Diaphragmatic dysfunction in sepsis due to severe acute pancreatitis complicated by intra-abdominal hypertension

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Abstract

Objective: This study aimed to examine the mechanism of diaphragmatic dysfunction in sepsis due to severe acute pancreatitis (SAP) with intra-abdominal hypertension (IAH) in a rat model.
Methods: The rats were assigned at random to four groups: (1) control (n = 5), (2) SAP (n = 5), (3) SAP+IAH (n = 5), and (4) SAP+IAH+SS-31 (n = 5). Length and force output of the diaphragm were analysed *in vivo*. Histopathological examinations were performed by haematoxylin–eosin. Oxidative stress levels related to protease in diaphragmatic mitochondria were detected with a colorimetric technique.
Results: In the septic rat model due to SAP complicated by IAH, myofibres were increased. Muscle contractile function was significantly lower in the SAP+IAH group compared with the SAP and control groups. Glutathione peroxidase and superoxide dismutase levels were significantly lower and malondialdehyde levels were higher in the SAP and SAP+IAH groups compared with the control group. Notably, SS-31 could reverse atrophy of myofibres in SAP+IAH rats, as well as contractile dysfunction and mitochondrial dysfunction in the diaphragm.
Conclusions: Diaphragmatic structure and biomechanics are altered in septic rats due to SAP and IAH. This finding is mainly due to an increase in release of mitochondrial reactive oxygen species.

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Introduction

Severe acute pancreatitis (SAP) is a common clinical disease, with a high rate of complications and mortality ranging from 10%–30%.[1,2] Studies have reported that the incidence of intra-abdominal hypertension (IAH) in patients with pancreatitis is as high as 50%.[3] SAP complicated by IAH is prone to sepsis and respiratory failure.[3,4]

SAP is one of the most common reasons for respiratory failure, whereas IAH precipitates exudation of pulmonary alveoli. SAP results in a longer length of hospitalization and higher mortality of sepsis.[5] The diaphragm is the most important inspiratory muscle, which accounts for approximately 80% of the volume of resting ventilation. However, there have been few studies on changes in the structure and function of the diaphragm in SAP, especially when SAP is accompanied by IAH. Therefore, this study aimed to examine the mechanisms of diaphragmatic dysfunction in SAP complicated by IAH in a rat model.

Methods

Rat model and study groups

Male Sprague–Dawley rats weighing 240–340 g were provided by the Experimental Animal Center of Zhejiang University. The study was approved by Zhejiang University Ethics Committee. All of the operations and experimental procedures were performed in accordance with the Regulations on the Administration of Laboratory Animals.

All of the rats were fed *ad libitum* and fasted for 12 h prior to the experiments. The rats were anesthetized intraperitoneally with 1% sodium pentobarbital (30 mg/kg). The SAP model was induced by retrograde infusion of 5% sodium taurocholate saline solution (0.1μ/100g, 0.1 ml/min) into the biliopancreatic duct. Thereafter, the abdomen was closed and 4–5 ml saline was injected into the back subcutaneously. IAH was raised by intraperitoneal insufflation with N_2 and a pressure of approximately 15 mmHg was maintained.

The rats were divided at random into four groups: (1) the control group (n = 5) in which rats received volume-matched saline rather than sodium taurocholate saline; (2) SAP group (n = 5); (3) SAP combined with IAH model (SAP+IAH) group (n = 5); and (4) SAP+IAH model and pretreatment with SS-31 (a mitochondrial-targeted antioxidant) (SAP+IAH+SS-31) group (n = 5). The first bolus dose (3 mg/kg) of SS-31 was subcutaneously infused at the onset of the experiment and then 0.5 mg/kg was injected every 3 h. Rats from each group were sacrificed at 12 h after treatment.

Measurement of muscle contractile function

The diaphragm with its central tendon and adhered ribs was quickly excised and submersed in Kerb's solution. This solution

was perfused with mixture 95% O_2 and 5% CO_2. Muscular strips (approximately 8 mm) containing parallel fibres from the lateral costal region of the diaphragm were dissected. The muscle bundle was mounted in a tissue dish at the optimal length (L0), which was the length that peak twitch tension (Pt) was achieved. The side of muscle strips was attached to a metal clamp that was mounted at the bottom of the tissue bath and the central tendon was connected with a tension transducer. Length and force output were obtained and analysed by the Biological Signal Collection System (Medlab-U/4c, Shanghai, China).

