Mitochondrial protein UCP1 mediates liver injury induced by LPS through EKR signaling pathway

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Abstract. – **OBJECTIVE:** Mitochondria are abundant in liver. The roles of mitochondrial protein in liver injury and related signaling pathways are still unclear. UCP1 is a novel mitochondrial transmembrane protein. Its expression pattern and function in liver still needs further investigation.

MATERIALS AND METHODS: A mouse model of liver injury was established by the treatment of LPS. UCP1 expression in the liver tissue was detected by Western blot and qRT-PCR. ERK signaling activity was tested by enzymatic activity kit. ATP production was evaluated by flow cytometry. Cell apoptosis was determined by Western blot and flow cytometry. ERK signaling pathway inhibitor, U0126, was used to pretreat mice. Liver tissue from sepsis patients was collected from the surgery.

RESULTS: Our data showed that the level of UCP1 was upregulated, ERK signaling was activated, ATP production was reduced, and cell apoptosis was enhanced in mice with liver injury model caused by LPS. U0126 intervention significantly suppressed UCP1 expression, inhibited ERK signaling pathway, enhanced ATP production, and restrained liver cell apoptosis in mice liver injury model. UCP1 increased, ERK signaling activated, and cell apoptosis elevated in the liver tissue of sepsis patients.

CONCLUSIONS: UCP1 plays a role in the liver tissue of mouse liver injury model and sepsis patients through the modulation of mitochondrial ATP production and cell apoptosis by ERK signaling pathway.

Key Words:
   Mitochondrial protein UCP1, ERK signaling pathway, LPS, Liver injury.

Introduction

LPS induced liver injury, which belongs to a kind of liver inflammatory response syndrome. It is characterized by high incidence, high case fatality rate, high cost, and increasing incidence[1,2]. Liver injury caused by LPS can lead to tissue hypoperfusion, septic shock, organ dysfunction, and death through multiple mechanisms[3-5]. Therefore, effective detection is the premise and foundation of the treatment of liver injury induced by LPS[6,7]. According to the current criteria, the treatment to the mice with severe inflammation became less effective[8]. At present, it is urgently needed to explore the biomarker with good specificity and sensitivity for early diagnosis, treatment, and prognosis of LPS-induced liver injury. Previous studies showed that biomarkers for LPS-induced liver injury mainly included the indicator of tissue perfusion, organ function, inflammatory cytokines, and liver tissue dynamics. However, the limitation was that all of these indicators were lack of specificity and high sensitivity[9,10]. Therefore, a better molecular biomarker is needed for the diagnosis of liver injury caused by LPS in clinic[11]. LPS-induced liver injury involves a variety of mechanisms, including the structure and function changes of mitochondria associated with inflammatory mediators[12,13]. Myocardial mitochondria are the important target of liver cell during liver injury. Following the development of biogenetics and the concept of cellular hypoxia, it was speculated that the mitochondrial energy metabolism had certain relationship with its own dynamics changes[14,15]. Mitochondrial protein UCP played an important role

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in mitochondrial function[16], which may be closely related to liver injury[17,18]. In this study, we intend to explore the potential relationship between UCP1 levels in liver cells and liver injury induced by LPS. As there is still lack of evidence about the relationship between UCP1 and LPS induced liver injury[19], we selected patients with liver injury from ICU and tested UCP1 level in the liver based on the data concerning mouse model of liver injury, in order to evaluate the value of UCP1 as molecular biomarker in liver injury induced by LPS and provide theoretical basis for the early diagnosis and prognosis of liver injury.

Materials and Methods

Experimental Model

Mouse liver injury model was established using LPS according to previous report[10]. Female C57BL/6 mouse at 6-8 weeks old was intraperitoneal injected by LPS at 25 μg/kg. The mouse injected by normal saline was treated as control. The experiment was approved by the Ethics Committee in our hospital conforming to animal welfare[10]. The liver tissue collected from the sepsis patients received surgery was used as experimental group, while the liver tissue obtained from liver operation due to traffic accident was treated as control. The experiment was approved by the Ethics Committee in Qingdao Municipal Hospital. All the subjects had signed informed consent.

