

Original Article

Histological alteration of pancreas in rats with sepsis

Jingzi Zhong^{1*}, Yanshu Xie¹, Lanshan Huang^{2*}, Gang Chen², Haixia Liao¹, Yiwu Dang², Roma Kajal Dewan¹, Dan Wei¹

Departments of ¹Pediatrics, ²Pathology, The First Affiliated Hospital of Guangxi Medical University, China. *Equal contributors and co-first authors.

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Abstract: Objectives: A sepsis rat model using the cecal ligation and puncture (CLP) was developed to study the histological alteration of the pancreas, and the pancreatic injury during the septic shock was evaluated. Methods: 36 healthy males specific-pathogen-free (SPF) Sprague-Dawley rats were divided into three groups: control group, sham operative (SOP) group and sepsis group, with 6 rats in each. Sepsis group underwent CLP approach, while the cecum was freed from the mesentery in SOP group, except for CLP approach. The pancreatic tissue was removed from living rats at different time spot, and the morphology alteration of pancreas by light microscope and electron microscope was assessed. Results: A septic shock rat model was successfully established using CLP approach. Pancreas didn't show necrosis neither in control group nor SOP group at each time spot by light microscope. However, scattered acinar cells showed necrosis at six hours post CLP in sepsis group, while most acinar cells showed necrosis at twenty-four hours post CLP. While, a large number of mitochondria, lysosomes and endoplasmic reticulum showed swelling, expansion at twenty-four hours post CLP in sepsis group by electron microscope, and diffuse vacuolar necrosis was found. Conclusion: Pancreatic damage may occur at the early stage of sepsis. Patients with sepsis might develop secondary pancreatic damage and should be aware of in clinic. The pathological alteration of pancreatic acinar cells would appear earlier than the mesenchyme. Timely intervention on the pancreas might help to reduce the mortality in critically ill patients with sepsis.

Keywords: Sepsis, pancreas, SOP, CLP, rat

Introduction

Sepsis is a systemic illness caused by a known or suspected microbial invasion of normally sterile parts of the body. Severe sepsis is accompanied with organ dysfunction or hypoperfusion or hypotension. Sepsis is considered as the leading cause of death in noncoronary intensive care units (ICUs). Multiple organ failure in sepsis substantially increases mortality. The dysfunction of organs such as lung, kidney, brain, and the haematopoietic and cardiovascular systems has been well characterized. Little attention has been given to the function of the exocrine pancreas in sepsis. Some animal studies showed that pancreatic blood flow decreases significantly more than regional flow during the septic stage [1, 2]. Tribl B ect described impairment of exocrine pancreatic function in critically ill patients with septic shock, which was not obtained in nonseptic controls [3]. A

prospective cohort study found an impairment of exocrine pancreatic function in sepsis, which increases with the severity of sepsis. Two pancreatic enzyme systems, amylase and the proteolytic enzymes trypsin and chymotrypsin, are strongly impaired in sepsis [4]. These evidence showed that pancreatic function may play an important role in sepsis. But only few studies have focused on it.

To our knowledge, the relationship between superior mesenteric artery blood flow and microcirculatory flow in the splanchnic organs during the development of septic shock has not been studied with techniques allowing dynamic measurements in intact organs over several hours.

In this study, we developed a rat model of sepsis using the cecal ligation and puncture (CLP) to study the histological alteration of the pan-

creas in rat, so as to assess if there was any pancreatic injury during the septic shock.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Animals

Healthy male specific-pathogen-free (SPF) Sprague-Dawley rats (250-300 g, 8-12 weeks old) were fasted overnight but allowed free access to water. All the rats were obtained from the experimental animal center of Guangxi Medical University. The rats were anaesthetized with intraperitoneal injection of 10% chloral hydrate (3 ml/kg). They were randomly divided into three groups: the control group (n=6), the sham operative (SOP) group (reassigned to 4 subgroups according to time three hours, six hours, twelve hours, twenty-four hours, each subgroup n=6), and the sepsis group (reassigned to 4 subgroups according to time three hours, six hours, twelve hours, twenty-four hours, each subgroup n=6).