The diaphragm was directly stimulated with wire electrodes on both sides of the muscle strip, with stimuli of 2 ms and 100 Hz. To ensure a maximum twitch tension response, the bundle was stimulated at 1.3 times the maximum (20 V). Each stimulus interval was 2 min, and both results were measured twice and the mean was recorded. The data of Pt, maximal titanic tension (Po), the maximal rate of contraction (+DT), and the maximal rate of relaxation (−DT) were recorded.

Histopathological examination

For histopathological analysis, parts of the diaphragm were cut free of the ribs and were then fixed in 4% phosphate-buffered formaldehyde. Tissues were then embedded in paraffin and sliced at 4-μm thick for haematoxylin–eosin staining. A morphological examination of the diaphragm was performed under a light microscope (Olympus, Tokyo, Japan). The characteristics of normal muscle included polygonal fibres, an acidophilic cytoplasm, a plasma membrane, and peripheral muscle nuclei. Abnormal characteristics of muscle usually included an internal nucleus, necrosis, distorted boundary, and inflammatory cell infiltration.

Oxidative stress measurements in the diaphragm

The left diaphragm was removed of the ribs, central tendon, fat, and connective tissue, and was rinsed with cold phosphate-buffered saline. The tissue was then dried of excess moisture by a filter and weighed. The muscle was frozen by liquid nitrogen and stored at −80°C. A part of frozen diaphragm tissue was sliced and homogenized. Levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), cytochrome coxidase (COX), and citrate synthase (CS) in diaphragmatic mitochondria were detected with a colorimetric technique.[6]

Statistical analysis

All data are reported as mean ± standard deviation. Differences between groups were tested by the Student's t-test or one-way ANOVA. Statistical significance was defined as P <0.05. Statistical analyses were performed using IBM SPSS Statistics, Version 20.0 software (IBM Corp., Armonk, NY, USA).

Results

Muscle contractile function

Diaphragmatic Pt was significantly lower in the SAP+IAH group than in the SAP group (P < 0.05). However, in the SAP+IAH+SS-31 group, diaphragmatic Pt was improved compared with the SAP+IAH group (P < 0.05). The same finding was observed with diaphragmatic Po. The +DT was significantly slower in the SAP and SAP+IAH groups compared with the control group (both P < 0.05). However, SS-31 treatment significantly reversed impaired +DT compared with SAP+IAH without SS-31 and controls

Table 1 Pt, Po, +DT, and -DT values for the diaphragm in all of the groups

	Control	SAP	SAP+IAH	SAP+IAH+SS31
Pt (g/cm^2)	104.60±14.87	59.8±8.39	24.22±8.43$^{\&*}$	44.63±11.16$^{\&\#}$
Po (g/cm^2)	187.75±2.82	169.86±7.05	116.52±17.75$^{\&*}$	140.55±8.72$^{\&\#}$
+DT/dtmax (g/s)	1577.53±195.28	517.43±94.38$^{\&}$	469.81±84.76$^{\&}$	606.39±181.99$^{\&\#}$
−DT/dtmax (g/s)	911.29±223.17	666.49±46.38	399.39±60.99$^{\&*}$	448.37±60.99

Each value represents mean ± SD.
*$P < 0.05$ versus the SAP group, #$P < 0.05$ versus the SAP+IAH group, &$P < 0.05$ versus the control group.
Pt, peak twitch tension; Po, maximal titanic tension; +DT, maximal rate of contraction; −DT, maximal rate of relaxation; SAP, severe acute pancreatitis; IAH, intra-abdominal hypertension.
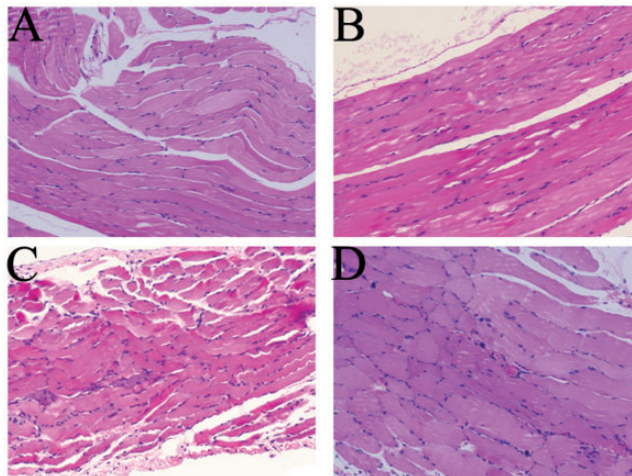
Figure 1. Morphological changes in the diaphragm in all groups of rats (×200)
A: control group; B: SAP group; C: SAP+IAH group; D: SAP+IAH+SS31 group.
SAP, severe acute pancreatitis; IAH, intra-abdominal hypertension.

