Efecto de la ulinastatina sobre el esteroide inflamatorio NLRP3 en la lesión renal aguda de sepsis

Effect of Ulinastatin on Inflammatory Steroid NLRP3 in Acute Renal Injury of Sepsis

Yanhui Cao*, Jiannan Zhang, Xianxin Kang, Wen Liu, Guangping Chang, Jianbing Wang, Yuanyuan Liu
Department of ICU, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China

*Correspondence author: Yanhui Cao, Email:icucaoyanhui@126.com

Resumen
Como inhibidor de la proteasa, la ulinastatina tiene la función de estabilizar la membrana lisosómica, regular la liberación de factores inflamatorios, mejorar la función inmune del cuerpo y puede usarse para el tratamiento de la pancreatitis aguda y crónica y otras enfermedades de insuficiencia circulatoria aguda. Este artículo analizó el efecto de la ulinastatina sobre el corpúsculo inflamatorio NLRP3 en la lesión renal aguda causada por sepsis. Los resultados mostraron que los niveles de proteína de NLRP3 y ASC, el nivel de expresión de m ARN y el nivel de caspasa-1 en suero en el grupo modelo y el grupo de tratamiento fueron significativamente más altos que los del grupo de control en blanco y el grupo de operación simulada (P <0,01). El grupo modelo tenía tejido renal NLRP3. Los niveles de proteína de ASC, el nivel de expresión de ARNm y el nivel de Caspase-1 en suero fueron significativamente más altos que los del grupo de tratamiento (P <0.01). Conclusión Está comprobado que la ulinastatina puede regular negativamente la expresión de NLRP3, ASC y Caspase-1 en la lesión renal aguda de sepsis, y tiene el efecto de regular la síntesis de inflamasoma NLRP3.

Palabras clave: sepsis; ulinastatina; inflamatorio Lesión renal aguda

Abstract
As a protease inhibitor, ulinastatin has the functions of stabilizing lysosomal membrane, regulating the release of inflammatory factors, improving the immune function of the body, and can be used for the treatment of acute and chronic pancreatitis and other acute organ circulatory failure diseases. This article analyzed the effect of ulinastatin on inflammatory corpuscle NLRP3 in acute kidney injury caused by sepsis. The results showed that the protein levels of NLRP3 and ASC, the expression level of m RNA and the level of caspase-1 in serum in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group (P < 0.01). The model group had renal tissue NLRP3. The protein levels of ASC, the level of m RNA expression, and the level of serum Caspase-1 were significantly higher than those in the treatment group (P < 0.01). Conclusion It is proved that ulinastatin can down-regulate the expression of NLRP3, ASC and Caspase-1 in acute kidney injury of sepsis, and has the effect of regulating the synthesis of NLRP3 inflammasome.

Key words: Sepsis; ulinastatin; inflammasome; acute kidney injury

1. Introduction

The incidence of acute kidney injury (AKI) in patients with sepsis is about 30%, and the mortality rate is over 40%. Most survivors still have different degrees of kidney damage[1]. As a protease inhibitor, ulinastatin has the functions of stabilizing lysosomal membrane, regulating the release of inflammatory factors, improving the immune function of the body, and can be used for the treatment of acute and chronic pancreatitis and other acute organ circulatory failure diseases[2-3]. Sepsis is caused by detached infected thrombus or bacterial emboli into the body's blood circulation, and caused by metastatic abscess inside the organ, which can involve multiple organs, of which acute kidney injury is a common serious complication[4]. Studies have shown that acute kidney injury in sepsis is the leading cause of death in critically ill patients with ICU. The mortality rate is about 32.1% to 55%, which is positively correlated with the severity of the disease. There is no specific treatment. Ulinastatin can inhibit a variety of proteases, reduce the chance of cell damage, promote the improvement of the body's microcirculation, and have a certain protective effect on the kidney[5-7]. Clinical study of mouse urinary kidney injury molecule-1 (KIM-1), atrial natriuretic peptide (ANP) and cystatin-c (CYS-C) and sepsis acute kidney injury have a significant correlation, the level of change can be Reflect the extent of disease...
As an important substance in the process of innate immune response, inflammasome is closely related to the occurrence and development of sepsis. Inflammatory bodies play an important role in fighting infection and eliminating pathogenic microorganisms by activating caspase-1[9-10]. Studies have shown that after knocking out the mouse caspase-1 gene, it exhibits obvious gram-negative bacteria including Escherichia coli, Salmonella typhimurium, T. facalis, and Shigella flexneri[11]. The susceptibility to the ability to resist Pseudomonas sylvesis and Candida albicans is also significantly reduced. Compared with wild-type heterozygous mice, the anti-infective ability of caspase-1 knockout mice was significantly decreased, and the mortality rate in sepsis was significantly increased[12-13].

