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ORIGINAL ARTICLE

Antioxidant Inhibits HMGB1 Expression and Reduces Pancreas Injury in Rats with Severe Acute Pancreatitis

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Abstract
Background Pathogenesis of severe acute pancreatitis is still unclear, which leads to a lack of proper treatment in severe acute pancreatitis therapeutic strategy.
Objective To investigate the effect of treatment with antioxidant pyrrolidine dithiocarbamate on pancreas injury in rats with severe acute pancreatitis and its possible mechanism.
Methods A total of 144 male Sprague–Dawley rats were randomly allocated into a sham operation group ($n = 48$), a severe acute pancreatitis group ($n = 48$), and a pyrrolidine dithiocarbamate-treated group ($n = 48$). All the rats were killed at 1, 3, 6, 12, 24, and 48 h after operation. The pancreas histopathologies were observed and serum amylase levels were tested. Meanwhile, the nuclear factor-κB activation, tumor necrosis factor-α levels and high-mobility group box protein-1 expression levels in pancreatic tissue were studied.
Results Animals receiving pyrrolidine dithiocarbamate had significantly improved pancreas histopathology and lower serum amylase levels ($p < 0.05$). In the severe acute pancreatitis group, pancreas tumor necrosis factor-α levels reached a peak at 6 h after operation and afterwards rapidly declined to normal levels. However, high-mobility group box protein-1 levels in pancreatic tissue increased remarkably at the 12th hour, reached a peak at 24 h, and maintained up to 48 h post-severe acute pancreatitis. Compared to the severe acute pancreatitis group, the pancreas nuclear factor-κB activity, tumor necrosis factor-α, high-mobility group box protein-1 levels in the pyrrolidine dithiocarbamate-treated group all remarkably decreased ($p < 0.05$).
Conclusions High-mobility group box protein-1 seems to act as a late cytokine mediator in the pathogenesis of severe acute pancreatitis. Pyrrolidine dithiocarbamate might inhibit the activation of nuclear factor-κB to blockade tumor necrosis factor-α, thereby indirectly suppressing the high-mobility group box protein-1 and reducing pancreatic tissue damage in rats with severe acute pancreatitis.

Keywords Severe acute pancreatitis · NF-κB · High-mobility group box protein-1 · Pyrrolidine dithiocarbamate

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Introduction

Severe acute pancreatitis (SAP) is characteristic of a serious pathogenetic condition with high mortality, and there is still no breakthrough in treatment. Numerous studies have emphasized the important role of proinflammatory cytokines in the mechanism that SAP develops systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF), and even death [1]. Despite their evident role in the pathogenesis of SAP, attempts to improve the prognosis of SAP by inhibiting 'early' Cytokines such as TNF-α, IL-1 have failed.

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Recent studies have suggested that high-mobility group box-1 protein (HMGB1), a DNA-binding intranuclear protein, is a late activator of the inflammatory cascade when released into the extracellular space [2]. It has even been speculated that HMGB1 might be a target for anti-inflammatory treatment in SAP [3]. NF-κB is a kind of DNA-binding protein, taking part in various kinds of inflammatory and immune responses by adjusting gene expression of cytokine and adhesion molecules [4, 5]. Research shows that antioxidant pyrrolidine dithiocarbamate (PDTC) as a potent inhibitor of NF-κB could suppress inflammation [6, 7]. We hypothesized that PDTC could inhibit tissue HMGB1 expression to prevent pancreas injury in rats with SAP. To test this hypothesis, we investigated the impact of PDTC administration on levels of HMGB1 and pancreas histopathology in rats with SAP.

Materials and Methods

Reagents

Sodium taurocholate (TCA) and PDTC were obtained from Sigma-Aldrich (St. Louis, MO). Antibodies to HMGB1, NF-κB P65, β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) were also purchased. TNF-α enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Sengxiong Biotechnology Co. (Sengxiong, Shanghai, China).

Animals

Male Sprague–Dawley (SD) rats weighing 210–230 g were maintained at 23°C on a 12-h light/dark cycle and allowed free access to water and standard laboratory chow. From 12 h before the start of the experiments, the animals were deprived of food but were allowed access to water. This study was conducted with the consent of the ethics committee for the use of experimental animals of the Animal Research Center of Wenzhou Medical College.