Establishment of a rat sepsis model

Those mice underwent CLP approach as previously mentioned [5]. We performed traditional disinfection of the abdomen and a median skin incision of approximately 2-3 cm was made to expose the abdominal cavity. The cecum was freed from the mesentery; the base of the cecum was ligated with a 3-0 suture, and punctured at two sites 3 mm apart by using a No. 9 needle. The intestinal canal was then restored to ensure smooth intestinal passage, the abdominal layers were sutured, the lost fluid was replenished, and the wounds were bandaged after disinfection with tamed iodine. Operation procedures for SOP group were the same as-mentioned above, except for the CLP approach.

Electron microscope specimen

We removed the pancreatic tissue (about the size of 1 mm × 1 mm × 1 mm) from living rats as soon as possible (within 1 minute), and fixed

in the 3% glutaraldehyde fixation liquid over two hours at 4°C, postfixed in 1% osmium acid one-two hours, washed (3 times × 15 min) by 0.1 mol/L phosphate buffer, dehydrated in a progressive ethanol and acetone solution: 50%, 70%, 80%, 90% ethanol, 1:1 mixed solution (90% ethanol: 90% propanol), 90% propanol, 100% propanol 3 times (15 min per grade); Then soaked with 1:1 acetone: embedding medium one hour or 1:3 acetone: embedding medium one-three hours or twelve hours, or embedding medium full penetration two hours; embedded in Epoxy 618 fifteen hours at 35°C, aggregation twelve hours at 45°C, trimmed twenty-four hours at 60°C; sectioned with LEICA UC7 ultramicrotome, and stained with uranyl acetate followed by lead citrate, then observed with H-600 microscopy and photographed.

Results

Ethology characteristic

Three hours post CLP, the rats appeared pilo-erection and with tarnished fur in the sepsis group. Six hours later, they started showing physical reaction and activity, and became frightened. Twelve hours later, poor reaction to stimulation and oliguria, even anuria was noted in the rats. On the other hand, three hours post operation, decreased activity was found, but no significant alteration in the hair color or urinary production in the SOP group. Six hours later, rats got better reaction and became more activity. Twelve hours later, all the reaction and activity became normal, and food intake was allowed.

Anatomy characteristic

Three hours post CLP, a little peritoneal hemorrhagic effusion and slight swelling in cecum were observed in the sepsis group. Six hours later, more peritoneal hemorrhagic effusion and obvious swelling in the cecum were observed. Twelve hours later, severe swelling and necrosis in cecum, intestinal adhesion, more bloody ascites with offensive odor, and gastric distension were observed. No significant alteration on the morphology of pancreatic tissue in each group grossly. In the SOP group, the color of cecum and tissue nearby was normal, and intestinal adhesion was slight. A little peritoneal hemorrhagic effusion was found. No