Liver Tissue Preparation

The liver tissue was extracted from the mouse or operation. The tissue was lysed and washed by normal saline and acetone for four times. Then, the homogenate was centrifuged at 150 g and stored at -20°C.

Caspase-3 Activity Detection

ERK signaling activity was detected by kinase activity kit. The caspase-3 activity was tested by microplate reader[20]. A total of 2×10^5 liver cells were added with lysate to release the protein. Then, the mixture was incubated with caspase-3 chromophoric substance at room temperature avoid of light for 20 min. At last, cells were tested on microplate reader at 492 nm[17].

ATP Production

Mitochondrial ATP production in liver tissue was detected by flow cytometry. The liver tissue was added with lysis to prepare the suspension.

Next, the mixture was added with detection fluid and tested on microplate reader at 560 nm to calculate the ATP concentration according to the standard curve.

Western Blot

The protein was extracted from the liver tissue and quantified. Next, the protein was separated by SDS-PAGE and analyzed by ImageJ software to obtain the gray value.

Real-time PCR

Total RNA was extracted from the liver tissue using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed for Real-time PCR[21]. Total RNA was reverse transcribed to cDNA using polyA at 42°C for 60 min. The PCR reaction system contained 1 μl cDNA, 2 μl primers, 4 μl dNTP, 2 μl loading buffer, and 1 μl Taq enzyme. The PCR reaction was composed of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s and 55°C for 30 s. The primer sequences were as follows.

UCP1, 5'-TTATTAGAGGGTGGGGTGGATTGT-3' and 5'- CCACCTAAATCAACCTCCAACCA-3'; actin, 5'-TTATTAGAGGGTGGGGCGGATCGC-3' and 5'- CCACCTAAATCGACCTCCGACCG-3'.

U0126 Pretreatment

ERK signaling pathway inhibitor U0126 was used to treat mouse through intraperitoneal injection at 10 μg/kg. Next, the mouse was intraperitoneal injected by LPS at 25 μg/kg. The mouse injected by normal saline was treated as control.

Statistical Analysis

All data analyses were performed on SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The measurement data were depicted as mean ± standard deviation. $p<0.05$ was considered as statistical significance.

Results

UCP1 Expression in Liver Cells from Mouse Liver Injury Model

As shown in Figure 1A, qRT-PCR results demonstrated that UCP1 mRNA significantly increased in liver cells from liver injury model compared with that in normal control ($p=0.0017$). As shown in Figure 1B and C, Western blot results revealed that the level of UCP1 protein obviously elevated in liver cells from liver injury model compared with that in normal control ($p=0.0027$).

ERK Signaling Activated, Mitochondrial ATP Production Reduced, and Cell Apoptosis Enhanced in Liver Injury Mouse

To further discuss the molecular mechanism of UCP1 in LPS induced liver injury model, we tested mitochondrial ATP production and cell apoptosis. As shown in Figure 2, our data exhibited that ERK signaling pathway was activated, mitochondrial ATP production was reduced, and cell apoptosis was enhanced in mice with liver injury.

The Influence of ERK Signaling Pathway Inhibitor U0126 Pretreatment

Next, to investigate the specific mechanism of UCP1 in liver injury induced by LPS, we detected ATP level and cell apoptosis in mice model pretreated by U0126. As shown in Figure 3, U0126 intervention significantly suppressed UCP1 expression, inhibited ERK signaling pathway, enhanced ATP production, and restrained liver cell apoptosis in mice, suggesting a key role of U0126 on ERK signaling pathway.

UCP1 Expression and Cell Apoptosis in the Liver Tissue from Sepsis Patients

To study UCP1 expression and cell apoptosis in sepsis patients, we collected the liver tissue from sepsis patients who received surgery. As shown in Figure 4, the level of UCP1 was increased, along with the increasing activation of ERK signaling pathway and elevation of cell apoptosis in the liver tissue of sepsis patients.