Animal Model

SAP model was induced by the improved Aho [8] method. The rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (60 mg/kg body weight). After entering the abdomen via a median epigastrium incision, confirming the bile-pancreatic and hepatic hilus common hepatic ducts, uncovering the pancreas, and identifying the duodenal papilla inside the duodenum duct wall, a No. 5 needle was used to drill a hole into the mesenterium avascular area. A segmental epidural catheter was inserted into the duodenum cavity via the hole and in a retrograde direction to the bile-pancreatic duct in the direction of the papilla. The microvascular clamp was used to nip the catheter head temporarily while another microvascular clamp was used to temporarily occlude the common hepatic duct at the confluence of the hepatic duct. After connecting the epidural catheter end with the transfusion converter, 5% sodium taurocholate 1 ml/kg was by retrograde transfused via the microinfusion pump (Medical Instrument Corporation of Zhejiang University, China) at a speed of 0.05 ml/min. Five minutes after injection, the microvascular clamp and epidural catheter were removed. After checking for bile leakage, the hole in the duodenum lateral wall was sutured. Finally, the abdomen was closed. The rats recovered from the anesthesia and were allowed access to water.

Animal Grouping

A total of 144 clean-grade healthy male SD rats were randomly allocated into a sham operation (SO) group ($n = 48$), an SAP group ($n = 48$) and a PDTC-treated group ($n = 48$). The groups were then randomly divided into 1, 3, 6, 12, 24, and 48-h groups, with eight rats in each group. In the SO group, nothing was injected into the biliopancreatic duct and the remaining procedure was the same as the SAP group. The PDTC-treated group was injected with PDTC intraperitoneally 1 h before the initiation of the SAP model at a dose of 30 mg/kg and the other two groups were administrated intraperitoneally with normal sodium of equivalent volume. All the rats were killed under anesthesia at 1, 3, 6, 12, 24, and 48 h after establishment of the model. Blood was collected by inferior caval vein puncture. One segment of pancreas was removed immediately, frozen in liquid nitrogen, and stored in a refrigerator at −70°C; another segment of pancreatic tissue was fixed by immersion in 10% formalin and embedded in paraffin wax for cutting sections.

Histological Analysis

The pancreas sections were stained with hematoxylin and eosin (H&E) for tissue morphological observation. For histological examination of the pancreatic tissue, the pancreas (20 random fields on each slide) of eight rats from each group at every time point was examined and scored for edema, acinar necrosis, hemorrhage, and inflammation by an experienced pathologist who was blind as to the treatment according to Schmidt's standard method [9] under a light microscope (Olympus, Japan).

Measurements of Serum Amylase Levels

The collected blood was conserved at room temperature for 10 min and serum was separated from blood by centrifugal separation for 3,000 rpm/min, at 4°C, 15 min. Serum amylase (AMY) levels were determined using AMY kits

(Nanjing Jiancheng Biotechnology, Nanjing, China) by automated clinical biochemistry analysis equipment (Hitachi, Japan).

Immunohistochemical Analysis

Pancreas tissue was obtained from animals in SO, SAP, and PDTC-treated groups at 24 h after establishment of model. The tissue samples were fixed immediately in 4% paraformaldehyde at 4°C overnight. Sections embedded in paraffin wax were de-waxed, rehydrated in gradient alcohol, and endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 min, and then incubated in normal goat serum and followed by using polyclonal HMGB1 antibody (diluted 1:100) overnight at 4°C. The sections were then incubated with secondary antibody using donkey anti-goat IgG. At last, the sections were visualized by incubating with diaminobenzidine (DAB) solution and weakly counter-stained with hematoxylin.

