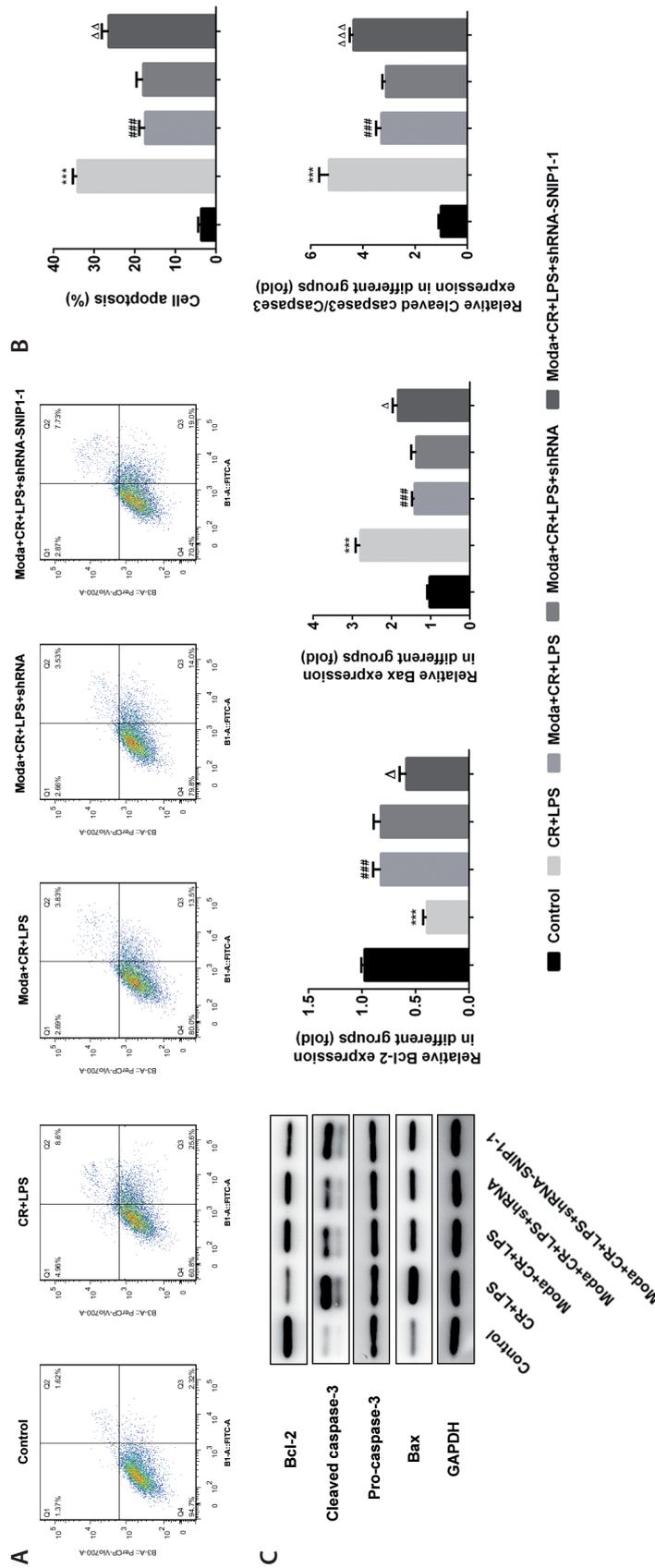


Figure 6. A, B. SNIP1 significantly affected the effects of modafinil on cell apoptosis. **C.** SNIP1 silence markedly reversed the effects of modafinil on apoptosis-related proteins in CR and LPS-stimulated AR42J cells. All data shown as mean \pm SD, $n = 10$. *** $p < 0.001$ vs. Control group; ## $p < 0.001$ vs. CR+LPS group; Δ $p < 0.5$, $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.001$ vs. Moda+CR+LPS+shRNA group. CR+LPS, cerulein (0.5 nM) and lipopolysaccharide (1.0 μ g/ml); Moda, modafinil. (For more details see Methods).

significantly hampered by administration of modafinil. In AP cell model, modafinil markedly reduced cell impairment, apoptosis, inflammation and oxidative stress *via* regulating SNIP1 levels. These findings replied that modafinil might inhibit TGF- β pathway, therefore regulating the pathway affecting cellular activities *via* SNIP1. However, this hypothesis needs further study. Although there is a controversy about the functions of TGF- β in apoptosis and inflammation in pancreas disease, more and more studies have indicated that the inhibition of TGF- β pathway reduces pancreas damage in AP (Zhou et al. 2019). Moreover, TGF- β , TNF- α and ROS levels mediate the progression of apoptosis. Finally, Bcl-2 was increased, along with decreased caspase-3 levels (Schuster and Krieglstein 2002).

A study has suggesting that modafinil significantly reduces MDA and NF- κ B levels in animal models and exerts protective effects (Yousefi-Manesh et al. 2019a, 2019b). The result was similar to the finding in our study. MDA and SOD levels are frequently used to evaluate the oxidative stress levels in AP (Tasci et al. 2019). The increased ROS production beyond anti-oxidative abilities mostly appears in activated neutrophils, which infiltrates pancreas, activates digestive enzymes in acinar cells and results in the self-digestion of pancreas (Tsai et al. 1998; Andican et al. 2005). In our study, MDA levels were decreased but SOD levels were increased, indicating that oxidative stress was decreased by modafinil treatment to some degree. In AP rat model, SNIP1 levels in pancreatic tissue were significantly down-regulated, as the GEO database showed. After modafinil treatment, the expression of SNIP1 was significantly recovered, along with decreased inflammation, oxidative stress and cell apoptosis. In AP cell model, the SNIP1 knockdown inhibited the protective effects of modafinil *via* repressing inflammation, oxidative stress and apoptosis. The results indicated that modafinil might develop significantly therapeutic effects by regulating SNIP1 levels. The study showed that modafinil ameliorated pancreatic injury and inflammation through upregulating SNIP1 through an *in vivo* and *in vitro* study. Modafinil could be a potential therapeutic drug in decreasing pancreatic injury in AP.

Conflict of interest. The authors declared no conflict of interest.

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