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Original Research Article

Abietic acid ameliorates pancreatic injury in acute pancreatitis by modulating MAPK pathway

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Abstract

Purpose: *To examine the effect of abietic acid (AA) in the treatment of acute pancreatitis (AP) in mice.*
Methods: *C57BL/6J mice were randomly assigned to 4 groups: sham, AP, AP + 20 mg/kg AA, and AP + 40 mg/kg AA groups. To induce AP mouse model, the mice received intraperitoneal (IP) injections of cerulein. The extent of pancreatic tissue damage was evaluated by histological examination. Serum ALT, lipase, and amylase levels were determined by commercial kits while TUNEL assay was used to assess the apoptosis of pancreatic cells. The contents of Bax, Caspase-3, Bcl-2, p-ERK/ERK, p-JNK/JNK, and p-P38/P38 in pancreatic tissues were evaluated by western blot while the contents of IL-6, TNF-α, IL-1β, nitrite, MDA, and GSH in the tissues were evaluated by enzyme-linked immunosorbent assay (ELISA) kits.*
Results: *AA relieved pancreatic damage and reduced ALT, lipase, and amylase levels in cerulein-treated AP mouse ($p < 0.001$). AA repressed apoptosis of pancreatic cells in cerulein-induced AP mouse, and inhibited oxidative stress and inflammatory response in the mice by reducing IL-6, TNF-α, IL-1β, nitrite, and MDA contents; it also enhanced the levels of GSH in the tissues ($p < 0.001$). In addition, AA inhibited MAPK pathway activity in the mice ($p < 0.001$).*
Conclusion: *AA ameliorates pancreatic damage, pancreatic cell apoptosis, oxidative stress, and inflammation in cerulein-induced AP mouse by inhibiting MAPK pathway. This study offers a new potential drug for the management of AP and expands the relevant molecular regulatory mechanisms.*

Keywords: *Abietic acid, Pancreatic injury, Acute pancreatitis, MAPK pathway, Cerulein*

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INTRODUCTION

Acute pancreatitis (AP) is an illness caused by acute inflammation of pancreatic tissue, which often results in damage to the pancreatic tissue and an inflammatory response that can cause symptoms such as abdominal pain, fever, nausea, vomiting, and diarrhea [1]. The symptoms of AP include sudden abdominal pain, nausea, vomiting, diarrhea, fever, and others. Severe AP cases may result in serious complications such as pancreatic necrosis, bleeding, infection, and organ failure [2]. Therefore, timely treatment of AP patients is very important. However, current clinical therapeutic drugs have limited effects and therefore, there is

a need to discover more operative management methods.

Chinese herbs have been performed to treat human sicknesses for thousands of years, and have recently attracted increasing attention in drug research [3,4]. Abietic acid (AA) is a natural organic compound belonging to the class of abietane diterpenes. It is commonly found in coniferous trees, particularly in the resin of pine trees [5]. AA is a colorless to light yellow crystalline solid with a characteristic odor, and has potential applications in the medical field. Studies have shown that AA has anticancer effects, which can impede tumor cell growth and stimulate tumor cell apoptosis [5]. Besides, AA has a certain anti-inflammatory effect, which can relieve inflammation and pain [6]. AA also regulates the immune system, promotes the proliferation and activity of immune cells, and helps to improve the body's immunity [6]. The application of AA in the medical field still requires further research and exploration. This study, however, hypothesizes that AA may ameliorate pancreatic injury in AP.

To confirm this hypothesis, an AP mouse model was built by cerulein induction.

EXPERIMENTAL

Animals

Laboratory mice of the C57BL/6J breed (25 - 30 g; male; 8 - 10 weeks old) were gotten from Shanghai Laboratory Animal Company (SLAC, Shanghai, China). They were reared adaptively in a specific pathogen-free animal facility for one week and exposed to water and food at random throughout the research. All animal tests were implemented consistent with the Guidelines for the Care and Use of Laboratory Animals [7]. The procedure for the mice tests was permitted by the Ethics Committee of Lishui People's Hospital, Lishui, China (approval no. 2022002).

