

## **Expression of thrombomodulin in lung tissue cells of ALI/ARDS rats induced by severe sepsis.**

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### **Abstract**

**Objective:** To investigate the expression of Thrombomodulin (TM) in lung tissue cells of model rats by using the ALI/ARDS rats induced by severe sepsis as a research model.

**Methods:** The ALI/ARDS (Acute Lung Injury/Acute Respiratory Distress Syndrome) model rats were prepared by the cecal ligation and puncture method in combination with 0.9% sodium chloride saline solution lavage, and 22 rats were randomly divided into the sham operation group and ALI/ARDS group. After successful modeling, rats were sacrificed in 1 h, 2 h and 3 h, and quantitative lung tissues were used to prepare the tissue homogenate, and then the expression of TM was measured by Enzyme-Linked Immunosorbent Assay (ELISA).

**Results:** The expressions of TM in the sham operation groups were (541 ± 15 ng/ml), (733 ± 27 ng/ml), (671 ± 34 ng/ml) at 1, 2, and 3 h respectively, in the ALI/ARDS group, the expressions of TM in 1 h, 2 h and 3 h after lavage were (16790 ± 155 ng/ml), (10034 ± 281 ng/ml) and (11698 ± 195 ng/ml), respectively. By comparison, the expression in the ALI/ARDS group was significantly higher than that of the control group, showing significant difference between groups (P<0.05). There was no significant difference in all time points in the groups (P>0.05), but the animals in the two groups showed a significant increase in stress at 1 h, and continued decrease at 2 h, and then increase at 3 h.

**Conclusions:** The expression of TM in the lung tissue cells of ALI/ARDS rats induced by severe sepsis was high, indicating that it has obvious effect of anticoagulation and wide application values in the clinical treatment, thus, it is worthy of promotion.

**Keywords:** Thrombomodulin (TM), Severe sepsis, ALI/ARDS rats.

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### **Introduction**

Clinically the sepsis refers to a kind of systemic inflammatory response syndrome (referred to as SIRS) after microbial infection of the body, of which, trauma, shock, burns, infections, surgery are the common complications of patients in the ICU, and if severe, it can cause septic shock, a variety of organ failure syndrome, namely severe sepsis. The severe sepsis refers to multiple organ dysfunction, low flow or abnormal perfusion, hypotension, accumulated blood lactic acid, low urine output and change of mental state [1]. At present, the morbidity shows a dramatic increase trend, with high mortality rate, and it is reported that its mortality rate exceeds that of the myocardial infarction. The pathogenesis and mechanism of sepsis is complicated, and its possible reasons are that a variety of inflammatory mediators of inflammatory cell nuclei are released to activate its coagulation system and produce different degrees of inhibition on the fibrinolytic system and physiological anticoagulant system, so that the blood flows in a hypercoagulable state, making it easy to form microthrombus in the microvessels to block the

microcirculation, which results in sepsis and further produces severe sepsis [2].

TM, also known as thrombin regulatory protein, is a binding of thrombin and high-adhesion receptor on the endothelial cells, to activate rapid activation of proteins and involve in the intravascular coagulation regulation process, to cause a specific change of thrombin substrate. Vascular endothelial cells can express the TM under the co-induction of endotoxin or inflammatory factors, but it is not reported its expressions in lung tissue cells of ALI/ARDS rats induced by severe sepsis. The abnormal coagulation function is a mechanism for the occurrence and progression of sepsis and one of important features of existence of SIRS in microvascular microthrombus; and it is reported that the coagulation abnormality are positively related to the inflammation, and coagulation dysfunction is a mechanism of ALI/ARDS progression [3,4]. Therefore, the blood coagulation control is used as a new means for the treatment of severe sepsis. In this study, we observed and investigated the expression of TM in lung tissue cells of ALI/ARDS rats induced by severe sepsis.

## Methods and Materials

### General information

**Kits and instruments:** The TM ELISA kits were purchased from Shanghai Westang Bio-tech Co., Ltd.

TRK-200C small animal breathing machines were purchased from Jiangsi TELI Anesthesia Co., Ltd.

### Test animals and grouping

22 clean SD rats were purchased from Fudan University Shanghai Medical College Experimental Animal Center, and their body weights were in the range of 200-250 g; and the animals were randomly divided into the sham operation group and ALI/ARDS group, 11 animals each group, all animals in the two groups were observed and recorded in 1, 2, and 3 h after modeling.

### Method

**Animal model:** In this experiment, the rat models of severe sepsis were made by cecal ligation and perforation. SD rats were anesthetized, and then under the sterile environment, laparotomy was performed in the middle of incision and then the cecum was moved out of the abdominal cavity. The distal 1/3 part of the cecum was ligated, and the needle injection was carried out at the two sites about 3 cm from the cecum free end to pierce the cecum and squeeze the intestinal contents out, and then reset the cecum and suture the abdominal incision, then rats were placed in the cage to have activities and usual diets. In the sham operation group, the root of cecum was not ligated and rats were placed in the cage directly to have usual diets. Rats in 6-12 h after cecal ligation and perforation would produce a systemic inflammatory response, and in 16 h after experiment, rats accepted the second strike to enter the ALI/ARDS models induced by severe sepsis. In 16 h, the trachea was cut, 2 ml of saline was used to have alveolar lavage for the ALI/ARDS model rats. The rats with  $\text{PaO}_2/\text{FiO}_2 < 300$  were included in this experiment as a marker of ALI/ARDS models. Rats received the auxiliary respiration by the respirator, with 100% oxygen concentration, respiratory rate of 90 times per min and tidal volume of 1 ml.