(both $P < 0.05$). There was no significant difference in +DT in the SAP+IAH group compared with the SAP group. Nevertheless, the –DT in the SAP+IAH group was significantly lower than that in the SAP group ($P < 0.05$) (Table 1).

Histology of the diaphragm

Twelve hours after establishment of the models, no obvious oedema, dissolution, or necrosis was observed in the control group. In the SAP group, muscle fibres showed distortion and a fuzzy boundary.

In the SAP+IAH group, muscle fibres were increased, and the nuclei varied in size and shape from cell to cell. In contrast, in the SAP+IAH+SS-31 group, distortion of muscle fibres was improved compared with the other groups (Figure 1).

Oxidative stress measurements in the diaphragm

GSH-Px and SOD levels were significantly lower in the SAP and SAP+IAH groups compared with the control group (both $P < 0.05$, Figure 2a/b). Moreover, GSH-Px
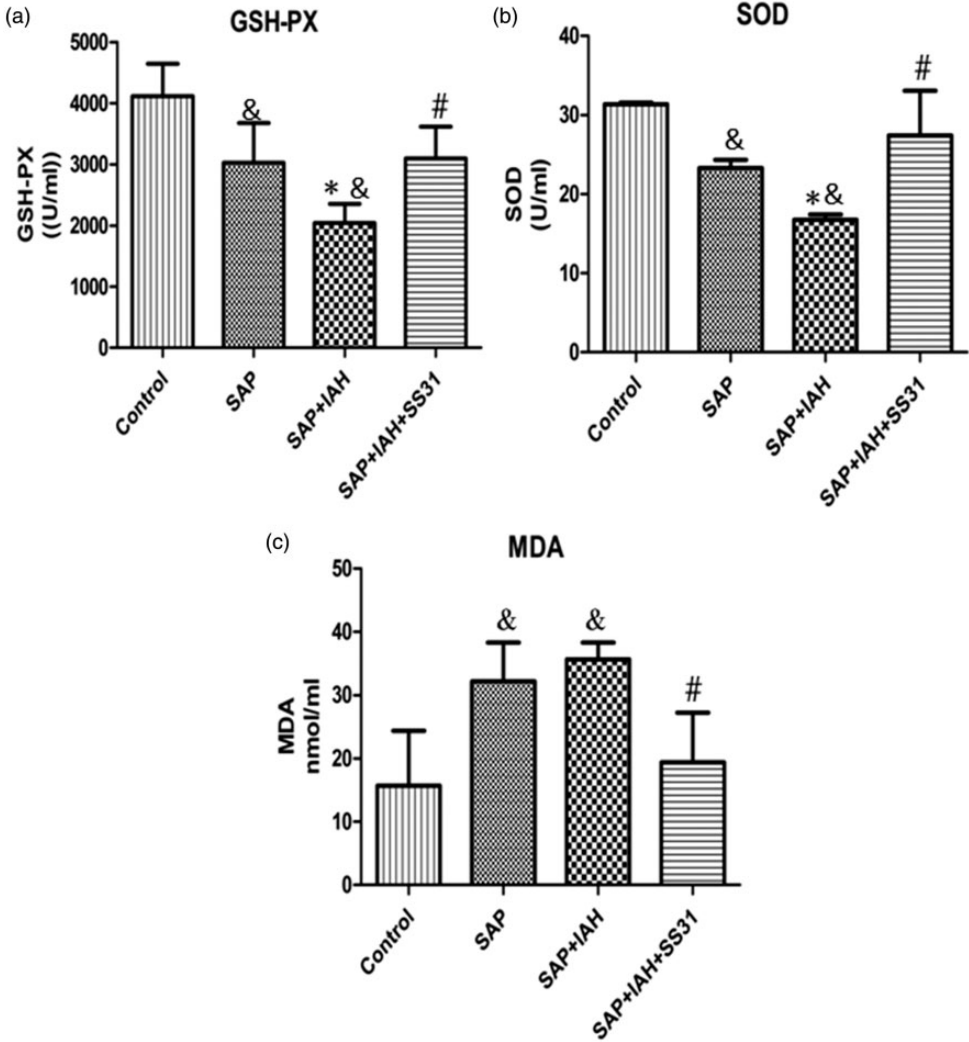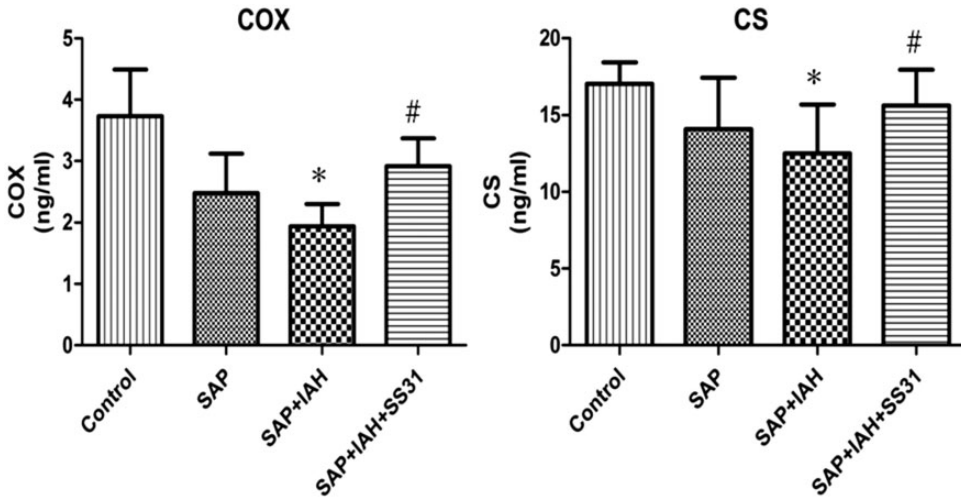
Figure 2. GSH-Px, SOD, and MDA values in mitochondria of the diaphragm
*P < 0.05 versus the SAP group, #P < 0.05 versus the SAP+IAH group, &P < 0.05 versus the control group.
SAP, severe acute pancreatitis; IAH, intra-abdominal hypertension; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

