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Inhibition of macrophage migration inhibitory factor attenuates inflammation and fetal kidney injury in a rat model of acute pancreatitis in pregnancy

Man Li[a,1], Jia Yu[a,1], Liang Zhao[a,b], Fang-chao Mei[a], Yu Zhou[a,b], Yu-pu Hong[a], Teng Zuo[a], Wei-xing Wang[a,*]

[a] Department of General Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, China
[b] Central Laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, China

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ABSTRACT

Acute pancreatitis in pregnancy (APIP) is a severe disease during pregnancy that mostly occurs during the third trimester. It can lead to additional complications including preterm delivery and high fetal mortality. In this study, we investigated the protective effects of (*S*, *R*)-3-(4-hydroxyphenyl)-4, 5dihydro-5-isoxazole acetic methyl ester (ISO-1), an inhibitor of macrophage migration inhibitory factor (MIF), on fetal kidney injury associated with the maternal acute necrotizing pancreatitis (ANP) and its potential mechanisms in a rat model. The APIP rat model was induced by retrograde infusion of sodium taurocholate saline solution into biliopancreatic duct. ISO-1 was given by intraperitoneally injection 30 min before the model was induced. The levels of maternal serum amylase, lipase, tumor necrosis factor-α (TNF-α) and interleukins (IL)-1β were measured. Maternal pancreas and fetal kidney injury were evaluated, and the expressions of MIF, phospho-p38MAPK (p-p38), nuclear factor-κB (NF-κB), TNF-α, IL-1β in fetal kidneys were detected. The results showed that fetal rats exhibited obvious acute kidney injury during APIP, and pregnant rats pretreated with ISO-1 notably attenuated the lesions. ISO-1 also significantly reduced the expression of MIF and the activations of p38MAPK, NF-κB, as well as the levels of TNF-α and IL-1β. These results indicated that ISO-1 could attenuate fetal kidney injury in pregnant rats with ANP by inhibiting MIF mediated p38MAPK/NF-κB signal pathways to reduce inflammatory response.

1. Introduction

Acute pancreatitis in pregnancy (APIP) can result in a high incidence of fetal risks, including preterm labor, small for gestational age, and intrauterine fetal death [1,2]. In recent years, the situation has been improved with the progressive management in specialized neonatal intensive care units and the progress in earlier diagnosis. However, the mortality rate for fetuses still reaches up to 18% [3]. In consideration of the protection for fetus, drug usage is limited in expectant mother and new emerging therapeutic strategies without adverse effects on the fetus should be recommend in APIP.

Acinar injury due to autodigestive processes stimulates an inflammatory response with infiltration of neutrophils and macrophages, and release of cytokines TNF-α and IL-1β, 6, 8 [4]. It will lead to a systemic inflammatory response syndrome (SIRS) if the inflammatory reaction is marked, which can finally lead to distant organ damage and multiple organ dysfunction syndrome [5]. Macrophage migration inhibitory factor (MIF) is a kind of protein that has a broad range of immunomodulatory functions. It is an important inflammatory regulator mainly produced and stored in macrophages and T cells which can induce the release of inflammatory mediators when subjected to stress and then promote inflammatory response [6]. Researches have shown that MIF is involved in the pathogenesis of various inflammatory disease, including sepsis and pancreatitis [7,8]. ISO-1 is an inhibitor of MIF that can suppress the pro-inflammatory activities by targeting and binding to the tautomerase active site. Treated with ISO-1 intraperitoneally dose-dependently inhibited the release of TNF-α and the activation of NF-κB induced by endotoxin in severe sepsis mice model [9]. In previous study, we have found that ISO-1 can suppress inflammatory response and attenuates maternal lung injury in rats with

* Corresponding author at: Department of General Surgery, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan 430060, Hubei Province, China.
E-mail addresses: liman192@whu.edu.cn (M. Li), yogaqq116@whu.edu.cn (J. Yu), zlnet@whu.edu.cn (L. Zhao), zhouyu0723@whu.edu.cn (Y. Zhou), hongyupu@whu.edu.cn (Y.-p. Hong), sate.llite@163.com (W.-x. Wang).

[1] These authors contributed equally to this work.

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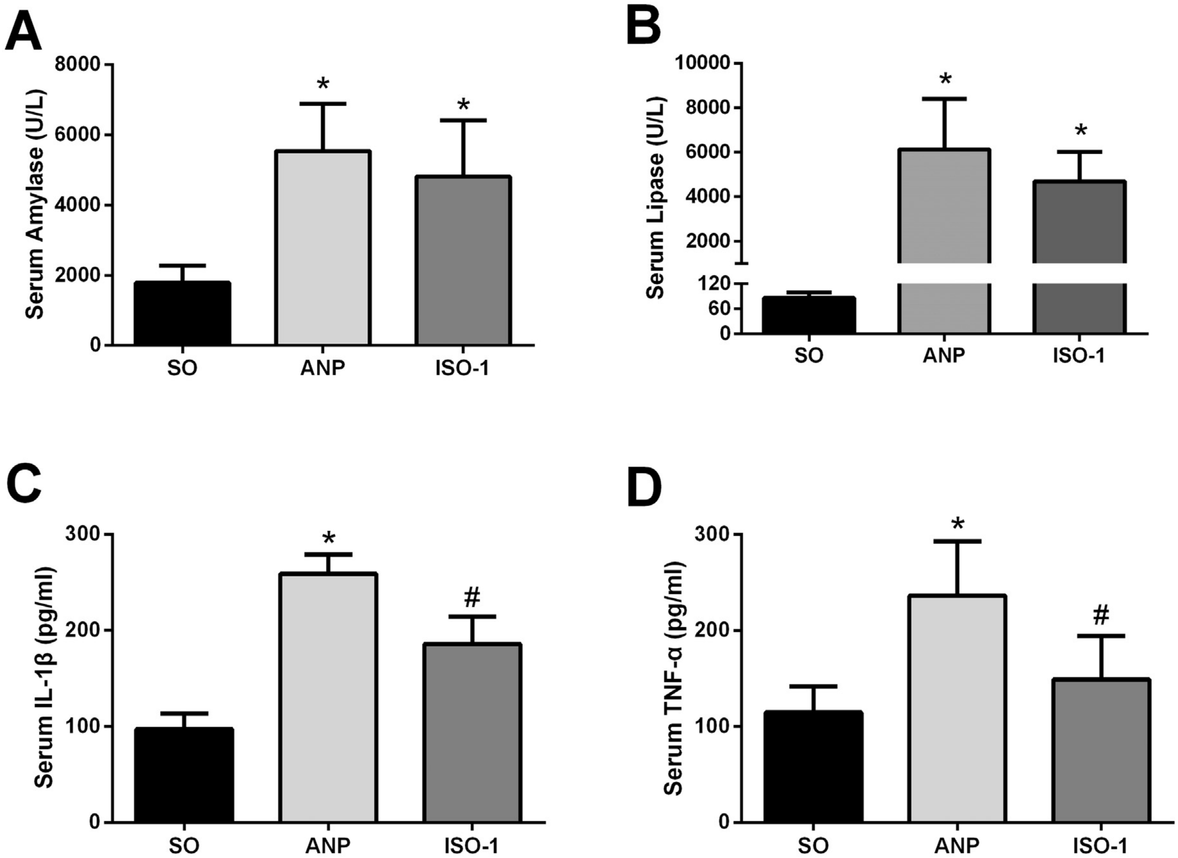