Western Blot of NF-κB P65 and HMGB1

Pancreatic protein was extracted by nuclear and cytoplasmic extraction reagents according to the manufacture's instructions (Beyotime Institute of Biotechnology, Shanghai, China). Nuclear extracts of NF-κB p65 and proteins of HMGB1 extracted from the pancreas were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 10% gel and then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were incubated with NF-κB P65 primary antibody (1:1,000) and HMGB1 primary antibody (1:200) at 4°C overnight followed, respectively, by HRP-conjugated secondary antibody (1:8,000, Beijing Zhongshan Biotechnology, Zhongshan, Beijing) and secondary antibody (1:6,000, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. Blots were subsequently developed using an enhanced chemiluminescence detection kit (Beyotime Institute of Biotechnology, Shanghai, China). The ECL-treated membrane was then exposed further to film. The film was scanned. The band concentration was calculated by the quantification of the integrated optical density of the appropriate band. Membranes were then stripped using 0.2 M NaOH solution for 30 min at room temperature and reprobed with a standard: β-actin. NF-κB P65 and HMGB1 was normalized to β-actin.

Evaluation of TNF-α Protein in Pancreas

The separated pancreatic tissue was homogenated and centrifuged and the supernatant was collected for the test. The total protein concentration in the supernatant was examined using enhanced BCA protein assay kits. TNF-α protein was measured by the enzyme-linked immunosorbent assay (ELISA) following the instruction of the kits. Finally, TNF-α levels in pancreatic tissue were determined with the ratio of TNF-α protein/total protein.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Comparison between groups was performed with analysis of variance (ANOVA) and Student–Newman–Keuls test (q-test). Simple linear correlation analysis was applied to investigate the relation between two parameters. Statistical significance was assumed at a value of $p < 0.05$.

Results

Effect of PDTC on Pancreatic Tissue

In the SO group, except for edema and a few inflammatory cells, a lobule structure existed, and mesenchyma was clear. In the SAP group, congestion, edema, and hemorrhagic and necrotic spots were found sporadically in the pancreatic tissue, with cell membrane dissolved and nucleus having disappeared. Acinus and lobule structures were not clear. Around the necrotic focus, numerous inflammatory cells infiltration could be observed in the pancreas. Moreover, these pathological damage aggravated with the progress of SAP. When compared to the SO group, the difference was significant ($p < 0.05$). In the PDTC-treated group, the degree of histological alterations were alleviated as compared to the SAP group ($p < 0.05$) (see Fig. 1, Tables 1 and 2).

Effect of PDTC on Serum Levels of AMY

Compared to the SO group, serum AMY levels in the SAP group obviously increased 3–24 h each time point after the induction of pancreatitis ($p < 0.05$). AMY levels in the PDTC-treated group were evidently lower than those in the SAP group at 6 and 12 h after the operation ($p < 0.05$) (see Table 3).

Effect of PDTC on NF-κB Activation in Pancreas

In contrast to the SO group, levels of NF-κB p65 from nuclear extracts in the pancreatic tissue, which indicated activation of NF-κB, had markedly increased 1 h after the establishment of the model, reached a peak at 3 h, and then decreased gradually in the SAP group ($p < 0.05$). However, levels of NF-κB p65 in the PDTC-treated group were distinctly lower than those in the SAP group at 1, 3 and 6 h after operation ($p < 0.05$) (see Fig. 2, Table 4).

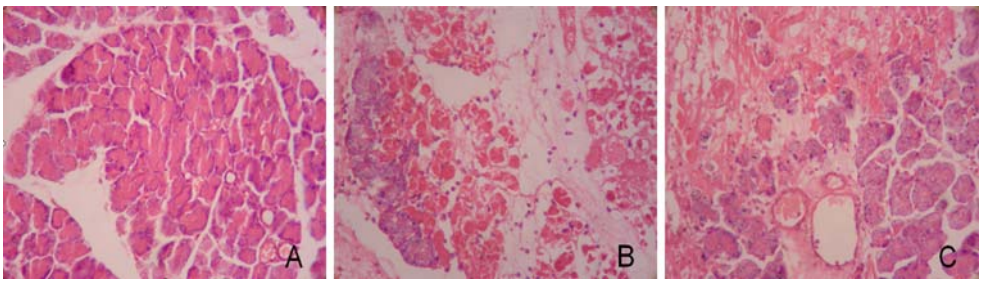

Fig. 1 Effects of pyrrolidine dithiocarbamate (PDTC) on pancreas injury in taurocholate (TCA)-treated rats. Pancreatic tissue was obtained from rats in the sham operation group 24 h after operation (**a**, magnification ×200 HE), rats in the SAP group 24 h after operation (**b**, magnification ×200 HE), and rats in the PDTC-treated group 24 h after operation (**c**, magnification ×200 HE)