and SOD levels in the SAP+IAH group were significantly lower than those in the SAP group (P < 0.05, Figure 2a/b). After supplementation of a mitochondrial-targeted antioxidant, SS31, GSH-Px and SOD levels were significantly improved compared with the SAP+IAH group (both P < 0.05, Figure 2a/b). MDA levels

were significantly higher in the SAP and SAP+IAH groups compared with the control group (both P < 0.05, Figure 2c). MDA levels were significantly lower in the SAP+IAH+SS-31 group compared with the SAP+IAH group (P < 0.05, Figure 2c).

Moreover, COX and CS levels in the SAP+IAH group were significantly lower

Figure 3. COX and CS values in mitochondria of the diaphragm
*P < 0.05 versus the SAP group, #P < 0.05 versus the SAP+IAH group.
SAP, severe acute pancreatitis; IAH, intra-abdominal hypertension; COX, cytochrome coxidase; CS, citrate synthase.

than those in the SAP group (both P < 0.05, Figure 3). Treatment with SS-31 partially reversed the SAP+IAH-induced decrease in COX and CS levels (both P < 0.05, Figure 3).

Discussion

In this study, we demonstrated that SAP complicated by IAH could change the structure and biomechanics of the diaphragm in a rat model, and thus deteriorate respiratory function. Moreover, this was associated with an increase in mitochondrial reactive oxygen species (ROS).

