

Effect of SB203580 on pathologic change of pancreatic tissue and expression of TNF- α and IL-1 β in rats with severe acute pancreatitis

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Abstract. – OBJECTIVE: This study aimed to investigate the effect of SB203580 which is the inhibitor of p38 mitogen-activated protein kinase on pathologic change of pancreatic tissue and expression of tumor necrosis factor- α (TNF- α) and interleukin-1-beta (IL-1 β) in rats with severe acute pancreatitis (SAP), Hematoxylin-eosin (HE) staining and immunohistochemistry were carried out in the present study.

METHODS: Forty-five male Wistar rats were divided into three groups: the SAP group (N=15), SB203580-treated group (SB group) (N=15), and the control group (N=15). Severe acute pancreatitis was induced by injection of sodium taurocholate into the pancreatic duct. For SB203580-treated group, SB203580 were administered via intraperitoneal injection (10 mg/kg). Serum amylase activity was measured 6, 12 and 24 hours respectively after the operation. The pancreas tissue were stained with HE for histopathological evaluation and the expression of TNF- α and IL-1 β in the pancreatic tissue were determined through inferior vena cava.

RESULTS: The results show that the level of amylase in SAP group was higher than that in the other groups. Further, the pancreas tissues of SB group rats were observed more mildly edematous, hemorrhagic and with monocytes infiltration. Based on immunohistochemical staining, the expression of TNF- α and IL-1 β in SAP rats were significantly increased than those of the control group. However, those of SB203580-treated group were more significantly reduced than those of SAP group ($p < 0.05$).

CONCLUSIONS: Those data suggest that SB203580, down regulating the expression of pro-inflammatory mediators such as TNF- α and IL-1 β , then through p38 MAPK signaling pathway inhibition, plays an important role in the treatment of SAP.

Key Words:

p38 Mitogen-Activated Protein Kinases, Tumor Necrosis Factor- α , Interleukin-1beta, SB 203580, Pancreatitis.

Introduction

Acute pancreatitis is usually a mild and self-limiting disease, but in a minority of the cases it develops into a severe disease, with high mortality¹. Death of severe acute pancreatitis (SAP) occurs biphasically with two different causes. Early death results from acute consequences of the pancreatic inflammatory process; thus, may lead to a systemic inflammatory response syndrome (SIRS) causing damage to remote organs and ultimately: multiple organ failure (MOF)². Later death is mainly caused by sepsis, especially infected pancreatic necrosis³.

The serum inflammatory mediators appear to play a critical role in the pathogenesis of pancreatitis and more so of the subsequent inflammatory response¹. TNF- α and IL-1 β are thought to play an important role. Levels of both pro-inflammatory mediators are elevated upon the onset and during the progress of acute pancreatitis^{4,5}.

The p38 mitogen-activated protein kinase (MAPK) is one of three major MAPK signaling pathways which is triggered by a wide range of stimuli such as proinflammatory cytokines (TNF- α , IL-1 β)^{3,6}. However, no data on the effects of p38 inhibition on the course of experimental pancreatitis is available so far. This study was to investigate the expression of pro-inflammatory mediators TNF- α and IL-1 β in rats with SAP, and the effects of p38 inhibition with SB 203580 on the expression of TNF- α and IL-1 β .

Methods and Materials

Research Design

Forty-five pathogen-free male Wistar rats weighing 200-300 g were provided by Laborato-

ry Animal Center of Anhui Medical University of China. Animals were fasted overnight except for free access to water. SB203580 (10 mg/ml) was supplied by Selleck Company (Houston, TX, USA). All studies were performed in accordance with the guide of the Committee on Care and Use of Laboratory Animals.

Forty-five male Wistar rats were divided at random into three groups which serve as control group, SAP group and SB203580-treated group (SB group), respectively. Each group (15 rats) was divided into three time points: 6, 12 and 24 hours (five rats for each time point).

All the rats were then anesthetized with 2.5% pentobarbital (0.1 mL/100 g body weight intraperitoneally). A midline laparotomy was performed, followed by the ligation of the bile-pancreatic ducts close to the liver and duodenum. Then in SAP group, the pancreatic duct was retrogradely injected 5% sodium taurocholate (0.1 mL/100 g body weight) for 1 minute and stagnant for 4 minutes. The SB203580-treated group rats were then treated with SB203580 via intraperitoneal injection (10 mg/kg) immediately after closing the incision. For control group, sham operation was performed with the injection of normal saline into the pancreatic duct and the peritoneal cavity (without sodium taurocholate and SB203580 administered).