Table 1 Pathological score of pancreatic tissue in rats of three groups

Group	*n*	Time (h)		
		3	6	12
SO	8	3.46 ± 0.15	3.52 ± 0.19	4.04 ± 0.25
SAP	8	8.79 ± 0.96$^{\#}$	11.21 ± 0.92$^{\#}$	12.71 ± 0.96$^{\#}$
PDTC	8	7.48 ± 0.46$^{\#}$*	9.52 ± 0.91$^{\#}$*	11.29 ± 0.74$^{\#}$*

Results are expressed as mean ± SD. The number of rats in each group at every time point was 8

$^{\#}$ $p < 0.05$ vs. SO group; * $p < 0.05$ vs. SAP group

Effect of PDTC on TNF-α Levels in Pancreas

TNF-α levels in pancreatic tissue obviously increased 1 h after the induction of SAP, and reached peak at 6 h, but rapidly declined towards normal levels at 12th hour, and then maintained up to 24 and 48 h post operation in low levels. Compared with the SO group, TNF-α levels in the SAP group were significantly higher 1, 3 and 6 h after operation ($p < 0.05$). However, TNF-α levels in PDTC-treated group were remarkably lower than those in the SAP group at 3 and 6 h post-operation ($p < 0.05$) (see Fig. 3).

Correlation Analysis Between NF-κB Activation and TNF-α Levels

After statistical analysis, the correlation coefficient demonstrated a significant positive correlation between the activation of NF-κB and TNF-α levels in pancreatic tissue of the SAP group at 1–12 h after the establishment of model, $r = 0.631$, $t = 4.628$ ($p < 0.05$).

Effect of PDTC on HMGB1 Expression in Pancreas

Immunohistochemical analysis showed that HMGB1 was slightly expressed in pancreatic tissue in the sham operation (SO) group, strongly expressed in the SAP group, and

Table 2 Pathological score of pancreatic tissue in rats of three groups 24 h after operation

Group	*n*	Pathological score			
		Edema	Inflammation	Hemorrhage	Acinar necrosis
SO	8	2.98 ± 0.56	0.36 ± 0.25	0	0
SAP	8	3.71 ± 0.29$^{\#}$	3.22 ± 0.63$^{\#}$	3.73 ± 0.56$^{\#}$	3.62 ± 0.51$^{\#}$
PDTC	8	3.22 ± 0.53$^{\#}$*	2.53 ± 0.41$^{\#}$*	3.27 ± 0.42$^{\#}$*	3.06 ± 0.47$^{\#}$*

Results are expressed as mean ± SD. The number of rats in each group at every time point was 8

$^{\#}$ $p < 0.05$ vs. SO group; * $p < 0.05$ vs. SAP group

Table 3 Serum amylase levels in rats of three groups

Group	*n*	Time (h)			
		3	6	12	24
SO	8	1386.67 ± 166.33	1646.51 ± 120.34	1126.21 ± 71.64	1108.67 ± 86.13
SAP	8	3584.72 ± 595.54$^{\#}$	6452.79 ± 1065.18$^{\#}$	4436.26 ± 354.91$^{\#}$	5021.03 ± 722.52$^{\#}$
PDTC	8	3512.56 ± 846.32$^{\#}$	3392.16 ± 279.40$^{\#}$*	3662.46 ± 247.58$^{\#}$*	4436.26 ± 416.23$^{\#}$

Results are expressed as mean ± SD. The number of rats in each group at every time point was 8

