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UCP2 in early diagnosis and prognosis of sepsis

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Abstract. – OBJECTIVE: Sepsis, a systemic inflammatory response syndrome caused by infection, is a serious threat to the lives of patients. Sepsis can cause tissue hypoperfusion and septic shock which leads to organ dysfunction and death via a variety of mechanisms. Mitochondrial protein (UCP2) involves in immune response, regulation of oxidative stress, and maintenance of mitochondrial membrane potential as well as energy production. However, the role of UCP2 in sepsis remains to be further explored.

PATIENTS AND METHODS: A total of 156 patients with sepsis from our hospital were included in this study (69 patients with sepsis and 87 patients with severe sepsis). A total of 69 healthy volunteers were included as controls. Levels of UCP2 in blood cells before and after treatment were measured using RT-PCR and Western blot. The correlation between levels of UCP2 and sepsis was analyzed.

RESULTS: The level of UCPPC2 in blood cells of sepsis patients was significantly higher than that of healthy controls at both mRNA level and protein level. The expression level of UCP2 in blood cells of sepsis patients was significantly reduced after treatment, compared to that before treatment. No significant difference was found in the level of UCP2 in blood cells of healthy controls before and after treatment ((*p*=0.45). Also, the level of UCP2 in blood cells of patients with severe sepsis was significantly higher than that of patients with sepsis at the protein level (*p*<0.05). Moreover, a positive correlation was found between the level of UCP2 protein and the severity of sepsis.

CONCLUSIONS: UCP2 in blood cells might be a specific biomarker for sepsis and the level of UCP2 is positively correlated with the severity of sepsis.

Key Words:
Sepsis, UCP2, Diagnosis.

Introduction

Sepsis is a systemic inflammatory response syndrome caused by infection[1,2]. Sepsis can cause tissue hypoperfusion and septic shock by a variety of mechanisms, leading to organ dysfunction and death[3-5]. Therefore, diagnosis and prognosis of sepsis are the premise and basis for the treatment of sepsis[6,7]. According to the current sepsis diagnosis standards, severe inflammation has occurred at the time of being diagnosed, which has adverse effects on the treatment[8]. Currently, researchers are trying to identify biomarkers with a high specificity and sensitivity for early diagnosis, treatment, and prognosis of sepsis.

Previous studies showed some biomarkers of sepsis including tissue perfusion index, indices of organ function, inflammatory factors, and hemodynamic indices. However, the specificity and sensitivity of these indicators are not satisfactory[9,10]. Therefore, biomarkers with higher specificity and sensitivity are needed for better diagnosis of sepsis[11]. The pathogenesis of sepsis involves the changes of structure and function of mitochondria, which plays an important role in mitochondrial dysfunction[12,13]. Cardiomyocytes have abundant mitochondria, which work cooperatively to provide energy. Cardiomyocytes' mitochondrion is an important target in septic myocardial injury. With the development of concepts of bioenergetics and disease-caused cell hypoxia, it is hypothesized that mitochondrial energy metabolism is associated with its own dynamic changes[14,15]. UCPs are present on the mitochondrial inner membrane[16]. An elevated level of UCPs can cause heart failure or myocardial injury. Numerous studies[17,18] showed that UCP2 involved in the regulation of inflammation, regulation of oxidative stress, maintenance of mitochondrial membrane potential and energy production, which may be related to the pathophysiology of sepsis. The present study was designed to investigate the level of UCP2 in blood cells of patients with sepsis and the relationship between UCP2 level and sepsis.

The relationship between UCP2 and sepsis is unclear[19]. The expression level of UCP2 in blood cells of sepsis patients and healthy controls was measured at both mRNA and protein levels using RT-PCR and Western blot, respectively. The relationship between UCP2 and sepsis was analyzed

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to provide a theoretical basis whether UCP2 can be used as a molecular marker for the early diagnosis and prognosis of sepsis.