Discussion

LPS seriously threatens mammal's life via the cause of liver inflammation, among which, the

Figure 1. UCP1 expression in liver cells from mouse liver injury model. *(A)* qRT-PCR detection. *(B)* Western blot detection. *(C)* Western blot analysis. *$p<0.05$, compared with control.

function of mitochondrial uncoupling protein (UCP1) in liver injury induced by LPS remains to be further investigated. Early diagnosis and treat-
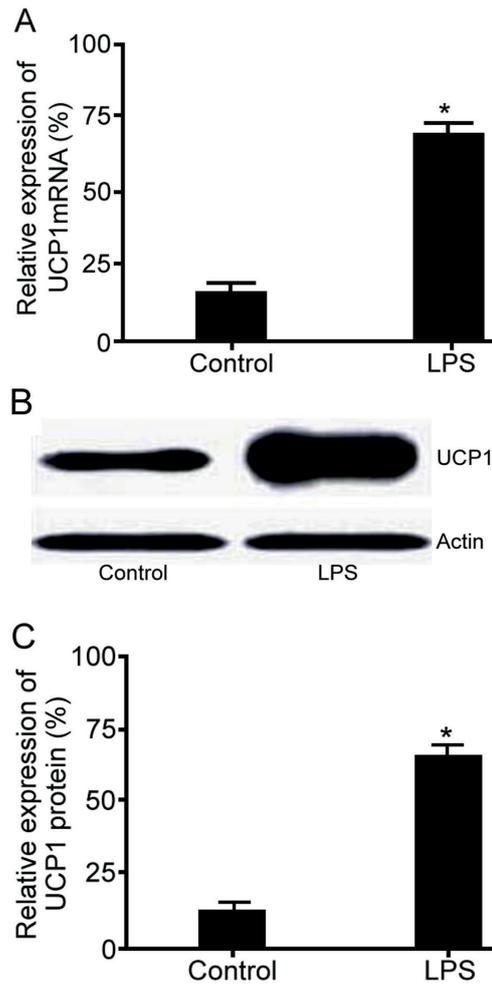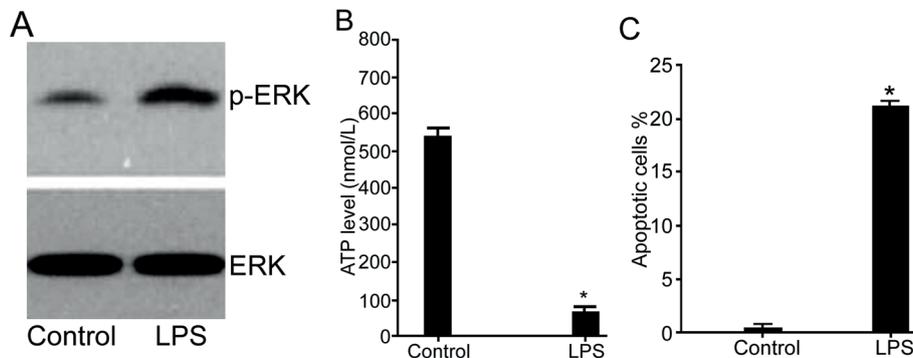
Figure 2. ERK signaling activated, mitochondrial ATP production reduced, and cell apoptosis enhanced in liver injury mouse. *(A)* Western blot detection. *(B)* ATP production. *(C)* Cell apoptosis detection.
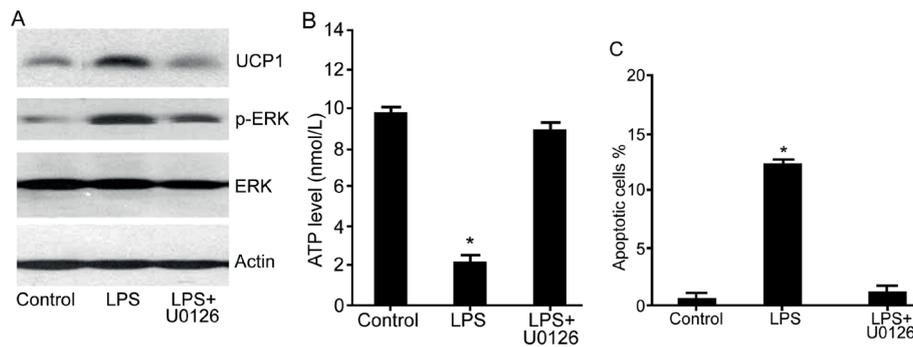
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Figure 3. The influence of ERK signaling pathway inhibitor U0126 pretreatment. *(A)* Western blot detection. *(B)* ATP production. *(C)* Cell apoptosis detection. *$p<0.05$, compared with control.
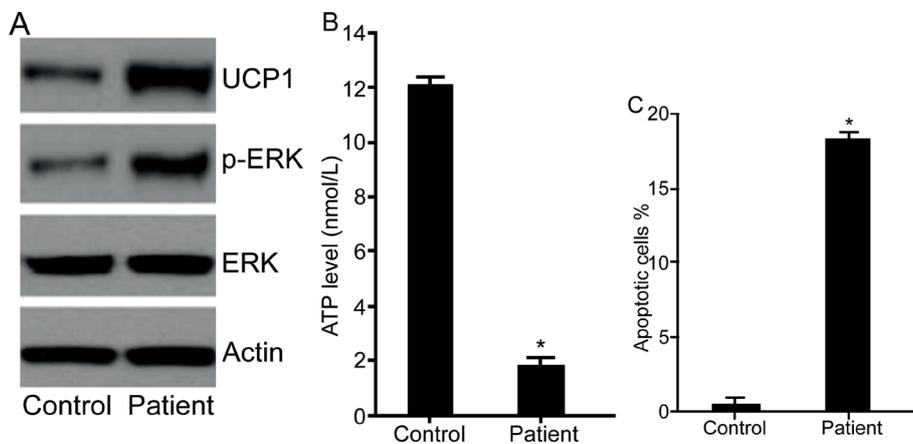

