Glycyrrhizin Suppresses the Expressions of HMGB1 and Relieves the Severity of Traumatic Pancreatitis in Rats

Ke Xiang1,2*, Long Cheng2*, Zhulin Luo2, Jiandong Ren3, Fuzhou Tian2*, Lijun Tang2, Tao Chen2, Ruiwu Dai2

1. The Third Military Medical University, Chongqing, P. R. China, 2. Department of General Surgery, Chengdu Military General Hospital, Chengdu, P. R. China, 3. Department of Pharmacy, Chengdu Military General Hospital, Chengdu, P. R. China

*tfz3006101@163.com

These authors contributed equally to this work.

Abstract

Background: High mobility group box 1 (HMGB1) plays important roles in a large variety of diseases; glycyrrhizin (GL) is recognized as an HMGB1 inhibitor. However, few studies have focused on whether glycyrrhizin can potentially improve the outcome of traumatic pancreatitis (TP) by inhibiting HMGB1.

Methods: A total of 60 male Wistar rats were randomly divided into three groups (n=20 in each): Control group, TP group and TP-GL group. Pancreatic trauma was established with a custom-made biological impact machine-III, and GL was administered at 15 minutes after the accomplishment of operation. To determine survival rates during the first 7 days after injury, another 60 rats (n=20 in each) were grouped and treated as mentioned above. At 24 hours of induction of TP, the histopathological changes in pancreas were evaluated and serum amylase levels were tested. Serum tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and HMGB1 were measured using enzyme linked immunosorbent assay. HMGB1 expressions in pancreas were measured using immunohistochemical staining, Western blot and Real-Time PCR analysis.

Results: Serum levels of HMGB1, TNF-α and IL-6 were increased dramatically in TP group at 24 hours after induction of TP. However, these indicators were reduced significantly by GL administration in TP-GL group comparing with TP group (P<0.05). Meanwhile, survival analysis showed that the seven-day survival rate in TP-GL group was significantly higher than that in TP group (85% versus 65%, P<0.05). GL treatment significantly decreased the pancreatic protein and mRNA expressions of HMGB1 and ameliorated the pancreatic injury in rats with TP.
Conclusions: Glycyrrhizin might play an important role in improving survival rates and ameliorating pancreatic injury of TP by suppression of the expressions of HMGB1 and other proinflammatory cytokine.

Introduction

Although pancreatic trauma is rare, occurring in only 2% to 5% of trauma victims, it is often imperceptible and intractable with a higher morbidity and mortality. Most pancreatic injuries in China are due to blunt abdominal trauma, such as motor vehicle crashes, falls, bicycle handlebar injuries, etc., while in Western countries, pancreatic injuries are due to penetrating abdominal trauma. The incidence of pancreatic trauma accounts for 5% of closed abdominal trauma and 2%–6% of abdominal penetrating trauma [1]. As early signs and symptoms of pancreatic trauma are not obvious, it is often noticed until trauma-induced acute pancreatitis is presented. Trauma-induced acute pancreatitis, also referred to as traumatic pancreatitis (TP), are often followed by some serious complications, such as systemic inflammatory response syndrome (SIRS), shock, multiple organ failure (MOF), acute pancreatitis (AP), etc [2]. Although the pathogenesis and treatments of acute pancreatitis induced by other causes have been widely researched, there are few researches on the treatments of trauma-induced acute pancreatitis.

High mobility group box 1 (HMGB1) is an intranuclear highly conserved nonhistone chromosomal protein that functions as a stabilizer of nucleosome structure and regulator of the genes transcription [3–4]. HMGB1 can be actively or passively released from cells and plays important roles in a large variety of diseases, such as trauma, burn, ischemia-reperfusion injury, sepsis, transplantation, surgical stress, shock, even in the cancer [5–8]. A strong correlation is found between HMGB1 levels and severity of AP, accordingly, it has been speculated that HMGB1 might be a target for anti-inflammatory treatment in AP [9–11]. Thus, inhibitors of HMGB1 were investigated to explore potential new treatment strategy for AP.

Recently, Glycyrrhizin (GL) was recognized as an HMGB1 inhibitor, which binds directly to HMGB1 and inhibits its cytokine activities [12]. GL, a main active ingredient in licorice root, is usually administered to treat patients with chronic hepatitis [13]. This compound is associated with numerous pharmacologic effects, including anti-inflammatory, antiviral, antitumor, and hepatoprotective activities [14]. However, the roles and mechanisms of GL in the treatment of AP, especially trauma-induced AP, were not investigated previously.

