High-mobility group box 1 protein and its role in severe acute pancreatitis

Xiao Shen, Wei-Qin Li

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Correspondence to: Wei-Qin Li, MD, PhD, Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, No. 305 Zhongshan East Road, Nanjing 210002, Jiangsu Province, China. njzy_pancrea@163.com

Telephone: +86-25-84803956
Fax: +86-25-84803956
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Abstract

The high mobility group box 1 (HMGB1), which belongs to the subfamily of HMG-1/-2, is a highly conserved single peptide chain consisting of 215 amino acid residues with a molecular weight of approximately 24894 Da. HMGB1 is a ubiquitous nuclear protein in mammals and plays a vital role in inflammatory diseases. Acute pancreatitis is one of the most common causes of acute abdominal pain with a poor prognosis. Acute pancreatitis is an acute inflammatory process of the pancreas (duration of less than six months), for which the severe form is called severe acute pancreatitis (SAP). More and more studies have shown that HMGB1 has a bidirectional effect in the pathogenesis of SAP. Extracellular HMGB1 can aggravate the pancreatic inflammatory process, whereas intracellular HMGB1 has a protective effect against pancreatitis. The mechanism of HMGB1 is multiple, mainly through the nuclear factor-κB pathway. Receptors for advanced glycation end-products and toll-like receptors (TLR), especially TLR-2 and TLR-4, are two major types of receptors mediating the inflammatory process triggered by HMGB1 and may be also the main mediators in the pathogenesis of SAP. HMGB1 inhibitors, such as ethyl pyruvate, pyrrolidine dithiocarbamate and Scutigera subspinipes mutilans, can decrease the level of extracellular HMGB1 and are the promising targets in the treatment of SAP.

Key words: High mobility group box 1 protein; Inhibitors; Inflammation; Severe acute pancreatitis; Nuclear factor kappa B

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Core tip: The newly discovered high mobility group box 1 protein is a ubiquitous nuclear protein that exists extensively in mammals. More and more studies have shown its vital role in inflammation. This paper is the first to reveal the bidirectional effect of this protein in the pathogenesis of severe acute pancreatitis and its role as a potential treatment target.
INTRODUCTION

The high mobility group box 1 protein (HMGB1), an important chromatin protein, is encoded by the Hmgb1 gene in humans\(^1,2\), and this protein was called amphoterin and was discovered 40 years ago\(^3\). This protein belongs to the high mobility group family and has an important role in mediating inflammation\(^3,4\). It has been shown that serum levels of HMGB1 are elevated in several inflammatory diseases, including sepsis, mechanical trauma, acute myocardial infarction, acute respiratory distress syndrome, hepatic injury, rheumatoid arthritis, and stroke\(^5-9\).

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas, and severe acute pancreatitis (SAP) is a severe type of acute pancreatitis associated with high mortality rates\(^10\). Recently, more and more studies have shown that HMGB1 may have a role in the SAP process. The aim of this review is to clarify the relationship between HMGB1 and SAP and to determine how HMGB1 affects the pathogenesis of SAP.

BRIEF INTRODUCTION OF HMGB1

High mobility group (HMG) proteins are a family of non-histone nuclear proteins that have a role in transcription, replication, recombination, repair, and other DNA-associated activities. HMG-1/-2, HMG-Y, and HMG-14/-17 are three subfamilies of HMG proteins\(^2\). HMGB1, which belongs to the subfamily of HMG-1/-2, is a highly conserved single peptide chain consisting of 215 amino acid residues with a molecular weight of approximately 24894 Da (Figure 1). The N terminal of the protein is composed of lysine that is rich in positive charge. The C terminal, also known as the acidic tail, is composed of aspartic acid and glutamic acid that are rich in negative charge. HMGB1 consists of the following three domains: A box (amino acid residues 9-79), B box (amino acid residues 95-163) and an acidic C-terminal tail (the receptor binding site, amino acid residues 186-215)\(^2,11-14\). Functional analysis has shown that the B box plays a major role in inflammation, and that the A box is the antagonistic site of the B box\(^16\). Both A and B boxes are able to bind to DNA and have a role in folding and distorting the double-stranded DNA. Generally, HMGB1 is ubiquitous in mammalian cells, and it is highly expressed in the liver, thymus, lymph tissue, testis, and in neonates\(^15\).