Cerulein-induced AP mouse model

All 24 C57BL/6J mice were randomly placed in 4 groups (6 mice per group): sham, AP, AP + 20 mg/kg AA, and AP + 40 mg/kg AA groups. Cerulein (Solarbio, Beijing, China) and AA (Solarbio) was dissolved in normal saline (Solarbio). To establish the AP mouse model, the mice in AP, AP + 20 mg/kg AA, and AP + 40 mg/kg AA groups received intraperitoneal (IP) injections of cerulein (50 μg/kg). Cerulein treatment was administered twice a day (10 h interval) for 7 consecutive days. Sham group mice were injected IP with an equivalent volume of normal saline. Mice in AP + 20 mg/kg AA and AP + 40 mg/kg AA groups were pre-treated with AA orally for 7 days (20 and 40 mg/kg) prior to cerulein treatment. Meanwhile, the mouse in AP group received the same amount of normal saline orally. All the mice were anesthetized utilizing isoflurane (Solarbio), and sacrificed via CO_2 inhalation at 8 h after the last cerulein injection. Pancreatic tissue and serum from the mice were gleaned for subsequent experiments.

Histological examination

The pancreatic tissues were fixed with buffered formalin (10 %; Solarbio) for 24 h and immersed in paraffin (Solarbio). Following this, the samples were sectioned (5 μm) and treated with hematoxylin and eosin (H&E, Solarbio). Histological inspection was implemented through a blinded observer utilizing scoring systems adapted from earlier procedures [8]. The images were obtained utilizing a light microscope (Leica, Wetzlar, Germany) at ×100 magnification.

Determination of ALT, lipase, and amylase levels

ALT, lipase, and amylase concentrations in serum samples were determined using an ALT assay kit (ab241035; Abcam, Cambridge, MA, USA), lipase assay kit (ab102524; Abcam), and amylase assay kit (ab102523; Abcam), following the kit manufacturers' protocol.

TUNEL assay

The pancreatic sample sections (5 μm) were deparaffinized using xylene (Solarbio) twice, and then dehydrated using ethyl alcohol (Solarbio). Following this, the proteins were digested with protease K (Solarbio). Thereafter, TUNEL in Situ Cell Death Detection Kit (Solarbio) was employed to appraise cell apoptosis. The samples were treated with TUNEL solution for 1 h in the dark, and then exposed to antifade reagent. The images were monitored through a fluorescence microscope (Leica) at ×200 magnification.

Western blot

The pancreatic samples were reaped and lysed in RIPA buffer (Solarbio). The lysates were centrifuged and the supernatant collected. The denatured proteins were detached using SDS-PAGE, then transferred to PVDF membranes (Solarbio). After blocking, the antibodies against Bax (ab32503; 1:1000; Abcam), Bcl-2 (ab182858; 1:1000; Abcam), Caspase-3 (ab184787; 1:1000; Abcam), p-ERK (ab229912;

1:1000; Abcam), ERK (ab32537; 1:1000; Abcam), p-JNK (ab307802; 1:1000; Abcam), JNK (ab199380; 1:1000; Abcam), p-P38 (4511; 1:1000; CST, Danvers, Massachusetts, USA), P38 (54470; 1:1000; CST), and anti-β-actin (ab8226; 1:1000; Abcam) were utilized. The next day, the membranes were treated with goat anti-rabbit IgG (ab205718; 1:2500; Abcam, Cambridge, MA, USA) for 1 h. The protein signals were appraised via an ECL-Plus kit (Invitrogen, Carlsbad, CA, USA) and protein quantification was carried out with ImageJ software.

Enzyme-linked immunosorbent assay (ELISA)

The mouse IL-6 ELISA kit (ab222503; Abcam), mouse TNF-α ELISA kit (ab208348; Abcam), mouse IL-1β ELISA kit (ab197742; Abcam), Griess Reagent Nitrite Measurement Kit (13547; CST), mouse MDA ELISA kit (ab118970; Abcam), and mouse GSH ELISA kit (ab112132; Abcam) were employed to appraise the IL-6, TNF-α, IL-1β, Nitrite, MDA, and GSH contents in pancreatic tissues in line with the kit manufacturer's protocol.