Accordingly, we hypothesize that glycyrrhizin may potentially improve the outcome of traumatic pancreatitis by inhibiting HMGB1. We have developed an experimental model of isolated traumatic pancreatitis [15] in rats and have been interested in the mechanisms and therapies of traumatic pancreatitis [16].
Materials and Methods

2.1. Animals

Male Wistar rats (250 g ± 30 g) were purchased from the Experimental Animal Center, Third Military Medical University (Chongqing, China). All animals were bred and housed in standard cages in a climate controlled environment with an ambient temperature of 22 ± 1°C and a 12-h light-dark cycle for 7 days before experiments. The animals were fed standard laboratory chow and water. The rats were fasted for 12 h before the experiment. Animals used in the present study were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Third Military Medical University and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The protocol of the current study was approved by this committee (Permit Number: KY-2011065).

2.2. Establishment of Traumatic Pancreatitis Model

Traumatic pancreatitis was induced according to our previous reports with some modifications [15]. Briefly, all rats were anesthetized with an intraperitoneal injection of 2.5% sodium pentobarbital (30 mg/kg, Sigma, USA) before the operation. Then, the rat was fixed in the supine position onto a wooden board. The pancreas was completely exposed through a midline incision after shaving and disinfection. Next, a plastic shim was placed in the rear of the impact site. The pancreas was impacted using compressed air with a single 400 kPa pressure, which was produced by a custom-made biological impact machine-III (Daping Hospital, Third Military Medical University, Chongqing, China). After the impact, the pancreas was carefully put back and the abdomen was closed. All the rats were consecutively monitored every 6 hours and received meloxicam (Boehringer Ingelheim, France) via the tail vain (2 mg/kg once daily for 2 days) as a postoperative analgesic. The rat was allowed to drink water freely but could not eat anything for 24 hours after recovering from the anesthesia.

2.3. Experimental Design

A total of 60 rats were randomly divided into three groups (n=20 in each): (1) sham operation control group (Control), in which animals underwent only laparotomy; (2) TP group; (3) TP-GL group, in which TP rats received GL. GL (0.2%, 6 mg/kg, saline as solvent, Minophagen Pharmaceutical Co, Tokyo, Japan) was administered intravenously via the tail vein at 15 minutes after the abdomen was closed. All the rats were consecutively monitored every 6 hours and received meloxicam (Boehringer Ingelheim, France) via the tail vain (2 mg/kg once daily for 2 days) as a postoperative analgesic. The rat was allowed to drink water freely but could not eat anything for 24 hours after recovering from the anesthesia.
or extreme reluctance to stand which persists for 24 hours, assuming that the animal has recovered from anesthesia. (3) Moribund state: measured by a lack of sustained purposeful response to gentle stimuli (example of purposeful response—weak attempt to get up; if rat is on its side, attempts should be asymmetrical in nature). (4) Infection: infection involving any organ system (either overt, or indicated by increased body temperature) and is accompanied by systemic signs of illness. (5) Signs of severe organ system dysfunction: such as severe vomiting or diarrhea, obstruction, peritonitis, anuria, oliguria, paralysis of one or more extremities, pain unresponsive to analgesic therapy, locomotor dysfunction, etc.

Serum samples were separated from heart blood by centrifugation (1800 g for 15 minutes at 4 °C) and stored in −20 °C refrigerator. The pancreatic tissue was divided into two parts: one part was fixed in 10% neutral formalin for histopathological analysis and embedded in paraffin wax for cutting sections; another part was removed immediately, frozen and stored in liquid nitrogen.

2.4. Measurements of Serum Amylase Levels
The levels of serum amylase were determined using an enzyme-based colorimetric assay on a fully automated Hitachi 7170 biochemistry analyzer (Hitachi, Tokyo, Japan).

2.5. Determination of Tumor Necrosis Factor-α, Interleukin-6 Levels and HMGB1 in Plasma
The serum concentrations of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and HMGB1 were measured with commercially available enzyme-linked immunosorbent assay kits (TNF-α, IL-6: R&D Systems, Minneapolis, MN; HMGB1: Shino-Test Corp, Sagamihara, Japan) according to the instructions of the manufacturers.