HMGB1 belongs to the family of damage-associated molecular pattern molecules, which can be recognized by pattern recognition receptors and initiate an immune response in the noninfectious inflammatory response\(^16\). As a nuclear protein, HMGB1 plays a vital role in nucleosome stabilization and DNA transcription. However, HMGB1 can also be released extracellularly under stress. Extracellular HMGB1 is known to affect certain cellular signal transduction pathways\(^17-19\). It is well known that extracellular HMGB1 is an important pro-inflammatory cytokine\(^20\). Although the exact intracellular signaling transduction mechanism of HMGB1 is not clear, it has been reported that receptors for advanced glycation end-products (RAGE) and toll-like receptors (TLR) are two major types of receptors mediating the inflammatory process triggered by HMGB1\(^21\).

SAP

AP is defined as an acute inflammatory process of the pancreas (duration less than six months) that affects other regional tissues or remote organ systems\(^10\). Of these, the lungs and kidneys are the most affected organs. Acute lung injury or acute respiratory distress syndrome can occur immediately or during the later course of pancreatitis, as well as acute kidney injury or acute renal failure. AP is often caused by biliary tract diseases, alcohol abuse, trauma, surgery, overeating, metabolic disorders (e.g., hypercalcemia and hyperlipidemia), infection, or other related factors. The typical symptoms of AP are sudden-onset upper abdominal pain often radiating to the back, and a significant elevation of serum lipase or amylase (three times above normal)\(^22\). According to the severity of the disease, AP can be divided into the following three degrees: mild, moderately severe, and severe. The latest classification also includes a new categorization of critical AP, which describes infected pancreatitis with persistent organ failure\(^23\).

SAP is characterized by the existence of either infected pancreatic necrosis or persistent organ failure, and has a global mortality of 15%-30%\(^22,24\). Organ failure is defined as shock (systolic blood pressure < 90 mmHg), pulmonary insufficiency (PaCO\(_2\) ≤ 60 mmHg), renal failure (serum creatinine level > 177 µmol/L or 2 mg/dL after resuscitation\(^25\)), or gastrointestinal bleeding > 500 mL per 24 h. In the
Recent updated classification of AP, the definition of organ failure differs slightly and is based on the failure of the following three organ systems: cardiovascular (need for inotropic agent), renal (serum creatinine level $\geq 171$ $\mu$mol/L or $\geq 2$ mg/dL) and respiratory ($\text{PaO}_2$/FiO$_2$ $\leq 300$ mmHg or 40 kPa$^{[23]}$). Organ failure lasting more than 48 h is considered to be persistent and is the main feature of SAP. Organ failure can be either single or multiple. Patients with organ failure are more likely to develop local complications$^{[22]}$. Patients with SAP always develop systemic inflammatory response syndrome (SIRS). SIRS can be diagnosed with the presence of two or more manifestations$^{[26,27]}$ (listed in Table 1).

<table>
<thead>
<tr>
<th>Finding</th>
<th>Value</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>$&lt;36$ °C or $&gt;38$ °C</td>
</tr>
<tr>
<td>Heart rate</td>
<td>$&gt;90$ beats/min</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>$&gt;20$/min or $\text{PaCO}_2$ $&lt;32$ mmHg ($4.3$ kPa$^{[23]}$)</td>
</tr>
<tr>
<td>WBC</td>
<td>$&lt;4 \times 10^9$/L ($&lt;4000$/mm$^3$), $&gt;12 \times 10^9$/L ($&gt;12000$/mm$^3$), or $&gt;10$% bands</td>
</tr>
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</table>

WBC: White blood cell.

The main feature of SAP is necrosis of the pancreas. Regardless of the etiology, the pathogenesis of SAP is mainly due to the autodigestion of the pancreas by pancreatic juice and trypsinogen activation. Normally, the pancreas uses the following defense mechanisms against autodigestion: (1) the protective layer in the epithelial of pancreatic duct composed of mucopolysaccharides; (2) pancreatic acini can prevent the invasion of pancreatic enzymes inside of the cells; and (3) the blood flow into the pancreas contains substances that can neutralize pancreatic enzymes. Furthermore, the majority of pancreatic enzymes, such as trypsins, are secreted in the form of zymogens (non-activated pancreatic enzymes). However, these aforementioned defense mechanisms can be destroyed in a number of pathologic conditions, for example, the obstruction of the pancreatic duct or the invasion of acini by infected bile. Both of these conditions can lead to increased pressure in the pancreatic duct, rupture of pancreatic acini and a sudden, explosive release of all the pancreatic enzymes, including trypsin, pancrelipase and amylopsin. These mechanisms result in the autodigestion of the pancreas$^{[28-31]}$.