Serum Amylase Activity and Histology

Six hours after SB203580 or normal saline treatment, five rats for each groups were operated again and serum amylase activity was measured by a chromogenic method with the Phadebas amylase test through inferior vena cava. The pancreas were rapidly removed and fixed in 10% neutral phosphate buffered formalin for histological study.

The specimens were embedded in 10% formaldehyde, stained with hematoxylin-eosin and evaluated in the optic microscopy. Pancreas tissue samples were examined by a pathologist who was kept unaware of the source of specimens, evaluated morphologic alterations following edema, hemorrhage, inflammation and necrosis of the pancreas, each graded from 0-3⁷.

When the time get to 12 hours after SB203580 or normal saline treatment, the other five rats for each group were sacrificed. Pancreas tissue samples were also observed under the optic microscopy. The procedure was the same for 24 hours time point.

Immunohistochemical Staining

Frozen and paraffin-embedded pancreas biopsy sections (5 μ m) were subjected to immunohistochemistry using standardized avidin-biotin peroxidase methodology. Before immunohistochemical labeling, paraffin sections were deparaffinized, rehydrated and boiled in a pressure cooker containing citrate buffer (pH 6) for 2 minutes. The sections were then cooled, rinsed in phosphate buffered saline (PBS) and processed for immunohistochemistry. All sections were examined and photographed under blind conditions.

The positive cell judgement standard is pale brown particles appear in cytoplasm. Semi quantitative analysis was done and the optical density (OD) value of positive region was calculated using image pro plus 6.0 analyzing software under 400 times of the optical microscope (five visual field for each pathological section). The OD value of the negative region was calculated as background. Phosphate buffered saline (PBS) was used to replace the first antibody as the blank control.

Statistical Analysis

All values are expressed as the mean $\pm$ SD. The method of Student's *t*-test was used to test the significance of their differences ($p < 0.05$) which was considered statistically significant.

Results

Serum Amylase Detection

The level of serum amylase was increased, which confirmed the diagnosis of acute pancreatitis. Table I shows the different values of amylase in each group. SAP group had higher level of amylase when compared to that of the other groups. According to the statistical analysis, there was significant difference between the SAP group and the control group ($p < 0.05$). However, after the rats were treated with SB203580, their level of amylase decreased significantly (Table I).

Pathological Examination

Furthermore, the findings of the histopathological analysis showed interstitial edema, parenchyma hemorrhage and necrosis, inflammatory infiltration of neutrophils into the pancreatic tissue. The changes became severer with the prolongation of time. The pancreas tissues of SB group rats at 12h and 24h were observed more mildly edematous, hemorrhagic and monocytes infiltration and the scores of pancreas tissues reduced significantly (Table II, Figure 1).

Table I. Serum amylase of SAP rats (n = 5 for each time points, $\bar{x} \pm s$).

Groups	Time (h)		
	6 h	12 h	24 h
Control	961 $\pm$ 133	974 $\pm$ 140	973 $\pm$ 137
AP	1401 $\pm$ 105*	1772 $\pm$ 179*	1997 $\pm$ 217*
SB	1271 $\pm$ 104*	1124 $\pm$ 88*. [#]	1107 $\pm$ 153 [#]

Compared to the control group, * $p < 0.05$. Compared to the SAP group, [#] $p < 0.05$.

Immunohistochemical Studies

Moderately positive expression of TNF- α and IL-1 β in pancreas tissues of the rats could be examined by immunohistochemistry. The expressions of TNF- α , IL-1 β became stronger in the pancreas tissues of SAP rats with the time prolonged. Positive expressions were weaker in SB group rats at 24 hours time point compared with the SAP group ($p < 0.05$) (Table III-IV, Figure 2).