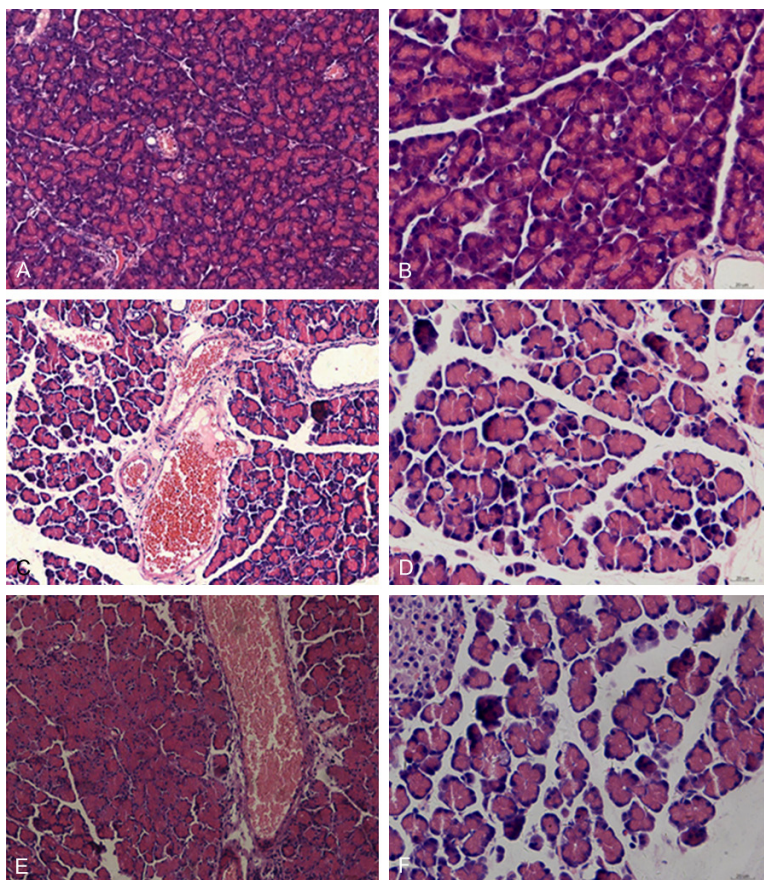


Figure 1. Pathologic characteristics of pancreas in control group and sham surgery group in rat model. A, B. Common appearance of control group 200 ×, 400 × magnification; C, D. Sham surgery group at 6 h 200 ×, 400 × magnification, hydropic degeneration in focal acinar cells with interlobular edema and congestion; E, F. Sham surgery group at 24 h 200 ×, 400 × magnification, degeneration of focal acinar cells with slight acute inflammatory cellular infiltrate.

significant alteration was found on the color and shape of the pancreas at each time point.

Pathologic characteristic

In the control group, pancreatic lobules could be seen and the acinar architecture was intact. Connective tissues and blood vessels could be seen in the stromal and peripancreatic fat was normal (**Figure 1A, 1B**).

In the SOP group, at the time of three hours post operation, interstitial edema occurred, dilated and congested vessels could be seen while there was neither degeneration and necrosis of acinar cells, nor inflammatory infiltrate. At six hours post operation, hydropic degeneration could be seen in focal acinar cells, interlobular edema and congestion were greater in degree while there was no inflamma-

tory infiltrate (**Figure 1C, 1D**). At twelve hours post-op, other than the appearance at six hours, slight acute inflammatory cellular infiltrate occurred in the interlobule spaces. At twenty-four hours post-op, the signs of edema were seen in intracinar spaces, degeneration of focal acinar cells and congestion were present, and slight amount of acute inflammatory cellular infiltrate was seen (**Figure 1E, 1F**). No necrosis of peripancreatic fat was found post -SOP.

In the sepsis group, at the time of three hours post CLP, hydropic degeneration could be seen in focal acinar cells, and small number of acinar cells showed appearance of lysis and necrosis. The necrotic cells distributed scatteredly and several acini were involved. Interlobular edema and congestion were seen and there showed slight acute inflammatory cellular infiltrate interlobule and interacinus (**Figure 2A, 2B**). At time of six hours post CLP, increased number of acinar cells showed necrosis and distributed focally. The acinar archi-

tecture was partially disrupted (**Figure 2C, 2D**). At time of twelve hours, interstitial inflammatory infiltrate was greater in degree than at six hours with predominance of neutrophils while the involved region of necrosis was similar (**Figure 2E, 2F**). At time of twenty-four hours post-op, the acinar architecture was markedly disrupted and most acinar cells showed necrosis. The nucleus disappeared and abundant vacuoles were seen in the cytoplasm (**Figure 2G, 2H**). There were scattered suppurative foci at the peripancreatic area with dominant neutrophils and fibrinous exudates. No necrosis of peripancreatic fat was found post CLP.

Ultrastructure changes of pancreatic cells

In the control group, the cellular ultrastructures were normal. Nuclear membrane was intact, no shrinkage and cracking was found. Chromatin