Autophagy is the primary cellular degradative pathway in AP. Recent studies have shown that autophagy is impaired in pancreatitis as a result of defective lysosomes, involving mainly the following three major autophagic pathways: chaperone-mediated autophagy, microautophagy, and macroautophagy$^{[32,33]}$. In addition, the following enzyme systems are also activated in AP: (1) collagenases allow the spread of inflammation; (2) elastases can damage the walls of blood vessels and cause bleeding; (3) ubiquitin-proteasome complex can further extend tissue necrosis; and (4) lipases can cause necrosis of adipose tissue around the pancreas (such as mesenteric root, lesser omental bursa, retroperitoneal space, renal artery, both sides of aorta, pelvic cavity, etc.). Calcium can combine with the fat necrosis and lead to the formation of saponification spots, which is one of the reasons for hypocalcemia in patients. Meanwhile, the decomposed and necrotic pancreatic tissue can produce vasooactive substances, including kallikrein, bradykinin and prostaglandin. These substances can decrease the tension of pericardial blood vessels, and coupled with a substantial amount of peripancreatic exudation and a sharp drop in blood volume as well as blood pressure, the pre-existing circulatory disorder and renal damage can further deteriorate. In addition, the myocardial depressant factor in the necrotic toxin can cause further damage to cardiopulmonary function. Organ dysfunction may also involve the liver and central venous system. All of these lesions can be referred to “enzymatic shock.” As a result of activation of all the enzymes, the damaged acinar cells will consequently lead to necrosis and inflammation$^{[34]}$.

The progression of AP can be divided into the following three phases: local acinar injury, systemic response, and generalized sepsis$^{[35]}$. Although the exact pathogenesis has not been completely revealed, activation of nuclear factor (NF)-κB is a key link$^{[36]}$. NF-κB plays a vital role in various stages of pancreatitis via mediation of the inflammatory process$^{[37]}$, and NF-κB activation is considered to be independent of trypsinogen activation in the pathogenesis of AP$^{[38,39]}$. Moreover, the intracellular Ca$^{2+}$ signaling pathway and protein kinase C may trigger the early activation of NF-κB in pancreatic acini$^{[40]}$. A great amount of pro-inflammatory mediators can be released as a result of NF-κB activation during pancreatitis, including numerous types of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-6 and IL-18, various chemokines such as IL-8, macrophage inflammatory protein-1, growth-related onco­gene-α and monocyte chemoattractant protein-1, reactive oxygen species, reactive nitrogen species, platelet-activating factor and adhesion molecules$^{[40-44]}$. Extra-pancreatic NF-κB activation can also be seen in the liver, lungs, endothelium and peripheral blood monocyte-macrophages$^{[45-47]}$.

In mild AP, inflammatory reaction as well as pro-inflammatory mediators is always confined to the pancreas. In SAP, the inflammation of the pancreas can be further exacerbated and cause a SIRS, which is an amplified and overwhelming inflammatory response. Major pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, are then released into the circulation and can cause remote organ injury$^{[48,49]}$. Activated circulating neutrophils and...
ROLE OF HMGB1 IN INFLAMMATION

HMGB1 was first reported by Wang et al\cite{4} as a mediator for endotoxin lethality in animal models. Later, the authors considered HMGB1 to be a potential late inflammatory mediator involved in the pathogenesis of sepsis\cite{56}. HMGB1 is derived from the secretions of certain immune cells (such as monocytes) and non-immune cells (such as epithelial cells), as well as passive release from necrotic and apoptotic cells\cite{4,19,57-60}. HMGB1 can mediate inflammation via receptors of innate immune systems such as TLR and RAGE in the pathogenesis of many inflammatory diseases, including sepsis, pancreatitis and arthritis\cite{61,62}.

TLRs are a family of proteins that play a vital role in the innate immune system. TLRs are named for their resemblance to the protein encoded by the Toll gene discovered by Christiane in 1985\cite{63}. TLRs belong to the family of pattern recognition receptors and respond to the structurally conserved molecules derived from various germs, the so-called pathogen-associated and damage-associated molecular pattern. Thirteen TLRs (named TLR1 to TLR13) have been found in mammalian species\cite{25,64,65}.