Discussion

The serum levels of proinflammatory cytokines, including TNF- α , IL-1 β have been reported to be significantly higher in severe acute pancreatitis compared with mild pancreatitis⁸. Levels of TNF- α and IL-1 β both are elevated upon the onset and during the progress of acute pancreatitis⁹. Both TNF- α and IL-1 β are thought to play an important role in acute pancreatitis. Blockade of the TNF and IL-1 receptor before or soon after induction of pancreatitis is associated with decreased severity of pancreatitis and reduced intrinsic pancreatic damage¹⁰.

Pro-inflammatory mediators TNF- α and IL-1 β also significantly correlated with the onset of bacteraemia. The Cox risk analysis model revealed that IL-1 β and TNF- α were independent predictors of bacteraemia whereas they were not significantly associated with mortality as the sole

outcome¹¹. In human subjects, the role of TNF- α and IL-1 β as predictors of pancreatitis severity is poor. In case of bacteraemia, cytokine production may become more systemic and result in higher plasma levels of TNF- α and to a greater extent IL-1 β .

p38MAPK plays an important role in the inflammatory response. After p38MAPK is phosphorylated, it activates many transcription factors, which regulate a variety of target gene expression including proinflammatory cytokines gene¹².

During early phase of SAP, p38MAPK inhibitor has shown has protective effect on pancreatic injury¹³. Studies have shown that, p38MAPK activity rapidly ascending in the pancreatic tissue of SAP rats^{14,15}. Blinman et al¹⁶ demonstrated that the activity of p38MAPK enhanced in pancreatic acinar cell *in vitro* acute pancreatitis model; p38 inhibition reduced the production of cytokines.

Our study shows that SB203580 could ameliorate the pancreatic injury effectively. The pancreas tissues of SB group rats were observed more mildly edematous, hemorrhagic and with monocytes infiltration. Moreover, there were more positive expression of TNF- α and IL-1 β in the pancreatic tissue of SAP rats compared with the control group. The p38MAPK inhibitor SB203580 was also observed reduce the expression of TNF- α and IL-1 β .

Table II. Pancreas tissues scores ($\bar{x} \pm s$).

Groups	n	Time (h)		
		6 h	12 h	24 h
SAP	5	10.0 $\pm$ 1.58	12.8 $\pm$ 1.92	15.4 $\pm$ 1.14
SB	5	7.4 $\pm$ 1.14*	10.2 $\pm$ 1.48*	12.0 $\pm$ 1.58*

*Compared to the SAP group, $p < 0.05$.