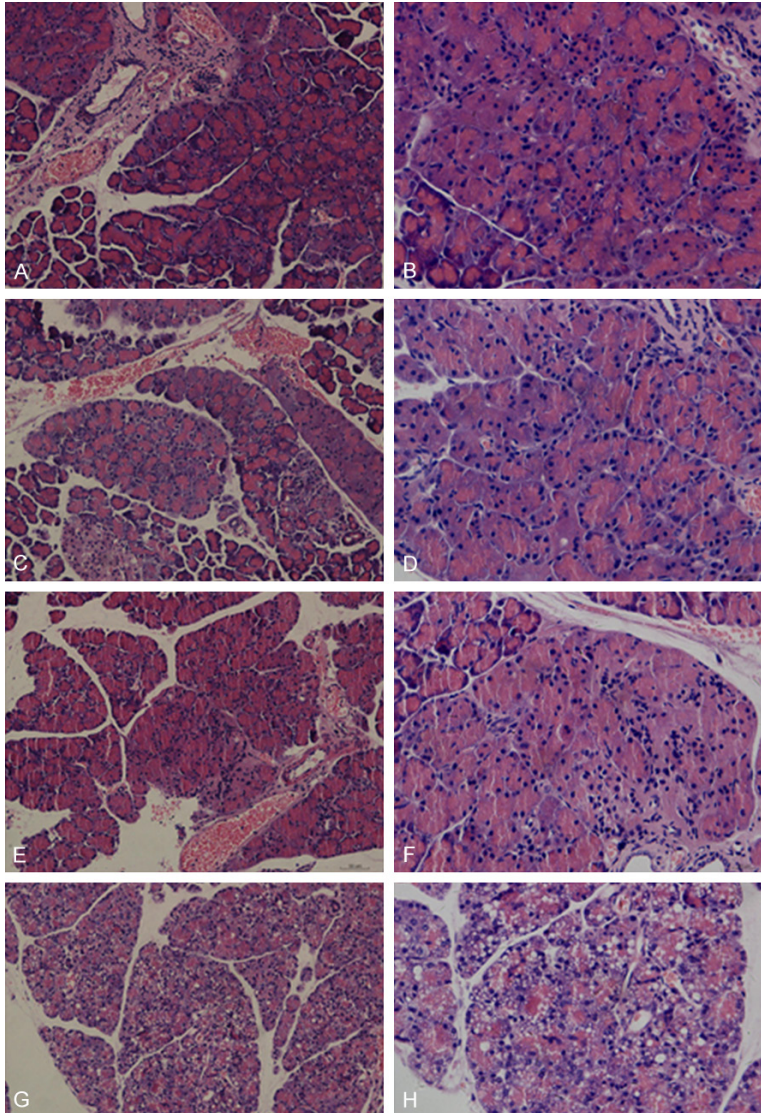


Figure 2. Pathologic characteristics of pancreas of sepsis group in rat model. A, B. 3 h post CLP approach 200 ×, 400 × magnification, hydropic degeneration in focal acinar cells and necrosis in small number of acinar cells; C, D. 6 h post CLP approach 200 ×, 400 × magnification, necrosis distributed focally in increased number of acinar cells; E, F. 12 h post CLP approach 200×, 400× magnification, necrosis and interstitial inflammatory infiltrate with predominance of neutrophils; G, H. 24 h post CLP approach 200 ×, 400 × magnification, necrosis in most acinar cells with abundant vacuoles in the cytoplasm. CLP, cecal ligation and puncture.

was well-distributed. The cell organelles were intact, including mitochondria, lysosomes, endoplasmic reticulum, no swelling and necrosis (**Figure 3A, 3B**). In the sham operative group, the cellular ultrastructures were normal as control group in three hours after CLP. Six hours and twenty-four hours later, nuclear membrane was intact. Swelling can be found in some of the mitochondria, lysosomes, but not

in endoplasmic reticulum. The morphology of zymogen granules was normal, and with no vacuoles. There was no obvious difference in pathology between six hours (**Figure 3C, 3D**) and twenty-four hours subgroups (**Figure 3E, 3F**). In the sepsis group, Nuclear membrane was intact in three hours after CLP. Swelling and Cracking can be found in some of the mitochondria, while only swelling was existent in lysosomes (**Figure 4A, 4B**). In the six hours later, nuclear membrane was still intact. Swelling, cracking and vacuolar necrosis can be found in some of the mitochondria and lysosomes. No swelling was found in endoplasmic reticulum (**Figure 4C, 4D**). In the twelve hours later, karyopyknosis and chromatin condensation were found. Swelling, cracking and vacuolar necrosis can be found in more mitochondria and lysosomes than six hours later. Swelling was found in endoplasmic reticulum (**Figure 4E, 4F**). In the next twenty-four hours later, karyopyknosis and chromatin condensation were the same as twelve hours. Swelling, cracking and vacuolar necrosis can be found in more and more mitochondria and lysosomes (**Figure 4G, 4H**).