2.6. Histopathological Analysis
The pancreas sections were stained with hematoxylin and eosin (H&E) and scored for edema, parenchymal necrosis, hemorrhage, and inflammation infiltration by two experienced pathologists who were blinded to the grouping and treatment according to Rongione’s method [17] under a light microscope (CH20, Olympus, Japan). (a) Edema: 0=null; 1= diffuse expansion of interlobular septa; 2= 1 + diffuse expansion of interlobular septa; 3=2+interacinous space broadened; 4=3+ intercellular space broadened. (b) Necrosis: 0= null; 1=1 to 4 necrotic cells/high-power field; 2= 5 to 10 necrotic cells/high-power field; 3= 11 to 15 necrotic cells/high-power field; 4= ≥16 necrotic cells/high-power field. (c) Hemorrhage: 0=null; 1= 1 to 2 points; 2= 3 to 5 points; 3= 6 to 7 points; 4= ≥8 points. (d) Inflammatory cell infiltration: 0= null; 1= around ductal margin; 2= in parenchyma <50% of lobules; 3= in parenchyma 51% to 75% of lobules; 4= in parenchyma >75% of lobules. Five fields of each section were counted, and the average scores of these 5 fields were the pathological injury scores of this section.
2.7. Immunohistochemical Analysis of HMGB1

Paraffin sections of pancreas tissue were de-waxed, rehydrated in gradient alcohol, and endogenous peroxidase activity was blocked with 3% H₂O₂ for 10 min. High-temperature antigen retrieval involved boiling the slides in citrate buffer (0.01 M, pH 6.0) for 20 minutes. The sections were incubated in normal goat serum at room temperature for 10 minutes and followed by incubation with polyclonal HMGB1 primary antibody (Biosynthesis biotechnology Co Ltd, Beijing) overnight at 4°C. At last, the sections were incubated with biotinylated secondary antibodies for 1 h at room temperature and then treated with streptavidin-peroxidase complex and visualized by incubating with diaminobenzidine (DAB) solution. Finally, sections were counterstained with hematoxylin.

2.8. Western Blot Analysis

Pancreatic protein was extracted by nuclear and cytoplasmic extraction reagents according to the manufacture’s instructions (Beyotime Institute of Biotechnology, Shanghai, China). Protein concentration was determined using a commercial BCA protein assay kit (Pierce, Rockford, IL). For the immunoblotting analysis, proteins were separated by a 4% to 8% polyacrylamide gel and transferred by electrophoresis to polyvinylidene difluoride membranes (Millipore, Bedford, MA). For nonspecific bindings, membranes were blocked with 5% nonfat milk in TBS containing 0.1% Tween 20 overnight at 4°C. Then, the membranes were incubated with a diluted solution of anti-HMGB1 antibody (1:200, Santa Cruz, CA) or anti-GAPDH antibody (1:1000, Abcam, Cambridge, MA) at 4°C overnight. After incubation with the secondary antibody, anti-immunoglobulin G horseradish peroxidase conjugate (Bio-Rad, Hercules, CA), the membrane was exposed to a chemiluminescent reagent (Amersham Biotechnology Pharmacia, Piscataway, NJ). Specific protein bands were photographed, The band concentration was calculated by the quantification of the integrated optical density of the appropriate band using Quantity One software (Bio-Rad, Hercules, CA).

2.9. RNA extraction and Real-Time PCR

Total RNA was extracted with Trizol reagent (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer’s instructions. The purity of RNA was verified by ethidium bromide staining on 1% agarose gels, and the integrity of RNA was verified by the presence of well-defined 28S and 18S rRNA bands. The purity of RNA was also quantified spectrophotometrically by a 260/280 ratio. Total cDNA was synthesized from the isolated total RNA using a reverse transcriptional system. Briefly, 5 μg of total RNA was reverse transcribed using 0.5 μg oligo (dT) 15 U avian myeloblastosis virus reverse transcriptase (Biouniquer Technology CO, LTD). The primers for quantitative real-time detection were as follows: 5’-TGCTGCATATCGAGCTAAAGG- 3’ and 5’-CCATACTGTACCAGGCAAGGT- 3’ for HMGB1 (399 bp), 5’-ACGGTCAG-
GTCATCACTATCG- 3’ and 5’ - GCCATAGAGGTCTTTACGGATG- 3’ for β-actin (155 bp). The real-time PCR reaction was performed in LightCycler systems according to the manufacturer’s instructions. In each PCR reaction of 2 μL complementary DNA, a final volume of 20 μL was used containing 0.8 μM of forward and reverse primers and 10 μL of SYBR Premix Ex Taq (TaKaRa). For relative quantification we used external standard curves. External standards were prepared by serial dilution (1:10^2 to 1:10^5) of cDNA. Melting curve analysis and electrophoresis on the agarose gel were used to ensure the specificity of the amplified products. The mRNA copy numbers of the transporter genes were normalized to those of rat β-actin.

