The inhibiting effect of intravenous anesthesia on adrenal gland functions under the sepsis condition

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Abstract
This study aims to explore the effect of intravenous anesthesia on the adrenal gland functions of rats with sepsis as well as on their lungs and adrenal gland tissues in order to provide a theoretical reference for the cure of sepsis. Female Sprague Dawley (SD) rats were taken as the research objects in this study. Venous channels of rats were constructed by catheterization through the external jugular vein, and the cecal ligation and puncture technique was adopted to duplicate the sepsis rat models. The level of tumor necrosis factor-α (TNF-α) in serum was detected using enzyme-linked immunosorbent assay (ELISA), and necrocytosis was observed by the fluorescent staining method. The results showed that the survival rates of groups A, B, C, and D were 100%, 60%, 60%, and 50%, respectively, while their concentrations of TNF-α in serum were 101.26 ± 43.38, 1398.68 ± 178.56, 451.16 ± 78.68, and 649.83 ± 98.56 pg/mL, respectively. Results of fluorescent staining showed that the number of living cells per unit view in group A was 1428 ± 166 and those of groups B, C, and D were 175 ± 56, 618 ± 76, and 468 ± 55, respectively. Besides, it was found that changes of inflammatory pathology of lung tissues of each group were significant. In conclusion, etomidate does not affect the survival of sepsis rats and does not exacerbate lung tissue inflammation in sepsis rats. Instead, it can inhibit TNF-α in serum of sepsis rats, as well as the apoptosis of adrenal cells in sepsis rats.

Keywords
adrenal gland, etomidate, intravenous anesthesia, necrosis factor, sepsis

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Introduction
Sepsis refers to a kind of systemic inflammatory response syndrome (SIRS) resulting from pathogenic microorganism-induced inflammation,¹ which is a severe complication of acutely and critically ill patients after infection, severe burn or trauma, shock, and major surgery. Moreover, it can progress to septic shock and multiple organ dysfunction syndrome (MODS)² and even multiple organ failure (MOF)³ and death. Sepsis and severe sepsis tend to be found in children due to their immature immune systems and tissues and organs, which have become a kind of disease that has high morbidity and fatality rates and severely harms children’s health.⁴ Liu et al.⁵ discovered that, in liver transplantation, ulinastatin (UTI) could relieve the acute inflammatory reaction of the body to protect liver cells. Chen et al.⁶ carried out an experiment and found that, at the early
stage of rabbit renal ischemia–reperfusion injury (I/R) model, UTI could relieve the renal tubule lesion and have significant improvement effect on renal dysfunction. On the contrary, Guo et al. reported that the anoxia preconditioning using dexmedetomidine had a direct protective effect on the left ventricular dysfunction caused by hypoxia reoxygenation; however, the hypoxic postconditioning had no such effect.

Using the cecal ligation and puncture (CLP) technique, the sepsis acute kidney injury (AKI) juvenile rat model which was closely related to the sepsis case in clinic was constructed in this study. In the experiment, general conditions of rats, changes of renal tissue forms, and expression level of tumor necrosis factor-α (TNF-α) were observed. In addition, the effect of UTI on TNF-α was also observed, thus discussing the protective effect and mechanism of UTI on AKI of sepsis juvenile rats and providing the pathogenesis and treatment of sepsis-induced AKI with new ideas and references.

**Experimental materials and grouping**

**Experimental materials**

Materials used in the experiment included female adult Sprague Dawley (SD) rats (200–240 g), etomidate, sevoflurane, phosphate-buffered saline (PBS), propidium iodide (PI), Hoechst 33342 staining solution, formalin, dimethylbenzene, absolute ethyl alcohol, heparin saline, antalgic compound brine, antifade mounting medium, eosin stain, sterile gauze, recovery cage, 21 G syringe needle, 4-0 silk yarn vacuum coagulation serum separation tube, centrifuge tube, freezing microtome, anti-stripping glass slide, dyeing kit for cell apoptosis and necrosis, fluorescence microscope, PE-50 catheter, silicone tube, TNF-α kit, and microplate reader.

**Experimental grouping**

Group A: Rats in this group only received sham surgery, and there was no operation on the intestinal tube or any drug intervention, except for the injection of the same amount of normal saline.