Statistical analysis

Statistical analysis was conducted utilizing SPSS 22.0 (SPSS Inc, Chicago, IL, USA). All tests were repeated three times, and the data expressed as mean ± SD. Paired or multiple comparison was implemented using Student's *t*-test or analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

AA relieved pancreatic damage in AP mouse

Histological examination of the pancreatic tissues confirmed that acinar cell vacuolation, focal acinar necrosis, interlobular and intraclobular edema, as well as perivascular and rare diffuse infiltration of the inflammatory cells were observed in the AP group mice versus the sham group (Figure 1 A). Compared with AP group, acinar cell vacuolation, tissue edema, and inflammatory cell infiltration were diminished in group AP + 20 mg/kg AA mice. The alleviative effect of 40 mg/kg AA treatment on pancreas injury was more obvious than that of AP + 20 mg/kg AA group (Figure 1 A). Moreover, the histopathological score was enhanced in the AP group, but reduced by AA management in a dose-dependent mode (Figure 1 B). Besides, the ALT, lipase, and amylase levels in the serum of mice were boosted in the AP group, but abridged by AA management in a dose-dependent mode (Figure 1 C). Therefore, the result confirmed that AA relieved pancreatic damage in the cerulein-induced AP mouse.

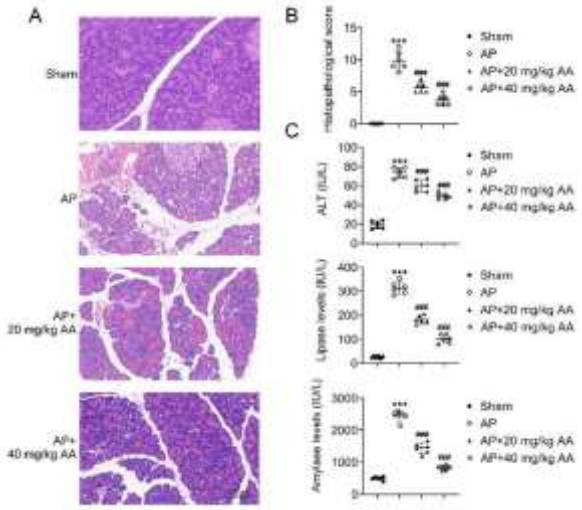

Figure 1: AA relieved pancreatic damage in AP mouse. (A) Images of H&E-stained pancreatic tissues section. Scale bar: 200 μm; (B) Histopathological score. (C) The ALT, lipase, and amylase levels were examined by commercial kits. Compared with sham group, $^{***}p < 0.001$; compared with AP group, $^{\#\#\#}p < 0.001$

AA inhibited apoptosis of pancreatic cells in AP mouse

Here, the influence of AA on apoptosis of pancreatic cells was inspected. The consequences presented that the apoptosis of the pancreatic cells increased in the AP group, was reduced by AA management in a dose-dependent mode (Figure 2 A). Next, the apoptosis-linked protein levels in the pancreatic tissue were examined. The Bax and Caspase-3 contents were enhanced, but the Bcl-2 content was reduced in pancreatic tissues after cerulein induction, while this influence was abridged by AA management in a dose-dependent mode (Figure 2 B). Therefore, the result illustrated that AA repressed the apoptosis of pancreatic cells in cerulein-induced AP mouse.

AA inhibited oxidative stress and inflammatory response in AP mouse

In this part, the influence of AA on oxidative stress and inflammatory response of pancreatic cells was analyzed. The result confirmed that the levels of proinflammatory factors (IL-6, TNF-α, and IL-1β) in pancreatic tissues were boosted after cerulein induction, while this influence was abridged by AA management in a dose-dependent manner (Figure 3 A).