2.10. Statistical Analysis

Statistical analyses were performed using SPSS Windows 17.0 statistical analysis software (Chicago, Ill). The data of serum cytokines are presented as median (interquartile range) and the other results are presented as mean ± standard deviation (SD). Groups were compared using Mann–Whitney U-tests. In the mortality study, time-to-survival data were analyzed by the Kaplan-Meier method and compared with the log-rank test. P values less than 0.05 was considered statistically significant.

Results

3.1. Survival Analysis

The survival curves are shown in Fig. 1. Only one rat died in the first 24 h (probably due to anesthetic accident) and the rest survived in the next 6 days in the Control group. The survival rate over the first 24 h in the TP group was 80% (16/20 rats), and that in the TP-GL group was 85% (17/20 rats). The survival rate was 45% (9/20 rats) at 7 days after impact injury in the TP group, and that in the TP-GL group was 65% (13/20 rats). The final cause of early death for the rats was abdominal bleeding and the late was abdominal infections. Seven-day survival of the TP-GL group was significantly higher than that of the TP group (P<0.05, Fig. 1), indicating that GL might be involved in the improvement of the survival rate of TP rats.

3.2. Serum Amylase Levels

At 24 h after induction of impact injury, serum amylase levels of TP group and TP-GL group were higher than those of the Control group. The increased amylase levels of two groups confirmed the effectiveness of this compressed air impact induced TP. The amylase levels of TP-GL group were slightly lower than those in TP group, but there were no statistical significance. This finding indicated that GL had no inhibitory effect on pancreatic enzymes (Fig. 2).
3.3. Serum Tumor Necrosis Factor-α and Interleukin-6 Levels

Serum TNF-α and IL-6 levels were measured at 24 hours after impact injury. Remarkably high levels of TNF-α and IL-6 were found in TP and TP-GL group, compared with Control group (P<0.05). Meanwhile, both of the two proinflammatory cytokines revealed a significant decrease in TP-GL group compared with TP group (P<0.05, Fig. 3). These results suggested that GL might play a potential role in inhibiting the inflammatory reaction during trauma-induced acute pancreatitis.

3.4. Serum HMGB1 Levels

The marked elevation of serum HMGB1 levels was observed in TP and TP-GL group compared with Control group (P<0.05). Moreover, the serum HMGB1 levels of TP-GL group showed obvious reduction compared with TP group (P<0.05, Fig. 4). These findings indicated that GL administration might be
associated with the inhibition of HMGB1 release during trauma-induced acute pancreatitis.

3.5. Pancreatic Histological Scores of Injured Pancreas

In TP and TP-GL group, edema, necrosis, hemorrhage and inflammation were found obviously in the pancreatic tissue (Fig. 5B and 5C). However, these pathological damages showed lighter in TP-GL group than those in TP group. Histopathologic scores were significantly higher in TP and TP-GL group; however, the scores of TP-GL group were less than TP group. Histopathological examination of the pancreas revealed that GL ameliorated TP injury in TP-GL group (Fig. 5D).
3.6. Effect of GL on HMGB1 Expression in Pancreas

Immunohistochemical analysis showed that HMGB1 was slightly expressed in pancreatic tissue in Control group (Fig. 6A), strongly expressed in the TP group (Fig. 6B), and expressed at an intermediate level in the GL-treated group (Fig. 6C). Meanwhile, Western blot analysis revealed that the expression of TP and TP-GL group is higher than the Control group; however, compared to TP group, HMGB1 expression levels in the TP-GL rats were evidently lower than those at 24 h after impact injury (Fig. 6D). Likewise, Real-Time PCR analysis showed that among the GL treated rats, HMGB1 expression levels were evidently lower than those in the TP rats at 24 h after impact injury (Fig. 6E). These results indicated that GL administration might be associated with the suppression of HMGB1 expression in pancreatic tissues during trauma-induced acute pancreatitis.
Discussion