Group B: Rats in this group only received CLP surgery and no drugs, except for the injection of the same amount of normal saline.

Group C: Rats in this group received CLP surgery and were given 1 mL (0.6 mg/kg) of etomidate through the catheter of the external jugular vein right after the surgery.

Group D: Rats in this group received CLP surgery and were given 1 mL (1.8 mg/kg) of etomidate through the catheter of the external jugular vein right after the surgery.

**Experimental methods**

**Treatment before experiment**

The experiment in this study was carried out according to relevant provisions of the ethics committee; all steps and instruments used during the surgery were sterile. The weight of each selected female adult SD rat (weighing from 200 to 240 g) was recorded before the surgery. Sevoflurane was used for anesthesia of rats; hairs on the nape of each rat were shaved and the skin was cut off a little bit; each rat was fixed on the operating table and 2%~3% sevoflurane was used again for anesthesia and to ensure the surgery.

**Venous channel surgery**

1. The position which was 1 cm above the sternal angle was cut open a little bit vertically using a scalpel.

2. An external jugular vein (about 1 cm) was separated layer-by-layer and its telecentric end was ligated. Then, a small opening was cut on the telecentric end and a polythene (PE) catheter was inserted into the vein through the opening. When the blood was pumped back into the catheter, the catheter was ligated and fixed.

3. The PE catheter was then washed with 0.5 mL of heparin saline (10 U/mL), injected into the PE catheter and sealed by an obturating cup.

4. An anesthesia spinal needle was punctured subcutaneously till the cut and was then led through the nape by the PE catheter.

5. The neck incision was then sutured by silk thread and the wound was covered by a gauze. Then, 1 mL of post-operation antalgic compound brine (sufentanil injection and midazolam injection) was injected subcutaneously.
**CLP surgery**

1. For anesthesia, 10% chloral hydrate (3 mL/kg) was injected through the enterocoelia of the rat. Then, the rat was fixed and its body hairs on the belly were removed quickly. After that, the middle of the rat’s belly was cut open a little bit (about 1.5 cm) and the cecum was found and separated (the mesentery and blood vessels should not be damaged during this process).

2. Almost one-half of the cecum was ligated circumferentially using the number 4 suture line. The cecum was penetrated by a number 18 needle twice and the distance was about 3 mm. The distance between the first pinhole and the end of the cecum was about 3 mm; then the intestinal content was squeezed out and two rubber bands were put through the cecum to avoid pinhole occlusion.

3. After the surgery, the cecum was put back and the operative wound was sutured; bloodstains around the wound were cleaned using sterile cotton balls. The rat was then given an injection of normal saline (30 mL/kg) subcutaneously at the root of vastus medialis muscle for fluid resuscitation.

**Fluorescence staining**

1. Adrenal gland tissues of rats were put on a fixed support (coated with embedding medium) and then into the freezer of freezing microtome for quick freeze; later, it was sliced and the thickness was 7 µm.

2. The slices were adsorbed and fixed on anti-stripping glass slides. Then, the slice tissues were stained using the dyeing kit for cell apoptosis and necrosis, and apoptosis and necrosis of cells were observed using a fluorescence microscope.

3. About 5 µL of Hoechst 33342 staining solution and 5 µL of PI were added to 1 mL of cell staining buffer and well blended; then the mixture was put on ice.

4. A certain amount of PBS solution was used to wash slices once; after the slices were spun dry, they were added to the cell staining liquid and put on ice for 15 min of staining (away from light).

5. Under the dark condition, slices were washed with PBS again and wiped dry using filter papers for mounting using antifade mounting medium. After mounting, they were observed and photographed by a microscope immediately.

**Enzyme linked immunosorbent assay**

1. The serum sample was diluted to seven different concentrations (two times) which were transferred to eight 1.5 mL centrifuge tubes. The eighth tube was taken as the blank control.

2. Each well of the reaction plate was added with about 100 µL of rat serum and the reaction plate was then placed at 37°C for 120 min.