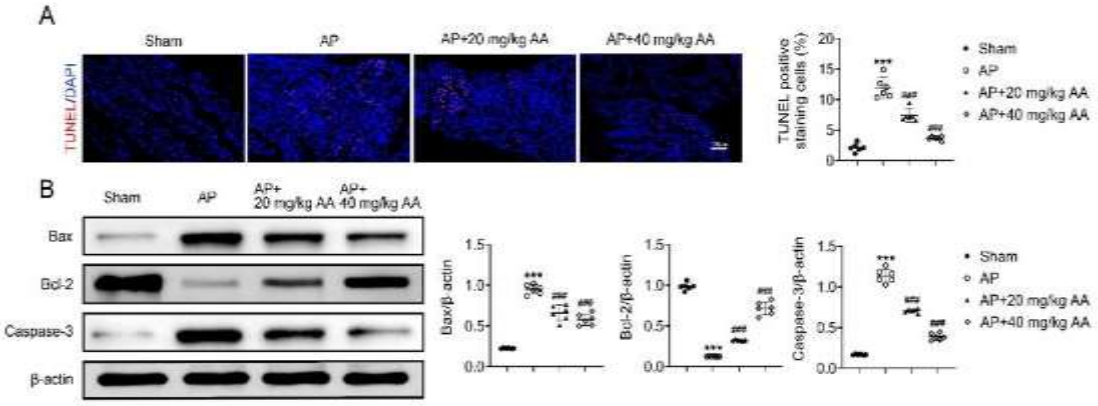

Figure 2: AA inhibited apoptosis of pancreatic cells in AP mouse. (A) Apoptosis of pancreatic cells; (B) Levels of Bax, Caspase-3, and Bcl-2. Compared with sham group, ***$p < 0.001$; compared with the AP group, $^{###}p < 0.001$

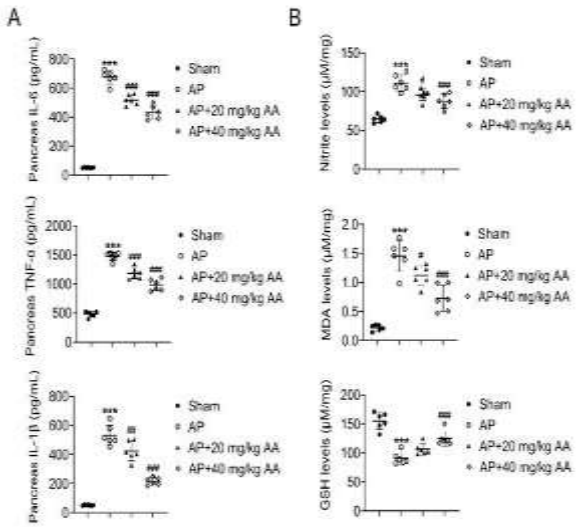

Figure 3: AA inhibited oxidative stress and inflammation in AP mouse. (A) Concentrations of IL-6, TNF-α, and IL-1β in pancreatic tissues. (B) Nitrite, MDA, and GSH levels in pancreatic tissues. Compared with sham group, ***$p < 0.001$; compared with AP group. $^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$

Additionally, the nitrite and MDA levels were increased, but the GSH level reduced in pancreatic tissues, while this influence was abrogated by AA management in a dose-dependent mode (Figure 3 B). These outcomes revealed that AA inhibited oxidative stress and inflammatory response in cerulein-tempted AP mouse.

AA inhibited MAPK pathway activity in AP mouse

The signaling pathways regulated by AA in AP mouse was revealed. The levels of the MAPK pathway linked factors in pancreatic tissues were determined. The contents of p-ERK/ERK, p-JNK/JNK, and p-P38/P38 levels in the pancreatic tissues were boosted in the AP group, but dwindled via AA management in a dose-dependent method (Figure 4). Hence, the result established that AA inhibited the MAPK pathway activity in cerulein-tempted AP mouse.

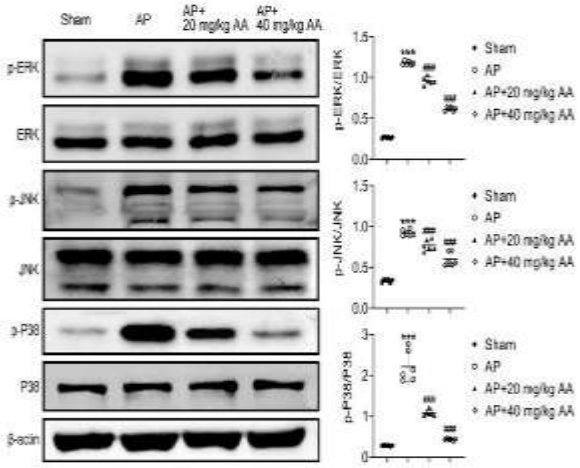

Figure 4: AA inhibited MAPK pathway activity in AP mouse. The p-ERK/ERK, p-JNK/JNK, and p-P38/P38 levels in pancreatic tissues. Compared with sham group, ***$p < 0.001$; compared with AP group, $^{###}p < 0.001$

DISCUSSION

AP is an acute abdominal illness with a mortality rate of 9-24 %, which can be increased to 47 - 69 % in patients with multiple organ dysfunction syndrome due to multiple organ failure of the lungs, liver, kidneys, and intestines [2]. Treatment of AP typically includes rest, fasting, fluid replacement, and medication. In severe cases, surgery may be necessary to remove necrotic tissues, drain fluids, or remove a portion of the pancreas. Preventive measures for AP include reducing alcohol consumption, quitting

smoking, weight control, eating a healthy diet, avoiding high-fat diets, and reducing overeating and other unhealthy habits [9,10].