As pancreatic injury is a rare complication during abdominal trauma, there are few researches focused on this disease. However, its higher morbidity and mortality prompt us to further investigate the pathogenesis and treatment strategy to improve its outcome. First of all, it is necessary to establish an animal model of TP for elucidating the pathogenesis and exploring potential effective treatment strategy for TP. For simulating clinic situation, our team developed a controllable rat model of TP and used a controlled compressed air to impact pancreas at a certain pressure [15]. Our new TP rat model was considered to closely simulate the pathogenesis of isolated TP without injury to other adjacent organs and can analyse the pancreas injury in different quantitative impact pressures. As a result, we believe that this model is superior to Modlin’s non-crushing vascular clamp method and Delany’s falling weight technique [18–19].

Through decades of investigation, AP has been considered as a life-threatening inflammatory disease, as the inflammatory mediator theory has indicated that the abnormal activation of pancreatin triggers the inflammatory cells and they release proinflammatory cytokines in the early stage of AP [20]. However, the
relationship between pancreatic trauma and the consequent uncontrolled systemic inflammatory response has remained elusive. Increasing evidence from recent studies has implicated that the inflammatory mediators and proinflammatory cytokines also play pivotal roles in the pathogenesis of TP and subsequent local and systemic complications. These inflammatory cytokines, leading to SIRS, MOF and even death, are associated with the severity of severe acute pancreatitis (SAP). Among them, TNF-α and IL-6 plays key roles in the pathogenesis of SAP and trauma \[7, 21–22\]. However, some studies found that levels of TNF-α, IL-1β, IL-6 in SAP or sepsis reached to a peak in the early several hours and then underwent subsequent decrease towards normal levels, while the inflammatory response and organs injury still sustained, indicating that some late proinflammatory mediators might contribute to the pathogenesis of SAP and sepsis. Consequently, the therapies of anti-TNF-α, IL-1β, and IL-6 were proved to be limited and disappointing \[23–24\], while it might be a promising strategy to explore new treatments targeted on the late proinflammatory mediators.

Unlike other proinflammatory cytokines, HMGB1 was recognized as a late-appearing inflammatory mediator, and it is secreted at peak about 20 hours after stimulation \[25–27\]. HMGB1 can bind to the receptor for advanced glycosylation end product (RAGE), Toll-like receptor 2 (TLR2), and Toll-like receptor 4 (TLR4) to enhance the inflammatory response \[28–30\]. HMGB1 was found to be up-regulated in many acute and chronic diseases \[6–8\] including SAP. Yasuda measured serum HMGB1 concentrations in 45 patients with SAP at the time of admission and found that the mean value of serum HMGB1 levels was significantly higher in patients with SAP than that in healthy volunteers. Also, Serum HMGB1 levels were significantly positively correlated with the Japanese severity score and Glasgow score. These results suggest that HMGB1 may act as a key mediator for inflammation and organ failure in SAP \[9\]. Cheng and his colleagues measured serum HMGB1 levels in rat models of SAP and found that serum HMGB1 levels were not significantly altered for the first 12 hours after SAP was induced. However, HMGB1 increased dramatically after 12 hours and reached the peak at 24 hours, on the basis of which our present study chose 24 h after impact as the detection time. Meanwhile, it was observed that HMGB1 could remain at a relatively high level for 72 hours \[11\]. As a result, compared to other proinflammatory cytokines, this characteristic of HMGB1 with delayed presence provides a wide and effective therapeutic window and become a unique target for anti-inflammatory therapy \[31–32\]. Therefore, inhibition of HMGB1 secretion or release becomes a new therapy method of TP.

Glycyrrhizin (GL), a natural compound of triterpene glycoside, is extracted from the licorice root which is widely cultivated throughout Europe and Asia and has been used medically for at least 2,500 years. Glycyrrhizin is commonly used in treating patients with liver diseases based on its anti-inflammatory and antiviral effects \[33\]. More recently, some studies indicated that GL could directly bind to HMGB1 protein by interaction with two arms of both HMG boxes and inhibited its cytokine activities by inhibition of HMGB1 chemoattractant and mitogenic activities \[12\]. Moreover, GL could reduce the serum level and gene expression of
HMGB1 and other proinflammatory cytokines and protect vital organs against porcine endotoxemia [24]. Our present study indicated that the glycyrrhizin was beneficial for the management of TP. As far as we know, the current study is the first report on the effect of GL in the treatment of TP.