Fig. 1. The levels of maternal serum pancreatic enzymes and cytokines. A: maternal serum amylase; B: maternal serum lipase; C: serum level of IL-1β; D: serum level of TNF-α. $^{*}P < 0.05$ *vs* SO group, $^{\#}P < 0.05$ *vs* ANP group (SO: sham operation group; ANP: acute necrotizing pancreatitis group; ISO-1: ANP + ISO-1 group).

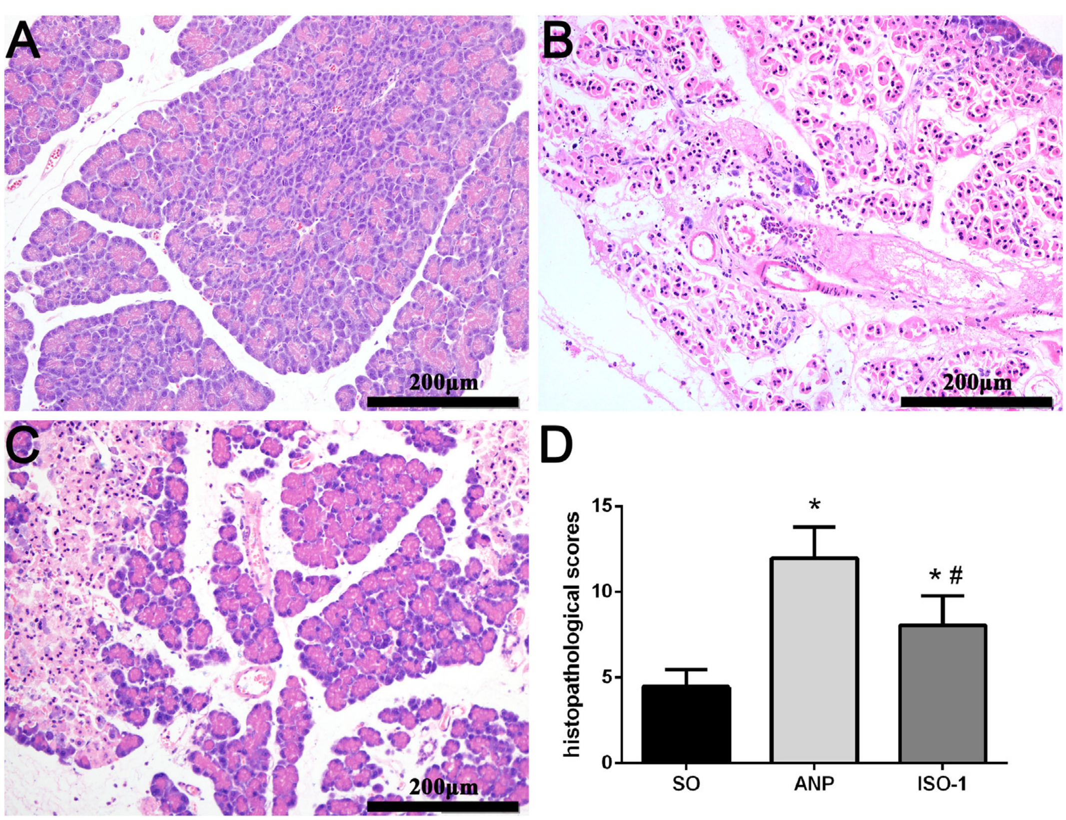

Fig. 2. Pathologic changes of maternal pancreas and the histopathological scores. A: SO group; B: ANP group; C: ISO-1 group; D: $^{*}P < 0.05$ *vs* SO group, $^{\#}P < 0.05$ *vs* ANP group. (H&E staining, original magnification: ×200).