TLR4 is the first target activated by extracellular HMGB1\cite{66-68}. TLR4 is the only TLR that uses four adaptors, myeloid-differentiation primary response protein 88 (MyD88), MAL, Toll/IL-1 receptor domain-containing adaptor-inducing IFN-β (TRIF), and TRAM and mediates their effects through the NF-κB and MAPK pathways. After TLR4 is combined by HMGB1 or forms a complex with HMGB1 and exogenous or endogenous molecules, it initially recruits MyD88 and thus activates the downstream NF-κB. Activated NF-κB is then transported from the cytoplasm to the nucleus and induces the expression of major inflammatory factors such as IL-1β and IL-6, interferon (IFN)γ, and TNF-α in the early phase\cite{66}. The aforementioned HMGB1-TRIF pathway can also trigger a second downstream pathway mediated by TRAF and leads to activation of type I interferon as well as a delayed activation of NF-κB\cite{69}, resulting in the release of cytokine and activation of macrophages. IL-1β, IL-6 and TNF-α are always released during the early stage of inflammation (the development of systemic inflammatory response) whereas HMGB1 is often released by macrophages 12-18 h after the onset of the inflammatory response and only mediates the late stage of the inflammatory process\cite{41}. The HMGB1 released extracellularly spurs a cascade of inflammatory reactions both in local and distant organs, ultimately resulting in multi-organ dysfunction. Other TLRs such as TLR2 and TLR9 also have a role in the inflammatory mediation by HMGB1\cite{17,70}. There are many overlaps between the TLR4-mediated pathway and TLR-2-mediated pathway, and the mechanism is almost the same\cite{17}.

RAGE is another important receptor of HMGB1. It is expressed on a variety of cells, such as monocytes and belongs to the immunoglobulin superfamily\cite{71,72}. Other than HMGB1, RAGE can also interact with diverse ligands, including S100 protein\cite{73,74}. Two major signaling pathways that are activated by RAGE are p38 MAPK and ERK1/2 pathways\cite{17}. Both pathways can lead to the phosphorylation and degradation of inhibitors of NFκB by IκB kinase and thus activate NFκB. Activated NFκB then transfers to the nucleus and results in increase in NFκB DNA binding, expression of various pro-inflammatory cytokines (e.g., TNFα, IL-1β, IL-6), proliferation of cells and chemotaxis.

ROLE OF HMGB1 IN PANCREATITIS

Extracellular HMGB1 was already known as a novel pro-inflammatory cytokine in humans\cite{56}. In the last decade, many studies have claimed a positive correlation between extracellular HMGB1 and SAP severity (Table 2).

Yasuda and colleagues\cite{75} were the first to report that the serum HMGB1 level was significantly increased within 72 h in patients with SAP. They compared forty-five SAP patients with eight healthy controls and found that the mean level of serum HMGB1 was nearly three times higher in patients with SAP and was positively related to the severity of SAP as well as organ dysfunction and infection. Furthermore, serum HMGB1 levels were positively correlated with serum lactate dehydrogenase, C-reactive protein, and total bilirubin and could estimate the prognosis of SAP patients; i.e., the higher the serum HMGB1 level, the worse the outcome. This result indicates that HMGB1 may be an important mediator in the pathogenesis of pancreatitis and organ dysfunction. To confirm this result further, they designed an experiment in mice. They chose thirty-eight female C3H/HeN mice and divided them into the following three groups: group A included eight mice with sham operations and intraperitoneal injections of normal saline; group B included twenty mice with SAP (induced by duodenal loop closure) and intraperitoneal injections of normal saline; group C included twelve mice with SAP and intraperitoneal operations.
injections of anti-HMGB1 neutralizing antibody\(^{[76]}\). Serum amylase levels were significantly decreased in mice injected with anti-HMGB1 neutralizing antibody compared with those injected with normal saline twelve hours after induction of SAP. The morphology of the pancreas and lungs changed significantly in SAP mice, but was ameliorated in groups injected with anti-HMGB1 neutralizing antibody. Similar changes were also seen in the liver and kidneys. These results demonstrated that blockade of HMGB1 attenuated the development of SAP and associated organ dysfunction. Subsequently, they conducted another small experiment and proposed a hypothesis called "HMGB1 circulation"\(^{[77]}\); HMGB1 is first produced by pancreatic and peritoneal macrophages during early SAP in response to inflammation and then partially released to the blood, thereby causing damage to remote organs. In turn, damaged organs can also release HMGB1 and cause a vicious circle. Kocsis \textit{et al.}\(^{[78]}\) also found a decrease in serum soluble RAGE in patients with severe pancreatitis compared with healthy controls and mild pancreatitis patients and revealed an inverse correlation between serum levels of soluble RAGE and HMGB1. In addition, they also found significantly elevated circulating DNA levels in patients with SAP and sepsis. This result was in contrast to the result of the study conducted by Bagul \textit{et al.}\(^{[79]}\) and Lindström \textit{et al.}\(^{[80]}\). Moreover, HMGB1 also contributes to the development of intestinal barrier dysfunction secondary to SAP, and HMGB1 levels in intestine were correlated with the severity of intestinal barrier dysfunction\(^{[81]}\). The study by Luan \textit{et al.}\(^{[82]}\) was consistent with that by Sawa \textit{et al.}\(^{[83]}\) and showed that downregulating HMGB1 levels using siRNA could inhibit NF-\(\kappa\)B activation, reduce inflammatory reaction and protect against SAP-associated lung injury.