In the present study, we found that GL can not only reduce the serum levels of TNF-α and IL-6, which were previously reported to reach to a peak in the early several hours, but also decrease the serum level of HMGB1 in rats at 24 hours after induction of TP. Moreover, it was showed that GL could also significantly inhibit the expression of HMGB1 in pancreas of TP. Although it has been reported that GL could suppress the proinflammatory activities of HMBG1, the mechanisms by which GL inhibited the expression of HMBG1 in local tissues or peripheral blood remained to be unclear. We presumed that the inhibition of HMGB1 expression might be associated with the alleviation of tissue inflammatory injuries after GL administration, as GL could extenuate the inflammatory reaction by inhibiting the activities of HMGB1 and other proinflammatory mediators. According to our present study, GL treatment obviously ameliorated pancreatic tissue injury and reduced the lethality of TP in rats. This finding suggested that GL might also exert its therapeutic effects on TP as HMGB1 inhibitor to extenuate the inflammatory reaction. However, the exact molecular mechanisms by which GL inhibits the expression of HMGB1 should be further elucidated.

In conclusion, the findings from our study indicate that glycyrrhizin can suppress HMGB1 and improve outcomes of traumatic pancreatitis in rats. Nevertheless, the definite mechanisms are still poorly understood. To clarify this, further basic and clinic investigations are required in the future.

**Acknowledgments**

We thank Dr. Yan Luo and Yi Jian (Department of Pathology, Chengdu Military General Hospital, Chengdu, China) for providing expert technical assistance.

**Author Contributions**

Conceived and designed the experiments: KX LC FZT. Performed the experiments: KX LC. Analyzed the data: LJT TC RWD. Contributed reagents/materials/analysis tools: ZLL JDR. Wrote the paper: KX LC.

**References**

1. Subramanian A, Dente CJ, Feliciano DV (2007) The management of pancreatic trauma in the modern era. Surg Clin North Am 87: 1515–32.
2. Levine RA, Bank MA (2011) Traumatic transection of the pancreas. A case of delayed presentation. JOP 12: 47–9.
3. Huang W, Tang Y, Li L (2010) HMGB1, a potent proinflammatory cytokine in sepsis. Cytokine 51: 119–26.
4. Bianchi ME, Manfredi AA (2007) High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. Immunol Rev 220: 35–46.

5. Tang D, Kang R, Zeh HJ 3rd, Lotze MT (2011) High-mobility group box 1, oxidative stress, and disease. Antioxid Redox Signal 14: 1315–35.

6. Levy RM, Mollen KP, Prince JM, Kaczorowski DJ, Vallabhaneni R, et al. (2007) Systemic inflammation and remote organ injury following trauma require HMGB1. Am J Physiol Regul Integr Comp Physiol 293: 1538–44.

7. Lantos J, Földi V, Roth E, Weber G, Bogár L, et al. (2010) Burn trauma induces early HMGB1 release in patients: its correlation with cytokines. Shock 33: 562–7.

8. Andrassy M, Volz HC, Igwe JC, Funke B, Eichberger SN, et al. (2008) High-mobility group box-1 in ischemia-reperfusion injury. Circulation 117: 3216–26.

9. Yasuda T, Ueda T, Takeyama Y, Shinzuki M, Sawa H, et al. (2006) Significant increase of serum high-mobility group box chromosomal protein 1 levels in patients with severe acute pancreatitis. Pancreas 33: 359–63.

10. Hidehiro S, Takashi U, Yoshifumi T, Yasuda T, Shinzuki M, et al. (2006) Blockade of high mobility group box-1 protein attenuates experimental severe acute pancreatitis. World J Gastroenterol 12: 7666–70.

11. Cheng BQ, Liu CT, Li WJ, Fan W, Zhong N, et al. (2007) Ethyl pyruvate improves survival and ameliorates distant organ injury in rats with severe acute pancreatitis. Pancreas 35: 256–61.

12. Mollica L, De Marchis F, Spitaleri A, Dallacosta C, Pennacchini D, et al. (2007) Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. Chem Biol 14: 431–41.

