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Ulinastatin prevents acute lung injury led by liver transplantation

Gang-Jian Luo, MD, PhD,[1] *Wei-Feng Yao, MD,*[1] *Ye He, MD, Chen-Fang Luo, MD, Xiao-Yun Li, MD, and Zi-Qing Hei, MD, PhD*[*]

Department of Anesthesiology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

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ABSTRACT

Background: Little is known regarding the effect of ulinastatin (UTI) on acute lung injury (ALI) induced by orthotopic liver transplantation. This study aims to investigate the protective effect of UTI on ALI induced by orthotopic autologous liver transplantation (OALT) in a rat model and to explore the potential underlying mechanism.

Materials and methods: Rats were randomly allocated into the following four groups ($n = 8$ each): (i) sham control group (group sham); (ii) model group (underwent OALT) (group model); (iii) low-dose UTI-treated group (group u1), with UTI (50 U/g) administered intravenously both before the portal vein was occluded and after liver reperfusion started; and (iv) high-dose UTI-treated group (group uh), with UTI (100 U/g) given in the same way as group ul. The lung pathologic parameters, lung water content, and levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, malondialdehyde (MDA), superoxide dismutase (SOD) activity, RanBP-type and C3HC4-type zinc finger-containing protein 1 (RBCK1), and peroxiredoxin-2 (Prx-2) were assessed 8 h after OALT was performed.

Results: According to histology, there was severe damage in the lung of group model accompanied by increases in the TNF-α, IL-1β, IL-6, and MDA levels and decreases in SOD activity and the expression of RBCK1 and Prx-2. UTI treatment significantly reduced the pathologic scores, lung water content, and TNF-α, IL-1β, IL-6, and MDA levels while restoring the SOD activity and expression of RBCK1 and Prx-2. Furthermore, compared with group u1, treatment with a high dose of UTI resulted in a better protective effect on the lung when assessed by the TNF-α, IL-1β, IL-6, and MDA levels and SOD activity.

Conclusions: UTI dose-dependently attenuates ALI that is induced by OALT in this rat model, which is mainly due to the suppression of the inflammatory response and oxidant stress, which may, in turn, be mediated by the upregulation of RBCK1 and Prx-2 expression.

1. Introduction

Acute lung injury (ALI) occurs in 34.2%–77.8% of patients undergoing liver transplantation [1,2] and is one of the main causes of death after orthotopic liver transplantation (OLT) due to serious complications [3]. The causes of ALI after liver transplantation are numerous and include ischemia–reperfusion, the substantial level of blood transfusion, endotoxin, and enterogenous inflammatory medium [4–8]. Recent research shows that uncontrolled lung inflammation [9]

[*] *Corresponding author*. Department of Anesthesiology, The Third Affiliated Hospital of Sun Yat-sen University, No. 600, Tianhe Road, Guangzhou, China 510630. Tel.: +86 020 85252297; fax: +86 020 85252305.
E-mail address: heiziqing@sina.com (Z.-Q. Hei).

[1] Gang-Jian Luo and Wei-Feng Yao contributed equally to this work.

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and oxidative stress [10] play important roles during lipopolysaccharide-induced ALI or trauma-induced ALI. However, to date, the mechanism of OLT-induced ALI has been poorly understood, and there have been no effective treatments for it.

Ulinastatin, a urinary trypsin inhibitor (UTI), is a protease inhibitor purified from fresh human urine [11], and it suppresses proteases such as chymotrypsin, trypsin, and elastase [12]. UTI also stabilizes the lysosomal membrane and suppresses the release of lysosomal enzymes [13]. Moreover, UTI is beneficial in the treatment of experimental acute pancreatitis and shock, and it has been used clinically as an anti-shock drug [12,13] in countries such as China and Japan; however, it is not approved in the United States of America. UTI is effective in relieving reperfusion injury in ischemic livers [14], intestines [15], and kidneys [16]. To the best of our knowledge, however, its efficacy on ALI induced by OLT still remains unknown.