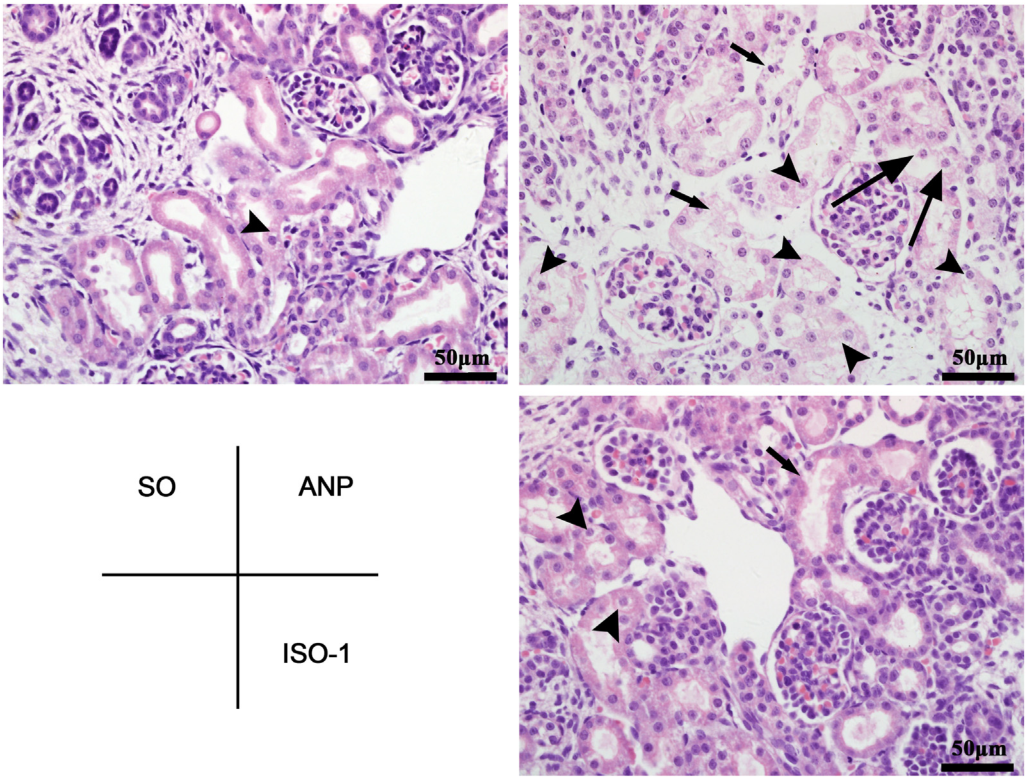

Fig. 3. Histopathological changes of fetal kidney tissues in each group. ANP group showed cytoplasmic vacuolation of tubular cells (arrowheads), desquamation of tubular epithelial cells (long arrows), and destruction of tubular architecture (short arrows); ISO-1 group showed mild vacuolation destruction of tubular architecture. (H&E staining, original magnification, ×400).

acute pancreatitis in pregnancy [10].

During pregnancy, fetuses can mount an immune response while preserving self-tolerance through intertwined mechanisms. Inflammatory cells or cytokines will transfer to the uteroplacental circulation then introduce a fetal inflammatory response, and it could be induced when fetuses suffer different insults [11,12]. Fetal kidneys are important organs associated with the growth environment and fetal metabolism, especially the production of urine and amniotic fluid. Infants and neonates suffered from systemic inflammation are thought to be at high risk of acute kidney injury (AKI) and associated with poor long-term outcomes [13]. Neonates with AKI have hypofunction of the tubular resulting in derangements of waste products such as serum creatinine [14], and even small changes in serum creatinine will increase the morbidity and mortality [15,16]. Therefore, it is of great importance to focus on the fetal acute kidney injury during maternal ANP and explore the protective measures or effective drugs for fetuses at the risk of AKI.

However, few studies have assessed fetal kidney injury induced by ANP in pregnancy and whether MIF inhibition by ISO-1 has protective effects has not been elucidated. In this study, based on the previous research [17], our aim was to explore the role MIF played and the effects of ISO-1 on inflammatory response as well as fetal kidney injury during the process of APIP.

2. Materials and methods

2.1. Reagents

Sodium taurocholate (STC) and dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich Corp (St. Louis, Mo, USA). The MIF inhibitor ISO-1 was purchased from Santa Cruz Biotechnology (CA, USA) and was dissolved in vehicle (5% DMSO) before administration. Isoflurane was from Abbott Laboratories (Shanghai, China). The MIF (Abcam Plc, Cambridge, UK), p38, p-p38 (CST, USA) antibodies were obtained for western blotting. β-Actin antibody was purchased from Abcam Plc (Cambridge, UK). Immunohistochemistry (IHC) was done with the following primary antibodies: NF-κB (CST, USA), TNF-α (Abcam Plc, Cambridge, UK) and IL-1β (Santa Cruz Biotechnology, USA).

2.2. Animals and experimental protocol

Twenty-four SPF pregnant Sprague-Dawley rats (gestation days, 17–19, weighing 370–450 g) were purchased from the experimental animal center of Wuhan University (Wuhan, China), housed in a temperature, humidity, and light controlled environment and fed a standard chow and water for 3 days. The study was approved by the Committee on Ethics of Animal Experiments of Renmin Hospital of Wuhan University (WDRM-20150606) and performed in compliance with the NIH standards (Guide for the Care and Use of Laboratory Animal, National Institutes of Health, revised 2011). A total of 24 rats were randomly assigned to three groups: (1) sham operation group (SO group, n = 8), (2) acute necrotizing pancreatitis group (ANP group, n = 8), (3) ANP + ISO-1 group (ISO-1 group, n = 8). Rats were fasted for 12 h before the modeling and anesthetized with isoflurane (induced with 4% isoflurane in 1.5 L/min oxygen in a sealed container and maintained with 2%–3% isoflurane in 1.5 L/min oxygen during surgery). ANP model was induced by retrograde infusion of 5% STC saline solution (0.6 mL/kg body weight) into biliopancreatic duct over 2 min while rats in the SO group underwent the same procedures without infusion into the duct. The ANP + ISO-1 group was given ISO-1 solution (3.5 mg/kg) by intraperitoneal injection 30 min before the ANP model was induced. All rats were given a saline (20 mL/kg body weight) injection subcutaneously for fluid loss after operation. Twelve hours after ANP induction or sham surgery, the rats in each group were re-anesthetized and euthanized.

2.3. Collection and analysis of maternal serum samples

Rats were laparotomized and fetal rats were obtained by cesarean section. Blood samples of pregnant rats were collected *via* postcava puncture and then centrifuged at 1500g for 10 min, and the supernate