$^{\#}$ $p < 0.05$ vs. SO group; * $p < 0.05$ vs. SAP group

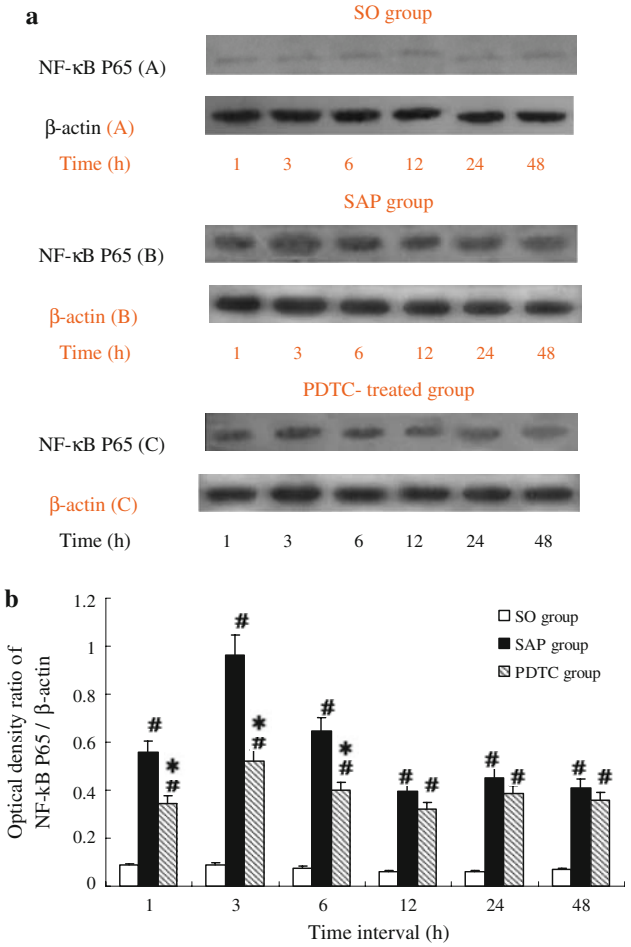

Fig. 2 Effects of pyrrolidine dithiocarbamate (PDTC) on NF-κB activation of pancreatic tissue in taurocholate (TCA)-treated rats. **a** Levels of NF-κB p65 and β-actin from nuclear extracts of pancreatic tissue in rats of three groups at each time point after operation was detected by Western blot and quantified by integrated optical density. *A* sham operation (SO) group, *B* SAP group, *C* PDTC-treated group. **b** NF-κB P65 was normalized to β-actin. # Significant difference from SO group animals ($p < 0.05$); * Significant difference from SAP group animals ($p < 0.05$)

Table 4 Pancreas NF-κB activity in rats of three groups (%)

Time (h)	*n*	Groups		
		SO	SAP	PDTC
1	8	8.62 ± 0.90	55.62 ± 3.78#	34.48 ± 3.27#*
3	8	8.97 ± 0.44	96.13 ± 9.61#	51.97 ± 4.38#*
6	8	7.52 ± 0.72	64.51 ± 5.34#	39.84 ± 3.53#*
12	8	5.87 ± 0.77	39.37 ± 3.22#	32.03 ± 3.02#
24	8	6.13 ± 0.59	45.06 ± 4.78#	38.56 ± 3.49#
48	8	6.78 ± 0.58	41.02 ± 3.86#	35.69 ± 3.35#

Results are expressed as mean ± SD. The number of rats in each group at every time point was 8

# $p < 0.05$ vs. SO group; * $p < 0.05$ vs. SAP group

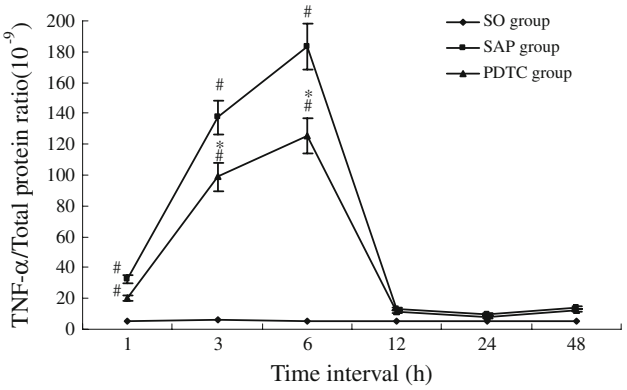

Fig. 3 Effects of pyrrolidine dithiocarbamate (PDTC) on TNF-α levels of pancreatic tissue in taurocholate (TCA)-treated rats. TNF-α protein of pancreatic tissue in rats of three groups 1, 3, 6, 12, 24, and 48 h after operation was examined by enzyme-linked immunosorbent assay (ELISA). Finally, TNF-α levels in pancreatic tissue were determined by the ratio of TNF-α/total protein. The number of rats in each group at every time point was 8. Results are expressed as mean ± SD. # Significant difference from SO group animals ($p < 0.05$); * Significant difference from SAP group animals ($p < 0.05$)