Discussion

Septic shock is the cause of ischemia, and the ischemia followed by reperfusion results in a breakdown of the microcirculation in many organs, the pancreas would be affected at the same time, and this is considered to be a critical factor in the pathogenesis of acute pancreatitis [6, 7]. Previous studies showed that the pancreas is highly sensitive to ischemia [8, 9]. Torgersen C's macroscopic postmortem study

Sepsis pathology

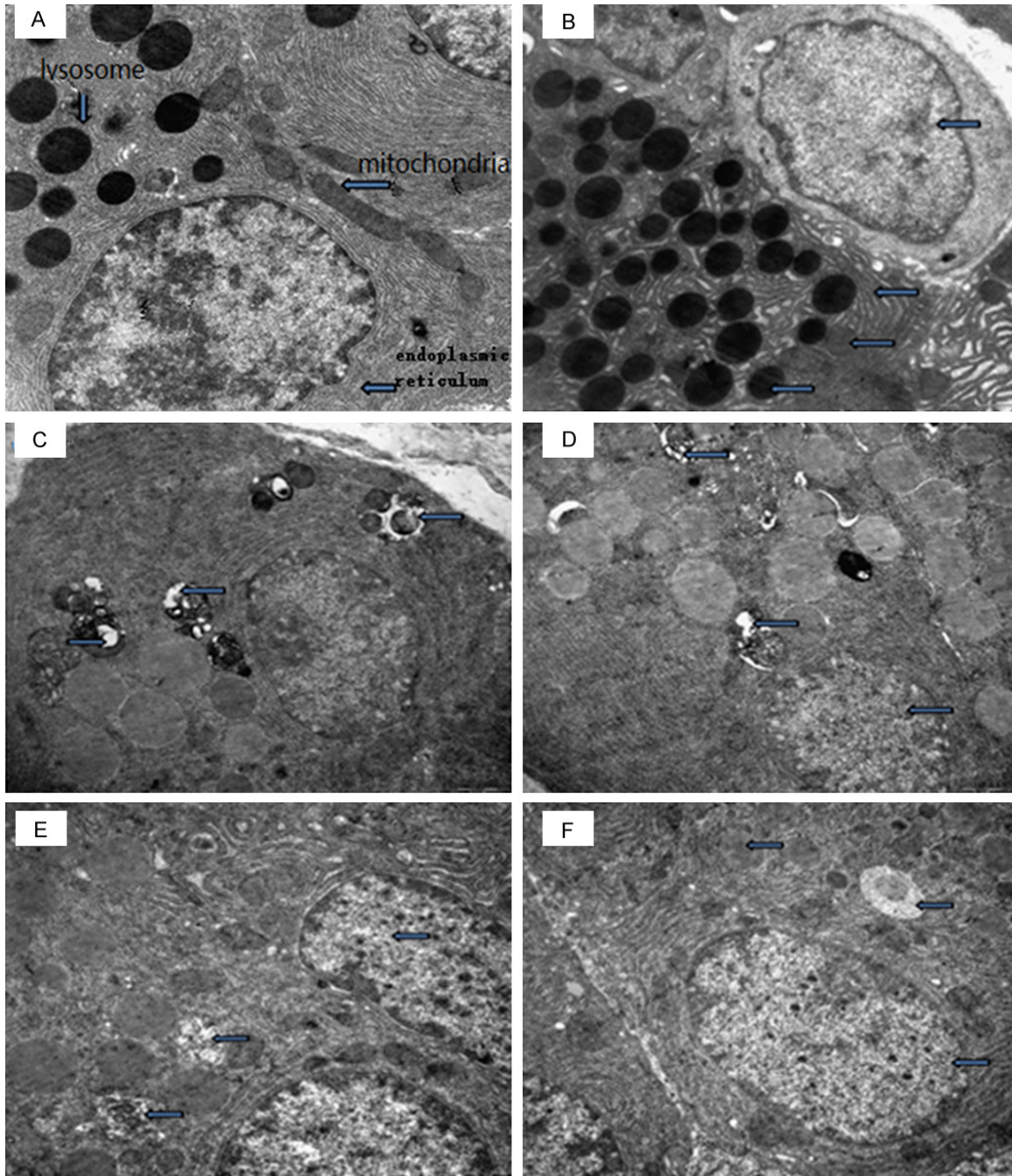


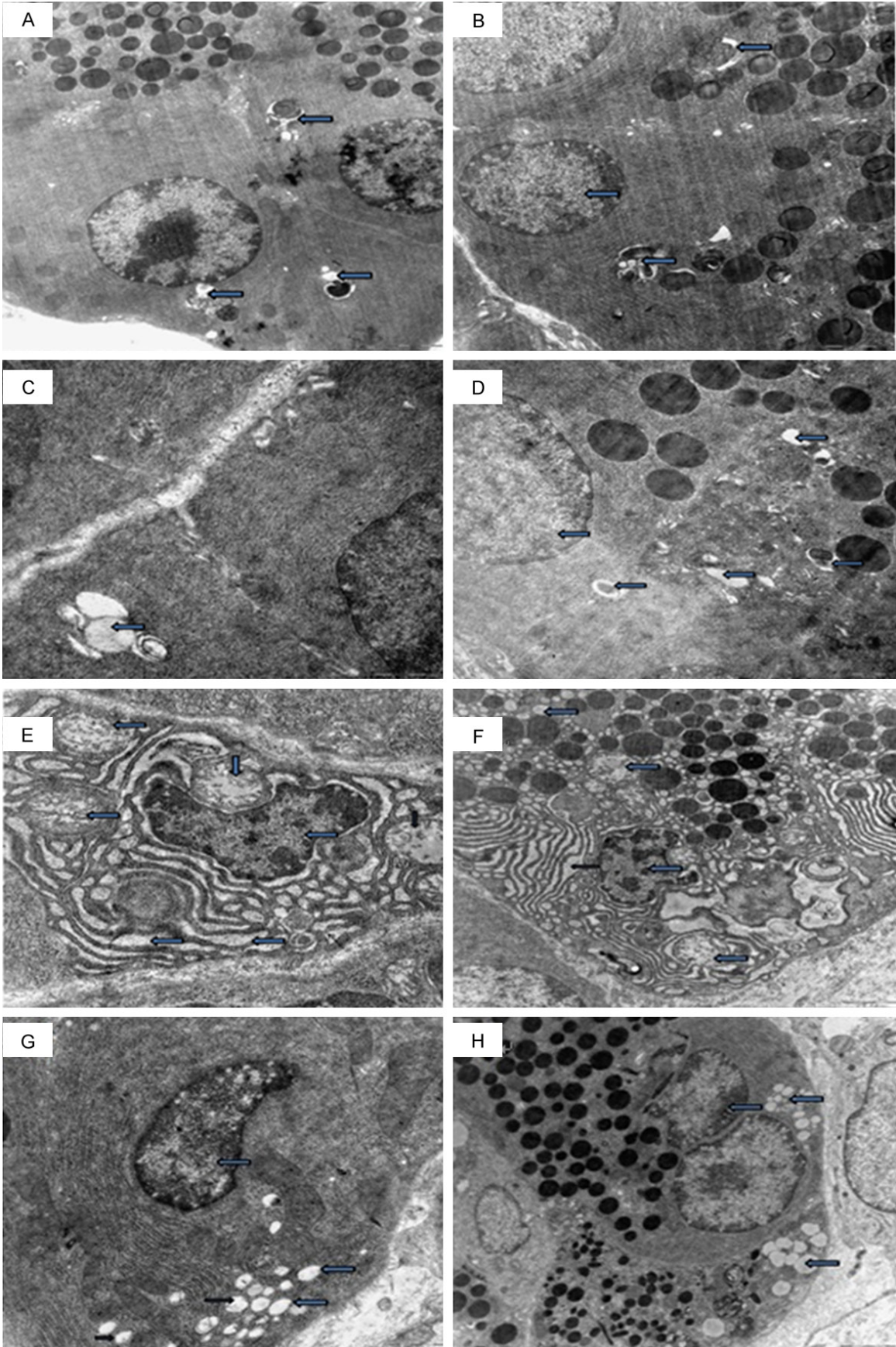
Figure 3. Ultrastructure changes of pancreatic cells of control group and SOP group. (A, B) Normal pancreatic cells: Nuclear membrane was intact, no shrinkage and cracking was found. Chromatin was well-distributed. Mitochondria were intact, no swelling and necrosis $\times 20,000$ (A), $\times 15,000$ (B) magnification. (C, D) SOP group six hours: Nuclear membrane was intact. Swelling can be found in some of lysosomes, but not in endoplasmic reticulum $\times 20,000$ magnification. (E, F) SOP group twenty-four hours: Nuclear membrane was intact. Swelling can be found in some of the mitochondria, lysosomes, but not in endoplasmic reticulum $\times 20,000$ (E), $\times 15,000$ (F) magnification.