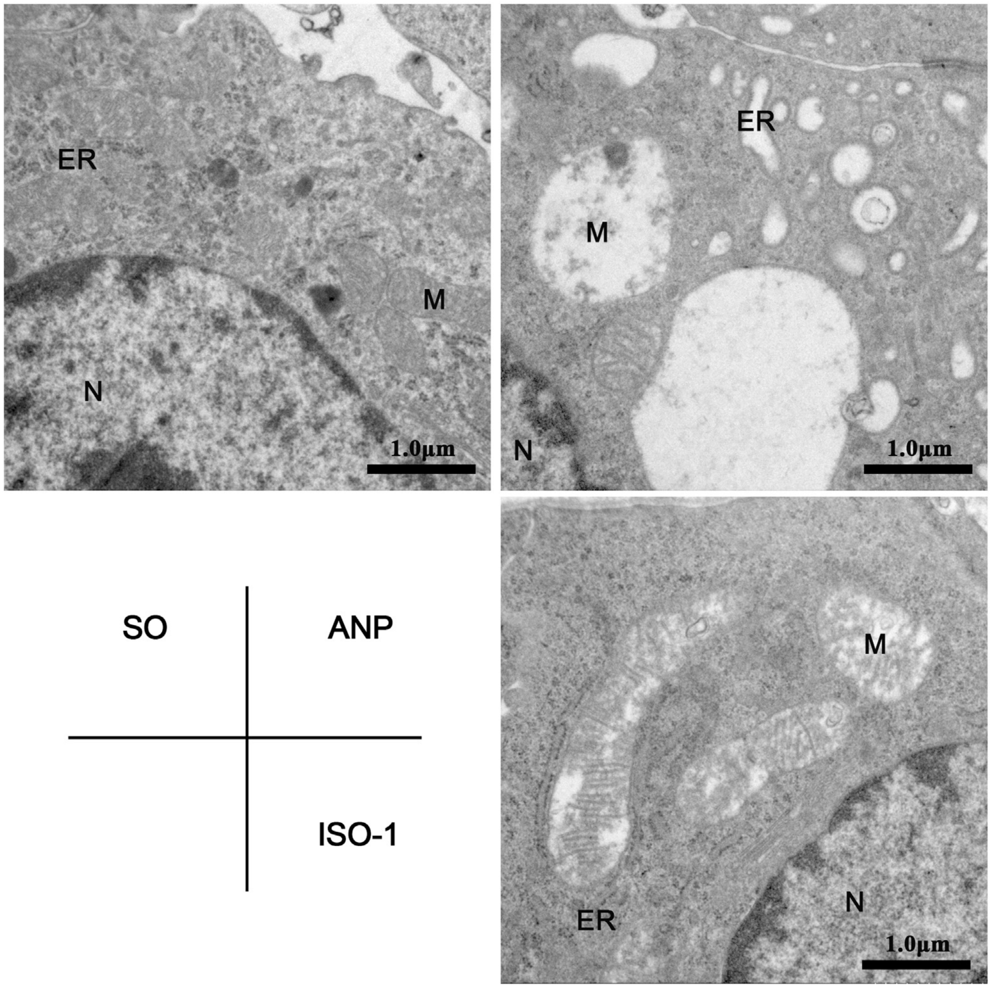

Fig. 4. Ultrastructural changes of tubular cells in fetal kidney of each group. ANP group showed severely dilated endoplasmic reticula and swollen mitochondria; ISO-1 group showed the mild dilatation of the endoplasmic reticulum and decrease of mitochondria damage. (N: nucleus; M: mitochondria; ER: endoplasmic reticulum; original magnification, ×5000).

was separated and stored at −80 °C until assay. Maternal serum amylase (AMY), lipase (LIP) levels were measured using computerized procedures by automatic biochemical analyzer (Olympus AU680; Olympus Diagnostics, Japan). The serum concentrations of TNF-α and IL-1β were detected with corresponding enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, China) according to the manufacturer's protocols, and all procedures were duplicated 3 times.

2.4. Histological evaluation

Pancreas and fetal kidneys were collected carefully and moved into the 4% paraformaldehyde immediately to fix for 24 h. Tissues were embedded in paraffin and cut into sections (4 μm) which were processed for hematoxylin and eosin (H&E) staining. H&E sections were evaluated by two observers blinded to the experimental treatment. The evaluation of pancreatic injury was conducted according to the pathological score system of Schmidt [18]. The different special histological markers of tissue injury were graded in 8 to 10 consecutive high-power fields. The score for each graded parameter was averaged and the total tissue damage was calculated by summing the averages. The evaluation of fetal kidney injury was conducted referring to the study of Paller [19]. Typical pathological changes of kidney tubules including cellular vacuolation, desquamation of tubular epithelial cells, and destruction of the tubular architecture.

2.5. Transmission electron microscopy (TEM) examination

Standard TEM was performed in order to assess the ultrastructural properties of renal tubular cells. Briefly, tissues from the fetal kidney cortex were fixed with 2.5% glutaraldehyde (0.1 mol/L phosphate buffer, pH 7.4) overnight at 4 °C, and then postfixed in a solution of 1% osmium tetroxide in the same buffer for 1 h at 4 °C. Tissues were dehydrated with ethanol and acetone, and embedded in epoxy resin. Ultrathin sections were cut on Leica EMUC7 ultramicrotome and mounted on pioloform-coated copper grids. Then the sections were stained with lead citrate and uranyl acetate and examined with a HT7700 transmission electron microscope (Hitachi, Japan) at 80 kV.

2.6. Western blot analysis

The levels of MIF and p-p38 were measured by western blotting analysis. Fetal kidneys were harvested and immediately frozen in liquid nitrogen. The procedures were completed in < 10 min to avoid phosphorylation status changes. Total proteins were extracted and equal sums of protein samples were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and then transferred onto polyvinylidene difluoride (PVDF) membrane. Membranes were blocked with blocking buffer (Tris-buffered saline containing 5% non-fat dry milk, 0.1% Tween-20) for 2 h at room temperature. Samples were incubated overnight in 4 °C with the primary antibodies. After washing with TBST (Tris-buffered saline containing 0.05% Tween-20), membranes were