Sepsis is more common in post-surgery, severe burns, multiple trauma, etc., can cause repeated episodes of chills, fever, anemia and other symptoms, and can cause acute damage to the kidneys, posing a serious threat to the patient's life safety. Early treatment of infection, nutritional support, and protection of the nerves can play a certain role in improving, but the effect is still poor. As a glycoprotein, ulinastatin is extracted from fresh urine and can have a variety of pharmacological effects[14]. There are many cytokines involved in the pathogenesis of sepsis acute kidney injury. KIM-1 is a transmembrane protein that is hardly expressed in normal tissues. The concentration of renal tissue after ischemia is significantly increased, and the proximal convoluted tubules are affected. It can be expressed continuously after damage. ANP receptors can be widely distributed in various organs of the body, but they are mostly concentrated in the kidney and adrenal cortex. They can bind to specific receptors and then act on the kidney through cascade amplification. The level can directly reflect the acute kidney injury of the body, and the accuracy[15]. CYS-C is mainly excreted by the kidney, its stability is good, pre-renal and other factors have no effect on CYS-C. When the glomerulus has tiny lesions, its filtration and reabsorption can produce abnormalities, resulting in an increase in its concentration, which can be reflected. The state of renal function damage caused by sepsis.

2. Materials and Methods

2.1 Laboratory Animals and Model Preparation

32 healthy adult male Sprague-Dawley rats aged 8 weeks, weighing 200-220 g, were purchased from the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. Mold was established by cecal ligation and perforation (CLP). Rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (40 mg/kg). A 1.5 cm longitudinal incision was made in the midline of the abdomen to find the cecum. The suture was ligated at the root and the 18-gauge needle was used. The cecum was punched through 3 times, and a small amount of intestinal contents was squeezed out. The 2 mm flap was placed to prevent the pinhole from closing. The cecum was also placed in the abdominal cavity, and the abdominal wall incision was sutured layer by layer. The subcutaneous injection of physiological saline (30 mL/kg) was given. shock.

2.2 Equipment and Reagents

Rat Caspase-1 quantitative enzyme-linked detection kit was purchased from Shanghai Senxiong Technology Industrial Co., Ltd.; Total RNA Extraction Kit II, produced by Guangzhou Jiebasi Biotechnology Co., Ltd. Reverse transcription was performed using Fermentas' Revert Aid First Strand c DNA Synthesis Kit. The PCR reaction was performed using Kang's Ultra SYBRMixture kit.

2.3 Animal Grouping and Interventions

Thirty-two healthy adult male Sprague-Dawley rats, aged 8 weeks, were randomly divided into 4 groups: 8 in the blank control group, 8 in the sham operation group, 8 in the model group, and 8 in the treatment group. The blank control group did not do any treatment, and anesthesia was performed 24 hours later and blood was taken from the abdominal aorta. The sham operation group underwent laparotomy after anesthesia, and the gastrointestinal tract was turned over and the abdomen was closed. After 24 hours, anesthesia was performed and blood was collected from the abdominal aorta. The model group was intragastrically administered with 2 ml of normal saline immediately after CLP, 6 h and 12 h after CLP, and then administered with normal saline 2 m L once, and anesthetized 24 h after CLP and blood was sacrificed from abdominal aorta. The treatment group was given ulinastatin Chinese medicine (10 g/kg) once a day after CLP, 6 hours and 12 hours after CLP, and then given ulinastatin Chinese medicine (10 g/kg) once a day. Anesthesia was given 24 h after CLP and blood was collected from the abdominal aorta.