In summary, serum and pancreatic levels of HMGB1 are increased significantly in patients with SAP and SAP-induced animal models. There are two possible mechanisms\(^{[75]}\). First, this phenomenon can be explained by the theory of "HMGB1 circulation." Second, as HMGB1 levels in the lungs and intestine were also increased in animal models and patients with SAP-associated acute lung injury and intestinal injury, HMGB1 may be released directly by the pancreas as well as damaged organs in SAP. Further research is needed to reveal the exact function of HMGB1 in SAP.

As mentioned before, HMGB1 has two functional

### Table 2 Relationship between high mobility group box 1 protein and severe acute pancreatitis

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Journal</th>
<th>Country</th>
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<th>Method</th>
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<tbody>
<tr>
<td>Yasuda \textit{et al.}(^{[83]})</td>
<td>Pancreas</td>
<td>Japan</td>
<td>Patients with SAP</td>
<td>Control group: healthy volunteers ((n = 8)); Experimental group: patients with SAP ((n = 45))</td>
<td>Serum HMGB1 levels were significantly increased in patients with SAP and were correlated with disease severity</td>
</tr>
<tr>
<td>Kocsis \textit{et al.}(^{[78]})</td>
<td>Pancreol</td>
<td>Hungary</td>
<td>Patients with AP</td>
<td>Control group: healthy volunteers ((n = 20)); AP group: patients with pancreatitis and divided into mild ((n = 32)) and severe ((n = 30)) subgroups; Sepsis group: patients with sepsis ((n = 20))</td>
<td>HMGB1 was significantly elevated in the plasma of SAP patients compared with healthy and mild pancreatitis patients, and was correlated with procalcitonin concentrations. There was an inverse correlation between the levels of sRAGE and HMGB1 in patients with SAP. Circulating DNA was significantly elevated in patients with severe pancreatitis or sepsis and was related to the severity scores</td>
</tr>
<tr>
<td>Lindström \textit{et al.}(^{[81]})</td>
<td>Pancreas</td>
<td>Finland</td>
<td>Patients with AP</td>
<td>Grade 0: mild AP ((n = 282)); Grade 1: SAP without organ failure ((n = 135)); Grade 2: SAP with organ failure ((n = 38))</td>
<td>Serum HMGB1 level is comparable in three groups, but sRAGE is significantly higher in AP patients who develop organ failure compared to AP patients who recover without organ failure</td>
</tr>
<tr>
<td>Yuan \textit{et al.}(^{[77]})</td>
<td>Pancreas</td>
<td>China</td>
<td>Male ICR mice</td>
<td>Control group: SAP mice ((n = 24)); Treatment group: SAP mice treated with recombinant HMGB1 A box protein 12 ((n = 12)) and 24 h ((n = 12)) after the modeling injection</td>
<td>HMGB1 A box can decrease the serum HMGB1 levels, attenuate organ dysfunction and improve the survival of SAP mice. Thus, it has a remarkable protective effect against pancreatitis and associated organ injury</td>
</tr>
<tr>
<td>Luan \textit{et al.}(^{[82]})</td>
<td>Immunobol</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>Control group: sham operation; SAP group: SAP-induced rats; Treated groups: SAP-induced rats treated with pRNA-U6.1/Neo-HMGB1 (containing siRNA targeting human HMGB1)</td>
<td>Downregulation of HMGB1 by using siRNA could inhibit the activation of NF-(\kappa)B in SAP rats so as to decrease the levels of downstream inflammatory cytokines, alleviate endothelial permeability and attenuate severe pancreatitis-associated acute lung injury</td>
</tr>
<tr>
<td>Kang \textit{et al.}(^{[84]})</td>
<td>Gastroenterol</td>
<td>United States</td>
<td>HMGB1 flox/ flox and Pdx1-Cre transgenic mice</td>
<td>AP group: AP-induced mice; Control group: administered with saline as a control</td>
<td>Deficiency of endogenous HMGB1 could escalate local inflammation through destabilization of the nucleus and enable rapid DNA and histone release, resulting in accelerated tissue injury and lethality, indicating that intracellular HMGB1 appears to have a protective effect against inflammation</td>
</tr>
</tbody>
</table>