13. Van Rossum TG, Vulto AG, Hop WC, Schalm SW (2001) Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis C. Am J Gastroenterol 96: 2432–7.

14. Asl MN, Hosseinzaah H (2008) Review of Pharmacological Effects of Glycyrrhiza sp. and its Bioactive Compounds. Phytother Res 22: 709–24.

15. Dai R, Chen G, Huang Z, Yan H, Lin N, et al. (2012) Establishment and characteristics of an animal model for isolated pancreatic trauma. J Trauma Acute Care Surg 73: 648–53.

16. Ren J, Luo Z, Tian F, Wang Q, Li K, et al. (2012) Hydrogen-rich saline reduces the oxidative stress and relieves the severity of trauma-induced acute pancreatitis in rats. J Trauma Acute Care Surg 72: 1555–61.

17. Rongione AJ, Kusske AM, Kwan K, Ashley SW, Reber HA, et al. (1997) Interleukin 10 reduces the severity of acute pancreatitis in rats. Gastroenterology 112: 960–7.

18. Modlin IM, Bilchik AJ, Zucker KA, Adrian TE, Sussman J, et al. (1989) Cholecystokinin augmentation of ‘surgical’ pancreatitis: benefits of receptor blockade. Arch Surg 124: 574–8.

19. Delany HM, Ali KB, Tocino AA, Teh EL, Steinberg JJ, et al. (1996) Traumatic pancreatitis: method and effects of i.v. fluids and Sandostatin. J Surg Res 60: 41–8.

20. Felderbauer P, Mager C, Bulut K, Belyaev O, Schmitz F, et al. (2005) Pathophysiology and treatment of acute pancreatitis: new therapeutic targets—a ray of hope. Basic Clin Pharmacol Toxicol 97: 342–50.

21. Laveda R, Martinez J, Munoz C, Penalva JC, Saez J, et al. (2005) Different profile of cytokine synthesis according to the severity of acute pancreatitis. World J Gastroenterol 11: 5309–13.

22. Lesina M, Wörmann SM, Neuhöfer P, Song L, Algül H. (2014) Interleukin-6 in inflammatory and malignant diseases of the pancreas. Semin Immunol 26: 80–7.

23. Zhang ZW, Zhang QY, Zhou MT, Liu NX, Chen TK, et al. (2010) Antioxidant inhibits HMGB1 expression and reduces pancreas injury in rats with severe acute pancreatitis. Dig Dis Sci 55: 2529–36.

24. Wang W, Zhao F, Fang Y, Li X, Shen L, et al. (2013) Glycyrrhizin protects against porcine endotoxemia through modulation of systemic inflammatory response. Crit Care 17: R44.

25. Wang H, Bloom O, Zhang M, Vishnubhatla JM, Ombrellino M, et al. (1999) HMGB-1 as a late mediator of endotoxin lethality in mice. Science 285: 248–51.

26. Wang H, Liao H, Ochani M, Justiniani M, Lin X, et al. (2004) Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med 10: 1216–21.
27. Ulloa L, Fink MP, Tracey KJ (2003) Ethyl pyruvate protects against lethal systemic inflammation by preventing HMGB1 release. Ann NY Acad Sci 987: 319–21.

28. Fiuza C, Bustin M, Talwar S, Tropea M, Gerstenberger E, et al. (2003) Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 101: 2652–60.

29. Yu M, Wang H, Ding A, Golenbock DT, Latz E, et al. (2006) HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 26: 174–9.

30. Park JS, Gamboni-Robertson F, He Q, Svetkauskaite D, Kim JY, et al. (2006) High mobility group box 1 protein interacts with multiple Toll-like receptors. Am J Physiol Cell Physiol 290: C917–24.

31. Yang R, Harada T, Mollen KP, Prince JM, Levy RM, et al. (2006) Anti-HMGB1 neutralizing antibody ameliorates gut barrier dysfunction and improves survival after hemorrhagic shock. Mol Med 12: 105–14.

32. Yuan H, Jin X, Sun J, Li F, Feng Q, et al. (2009) Protective effect of HMGB1 a box on organ injury of acute pancreatitis in mice. Pancreas 38: 143–8.

33. Gwak GY, Moon TG, Lee DH, Yoo BC (2012) Glycyrrhizin attenuates HMGB1-induced hepatocyte apoptosis by inhibiting the p38-dependent mitochondrial pathway. World J Gastroenterol 18: 679–84.