In some animal studies, UTI can attenuate ALI induced by endotoxin, oleic acid, and mechanical ventilation, which may be related to the inhibition of inflammatory cell infiltration and cytokine release. The anti-inflammatory effect is the main therapeutic effect of UTI [17–19]. In a previous study, we found that the concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8 in the serum increased in patients after OLT [20]. To directly address the role of UTI during ALI induced by orthotopic autologous liver transplantation (OALT), we analyzed the expression of the inflammatory cytokines (TNF-α, IL-1 and IL-6) and an oxidative product (malondialdehyde, [MDA]) and evaluated the activity of superoxide dismutase (SOD) in lung tissue.

Recent studies have shown that RanBP-type and C3HC4-type zinc finger-containing protein 1 (RBCK1) and peroxiredoxin-2 (Prx-2) are involved in the anti-inflammatory [21] and anti-oxidative effects [22]. Our previous comparative proteomic study of lung tissue in rats suffering from ALI after OALT revealed 20 differentially expressed proteins including RBCK1 and Prx-2 (data not shown). Interestingly, the two proteins were both downregulated. Furthermore, it is unknown whether the protective effect of UTI is due to the regulation of the two proteins.

Based on the aforementioned findings, we hypothesized that UTI could attenuate ALI in rats after OALT. Therefore, this experiment was designed to investigate the effect of different doses of UTI on ALI after OALT and explore the mechanism involved in these effects.

2. Materials and methods

2.1. Animals and establishment of the OALT model

The present study was approved by the Animal Care Committee of Sun Yat-sen University and was performed in accordance with National Institutes of Health guidelines for the use of experimental animals [23]. Thirty-two adult, pathogen-free male Sprague–Dawley rats weighing between 200 and 250 g were housed in separated cages in a temperature-controlled room with alternating 12 h light–dark cycles; the rats acclimated for 1 wk before the study. We used male rats to prevent any changes in the female hormones from confounding the experimental results. Food was removed 8 h before the study, but all animals had free access to water. A standard model of OALT was established as previously described [24].

2.2. Experimental protocol

Rats were randomly divided into four groups. For the sham group (group sham, $n = 8$), rats were anesthetized and an abdominal incision was made. We dissociated the portal vein and then sutured the abdominal incision. The other three groups received the following treatment respectively, both before the portal vein was occluded and after liver reperfusion started: saline (model group [group model], $n = 8$), low-dose UTI (25 U/g, Techpool Bio-Pharma Co, Guangzhou, China) (group ul, $n = 8$), and high-dose UTI (50 U/g) (group uh, $n = 8$). The time of the anhepatic phase, the operation duration, and the weight of animals are summarized in Table.

2.3. Disposal of specimens

Eight hours after OALT, the animals were anesthetized with chloral hydrate. After a midline laparotomy incision was performed, the rats were eviscerated and the abdominal aorta was exposed; a 24-gauge needle was used to cannulate the abdomen for blood collection. Next, the thorax was opened, and all surrounding tissues were dissected, after which the lungs were removed. The middle lobes of right lungs were weighed on an electronic scale, and the inferior lobes of the right lungs were fixed in 10% buffered formalin and embedded in paraffin for an histologic evaluation. The remaining lung tissues were quickly frozen in liquid nitrogen for preservation and transferred to a −80°C refrigerator for storage before subsequent protein expression analyses of RBCK1 and Prx-2 and measurements of the TNF-α, IL-1β, IL-6, and MDA levels and SOD activity.

2.4. Liver histologic evaluation

Formalin-fixed and paraffin-embedded tissue sections were cut to a thickness of 3 μm and stained with hematoxylin and eosin (H&E) for an histologic examination. Two pathologists, who were blinded to the treatment, used light microscopy to assess the degree of liver damage. The grading of the severity of hepatic injury was as follows: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting of cytoplasm vacuolization and focal nuclear pyknosis; grade 2, moderate to

Table – The time of the anhepatic phase, operation time, and weight of the animals ($n = 8$ in each group, $\bar{x} \pm s$).

Group	Weight (g)	Time of the anthepatic phase (min)	Operation time (min)
Sham	235.0 ± 10.6	—	—
Model	224.0 ± 20.1	21.3 ± 0.5	62.0 ± 3.1
Ul	217.0 ± 15.8	19.8 ± 1.2	66.0 ± 2.4
Uh	229.0 ± 7.5	20.2 ± 0.7	63.0 ± 3.3