found that a rate of 8.5% of surgical intensive care patients with sepsis suffered from acute pancreatitis [10].

In this study, we aimed to observed pancreatic tissue ultrastructural morphological changes at

different time points (three hours, six hours, twelve hours, twenty-four hours), and to learn more about the degree of damage to the pancreas when sepsis occurred in rats. We established an experimental model of severe sepsis, using the CLP approach and set the normal

Sepsis pathology



Sepsis pathology

Figure 4. Ultrastructure changes of pancreatic cells of sepsis group. (A, B) Three hours: Nuclear membrane was intact, Swelling and Cracking can be found in some of the mitochondria $\times 10,000$ (A), $\times 15,000$ (B) magnification. (C, D) Six hours: Swelling, cracking and vacuolar necrosis can be found in some of the mitochondria and lysosomes. No swelling was found in endoplasmic reticulum $\times 20,000$ (C), $\times 30,000$ (D) magnification. (E, F) Twelve hours: Karyopyknosis and chromatin condensation were found. Swelling, cracking and vacuolar necrosis can be found in more mitochondria and lysosomes, swelling was found in endoplasmic reticulum $\times 20,000$ (E), $\times 10,000$ magnification (F). (G, H) Twenty-four hours: karyopyknosis and chromatin condensation were found. Swelling, cracking and vacuolar necrosis can be found in more and more mitochondria and lysosomes $\times 20,000$ magnification.

control group and the sham group to control. In a rat model with CLP-induced septic shock, bacteria leaked to the internal organs, infected the abdominal cavity, and caused systemic infection and septic shock. In the present study, the rats that underwent CLP exhibited signs such as piloerection, curling, fatigue, and hypokinesia, low appetite, weight loss, shortness of breath, severe bleeding in snouts and inner canthi, and trembling. On the other hand, rats in the SOP group moved freely, responded sensitively, and exhibited normal drinking behavior. The hemodynamic changes and times in the sepsis group were similar to those observed in clinical human patients with sepsis. These results demonstrated that a septic shock rat model can be effectively established using the CLP approach.

Pathogenesis of pancreatic damage is a complex pathophysiological process with many factors involved, which may be mainly related to bacterial translocation and endotoxemia, visceral hypoperfusion and ischemia-reperfusion, vascular injury and thrombosis, metabolic inhibition. Also, it may be related to pancreatic self-digestion, inflammation, immunological damage and pancreatic microcirculation. Pathological changes of pancreatic tissue have not been fully studied during the development of sepsis. No one studied its biochemical markers and histopathological features. So the diagnostic criteria and prevention for acute pancreatitis in sepsis were unclear.

Our study shows the presence of different degrees of pancreas pathological changes in rats with sepsis. The longer the sepsis persists, and the more severe pathology changes. Using light microscope to observe pancreas in different hours of control group and sham group shows no necrosis. But in the sepsis group, sporadic acinar cells showed necrosis in the 6 hours, while most acinar cells showed necrosis in the twenty-four hours. The damage of the sepsis to acinar cells was progressively aggra-