expressed at an intermediate level in the PDTC-treated group (see Fig. 4). Meanwhile, Western blot analysis revealed that compared to the SO group, the expression of HMGB1 in pancreatic tissue of the SAP group obviously increased at the 12th hour, reached a peak at the 24th h, and maintained up to 48 h in higher levels following the establishment of the model ($p < 0.05$). Among the PDTC-treated rats, HMGB1 expression levels were evidently lower than those in the SAP group at 24 and 48 h after the induction of pancreatitis ($p < 0.05$) (see Fig. 5, Table 5).

Discussion

At present, pathogenesis of SAP is still unclear, which leads to the lack of proper treatment in SAP therapeutic strategy. However, the inflammatory mediator theory proposed recently provides a new clue for SAP research [1]. This theory explains that in the early stage of SAP, the abnormal-activated pancreatin damages adjacent pancreatic cells and leads to local infiltration of inflammatory cells. Activated inflammatory cells such as macrophage, neutrophil, and pancreatic acinar cells produce and release proinflammatory cytokines [10]. The rising degree of inflammatory cytokines is closely associated with the severity of SAP, and it is these inflammatory cytokines that result in SIRS, MOF, and even death. It is shown by study that among various inflammatory mediators related with SAP, TNF-α plays a key role in the pathogenesis of SAP [11]. However, in this research we found that TNF-α levels in pancreatic tissue immediately increased to a peak after the induction of pancreatitis, and then quickly decreased

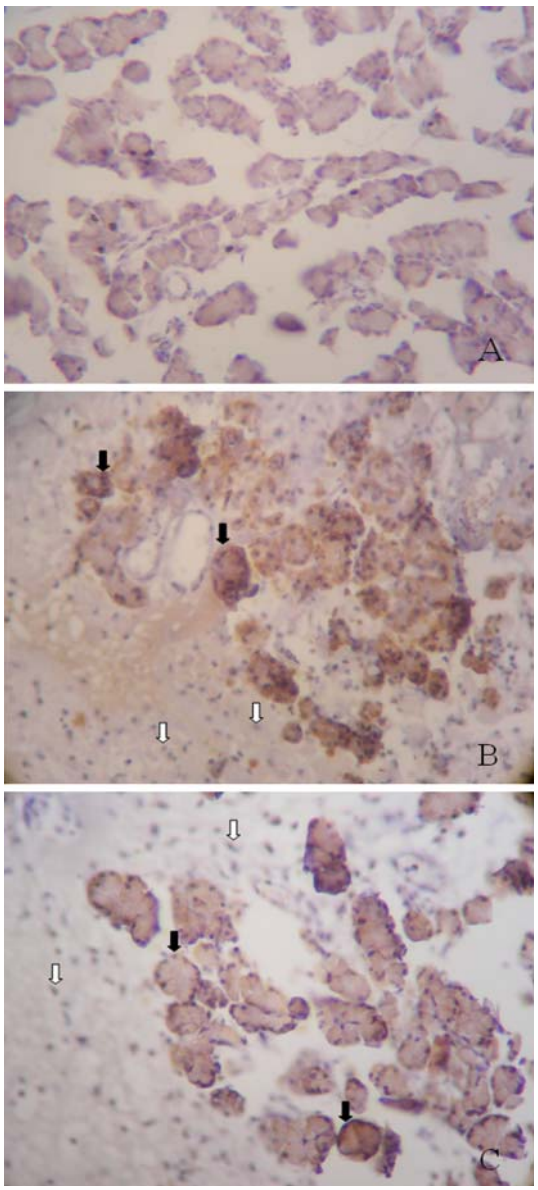

Fig. 4 Effects of pyrrolidine dithiocarbamate (PDTC) on HMGB1 protein expression of pancreatic tissue in taurocholate (TCA)-treated rats. Immunohistochemical (IHC) analysis to detect HMGB1 expression in pancreas from animals killed 24 h after operation in three groups. **a** Sham operation (SO) group (magnification ×200 IHC). **b** SAP group (magnification ×200 IHC). **c** PDTC-treated group (magnification ×200 IHC). *Black arrows* point to positive acinar cells and *white arrows* point to inflammation cells