Currently, there are several types of drugs commonly used in the treatment of AP [11]. They include analgesics such as opioid analgesics (e.g., fentanyl, etc.) and anti-inflammatory drugs (e.g., ibuprofen, naproxen, etc.), used to relieve severe pain caused by pancreatitis [11]; enzyme inhibitors including pancreatic enzyme inhibitors which reduce the activity of enzymes secreted by the pancreas, thereby reducing self-digestion and inflammation; and antibiotics; for patients with concurrent infections, broad-spectrum antibiotics such as ceftriaxone, fluoroquinolones, etc, can be used to prevent or treat secondary infections; Other drugs, such as cholinergic receptor antagonists, proton pump inhibitors, etc, can be used to control symptoms such as nausea, vomiting, and gastrointestinal reactions [11]. However, these drugs can only relieve the symptoms of AP patients, but not the pancreatic damage. Therefore, in this paper, the functions of AA in AP were examined.

AA is a natural product extracted from turpentine, and it possesses lots of biological properties, like antibacterial, anti-inflammatory, anti-tumor and so on. Besides, AA inhibits the growth of different tumor cells, like breast cancer and lung cancer [5]. AP sufferers have severe abdominal pain and elevated blood amylase, lipase, and ALT [12]. In the present study, the results confirmed that AA relieved pancreatic impairment and abridged the contents of ALT, lipase, and amylase in cerulein-tempted AP mice. These consequences pointed out that AA has the potential to treat AP. Wahab *et al* [6] revealed that AA mitigated the advancement of diabetic nephropathy by inhibiting oxidative stress, inflammation and apoptosis. In this study, it was also discovered that AA repressed apoptosis of pancreatic cells in cerulein-induced AP mouse, and this was comparable to the findings of Wahab *et al* [6].

Systemic inflammation increases the burden on organs and aggravates the severity of AP, bringing a second blow to patients. In addition, intracellular ROS accumulation also aggravates AP injury [13]. AA has anti-inflammatory effects and inhibits various inflammatory reactions, such as skin inflammation and rheumatoid arthritis [6]. Kang *et al* [14] confirmed that AA repressed IL-1β-tempted inflammatory response in osteoarthritis chondrocytes. Moreover, Yuan *et al* [15] illustrated that AA inhibited acetaminophen-tempted liver damage by lessening inflammation. In present study, it was found that AA inhibited oxidative stress and inflammatory reaction in cerulein-tempted AP mouse by reducing IL-6, TNF-α, IL-1β, nitrite, and MDA levels, and enhanced the level of GSH in pancreatic tissues. These conclusions were comparable with the findings of Wahab *et al* [6] and Kang *et al* [14] and Thummuri *et al* [16] who found that AA inhibits the activity of MAPK pathway. Inhibiting the activity of MAPK pathway inhibits the inflammatory response and apoptosis induced by cerulein and alleviates pancreatic injury [17,18]. The present study also found that AA inhibited the activity of MAPK pathway in cerulein-induced AP mouse, and this corroborates the findings of Thummuri *et al* [16]. Thus, it has been discovered in this work, for the first time, that AA has a therapeutic effect on AP.

Limitations of this study

There are some limitations in this research, however. The experiment was conducted on a mouse model, and the results obtained need to be further validated in clinical trials. A clinical trial is planned to further study the therapeutic effects of AA on AP.

CONCLUSION

AA mitigates pancreatic damage, pancreatic cells apoptosis, oxidative stress, and inflammation in cerulein-induced AP mouse by regulating MAPK pathway. The findings of this study indicate that AA is a potential drug for the management of AP.

DECLARATIONS

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None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hailin Ye and Debiao Pan designed the study and carried them out; Hailin Ye, Jun Wang, Jiaodan Mao supervised the data collection, analyzed and interpreted the data; Hailin Ye and Debiao Pan prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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