### Preparation of lung tissue specimens

After successful modeling of ALI/ARDS and 1, 2, and 3 h after operation in the sham operation group, all rats were killed. The lung tissues were precisely weighed and unit volume of 0.9% saline solution was added, to break and centrifuge at 3000 r/min for 10 min, and then the supernatant was extracted to prepare the tissue homogenate, stored in the refrigerator -20°C for standby.

### Evaluation criteria

The expression conditions of rats in the two groups were evaluated. The TM expression results were recorded in 1, 2 and 3 h after operation in different groups.

### Statistical processing

The statistical data obtained from the two groups were compared by SPSS 14.0 statistical analysis software. When the t test was used, measurement data were expressed as  $(\bar{x} \pm s)$ ; and the count data were expressed as ratio (%) and  $\chi^2$  test was used.  $P < 0.05$  was considered significant difference.

## Results

### Expressions of TM in the sham operation group

The results were shown in Table 1. As shown from the data, the expression levels of TM in 1 h, 2 h and 3 h in the sham operation group were  $(541 \pm 15 \text{ ng/ml})$ ,  $(733 \pm 27 \text{ ng/ml})$ ,  $(671 \pm 34 \text{ ng/ml})$  respectively. It showed a trend of dramatic increase in 1 h, continued decrease in 2 h and increase in 3 h, but the values at different time points had no great difference. It can be known from the data that the expressions of TIM 1 h, 2 h and 3 h in ALL/ARDS group are  $(16790 \pm 155 \text{ ng/ml})$ ,  $(10034 \pm 281 \text{ ng/ml})$  and  $(11698 \pm 195 \text{ ng/ml})$  respectively. It shows a trend of dramatic increase in irritability in 1 h, continued decrease in 2 h and increase in 3 h, but the values at different time points had no great difference. However, compared with the results in the sham operation group, the expression levels were increased dramatically, showing significant difference between groups ( $P > 0.05$ ).

**Table 1.** Expressions of TM in the sham operation group.

Group	Expression levels (ng/ml)		
	1 h	2 h	3 h
Sham operation group	$541 \pm 15$	$733 \pm 27$	$671 \pm 34$
ALI/ARDS group	$16790 \pm 155$	$10034 \pm 281$	$11698 \pm 195$
t	11.223	7.553	9.417
F	0.001	0.008	0.003

## Discussion

Recently, studies have reported that severe sepsis is caused by inflammatory response, microvascular thrombosis and endothelial cell dysfunction and the interactions between them. As one of the major causes of Multiple Organ Dysfunction Syndrome (MODS), Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) are characteristics of lesions in the lung site of MODS induced by severe sepsis. The endothelial cell dysfunction and disorder of the blood coagulation system play important roles in the occurrence and progression of ALI/ARDS [5].

ARDS is mainly manifested by injuries of pulmonary vascular endothelial cells and lung epithelial cells in patients, to increase the vascular permeability, result in pulmonary edema and a series of clinical critical illness including hypoxemia, dyspnea and other complex diseases. The disease progression is rapid and the incidence is high, with complex mechanism. A large number of related studies have shown that the changes in

blood coagulation are involved in the physiological and pathological process of the ALI and ARDS, with main characteristics of the systematic and alveolar coagulation phenomena. With the inflammatory responses, systemic and alveolar coagulation hyperthyroidism occurs, and fibrin depositions in the alveolar and lung sites appear, resulting in lung tissue damage.

TM, as a kind of glycoprotein with corning activity and important regulatory factor of coagulation system, is mainly synthesized in the vascular endothelial cells and megakaryocytes, and mainly expressed on the surface of vascular endothelial cells. It is also expressed in the monocytes, platelets and smooth muscle cells, cardiomyocytes and cancer cells. TM functions as important anticoagulant cofactor to maintain vascular homeostasis and protect endothelial cells, etc., and plays an important role in regulating inflammation (vasculitis, sepsis, atherosclerosis), fibrinolysis and cell proliferation, etc. Therefore, it plays the role of anti-inflammation and anti-coagulation through direct and indirect ways, having significant guidance for the early diagnosis of vascular lesions and endothelial cell injury. TM is distributed in the arteries and veins, capillaries and endothelial cells of lymphatic vessels, especially wide presence in the human lungs, kidneys and placenta and other tissues and organs. At present, it has been reported that TM is used for the treatment of disseminated intravascular coagulation, neomycinemia, sepsis, and complications post hematopoietic stem cell transplantation clinically.

TM exists on the surface of the endothelial cells, but when combined with thrombin, it can greatly reduce the thrombin coagulation activity, increase the role of thrombin activating protein C. At this time, the inflammatory mediator can block the nuclear transcription process to slow down the synthesis of TM of endothelial cells.

## **Conclusion**

The study results showed that, the expression in the ALI/ARDS group was significantly higher than that of the sham

operation group, showing significant difference between groups ( $P < 0.05$ ). There was no significant difference in all time points in the groups ( $P > 0.05$ ), but the animals in the two groups showed a significant increase in stress at 1 h, and continued decrease at 2 h, and then increase at 3 h.

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