AP: Acute pancreatitis; HMGB1: High mobility group box 1 protein; SAP: Severe acute pancreatitis; sRAGE: Soluble receptors for advanced glycation end-products.
structures: the A box and B box. The A box is the main site mediating the anti-inflammatory process, while the B box is the main site for the pro-inflammatory response[83]. To study the effect of the A box in pancreatitis further, Yuan et al[84] designed a study in male mice. The SAP groups were divided into control and treatment groups. In the treatment group, the SAP mice were treated with a recombinant HMGB1 A box protein 12 and 24 h after modeling. The HMGB1 A box significantly improved the elevation of the serum levels of HMGB1 and pancreatic injury and alleviated other organ injury more than that in control group. As a result, the HMGB1 A box showed a protective effect against SAP and improved the survival rate. The latest study conducted by Kong et al[85] also confirmed the protective effect of HMGB1 A box in lung injury induced by AP.

A more recent study by Kang et al[86] demonstrated a protective effect of intracellular HMGB1 against inflammation, limiting AP in HMGB1 knockout mice. This finding indicates the complex role of HMGB1 in AP, i.e., endogenous HMGB1 derived from the pancreas itself can protect cells from activation of NF-κB, release of nucleosomes and DNA damage, thereby limiting the severity of pancreatic injury. In contrast, extracellular HMGB1 released from innate immune cells including macrophages and monocytes would aggravate the inflammatory response and increase pancreatic and remote organ damage.

EFFECT OF HMGB1 INHIBITORS IN AMELIORATING PANCREATITIS

Recently, some researchers studied the effect of HMGB1 inhibitors in preventing against SAP (Table 3). They thought HMGB1 inhibitors might be potential targets in ameliorating SAP. Anti-HMGB1 neutralizing antibody is an inhibitor for HMGB1. Ethyl pyruvate (EP), pyrrolidine dithiocarbamate (PDTC) and Scolopendra subspinipes mutians (SSM) are three potential HMGB1 inhibitors that may attenuate pancreatitis.

Anti-HMGB1 neutralizing antibody was reported to ameliorate the inflammatory reaction in the airway, liver and intestine and could even prevent bacterial translocation[87,88]. Sawa et al[89] reported that blockade of the high mobility group box-1 protein using the anti-HMGB1 antibody could attenuate SAP in the mouse model.

EP, which is derived from pyruvic acid, is an important intermediate product in glucose metabolism[89,90]. EP was first used as a potential treatment in rat models of reactive oxygen species-mediated acute renal failure[89]. EP is reported to inhibit lipopolysaccharide-induced NF-κB activation and then decrease HMGB1 levels in sepsis-induced mice and show its protective effect in all types of organ dysfunction[90,92-95]. Yang et al[96] first demonstrated that delayed treatment with EP could downregulate the inflammatory reaction and ameliorate the development of both local and distant organ dysfunction in an animal model of severe necrotizing pancreatitis. Subsequently, Cheng et al[97] and Yang et al[96] found that the anti-inflammatory effect of EP was via modulating HMGB1 and other inflammatory cytokine responses. Further research found that the mechanism of EP was by inhibiting the activation of NF-κB and downregulating serum HMGB1, TNF-α, IL-1β and other cytokines to ameliorate tissue injury and organ dysfunction in AP[98]. Acute lung injury is the most common extra-pancreatic complication leading to death in SAP patients. Studies showed that EP protected against the development of lung injury in SAP-induced mice[92]. Moreover, EP can attenuate other SAP-associated organ dysfunctions, such as liver and intestinal barrier injury in murine models[81,82,99,100].

PDTC is an antioxidant that can prevent induction of nitric oxide synthase. Some studies have shown that PDTC may be a potent inhibitor of NF-κB and play a role in suppressing the inflammatory process[101,102]. A recent study has shown that PDTC pre-administration can decrease HMGB1 levels and alleviate the inflammatory reaction in SAP rats by inhibiting NF-κB activation[103]. However, it was less effective when it was given 2 h after the induction of pancreatitis, therefore indicating that PDTC may indirectly inhibit HMGB1.