3. Then, each well of the reaction plate was washed by scrubbing solution 4–6 times and pressed dry using filter papers.

4. After that, each well was added about 50 µL of first antibody working solution (the reaction plate was fully mixed).

5. Later, the reaction plate was placed at 37°C for 60 min and step 3 was repeated; about 100 µL of enzyme-labeled antibody working solution (the reaction plate was fully mixed) was added to each well and the plate was placed at 37°C for 60 min.

6. Step 3 was repeated and 100 µL of substrate working solution was added to each well for 5–10 min of reaction (37°C, in dark).

7. Finally, each well was added with 50 µL of stop buffer from the TNF-α kit; the optical density value was measured using a microplate reader at 450 nm.

**Statistical analysis**

SPSS 19.0 software was used for data analysis; measurement data were expressed as mean ± SD (standard deviation); one-way analysis of variance was used for data comparison among multiple groups; Student–Newman–Keuls q (SNK-q) test was adopted for pairwise comparison; chi-square test was used for enumeration data; \( P<0.05 \) indicated statistical significance; and \( P<0.01 \) indicated significant difference.

**Experimental results and discussion**

**Characteristics of rats after CLP surgery**

All rats in the experiment could recover from anesthesia after CLP surgery, but some sick signs
occurred within 12 h after the surgery. For example, some rats became lazy and left the group, some were addictive to sleeping and refused to intake water, some rats’ hairs stood up, some rats had polypnea, some had scab-like effusion, some rats had oliguria or even anuria, and so on. In addition, obvious inflammatory response could be found and lungs of rats were injured, indicating that the CLP surgery–induced sepsis model was successfully constructed. The increased heart rate and accelerated breathing were due to the increase in arterial blood pressure caused by pain; then it decreased gradually and the body temperature of rats increased.

**Survival curves of rats**

Survival rates and survival curves of rats after experiment are shown in Figure 1.

Figure 1 shows that the survival rate of rats in group A after sham operation was 100%, indicating that the anesthesia, sterile surgical operations, incision of CLP surgery, and postoperative care in the experiment had no effect on the survival rate of rats. Survival rates of both groups B and C were 60%, while that of group D was the lowest, 50%. Statistical results indicated that there was no significant difference between every two groups.

**Detection of TNFs in rat serum**

This study adopted enzyme-linked immunosorbent assay (ELISA) to detect the level of TNF-α in serum of rats. Concentrations of TNF-α in groups A, B, C, and D were 101.26 ± 43.38, 1398.68 ± 178.56, 451.16 ± 78.68, and 649.83 ± 98.56 pg/mL, respectively. Detection results are shown in Figure 2.

Figure 2 shows that the concentration of TNF-α of group B was significantly higher than that of other three groups, indicating that the CLP surgery could result in an increase in TNF-α in serum of sepsis rats, and etomidate had an inhibiting effect on TNF-α in serum of rats after CPL surgery. No statistical difference was found between groups C and D.

**Observation of apoptosis of adrenal glands using fluorescent staining method**

Figure 3 shows the apoptosis of adrenal glands of rats under the same field of view, which were synthesized by fluorescence excitation under different wavelengths. The fewer the living cells, the more severe the adrenal gland damage.

As shown in Figures 3 and 4, the number of living cells of group A was significantly higher than other three groups, and the numbers of living cells of groups C and D were much higher than those of group B. The results indicated that the CLP surgery had an inhibiting effect on adrenal gland cells and etomidate had an enhancing effect on adrenal gland cells.

**Conclusion**

This study mainly analyzes the effect of intravenous anesthesia (etomidate) on survival rates of sepsis rats as well as its inhibiting effect on lung and adrenal gland tissues. Results indicate that etomidate has no effect on the survival rates of sepsis rats; etomidate has an inhibiting effect on TNF-α in serum of sepsis rats; etomidate will not have any influence on lung tissue inflammation of sepsis rats; and etomidate can reduce the apoptosis of adrenal gland cells of sepsis rats. The research carried out in this study can provide a theoretical reference for the treatment and cure of sepsis patients in China.
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Figure 3. Observation of adrenal glands using fluorescent staining method: (a) group A, (b) group B, (c) group C, and (d) group D.

Figure 4. Observation of adrenal glands using fluorescent staining method.
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