2.4 Specimen Collection

2% sodium pentobarbital (40 mg/kg) Anesthetized rats were intraperitoneally injected, the abdominal cavity was opened, the abdominal organs such as the intestines were opened, the peritoneum was opened, the
abdominal aorta was exposed, and blood was slowly drawn with a 10 mL sterile syringe. Dispense in a non-anticoagulated sterile test tube, let stand for 30 min, centrifuge at 3000 r/min for 15 min, take 200 μL of the upper serum, dispense into EP tube, and keep at -20 °C in the refrigerator. After the blood was collected from the rats, the kidney tissue samples were wrapped in tin foil and placed in a liquid nitrogen tank for storage.

2.5 Detection Indicators and Methods
The general condition of the rats within 24 hours after surgery was observed, including mental state, appetite, activity, appearance, coat color, feces, secretions and the like. The content of Caspase-1 in rat serum was detected by ELISA. The content of NLRP3 and ASC protein in renal tissue was detected by Western-Blot method. The expression of mRNA in renal tissue NLRP3 and ASC was detected by real-time PCR.

2.6 Statistical Processing
Apply SPSS 21.0 statistical software. It conforms to the normal distribution, and the measurement data is represented by \( \bar{x} \pm S \). The test level of the normality test is \( \alpha = 0.10 \), and the test level of variance analysis is \( \alpha = 0.05 \). Comparisons between the two groups were performed using an independent sample t test. \( P < 0.05 \) was considered statistically significant.

3. Result

3.1 Comparison of General Conditions of Each Group
The appearance, diet and activities of the blank control group and the sham operation group were normal. In the model group, the rats suffered from mental wilting, the activity was significantly reduced, the respiratory rate was significantly accelerated, the diet was significantly reduced, the abdomen appeared bulging, the secretions in the corner of the eyes appeared, and the excretion increased. The above symptoms of the rats in the treatment group were lighter than those in the model group, and the mental state and activity were significantly better than the model group. Abdominal bulging, secretion, excretion and the like were also superior to the model group.

3.2 Comparison of Serum Caspase-1 Levels in Rats of Each Group
See Table 1. The levels of Caspase-1 in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group. The level of Caspase-1 in the model group was significantly higher than that in the treatment group (\( P < 0.05 \) or \( P < 0.01 \)), and the levels of the blank control group and the sham operation group were significantly higher. Quite (\( P > 0.05 \)).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Caspase-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control group</td>
<td>8</td>
<td>13.30±2.68</td>
</tr>
<tr>
<td>Mock surgical group</td>
<td>8</td>
<td>12.56±3.60</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>49.16±6.70</td>
</tr>
<tr>
<td>Therapy group</td>
<td>8</td>
<td>26.46±1.88</td>
</tr>
</tbody>
</table>

3.3 Comparison of NLRP3 and ASC Protein Levels in Renal Tissues of Rats
See Table 2. The NLRP3 and ASC protein levels in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group. The NLRP3 and ASC protein levels in the model group were significantly higher than those in the treatment group (\( P < 0.05 \) or \( P < 0.01 \)), blank control group and sham operation. Group levels were comparable (\( P > 0.05 \)).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>NLRP3/GAPDH</th>
<th>ASC/GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control group</td>
<td>8</td>
<td>0.44±0.01</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>Mock surgical group</td>
<td>8</td>
<td>0.48±0.03</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>1.47±0.06</td>
<td>1.17±0.05</td>
</tr>
<tr>
<td>Therapy group</td>
<td>8</td>
<td>0.90±0.05</td>
<td>0.78±0.03</td>
</tr>
</tbody>
</table>

3.4 Comparison of mRNA Expression Levels of NLRP3 and ASC in Renal Tissues of Rats Each Group
See Table 3. The mRNA expression levels of NLRP3 and ASC in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group. The expression levels of MLRP3 and ASC in the model group were significantly higher than those in the treatment group.
group (P < 0.05 or P < 0.01). The blank control group and the sham operation group were equally level (P > 0.05).