Patients and Methods

Patients

A total of 156 patients with sepsis from Jan 2013 to Jan 2016 of Renji Hospital, School of Medicine, Shanghai Jiaotong University, were included in this study (69 patients with sepsis and 87 patients with severe sepsis). A total of 69 healthy volunteers were included as controls. Blood samples were collected from both sepsis patients and healthy controls. This study has been pre-approved by the Ethical Committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University. All subjects have signed the consent forms before recruitment in this study.

American Critical Care Medicine/American College of Chest Physicians standards (2016) were used for the inclusion and exclusion criteria[20, 21]. All patients were divided into sepsis and septic patients. Total score of APS is 71 points. Risk of death (R) is: (R/1-R) = -3.517 + (APACHE score × 0.146) +0.603+ points of patients assigned when entering ICU[22, 23].

In this study, the average age of 156 patients was 55.3 ± 18.4 years (range: 18-88 years) and the average age of healthy volunteers was 58.3 ± 17.4 years (range: 25-83 years). No significant difference was seen in the age between two groups.

Preparation of Blood Samples

10 ml venous blood was collect from each individual and centrifuged at 900 rpm for 7 min. Blood cells were collected and stored at -70°C for later use.

RT-PCR

Total RNA of blood cells was extracted and reversely transcripted into cDNA using RT-PCR[23]. Briefly, blood cells were collected, total RNA was extracted and reversely transcripted into cDNA followed by subsequent PCR reaction at: 95°C 5 min for pre-denaturation; 30 cycles of 95°C 30 s, 58°C 30 s, 72°C 30 s and a final extension at 72°C for 10 min.

Agarose Gel Analysis of RT-PCR Product

Electrophoresis was used to analyze RT-PCR products at 100 V for 20 min[24]. After the end of the electrophoresis, images were taken using gel imaging system to quantify the expression of genes.

Western Blot

SDS-polyacrylamide gel electrophoresis was carried out according to a conventional method. Briefly, sample concentrations were measured and the same amounts of protein samples were loaded to gel for electrophoresis. Proteins were then transferred to PVDF membranes overnight at 4°C. Membranes were then blocked and incubated with the first and secondary antibody to detect the expression of protein.

Statistical Analysis

Data were analyzed by SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard error. Statistical difference was considered significant at $p<0.05$.

Results

RT-PCR Analysis of UCP2 mRNA Levels in the Blood Cells of Patients with Sepsis

RT-PCR results showed that UCP2 mRNA level in the blood cells of patients with sepsis was significantly higher than that of healthy controls ($p=0.0026$). UCP2 mRNA level in the blood cells of patients with severe sepsis was significantly higher than that of patients with normal sepsis ($p<0.05$) (Figure 1).

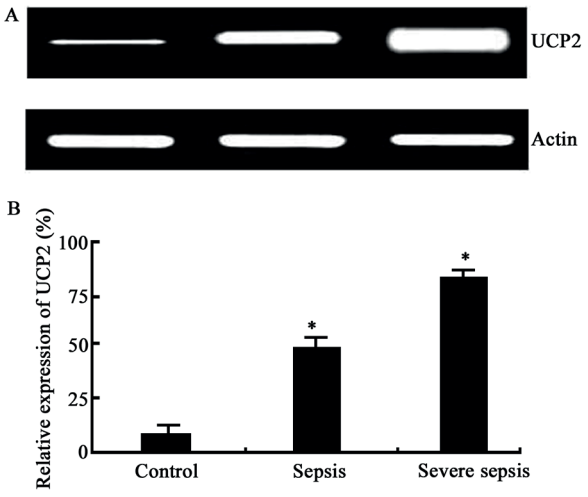

Figure 1. RT-PCR analysis of UCP2 mRNA level in blood cells of patients with sepsis. *Compared with controls, $p<0.05$.