vated. Pancreatic ultrastructure in the sham group rat had no change in three hours. But in the six hours, mitochondria and lysosomes were swollen, while nucleus was still intact, and zymogen granules were normal. No obvious vacuolar seen occasionally after six hours. No obvious different changes were found in twenty-four hours. Cell damage didn't aggravate as time goes by. Using electron microscope pancreas ultrastructure (nucleus, mitochondria, lysosomes, endoplasmic reticulum) of sepsis group in three hours shows the nucleus, endoplasmic reticulum is still intact, and some mitochondrial swelling, cracking, lysosomes swelling. After twelve hours nucleus, endoplasmic reticulum appears to change (nuclear condensation, chromatin condensation, swelling of the rough endoplasmic reticulum). Comparing with six hours, mitochondria and lysosomes change significantly, swelling and necrosis, vacuolar change, the number and volume of vacuoles increased significantly, the number of zymogen granules reduced. After twenty-four hours, a large number of mitochondria, lysosomes and endoplasmic reticulum swelling, expansion, showed a vacuolar necrosis. Pancreatic ultrastructural changes in rat with sepsis are mainly characterized by intact mitochondria and lysosomes, nucleus, endoplasmic reticulum in early changes. The longer the sepsis persists, the more serious changes would be found in mitochondria and lysosomes.

Pancreatic damage occurs in the early-stage of sepsis. The longer sepsis persists, the more serious damage to Pancreas. Mitochondrial swelling always is caused by lack of hypoxic cellular energy. In the early-stage of sepsis, it is still reversible, but severe mitochondrial swelling is irreversible. Severe mitochondrial matrix swelling and endoplasmic reticulum degranulation would cause cell damage. So our study suggests that we should be vigilant about patients with sepsis secondary to pancreatic damage in clinic. The damage of the pancreatic cells is earlier than the pancreatic tissue. Early

prevention of damage to the pancreas early might be able to reduce mortality in critically ill patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Dan Wei, Department of Pediatrics, The First Affiliated Hospital of Guangxi Medical University, China. Tel: 158078-00012; Fax: 07715356503; E-mail: 842412593@qq.com

References

- [1] Booke M, Hinder F, McGuire R, Traber LD and Traber DL. Nitric oxide synthase inhibition versus norepinephrine in ovine sepsis: effects on regional blood flow. *Shock* 1996; 5: 362-370.
- [2] Hildebrand LB, Krejci V, Banic A, Erni D, Wheatley AM and Sigurdsson GH. Dynamic study of the distribution of microcirculatory blood flow in multiple splanchnic organs in septic shock. *Crit Care Med* 2000; 28: 3233-3241.
- [3] Tribl B, Madl C, Mazal PR, Schneider B, Spitzauer S, Vogelsang H and Gangl A. Exocrine pancreatic function in critically ill patients: septic shock versus non-septic patients. *Crit Care Med* 2000; 28: 1393-1398.
- [4] Tribl B, Sibbald WJ, Vogelsang H, Spitzauer S, Gangl A and Madl C. Exocrine pancreatic dysfunction in sepsis. *Eur J Clin Invest* 2003; 33: 239-243.
- [5] Rittirsch D, Huber-Lang MS, Flierl MA and Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat Protoc* 2009; 4: 31-36.
- [6] Hoffmann TF, Leiderer R, Waldner H, Arbogast S and Messmer K. Ischemia reperfusion of the pancreas: a new in vivo model for acute pancreatitis in rats. *Res Exp Med (Berl)* 1995; 195: 125-144.
- [7] Klar E, Endrich B and Messmer K. Microcirculation of the pancreas. A quantitative study of physiology and changes in pancreatitis. *Int J Microcirc Clin Exp* 1990; 9: 85-101.
- [8] Spormann H, Sokolowski A and Letko G. Effect of temporary ischemia upon development and histological patterns of acute pancreatitis in the rat. *Pathol Res Pract* 1989; 184: 507-513.
- [9] Redha F, Uhlschmid G, Ammann RW and Freiburghaus AU. Injection of microspheres into pancreatic arteries causes acute hemorrhagic pancreatitis in the rat: a new animal model. *Pancreas* 1990; 5: 188-193.
- [10] Torgersen C, Moser P, Luckner G, Mayr V, Jochberger S, Hasibeder WR and Dunser MW. Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis. *Anesth Analg* 2009; 108: 1841-1847.