SSM is a polysaccharide that is extracted from Scolopendra. It was reported that SSM has many biologic effects including anti-inflammation as a traditional medicine[104-106]. A study by Jo et al[107] showed that SSM pre-treatment decreased cytokines, including HMGB1, TNF-α and IL-1β, by inhibiting c-Jun NH2-terminal kinase, p38 and NF-κB in AP mice. Therefore, SSM can attenuate the development of AP and related lung injury.

CONCLUSION

We reviewed all the studies on HMGB1 and SAP and drew several conclusions (Figure 2). First, extracellular HMGB1 is a vital mediator of inflammation and plays a major role in many inflammatory-related diseases. A number of studies have reported the increased levels of serum HMGB1 in SAP patients or models and showed its positive correlation with the severity of the disease. Second, decreasing HMGB1 levels by HMGB1 antibodies, the A Box or specific inhibitors can significantly decrease the release of related cytokines and reduce the inflammatory reaction in pancreatitis, thereby attenuating organ dysfunction and improving prognosis. Delayed EP administration is known to be an effective way to
### Table 3  Effect of high mobility group box 1 protein inhibitors in preventing against severe acute pancreatitis

<table>
<thead>
<tr>
<th>Ref.</th>
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<tbody>
<tr>
<td>Sawa et al.</td>
<td>World J Gastroenterol</td>
<td>Japan</td>
<td>Female C3H/HeN mice</td>
<td>Anti-HMGB1 neutralizing antibody</td>
<td>Group A: sham, laparotomy with saline injection (n = 6); Group B: SAP, SAP with saline injection (n = 20); Group C: HMGB1 Ab + SAP, SAP with anti-HMGB1 antibody injection (n = 12) Blockade of HMGB1 in the early phase is useful as a new therapeutic option against the inflammatory response and MODS in patients with SAP</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>Crit Care Med</td>
<td>United States</td>
<td>Male C57BL/6 mice</td>
<td>EP</td>
<td>RLS group: mice were injected with RLS; REPS group: mice were treated with REPS; Control group: mice were injected with PBS</td>
</tr>
<tr>
<td>Cheng et al.</td>
<td>Pancreas</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Group A: SAP-induced rats; Group B: moderate pancreatitis-induced rats; Group C: mild pancreatitis-induced rats; Control group: rats received the same dose of vehicle solution Delaysed treatment with EP downregulated the inflammatory response through decreasing the release of pro-inflammatory cytokines and attenuated the development of both local and distant organ dysfunction, improving survival in a murine model of severe necrotizing pancreatitis</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>World J Gastroenterol</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Group I: sham operation (n = 32); Groups II: SAP-induced rats and treated with EP (n = 32); Groups III: SAP-induced rats (n = 32) A strong correlation between levels of HMGB1 and severity of acute pancreatitis. Treatment with EP significantly protected against SAP lethality and ameliorated extrapancreatic tissue and organ injury or dysfunction in rats with SAP</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>J Surg Res</td>
<td>United States</td>
<td>Male C57BL/6 mice</td>
<td>EP</td>
<td>Control group: injected with PBS; EP group: SAP-induced mice and treated with EP; RLS group: SAP-induced mice and treated with RLS EP is able to inhibit NF-κB DNA binding, decrease the level of both early inflammatory cytokines such as TNF-α, IL-6, COX-2, and iNOS and late proinflammatory mediator (HMGB1), reduce inflammatory cells infiltration, and reverse hepatic oxidative stress, thus protecting hepatocytes from SAP-induced injury. Thus, treatment with EP ameliorates hepatocellular injury and redox stress in the setting of SAP</td>
</tr>
<tr>
<td>Luan et al.</td>
<td>Pancreas</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Control group: sham operation (n = 20); SAP group: SAP-induced rats (n = 32); EP-treated group: SAP-induced rats and treated with EP (n = 32) HMGB1 contributes to the development of gut barrier dysfunction after SAP. Intestinal HMGB1 levels were significantly increased in rats with SAP and were correlated with the severity of intestinal barrier dysfunction</td>
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<tr>
<td>Luan et al.</td>
<td>Pancreas</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Control group: sham operation (n = 8); SAP group: SAP-induced rats (n = 8); EP-treated group: SAP-induced rats and treated with EP (n = 8) EP administration inhibits NF-κB activation to suppress the expression of both early (TNF-α, IL-1β) and late (HMGB1) cytokines that mediate liver injury after SAP and reduces liver injury in SAP rats. Thus EP can provide durable protection against the deleterious effects of proinflammatory cytokines and HMGB1</td>
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<tr>
<td>Luan et al.</td>
<td>J Surg Res</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Control group: sham procedure (n = 48); SAP group: SAP-induced rats (n = 48); EP-treated group: SAP-induced rats and treated with EP (n = 48) EP attenuates taurocholate-induced pancreatitis and pancreas injury, decreases the taurocholate-induced pancreatic expression of TNF-α and HMGB1, alleviates neutrophil infiltration and lipid peroxidation in the pancreas and decreases NF-κB DNA binding activity as well</td>
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<tr>
<td>Luan et al.</td>
<td>Clin Exp Immunol</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>EP</td>
<td>Control group: sham procedure (n = 48); SAP group: SAP-induced rats (n = 48); EP-treated group: SAP-induced rats and treated with EP (n = 48) EP can reduce the lung permeability index in mice with LPS-induced acute lung injury in a dose-dependent way. The protective effect is associated with a reduction in both early (TNF-α and IL-1β) and late (HMGB1) cytokine levels by inhibiting NF-κB activity</td>
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<tr>
<td>Zhang et al.</td>
<td>Dig Dis Sci</td>
<td>China</td>
<td>Male Sprague-Dawley rats</td>
<td>antioxidant PDTC</td>
<td>Sham operation group (n = 48); SAP group (n = 48); PDTC-treated group (n = 48) HMGB1 is a late cytokine mediator that plays an important role in the pathogenesis of SAP. PDTC pre-administration might inhibit NF-κB activation to inhibit the production of HMGB1 and reduce pancreas injury in SAP rats; but PDTC was less effective when it was given 2 h after the induction of pancreatitis</td>
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</table>
inhibit HMGB1 release in the setting of SAP, whereas PDTC and SSM work only when administered in advance. A possible mechanism of these components is to inhibit the activation of NF-κB and reduce extracellular HMGB1 levels; hence, ameliorating the development of SAP. However, the exact mechanisms of these inhibitors still need verification through more fundamental studies. Lastly, the latest study detected the role of intracellular HMGB1 in inflammation and demonstrated its protective effect against pancreatitis in HMGB1 knockout mice. This research indicates that HMGB1 may have a bidirectional effect in the pathogenesis of SAP.