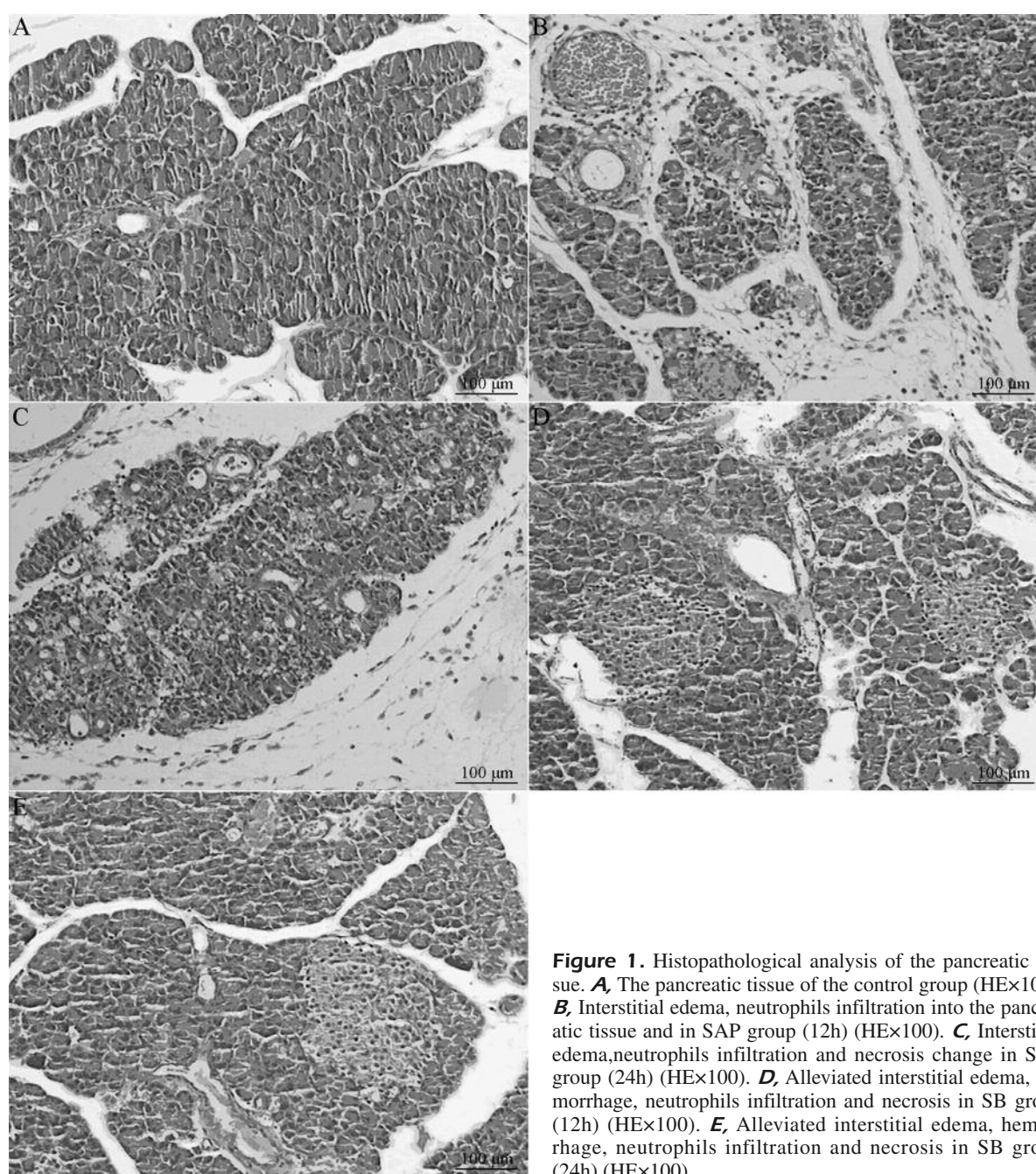


Figure 1. Histopathological analysis of the pancreatic tissue. **A**, The pancreatic tissue of the control group (HE×100). **B**, Interstitial edema, neutrophils infiltration into the pancreatic tissue and in SAP group (12h) (HE×100). **C**, Interstitial edema, neutrophils infiltration and necrosis change in SAP group (24h) (HE×100). **D**, Alleviated interstitial edema, hemorrhage, neutrophils infiltration and necrosis in SB group (12h) (HE×100). **E**, Alleviated interstitial edema, hemorrhage, neutrophils infiltration and necrosis in SB group (24h) (HE×100).

Table III. Semi-quantitative analysis on IL-1 β (OD value, $\bar{x} \pm s$).

Groups	n	IL-1 β		
		6 h	12 h	24 h
Control	5	0.403 $\pm$ 0.040	0.404 $\pm$ 0.035	0.400 $\pm$ 0.049
SAP	5	0.418 $\pm$ 0.046	0.488 $\pm$ 0.033*	0.552 $\pm$ 0.050*
SB	5	0.412 $\pm$ 0.046	0.444 $\pm$ 0.048	0.484 $\pm$ 0.027*.#

*Compared to the control group, $p < 0.05$. #Compared to the SAP group, $p < 0.05$.

Table IV. Semi-quantitative analysis on TNF- α (OD value, $\bar{x} \pm s$).

Groups	n	TNF- α		
		6 h	12 h	24 h
Control	5	0.378 $\pm$ 0.041	0.376 $\pm$ 0.018	0.378 $\pm$ 0.036
SAP	5	0.420 $\pm$ 0.040	0.498 $\pm$ 0.043*	0.562 $\pm$ 0.026*
SB	5	0.410 $\pm$ 0.020	0.434 $\pm$ 0.043*.#	0.472 $\pm$ 0.030*.#

*Compared to the control group, $p < 0.05$. #Compared to the SAP group, $p < 0.05$.

Conclusions

We observed that there is association between p38MAPK signaling pathway and the expression of pro-inflammatory mediators TNF- α and IL-1 β in a rat model of severe acute pancreatitis. Regu-

lating and blocking the expression of p38MAPK in the signaling pathways may become a new way in future treatment of SAP. In other words, the signal transduction pathway of p38MAPK could be the anti-inflammatory therapeutic targets in SAP treatment.