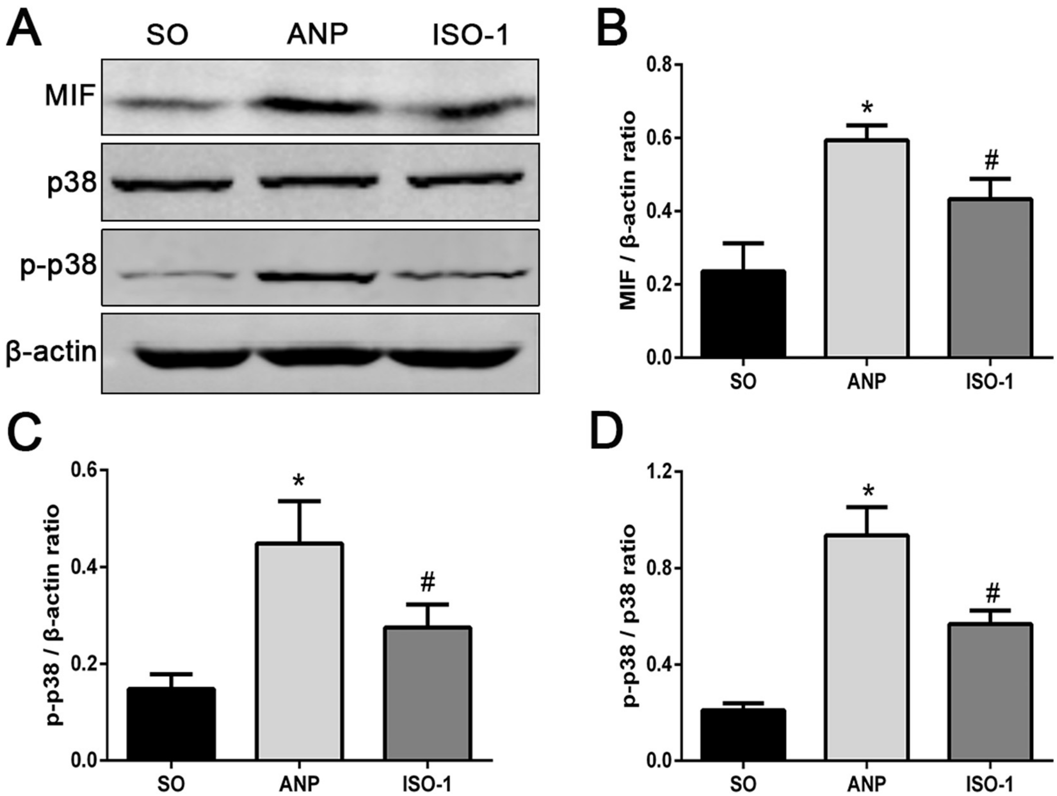

Fig. 5. Effect of ISO-1 on the expression of MIF and activation of p38MAPK in fetal kidney tissues. Densitometric analysis of the bands for the ratio of (B) MIF/β-actin, (C) p-p38/β-actin, and (D) p-p38/p38 was evaluated by the Quantity One software. β-Actin was used as internal control of cytoplasm. **P* < 0.05 *vs* SO group; #*P* < 0.05 *vs* ANP group.

incubated with goat anti-rabbit secondary antibodies (Pierce Biotechnology, Rockford, Ill) for 90 min at room temperature and scanned on Odyssey infrared imaging system. The protein bands were quantified with densitometry (Quantity One 4.5.0 software; Bio-Rad Laboratories, Hercules, Calif).

2.7. Immunofluorescence assay

The expression of p-p38 was further detected in the fetal kidney by immunofluorescence analyses. Briefly, after xylene deparaffinization and hydration, the slides were boiled for 10 min at 121 °C in a pressure cooker containing citrate buffer (10 mM, pH 9.0) for epitope retrieval. Then the slides were cooled to room temperature and rinsed in phosphate-buffered saline (PBS). After permeabilization with 0.2% Triton X-100 for 45 min, the sections were blocked with 10% normal donkey serum to avoid nonspecific fluorescence. Sections were incubated with the primary antibody overnight at 4 °C. Following the reacting with fluorescence-labeled secondary antibodies, nuclei were counter-stained with DAPI. The control group was performed with PBS substituted for the primary antibody. All sections were examined and photographed with automatic fluorescence microscope (Olympus Optical Ltd.), and the p-p38 positive cells ratio was calculated for the comparison in each group.

2.8. Immunohistochemical staining

Immunohistochemistry analysis was performed on the fetal kidney tissues. The thick tissue sections were dewaxed with xylene and hydrated in gradient ethanol (100, 95, 85, and 75%). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide solution for 12 min. Antigen retrieval was performed by heating the sections in 0.1% sodium citrate buffer in microwave oven at high power. The primary antibody was incubated at 4 °C overnight and then incubated with a second antibody while the control group was performed with PBS substituted for the primary antibody. The staining results were visualized using the 3,5-diaminobenzidine. All samples were observed and photographed using light microscope in a blind manner. Mean optical density (MOD) value was measured by Image Pro-Plus 6.0 system (Media Cybernetics, Inc., Rockville, MD, USA) for semi-quantitative analysis. The MOD of TNF-α and IL-1β expression of different groups were analyzed and compared. The ratio of positive nuclei was calculated for the comparison of NF-κB activation in each group.

2.9. Statistical analysis

Parametric results in the figures and text are expressed as mean ± standard deviation. Statistical measurements were made by using the SPSS 21.0 statistical software (SPSS Inc., Chicago, USA). The difference between all the groups was analyzed using One-way analysis of variance (ANOVA). A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. ISO-1 reduce the severity of ANP in pregnancy and levels of maternal serum inflammatory mediators

Maternal serum amylase and lipase levels in ANP group greatly increased 12 h after ANP was induced, and there were significant differences in compassion with those in SO group ($P < 0.05$). Pretreated with ISO-1 slightly reduced the serum levels of AMY and LIP compared with those in ANP group, but the differences were not significant (Fig. 1A, B). Changes of serum inflammatory mediators were in consistent with the AMY and LIP. Results showed that levels of TNF-α and IL-1β increased obviously in ANP rats but was reduced significantly in rats treated with ISO-1 (Fig. 1C, D, $P < 0.05$). The microscopic