In addition to inflammation, HMGB1 also plays a regulatory role in angiogenesis. It is now known that HMGB1 affects many angiogenesis-related conditions, such as cancer, proliferative diabetic retinopathy and wound healing, via the p35 pathway and is said to be a promising therapeutic target in many tumors including epidermal tumors, prostate cancer and colon cancer. The exact function of HMGB1 and its mechanism still need to be elucidated.

## Figures

### Figure 2 Strategies targeting high mobility group box 1 in severe acute pancreatitis

Severe acute pancreatitis (SAP) can be induced by gallstone, hypertriglyceridemia (HTG), alcohol and other causes. High mobility group box 1 (HMGB1) can be activated in the condition of pancreatitis by inflammation reactions. In turn, HMGB1 can further deteriorate pancreatitis through toll like receptors (TLR) and receptor for advanced glycation end products (RAGE). Anti-HMGB1 treatment is beneficial in SAP as described in the text by using anti-HMGB1 antibodies, HMGB1 antagonist A box, and some specific HMGB1 inhibitors such as ethyl pyruvate (EP), pyrrolidine dithiocarbamate (PDTC) and Scopolandra subsinuata multitins (SSM).

## References


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MDS: Multorgan dysfunction; ALT: Alanine transaminase; AP: Acute pancreatitis; AST: Aspartate transaminase; COS: Cyclooxygenase; Cr: Creatinine; EP: Ethyl pyruvate; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharide; NF: Nuclear factor; PDTC: Pyrrolidine dithiocarbamate; RAGE: Receptor for advanced glycation end products; SSM: Scopolandra subsinuata multitins; TNF: Tumor necrosis factor.

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**Figure 2** Strategies targeting high mobility group box 1 in severe acute pancreatitis. Severe acute pancreatitis (SAP) can be induced by gallstone, hypertriglyceridemia (HTG), alcohol and other causes. High mobility group box 1 (HMGB1) can be activated in the condition of pancreatitis by inflammation reactions. In turn, HMGB1 can further deteriorate pancreatitis through toll like receptors (TLR) and receptor for advanced glycation end products (RAGE). Anti-HMGB1 treatment is beneficial in SAP as described in the text by using anti-HMGB1 antibodies, HMGB1 antagonist A box, and some specific HMGB1 inhibitors such as ethyl pyruvate (EP), pyrrolidine dithiocarbamate (PDTC) and Scopolandra subsinuata multitins (SSM).


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