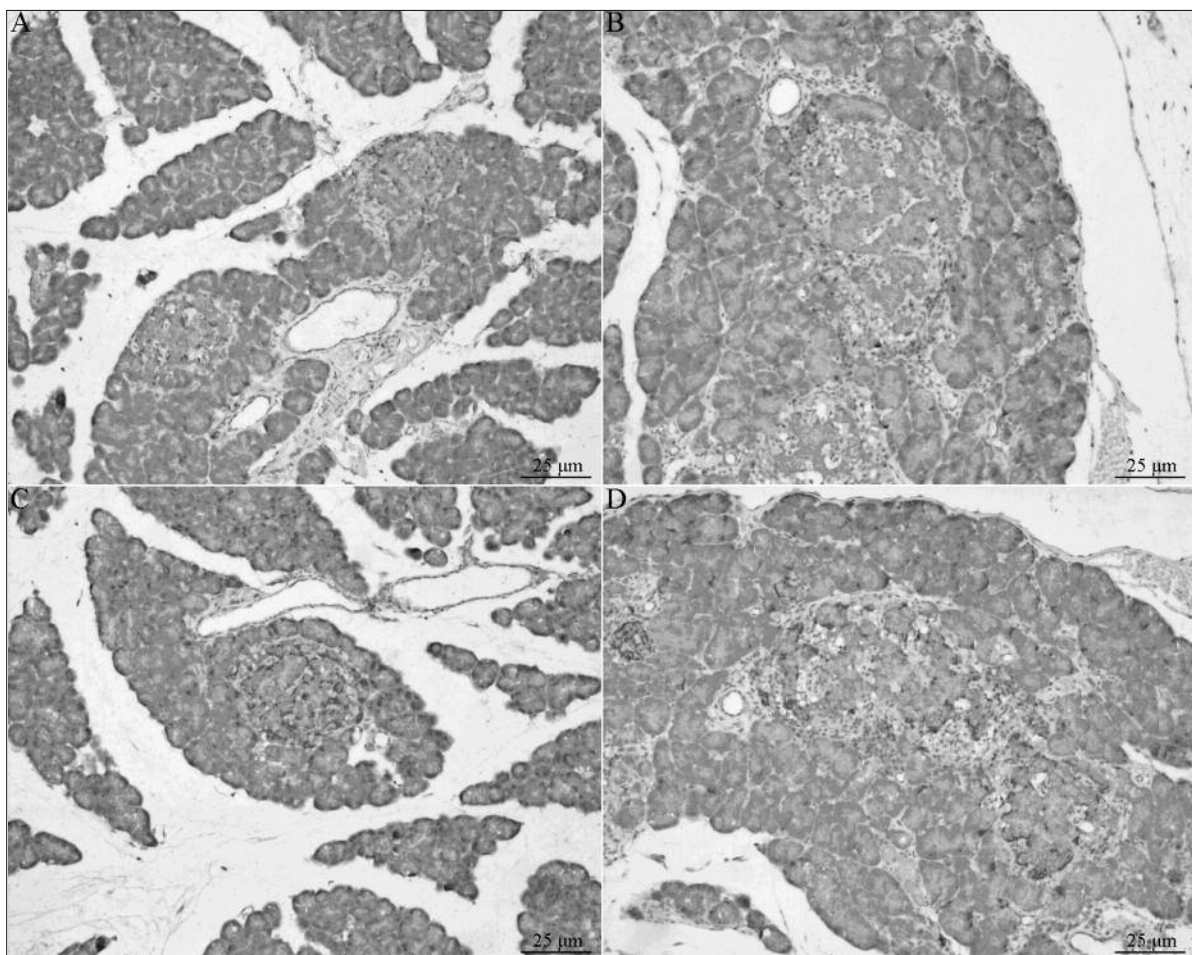


Figure 2. Immunohistochemical analysis of IL-1 β and TNF- α protein expression in the pancreatic tissue. **A**, IL-1 β expression in pancreas tissues of the control group ($\times 400$). **B**, IL-1 β expression in pancreas tissues of the SAP group (24h $\times 400$). **C**, TNF- α expression in pancreas tissues of the control group ($\times 400$). **D**, TNF- α expression in pancreas tissues of the SAP group (24h $\times 400$).

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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