IAH is a life-threatening complication, especially when abdominal compartment syndrome develops during the course of SAP.[3] A previous study found that cranial displacement of the diaphragm in patients with IAH leads to significantly limited activity. Patients with SAP usually have ascites. A previous study showed that intra-abdominal pressure variation was linearly related to the volume of ascetic fluid.[7]

Therefore, the volume of fluid in the abdominal compartment could also directly influence contractility of the diaphragm. In a study of a canine model, with a progressive increase in intra-abdominal pressure, the amplitude of diaphragmatic muscle fibres was decreased.[8] Furthermore, IAH caused increased oxidative damage to the abdominal rectus muscle with an increased pro-oxidant–antioxidant balance.[9] In our study, greater injury of the diaphragm occurred in rats with SAP complicated by IAH compared with the other groups. However, an antioxidant (SS-31) could reverse the diaphragmatic dysfunction to some extent, which indicated that these changes might have been induced by oxidative stress. Therefore, detecting the source of ROS in the diaphragm during IAH is important. We hypothesize that mitochondria are a major source of ROS. Therefore, release of mitochondrial ROS might play a dominant role in IAH-induced diaphragmatic oxidative stress and contractile dysfunction.

Mitochondria are important for energy metabolism, free radical generation, senescence, and apoptosis. A previous study showed that with an increase in intra-abdominal pressure, mitochondrial damage became increasingly serious (from mild swelling to ballooning, degeneration, and necrosis).[10] This finding indicated that IAH was associated with a change in structure and function of mitochondria. A recent study demonstrated that oxidative stress was a central component of ventilator-induced diaphragm dysfunction.[11] Oxidative stress was the result of an imbalance between oxidant and antioxidant production and oxidants, such as ROS, and could modify proteins, phospholipids, and DNA. This then led to cellular damage and organs dysfunction.[11] IAH can also cause imbalance of oxidation–antioxidation, thus promoting secondary injury mediated by oxidative stress.[12] Our study showed that the SAP+IAH group showed an obvious increase in biomarkers of oxidative damage in mitochondria of the diaphragm.

MDA levels are an indicator of lipid peroxidation. GSH can eliminate O_{2^-} and protect cells from oxidation, and has an important role in restoring other free radical scavengers and antioxidants. GSH-Px is critical for the GSH-redox cycle.[13] Similarly, SOD, a superoxide dismutase, is also an antioxidant and prevents oxidative stress in cells.[14] In the present study, MDA levels were significantly higher in the SAP+IAH and SAP groups compared with the control group. In contrast, SOD and GSH-Px levels were significantly lower in the SAP+IAH and SAP groups compared with the control group. These results indicated an imbalance of the oxidation–antioxidation system and oxidative stress of mitochondria in rats with SAP and IAH.

The tricarboxylic acid cycle is a common energy metabolism pathway in aerobic organisms, and is mainly distributed in mitochondria. COX and CS are important enzymes in the tricarboxylic acid cycle. COX is the terminal oxidase of the electron transport chain, and catalyses the transfer of electrons for a complete decrease in oxygen to support ATP synthesis.[15] In the current study, CS and COX levels were lower in the SAP+IAH group than in the SAP group. This finding indicated a disturbance in mitochondrial energy metabolism, and consequently, induced mitochondrial dysfunction.

SS-31 (D-Arg-2′6′dimethylTyr-Lys-Phe-NH2) is a synthetic aromatic cationic tetrapeptide, which selectively targets and concentrates at approximately1000-fold in the mitochondrial inner membrane.[16] Several studies in isolated mitochondria, animal models, and cultured cells have shown that SS-31 can selectively eliminate mitochondrial ROS and protect the function of mitochondria.[17–19] In our study, SS-31 could improve dysfunction of the diaphragm and mitochondria induced by SAP and IAH to some extent, which is consistent with previous studies.[19] This finding indicated that SS-31 might be useful in patients with SAP complicated by IAH and respiratory failure.

This study has some limitations. First, our study was limited by the small sample size and further studies were necessary with a larger sample. Second, our study showed that mitochondrial oxidative stress was abnormal, but mitochondrial structure and size were not evaluated. Similarly, we only evaluated some oxidative stress-related protease levels in mitochondria of the diaphragm, but further molecular mechanisms need to be examined. Finally, our positive results were only investigated in a model, and further evaluation is required in patients with SAP and IAH.

Conclusions

In conclusion, diaphragmatic structure and biomechanics are disordered in septic rats

because of SAP and IAH. This finding is mainly due to an increased in release of mitochondrial ROS.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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