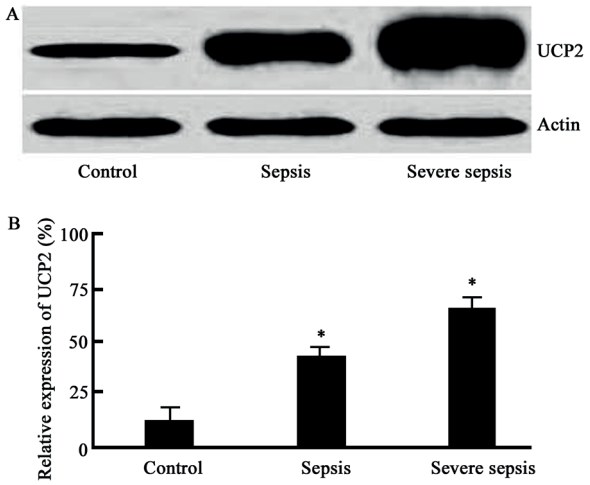

Figure 2. Western blot analysis of UCP2 protein expression in blood cells of patients with sepsis. *Compared with controls, $p<0.05$.

Western Blot Analysis of UCP2 Protein in the Blood Cells of Patients with Sepsis

Western blot results showed that UCP2 protein expression in the blood cells of patients with sepsis was significantly higher than that of healthy controls ($p=0.0014$). UCP2 protein level in the blood cells of patients with severe sepsis was significantly higher than that of patients with normal sepsis ($p<0.05$) (Figure 2).

Decreased UCP2 mRNA Levels in the Blood Cells of Patients with Sepsis After Treatment

As shown in Figure 3, after treatment, the level of UCP2 mRNA in the blood cells of patients with sepsis was decreased.

Decreased UCP2 Protein Level in the Blood Cells of Patients with Sepsis after Treatment

As shown in Figure 4, the level of UCP2 protein in blood cells of patients with sepsis was significantly decreased after treatment.

Level of UCP2 is Positively Correlated with the Severity of Sepsis

Correlation analysis showed (Figure 5) that there was a significantly positive correlation (R=95%) between the severity of sepsis and the level of UCP2 in the blood cells of patients with sepsis.

Discussion

Early diagnosis and treatment of sepsis are extremely important[25,26]. No effective biomarkers of sepsis are available[27]. Therefore, the present study was to investigate the possibility of using UCP2 as a biomarker for early diagnosis and prognosis of sepsis.

Sepsis is a highly complicated inflammatory process caused by clinical infections. The development and progression of sepsis will cause the body's immune response, which is one of the major causes of death in patients with sepsis[1,2]. According to the current diagnostic criteria for sepsis[20,21], the higher immune response can be found under general sepsis. Therefore, detection of some reliable markers must be performed for early detection and prognosis of sepsis. However, currently, identification of biomarkers for early diagnosis of sepsis is difficult.

Overexpression of UCP2 can significantly reduce mitochondrial membrane potential. Study of oxidative stress on the injury of myocardial cells with UCP2 overexpression showed that UCP2 can inhibit apoptosis. However, little is known about the use of UCP2 as a molecular marker for early diagnosis of sepsis.

Levels of lipids and proteins in blood samples are different, which may affect RNA extraction as inhibitors of PCR reaction, and might be introduced into RNA along with the process of RNA extraction. Small RNAs were added into PCR

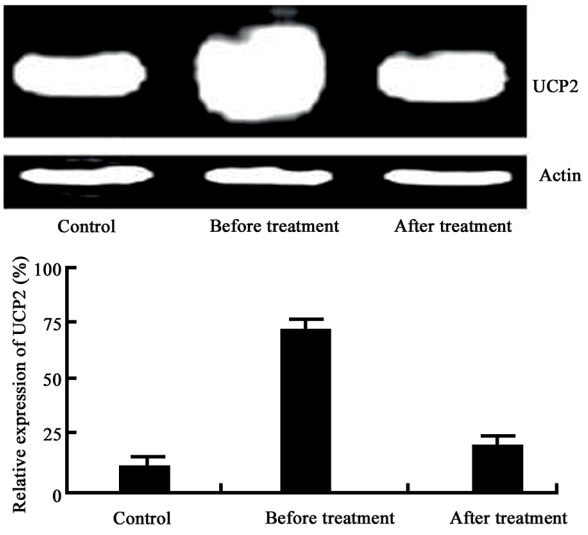

Figure 3. Decreased level of UCP2 mRNA in blood cells of patients with sepsis after treatment. *Compared with controls or before treatment group, $p<0.05$.