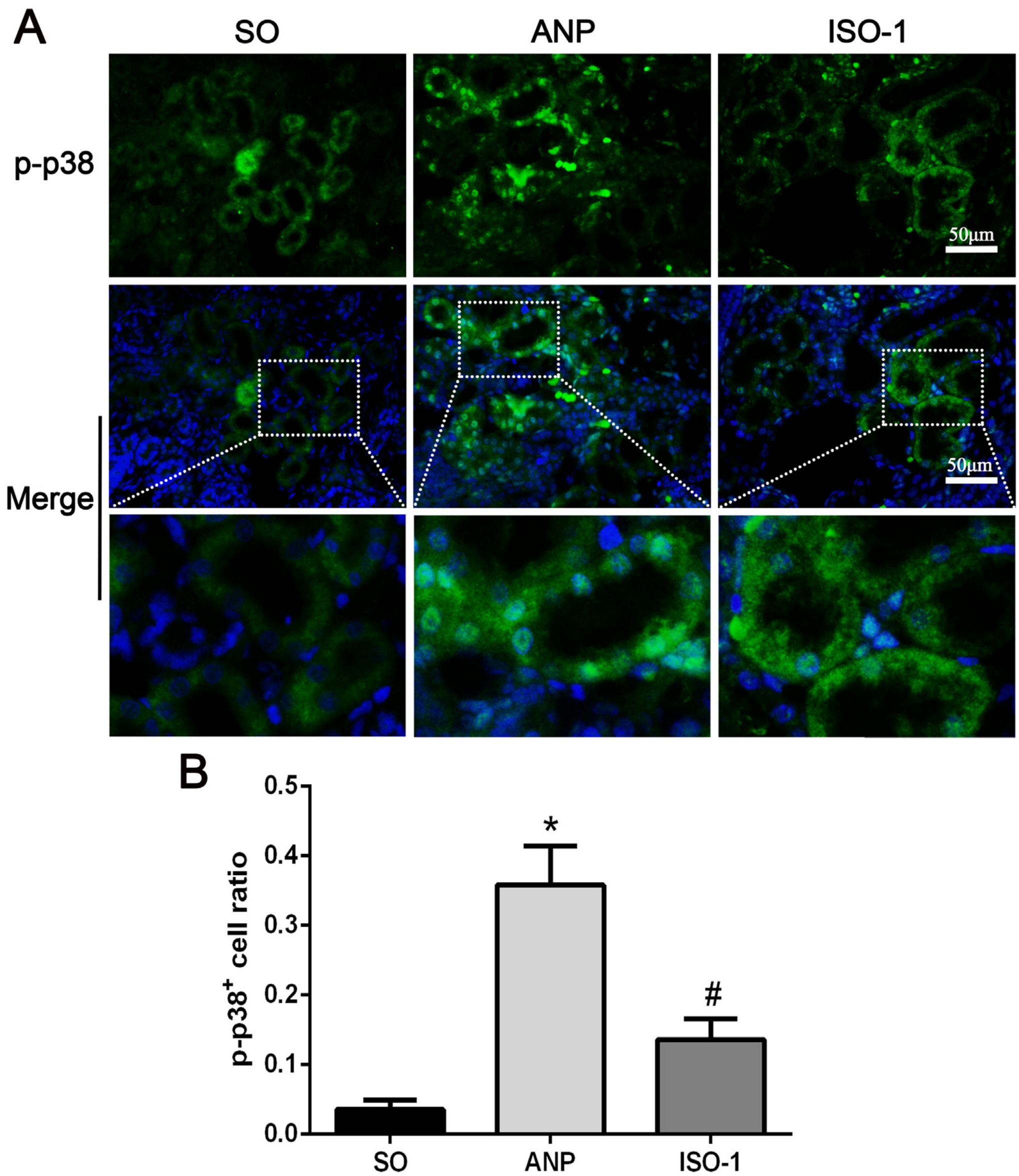

Fig. 6. ISO-1 reduce the expression of p-p38 in fetal kidney tissues. A. Representative immunofluorescence staining in fetal kidney sections from different groups (Immunofluorescence, original magnification, ×400). Underneath insets show higher magnified view of the selected fields. B. Quantitative analysis of p-p38 positive cells ratio and comparison in all groups. $^{*}P < 0.05$ *vs* SO group; $^{\#}P < 0.05$ *vs* ANP group.

investigations demonstrated major histological changes in pancreas on H&E staining in the different groups. As is shown in Fig. 2, pancreas in SO group showed normal acinar architecture except mild interstitial edema. In ANP group, massive areas of acinar necrosis, cytolysis, substantial inflammatory cell infiltration, and hemorrhage were observed, and there was significant difference in pancreatic histopathological scores in comparison with the SO groups. Compared with the ANP group, the extents of pancreatic tissue damage were significantly less and the histopathological scores decreased in ISO-1 group ($P < 0.05$).

3.2. ISO-1 mitigate the pathological injury of fetal kidney tubules

Representative changes of fetal kidneys are shown in Fig. 3. Fetal kidneys in SO group showed few renal tubule damages presented as certain cellular vacuolation of proximal tubules. Obvious pathological changes including cellular vacuolation, desquamation of tubular epithelial cells, and destruction of the tubular architecture could be observed in ANP group. Kidneys obtained from fetal rats in ISO-1 group demonstrated less histological features of tissue injury. Fig. 4 showed the ultrastructural changes of tubular cells from representative fetal kidney. The cells in SO group showed nearly normal morphology of nuclei, endoplasmic reticulum and mitochondria. In ANP group, severely dilated endoplasmic reticulum and swollen mitochondria in tubular cells were observed. And TEM examination has revealed the mild dilatation of the endoplasmic reticulum and decrease of mitochondria damage in ISO-1 group.

3.3. ISO-1 reduce the expression of MIF and inhibit p38MAPK, NF-κB activation in fetal kidney tissues

Our results showed weak expression of MIF in fetal kidneys of SO group. In contrast, the levels of MIF in ANP group were increased obviously and the expression was significantly reduced in fetal kidneys from ISO-1 group compared with the ANP group ($P < 0.05$). P38MAPK is activated by the process of phosphorylation during kinds of stimulations. We analyzed the activation of p38MAPK by western blotting and the results showed that the phosphorylation ratio in the ANP group was significantly higher than that of the SO group. In comparison with