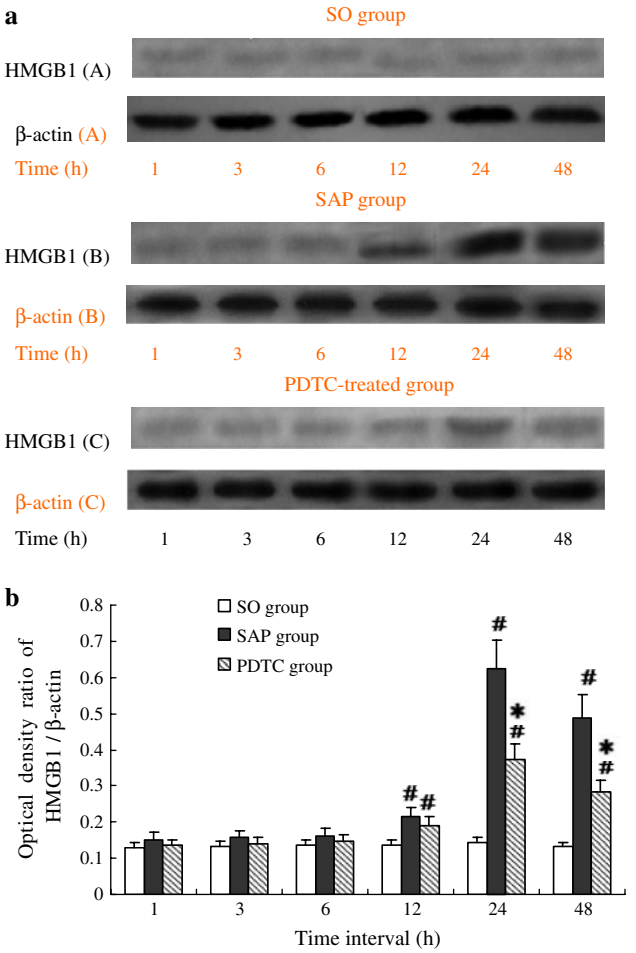

Fig. 5 Effects of pyrrolidine dithiocarbamate (PDTC) on HMGB1 protein expression of pancreatic tissue in taurocholate (TCA)-treated rats. **a** Levels of HMGB1 and *β*-actin in pancreatic tissue from rats of three groups at each time point after operation were detected by Western blot and quantified by integrated optical density. *A* sham operation (SO) group, *B* SAP group, *C* PDTC-treated group. **b** HMGB1 was normalized to *β*-actin. # Significant difference from SO group animals ($p < 0.05$); * Significant difference from SAP group animals ($p < 0.05$)

towards normal levels. However, the inflammatory response and pancreas injury still continued, which suggested some late mediators might contribute to the pathogenesis of SAP.

High-mobility group box protein-1 (HMGB1) is an intranuclear protein originally identified to be important in the regulation of genetic information [12]. Recently, it has been discovered to play a key role as a late-phase mediator in the pathogenesis of sepsis [2]. Once released from

Table 5 Pancreas HMGB1 levels in rats of three groups (%)

Time (h)	*n*	Groups		
		SO	SAP	PDTC
1	8	12.98 ± 1.37	15.23 ± 1.71	13.58 ± 1.34
3	8	13.22 ± 1.32	15.64 ± 1.83	13.96 ± 1.48
6	8	13.69 ± 1.12	16.21 ± 1.97	14.82 ± 1.59
12	8	13.57 ± 0.94	21.42 ± 3.31#	19.17 ± 2.78#
24	8	14.29 ± 1.54	62.36 ± 8.62#	37.16 ± 4.70#*
48	8	13.13 ± 1.47	48.75 ± 7.44#	28.29 ± 3.37#*

Results are expressed as mean ± SD. The number of rats in each group at every time point was 8

# $p < 0.05$ vs. SO group; * $p < 0.05$ vs. SAP group

necrotic cells, or secreted by activated monocytes or macrophages, HMGB1 can mediate cell-to-cell signaling by binding to the receptor for advanced glycosylation end product (RAGE) [13], Toll-like receptor 2 (TLR2) [14, 15], and Toll-like receptor 4 (TLR4) [14, 15] enhance the inflammatory response. HMGB1 has been found to be up-regulated in many acute and chronic inflammatory conditions including sepsis, acute lung injury, rheumatoid arthritis, and Sjögren's syndrome [2, 16–18]. In contrast to other known pro-inflammatory cytokines, its delayed kinetics allows a wide window of opportunity for therapeutic approaches. As a result, HMGB1 offers the hope of developing an anti-inflammatory therapy that is both practical and effective.