Figure 4. UCP1 expression and cell apoptosis in the liver tissue from sepsis patients. *(A)* Western blot detection. *(B)* ATP production. *(C)* Cell apoptosis detection. *$p<0.05$, compared with control.

ment were of prime importance for liver injury induced by LPS[22,23], but current molecular biomarker provided an inconvenient and unsatisfactory solution to evaluate this disease[24]. Therefore, this study aimed to explore the possibility of UCP1 as the biomarker in early diagnosis and prognosis of liver injury induced by LPS. LPS induced liver injury is a complicated inflammatory response process induced by infection. A variety of inflammatory cytokines interact with each other to induce intense immune response during the process of liver injury, thus leading to mouse death[1,2]. Under this circumstance, pro-inflammatory cytokines signaling pathways were activated to lead to the damage in organs and tissues of mammal and anti- anti-inflammatory strategy should be adopted to prevent inflammatory diseases[25-27]. Therefore, the application of reasonable detection method for early detection and prognosis is of great significance. In our study on the molecular mechanism of UCP1 in liver injury, we found

that UCP1 was upregulated, ERK signaling was activated, ATP production was reduced, and cell apoptosis enhanced in mouse model with liver injury induced by LPS. Also, with the treatment of U0126, the expression of UCP1 was significantly suppressed, ERK signaling pathway was inhibited, ATP production was increased, and liver cell apoptosis was decreased in mice. It suggested that UCP1 played its role in liver injury through the regulation of ERK signaling pathway, ATP production and cell apoptosis. Endotoxin injection in mouse showed the production of reactive oxygen species (ROS), sustained inflammatory activation and over expression of immune cytokines[14]. Moreover, levels of inflammatory cytokines were upregulated when ROS was activated[15,16]. Thus, the role of inflammatory cytokines in regulating reactive oxygen species and the influence of molecular target still needs further exploration. There are also deficiencies in this study. Firstly, the sample scale was small and larger scale was needed to

investigate the possibility of UCP1 as a practical biomarker in liver injury induced by LPS. Secondly, since most liver injury patients received chemotherapy and other treatments[28], the impact of treatment on UCP1 level is still to be further elucidated. Lastly, UCP1 as the target in animal liver injury model requires to be evaluated.

Conclusions

UCP1 exerted an essential effect on mice with liver injury and sepsis patients by regulating mitochondrial ATP production, cell apoptosis and ERK signaling pathway.

Conflict of interest
The authors declare no conflicts of interest.

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