Table 3: Comparison of mRNA Expression Levels of NLRP3 and ASC in Renal Tissues of Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>NLRP3 mRNA Relative expression</th>
<th>ASC mRNA Relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control group</td>
<td>8</td>
<td>1.49±0.31</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Mock surgical group</td>
<td>8</td>
<td>1.74±0.16</td>
<td>0.20±0.06</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>117.17±6.64</td>
<td>1.78±0.10</td>
</tr>
<tr>
<td>Therapy group</td>
<td>8</td>
<td>72.67±4.30</td>
<td>0.98±0.06</td>
</tr>
</tbody>
</table>

3.5 Kidney Damage

The degree of renal injury was aggravated within 3 days after treatment, but there was no significant difference between the two groups. However, with the increase of treatment time, the degree of renal injury was better in the observation group than in the control group (P < 0.05), see Table 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Example</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>44</td>
<td>12</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Before treatment</td>
<td></td>
<td>8</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>3d after treatment</td>
<td>9</td>
<td>19</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>5d after treatment</td>
<td>9</td>
<td>25</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Observation group</td>
<td>44</td>
<td>9</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Before treatment</td>
<td></td>
<td>8</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>3d after treatment</td>
<td>12</td>
<td>22</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5d after treatment</td>
<td>16</td>
<td>18</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

At present, sepsis is the most serious complication in clinically critically ill patients with infection, shock, and major surgery; its essence is that the systemic inflammatory response caused by infection causes multiple organ dysfunction. In addition, AKI as a common complication of sepsis, its high mortality and morbidity has become an independent risk factor affecting the prognosis of patients.

Ulinastatin is an important protease inhibitor in human urine. It inhibits serine protease activity and significantly inhibits proteolytic enzymes such as trypsin, thereby keeping its cells and tissues undigested. Studies have shown that ulinastatin, in addition to its broad anti-shock and anti-tumor effects, can also act as an oxygen free radical scavenger and membrane stabilizer, and can regulate the release of inflammatory cytokines, inhibit leukocyte migration and blood cell activation. Prevent further development of the inflammatory response. Liu Hong et al found that the expression levels of tumor necrosis factor-α and interleukin-6 in patients treated with ulinastatin were lower than those in the control group.

Inflammasome is a type of macromolecular, multiprotein complex induced by activated nucleotide-binding oligomeric domain-like receptors (NLRs) present in the cytoplasm of cells and is mediated. The important role of the body's innate immune response. The NLRP3 inflammasome is a member of the inflammatory family and its structure is composed of NLRP3, apoptosis-associated spot-like protein (ASC), and caspase-1 precursor (pro-caspase-1) assembly. of. NLRP3 is activated by recognition of pathogen-associated molecular patterns (PAMPs) or risk-related molecular patterns (DAMPs) in combination with ligands, induces NLRP3 inflammasome assembly, and promotes oligomerization, oligomerized pro-caspase-1 self-enzymatic hydrolysis to form a biologically active caspase-1, caspase-1 promotes the maturation of interleukin-1β precursor (pro-IL-1β) and interleukin-18 precursor (pro-IL-18), producing organisms Active IL-1β and IL-18 are secreted outside the cell to exert their biological effects. The NLRP3 inflammasome can be activated by a wide range of exogenous and endogenous stimuli. Microbial infections such as Sendai virus, influenza virus, adenovirus, Saccharomyces cerevisiae and Candida albicans, as well as some bacteria such as Staphylococcus aureus, Listeria monocytogenes, Shigella flexneri, etc. can induce NLRP3 inflammatory Activation of the body. In some cases, certain specific microbial components can also trigger the activation of NLRP3 inflammasomes, such as bacterial RNA, Plasmodium pigment crystals and many bacterial pore-forming toxins such as Nigerian, toxin, Aeromonas Lysin and Listeria Lysin.
The results showed that KIM-1, ANP, and CYS-C levels were significantly decreased after ulinastatin treatment, indicating that ulinastatin can reduce renal damage, which may be related to the stability of renal tubular and glomerular cell membranes. In the case of sepsis, the body produces a series of inflammatory factors, which can affect the activity of hydrolase and protease, leading to damage to tissues and organs such as kidney and kidney. IL-1 and TNF-α are typical pro-inflammatory factors that stimulate the body to initiate an immune response, participate in the entire pathogenesis of sepsis, and cause pathological damage to the kidney. IL-6 is a multifunctional cytokine that interferes with the balance of vascular endothelial cells, promotes vasoconstriction and clotting processes, and aggravates vascular endothelial cell damage. As an acute phase protein of the body, CRP can significantly increase in the body when it produces inflammatory stimuli, and its stability is good, and the level does not fluctuate due to drug factors.