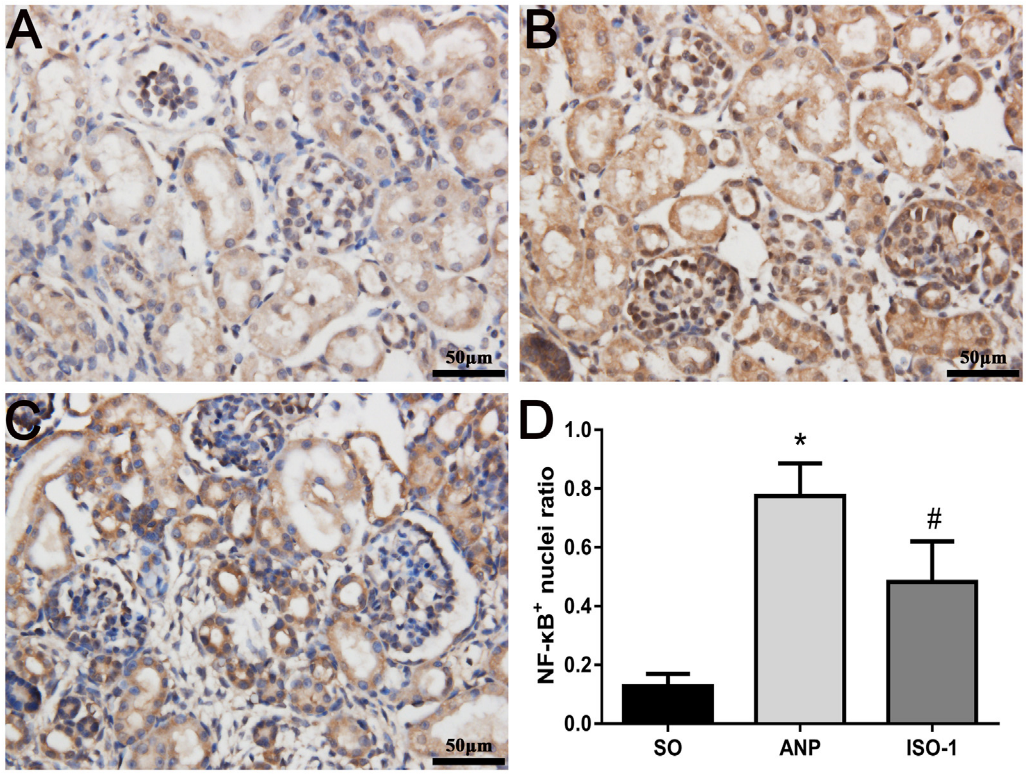

Fig. 7. Expression of NF-κB in fetal kidney tubules. A: SO group; B: ANP group; C: ISO-1 group. D: Comparison of NF-κB positive nuclei to total nuclei ratio in all groups. *$P < 0.05$ *vs* SO group; #$P < 0.05$ *vs* ANP group. (IHC staining, original magnification, ×400).

the ANP group, however, the phosphorylation level of p38MAPK was decreased in ISO-1 group and the difference was statistically significant ($P < 0.05$) (Fig. 5). In order to further evaluate the expression and localization of p-p38, immunofluorescence was applied and fetal kidney from ANP group showed distinct expression in cytoplasm and nuclei compared with the SO group. Moreover, in fetal kidney sections from the rats of ISO-1 group, both the expression in cytoplasm and nuclei were reduced significantly ($P < 0.05$) (Fig. 6). As is shown in Fig. 7, there was little positive immunostaining nuclei for NF-κB in specimens from SO group. But the positive expressions were markedly increased in ANP group and the positive immunostaining nuclei counts were significantly higher than the SO group. Compared with the ANP group, the NF-κB expressions in nuclei were reduced in the group pretreated with ISO-1, and semi-quantity analysis showed that the difference was statistically significant ($P < 0.05$).

3.4. ISO-1 inhibit the expression of TNF-α and IL-1β in fetal kidney tissues

Immunohistochemical staining was conducted to evaluate the expressions of cytokines including TNF-α and IL-1β in fetal kidney tissues. The SO group showed little expression of TNF-α and IL-1β. The expression of TNF-α was significantly increased in the ANP group in comparison to the control group ($P < 0.05$). In addition, IL-1β staining presented with a similar pattern to TNF-α, showing an increased diffuse positive reaction in the ANP group when compared to the SO group ($P < 0.05$). Both TNF-α and IL-1β showed significant decrease of their expression in the ISO-1group and lower values than the ANP groups studied ($P < 0.05$) (Fig. 8).

4. Discussion

Inflammatory and immune response are essential features of acute necrotizing pancreatitis and play a central role in the pathogenesis of tissue damage. The inflammatory response is related to increased synthesis and secretion of cytokines especially TNF-α and IL-1β, which are the primary members of the inflammatory cytokine family [20,21]. The production of TNF-α and IL-1β often occurs prior to appreciable changes in pancreatic histology and both are produced systemically during acute pancreatitis and not just within the pancreas, which are known to be toxic to many cell types and tissues [22–24]. In the present study, we built a rat model of acute necrotizing pancreatitis in pregnancy on the basis of previous research in placental injury [17]. Results showed that there were typical pathological changes of hemorrhage and necrosis of maternal pancreas tissues in ANP group and serum levels of AMY, LIP, TNF-α and IL-1β were also markedly increased, which indicated that the ANP model was successfully induced in pregnant rats. We also observed the pathological changes of fetal kidneys and found that the fetal kidneys from ANP group exhibited obvious kidney tissue injury. And that pregnant rats pretreated with ISO-1 notably attenuated the lesions of fetal kidneys as showed in microstructure and ultrastructure. These results incriminate that inflammatory response and inflammatory cytokines which could be reduced by ISO-1 play a vital role in fetal kidney injury during maternal acute necrotizing pancreatitis.

MIF is mainly released by monocytes and macrophages in response to infection and stresses. It is associated with activation of immune cells and the expression of pro-inflammatory mediators, thereby strongly promoting inflammatory and immune response [25]. MIF levels in ascitic fluids, serum and the lung were increased in hemorrhagic necrotizing pancreatitis model induced by the injection of taurocholic acid, and even serum MIF [26] is a good discriminator of severe acute pancreatitis accompanied by multiple organ failure [27]. In our experiments, we found that the expression of MIF in fetal kidney was significantly increased, which indicated that MIF might play an important role in the process of fetal kidney injury. MIF is a trimeric protein and the catalytic pocket is known to be essential for MIF's proinflammatory activity. Because inhibition of this site reduces some of the pro-inflammatory activities of MIF [28]. The compound (ISO-1) is a