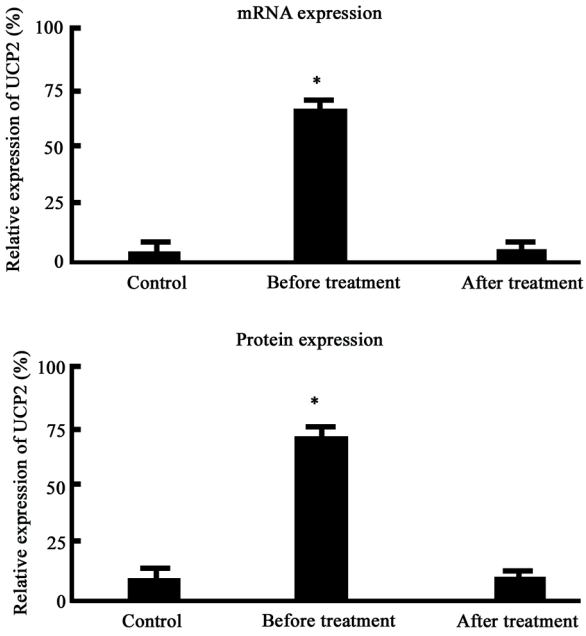

Figure 4. Decreased level of UCP2 protein in blood cells of patients with sepsis after treatment. *Compared with controls or before treatment group, $p<0.05$.

reaction to compete for the inhibitors of PCR reaction to eliminate its interference.

In this study, we found that levels of UCP2 mRNA and protein in blood cells of patients with sepsis were significantly higher than healthy controls. The levels of UCP2 mRNA and protein in blood cells of patients with sepsis were restored to normal levels after treatment (p=0.45, compared with healthy controls). Third, the level of UCP2 protein in blood cells of patients with severe sepsis was significantly higher than that of the patient with normal sepsis. The level of UCP2 protein had a positive correlation with the severity of sepsis. These data suggested that UCP2 might be a specific biomarker for sepsis.

Studies[14-16] showed that injection of bacterial endotoxin to mice induced production of ROS, TNF-a, IFN-r, and NF-kB. Via regulating the production of ROS by immune cells, inflammatory factors can affect oxidative stress and inflammation and antagonize oxygen free radicals; therefore, could be used to treat sepsis. However, the relationship between UCP2 and this pathway remains clear and needs to be further explored.

It is worth to mention that the sample size of this study is relatively small, many of the patients underwent chemotherapy and other treatments[28], and no sepsis animal model was used in this

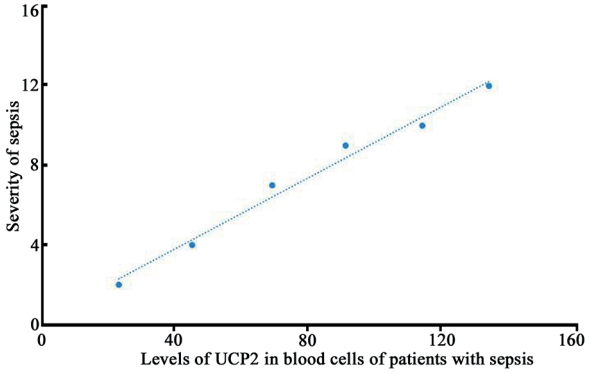

Figure 5. Correlation analysis of the severity of sepsis and level of UCP2 in blood cells of patients with sepsis.

study. All the shortcomings should be taken into consideration in the future study.

Conclusions

The present study showed that the level of UCP2 protein had a positive correlation with the severity of sepsis and UCP2 might be a specific biomarker for sepsis.

Conflict of interest

The authors declare no conflicts of interest.

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