Our results showed that being different from early cytokine mediators such as TNF-α, HMGB1 was produced at a relatively late stage by the pancreatic tissue in rats with SAP but stayed at elevated plateau levels for a relatively long time. Moreover, the inflammatory response and pancreatic tissue damage became worse as HMGB1 expression levels increased and were maintained, which suggested HMGB1 also acted as a late cytokine mediator in the pathogenesis of SAP.

Nuclear factor-κB (NF-κB) is a transcription factor that plays a pivotal role in the induction of genes involved in the response to injury and inflammation. The generation of TNF-α is directly adjusted by NF-κB as there are binding sites of NF-κB on the TNF-α promoter [19]. Research has proven that activation of NF-κB can result in increased expression of cytokines such as TNF-α, leading to immune and inflammatory responses in organisms [20]. Our results also showed that there was a significant positive correlation between the activation of NF-κB and TNF-α levels. However, now we cannot be sure whether there are binding sites of NF-κB on the HMGB1 promoter. So it is still unclear whether the generation of HMGB1 is directly adjusted by NF-κB.

Reactive oxygen species are important inducers in the activation of NF-κB, so antioxidants are studied to inhibit NF-κB. Recent evidence suggests that pyrrolidine dithiocarbamate (PDTC) as an antioxidant could prevent the IkB-[alpha] degradation to inhibit NF-κB activation, thereby ameliorating the tissue injury associated with experimental murine acute pancreatitis [6]. It has also been shown that NF-κB activation in the peritoneal as well as alveolar macrophages is dose-dependently inhibited by PDTC, consequently reducing fatal outcome in rats with SAP [7]. In our research, We found activation of NF-κB of pancreatic tissue had markedly increased 1 h after the induction of pancreatitis, reached a peak at 3 h, and then decreased gradually. So PDTC was injected intraperitoneally 1 h before the establishment of the model in order to prevent the activation of NF-κB. Moreover, Satoh [7] reported when PDTC was injected intraperitoneally 1 h before the induction of pancreatitis, the survival of rats was significantly improved. However, PDTC was less effective when it was given 2 h after the induction of pancreatitis, and no favorable effect was observed when it was given 6 h after the induction of pancreatitis.

In the present study, we have shown that the activation of NF-κB and TNF-α levels in pancreatic tissue were all remarkably alleviated and HMGB1 expression levels also significantly decreased after pretreatment with PDTC before induction of pancreatitis. At the same time, preadministration of PDTC resulted in lower serum amylase levels and reduced pancreatic pathological damage. What, then, is the mechanism by which PDTC protects against inflammatory injury? On one hand, what the article has noted, PDTC could inhibit NF-κB activation to directly reduce the transcription and expression of TNF-α. On the other hand, it has been shown that monocytes and macrophages stimulated by TNF-α can secrete HMGB1 [21, 22]. So, PDTC might inhibit NF-κB activation to reduce TNF-α levels and then to indirectly decrease the HMGB1 expression, although it is still unclear whether the generation of HMGB1 is directly adjusted by NF-κB. Our results suggest that the inhibition of early cytokine secretion by PDTC in SAP mediates the inhibition of HMGB1.

In conclusion, HMGB1 as a late cytokine mediator seems to play an important role in the pathogenesis of SAP and PDTC pre-administration might inhibit NF-κB activation to suppress the activation of the cytokine network, thereby inhibiting the production of HMGB1 and reducing pancreas injury in SAP rats. These findings suggest that PDTC might be a potential treatment for SAP. However, Grisham [23] previously reported that the dose of PDTC that fully inhibited the activation of NF-κB could aggravate pancreatic tissue damage of acute pancreatitis. So, further research is needed to better explore this possibility.

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