The results showed that serum levels of IL-1, IL-6, CRP, and TNF-α were significantly decreased after ulinastatin treatment, indicating that ulinastatin can reduce the level of inflammatory factors and alleviate the damage caused to the body. Foreign studies have shown that acute kidney injury is closely related to renal blood flow reduction. NO is a kind of vasodilator that can regulate the blood supply of the kidney and maintain the normal physiological function of the kidney. The decrease of its concentration can lead to pathological damage of blood vessels. To increase kidney damage. ET-1 can cause vasoconstriction, glomerular mesangial cells can form a large number of ET-1 receptors, reduce renal blood flow, induce acute kidney injury, and endotoxin can induce the body's ET-1 level to rise, making the renal blood vessels the shrinkage is enhanced. The results showed that the level of NO increased and the level of ET-1 decreased after ulinastatin treatment, indicating that ulinastatin can be beneficial to the recovery of vascular endothelial function in the body, which is beneficial to the improvement of blood circulation of the kidney, which may be related to the ability of the body to cause inflammatory factors. It expresses receptor inhibition and at the same time relieves ischemia-reperfusion injury in the kidney, thereby reducing its damage to endothelial cells. The ability of sepsis patients to clear the pathogens of the body is significantly reduced, and a large number of immune resuscitation products can be formed, resulting in disorder of immune function, resulting in a decrease in immunoglobulin levels. The results show that the level of immunoglobulin after ulinastatin treatment is higher than that of the conventional treatment group, indicating that ulinastatin can correct the body's immune dysfunction and enhance its ability to defend against disease. The APACHE-II scoring system can accurately assess the acute physiological state of the body and reflect the progress of the body. This result shows that the APACHE-II score is significantly reduced after ulinastatin treatment, indicating that ulinastatin can effectively control the progression of the disease. Slowing down the disease may be able to regulate the damage caused by cytokines in many ways, promote the improvement of the patient's internal environment, and facilitate the removal of renal toxicity of the patient, thereby improving the kidney damage caused by sepsis.

5. Conclusion

In this experiment, the NLRP3, ASC protein levels and m RNA expression levels in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group, indicating that the NLRP3 inflammasome level was significantly increased in the onset of sepsis. It plays an important role in the pathogenesis of sepsis. The NLRP3, ASC protein levels and m RNA expression levels in the treatment group were also significantly lower than those in the model group, indicating that ulinastatin can down-regulate the expression of NL-RP3 and ASC, thereby regulating the synthesis of NLRP3 inflammaosome. The caspase-1 levels in the model group and the treatment group were significantly higher than those in the blank control group and the sham operation group, while the caspase-1 level in the treatment group was significantly lower than that in the model group. This result indicates that ulinastatin has a down-regulation of Caspase-1 levels, which is consistent with its regulation of NLRP3 and ASC. At the same time, the results of this experiment also suggest that the degree of regulation of inflammatory response by regulating the inflammasome may become a target for the treatment of sepsis.

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References