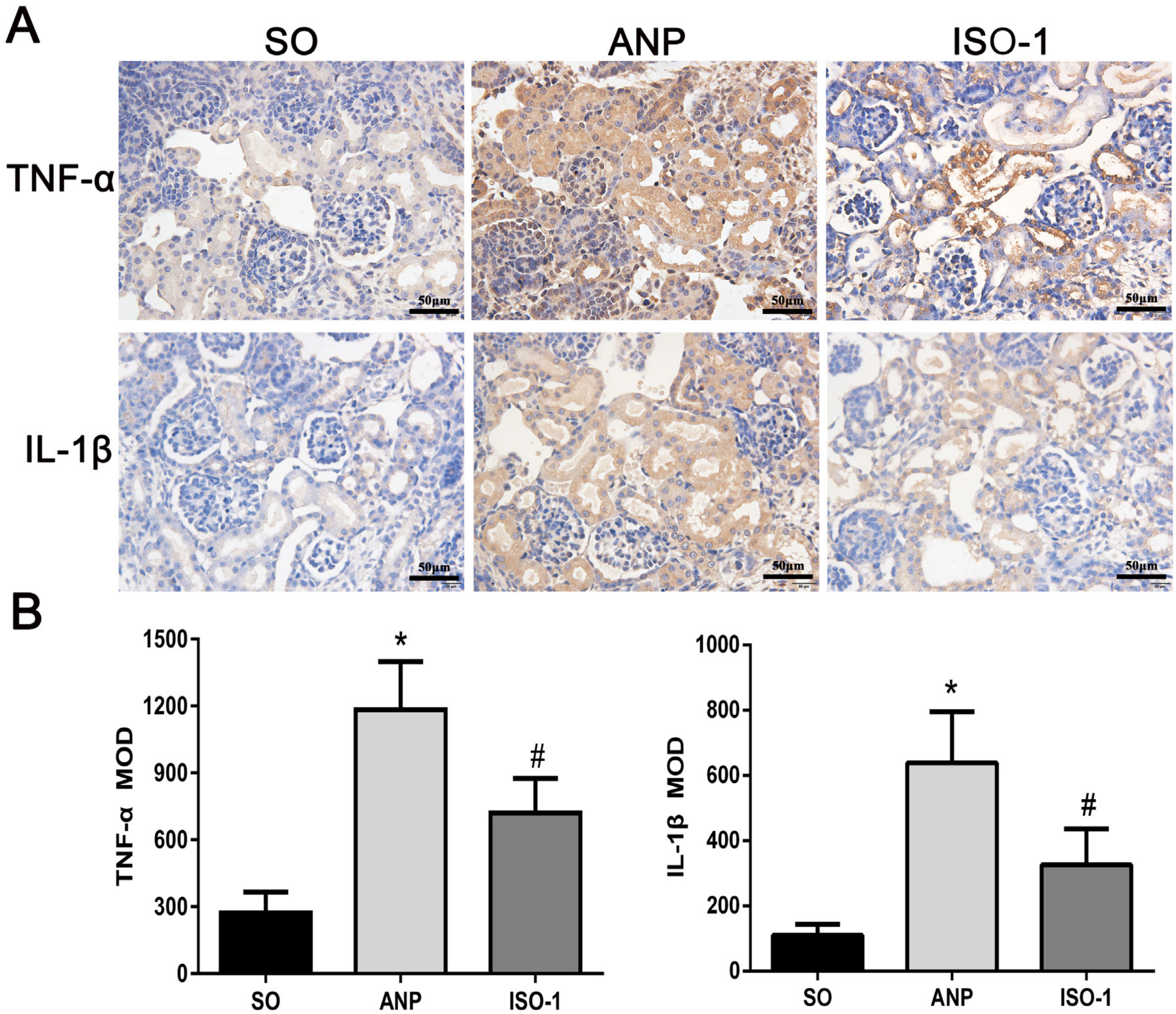

Fig. 8. Expression of TNF-α and IL-1β in fetal kidney. A: Changes of TNF-α and IL-1β expression; B: The MOD of TNF-α and IL-1β respectively. $^{*}P < 0.05$ *vs* SO group; $^{\#}P < 0.05$ *vs* ANP group. (IHC staining, original magnification, ×400).

topoisomerase inhibitor of MIF that can specifically bind to the catalytic pocket and inhibit the tautomerase activity which resulted in a significant reduction in the release of inflammatory cytokines and the inflammatory response [29]. In acute pancreatitis rat model, treated with anti-MIF antibody can attenuate the increase of pulmonary TNF-α levels [30]. This coincide with our results that treating pregnant rats with MIF inhibitor ISO-1 reduced the expression of TNF-α and IL-1β in fetal kidney tissues.

It has been indicated that MIF is a natural ligand for CD74 and it can interact with target cells by binding to the receptor. Stimulation of CD74 initiates a signaling cascade through the activation of mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK)1/2, protein kinase B and NF-κB, leading to leukocytic integrin activation, cell survival and induction of pro-inflammatory gene expression [31]. Mitogen activated protein kinases (MAPKs) signaling cascades are induced by pro-inflammatory cytokines and then have influences on the immune response and cell death [32,33]. P38MAPK is one of the MAPKs families that is involved in the severity of pancreatitis, and inhibition of p38MAPK decreased pancreatic and pulmonary injury induced by severe acute pancreatitis in rats [34]. When activated by pro-inflammatory cytokines or other mediators, p38MAPK will be phosphorylated for further inflammatory response while blocking the phosphorylation process could decrease pulmonary TNF-α production and prevent pulmonary injury induced by pancreatitis [35]. In addition, activated p38MAPK can mediate NF-κB activation to promote cytokines expression and aggravate inflammatory injury [36,37].

The transcription factor NF-κB plays an important part in human inflammation and immune regulation over the past several years [38]. As an early and central event in the process of inflammation during acute pancreatitis, NF-κB activation and translocation can medicate cytokines release by regulating the transcription of many genes including TNF-α and IL-1β [39–42]. Moreover, the up-regulation of these inflammatory mediators may also activate NF-κB and stimulate its translocation into the nuclei [43]. All of these signaling cytokines further activate cells with inflammatory property and interact with other cytokines, which finally result in the amplification of inflammatory response [44,45]. In this study, we observed distinct activation of p38MAPK, NF-κB as well as increased expression of TNF-α and IL-1β in fetal kidneys from ANP group. Furthermore, after the inhibition of MIF with ISO-1, the activation levels of p38MAPK/NF-κB in accordance with TNF-α, IL-1β were significantly decreased. These results indicated that ISO-1 could inhibit the pro-inflammatory effect of MIF to reduce the activation of p38MAPK and NF-κB. The amplification of inflammatory response in fetal kidney is disrupted by the pretreatment of ISO-1 and then relieves inflammatory injury. It was interesting to find that the expression of MIF in fetal kidney tissues was also down-regulated by ISO-1 treatment. As researches illustrated, MIF promotion of TNF-α and IL-1β can lead in turn to further MIF release [46]. Therefore, the downregulation of MIF may be associated with decreased levels of TNF-α and IL-1β.

In conclusion, our results demonstrate that ISO-1, an inhibitor of MIF, could attenuate inflammation and fetal kidney injury associated with the maternal acute necrotizing pancreatitis, which may be through inhibiting the activation of p38MAPK and NF-κB to reduce the expression of TNF-α and IL-1β. This study reveals the protective effects of ISO-1 on fetal inflammatory response and kidney injury during maternal acute necrotizing pancreatitis. Future studies will define the immune-inflammatory mechanisms and identify more molecular targets for treating pancreatitis patients during pregnancy.

Acknowledgments

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