Original Article
Therapeutic effect of ulinastatin combined with continuous blood purification in children with sepsis

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Abstract: Objective: To investigate the therapeutic effect of continuous blood purification (CBP) combined with ulinastatin in children with sepsis. Methods: A total of 100 children with sepsis were selected and divided into the CBP group and the CBP + ulinastatin group by complete randomization, with 50 cases in each group. The CBP group was given continuous blood purification treatment. The CBP + ulinastatin group was given ulinastatin in addition to the treatment given in the CBP group. The ulinastatin was administrated in the morning and evening, for one-hour at 20,000 U/kg ulinastatin plus 50 mL normal saline by microinfusion pump once for 7 d. Serum soluble triggering receptor expressed on myeloid cell-1 (sTREM-1), C-reactive protein, procalcitonin (PCT), acute physiology and chronic health evaluation (APACHE II) score, cardiac troponin I, creatine kinase-MB, CD8⁺ level, and CD4⁺/CD8⁺ ratio were measured in patients before and after 7-days of treatment. The total effective rate, incidence of adverse reactions and 28-day survival rate were statistically analyzed in the two groups. Results: After treatment, the levels of sTREM-1, C-reactive protein, PCT, cardiac troponin I, creatine kinase-MB, and APACHE II score in the CBP + ulinastatin group were lower than those in the CBP group, and CD8⁺ level and CD4⁺/CD8⁺ ratio in the CBP + ulinastatin group were higher than those in the CBP group (P<0.001). After treatment, the total effective rate of the CBP + ulinastatin group was higher than that of the CBP group (P<0.01). After treatment, the incidence of adverse reactions in the CBP + ulinastatin group was lower than that in the CBP group, but the difference was not statistically significant (P>0.05). After treatment, the 28-day survival rate in the CBP + ulinastatin group was higher than that in the CBP group (P<0.05). Conclusion: Ulinastatin combined with CBP has a significant therapeutic effect on the treatment of sepsis in children, which can reduce the inflammatory response, effectively decrease the levels of sTREM-1 and PCT, boost the immune system and protect cardiac function.

Keywords: Pediatric sepsis, ulinastatin, continuous blood purification, procalcitonin

Introduction
Sepsis has a high clinical incidence and is the leading cause of death in pediatric emergencies and in children in paediatric intensive care units (PICU). Early detection and effective treatment of this disease can improve survival outcomes [1]. Currently, assisted ventilation and vasoactive drugs are the main methods for clinical treatment of sepsis. CBP (continuous blood purification) has been demonstrated to be useful in the treatment of sepsis in children, which is widely used in treating children with severe sepsis, as it achieves good efficacy [2]. Septic shock is the leading cause of death in children with sepsis, and it is important to find effective treatments [3]. As a broad-spectrum protease inhibitor, ulinastatin can improve microcirculation, control disease development in patients, and protect the vital organs of the body [4]. At present, there are very few relevant studies on the treatment of sepsis in children; therefore, we studied the therapeutic effect of ulinastatin combined with CBP in children with sepsis and its effects on sTREM-1 and PCT, in order to provide a new perspective for the treatment of sepsis in children.

Materials and methods

Subjects
A total of 100 children with sepsis who received treatment in The Affiliated Hospital of Qingdao University from March 2019 to April 2020 were selected and divided into the CBP
group and CBP + ulinastatin group, according to complete randomization, with 50 cases in each group. This study was approved by the Ethics Committee of The Affiliated Hospital of Qingdao University.

Inclusion criteria: (1) Patients in line with the 2016 International Consensus Definition for Sepsis and Septic Shock [5]; (2) Patients with indications for CBP; (3) Patients with an APACHE II (acute physiology and chronic health evaluation) score of ≤90 points; (4) Patients' whose families were informed and signed the consent form.

Exclusion criteria: (1) Patients with severe organ diseases; (2) Patients with congenital immunodeficiency; (3) Patients who received treatment upon admission; (4) Patients with end-stage chronic diseases; (5) Patients with allergic reactions; (6) Patients with contraindications for the use of ulinastatin.

Methods

Treatment methods: Patients in both groups received treatment for the maintenance of a stable internal environment, anti-shock and anti-infection therapies. The CBP group received continuous blood purification treatment on the basis of conventional treatment using continuous renal replacement therapy (CRRT) machine (MEDICAL, Italy) and hollow fiber dialyzer (Baxter), with specific parameters configured as follows: replacement fluid rate 20-35 mL/kg/h, blood flow rate 3-5 mL/kg/min, total exchange volume of dialysate 0.5-8.0 L/d, and concentrations of electrolytes including sodium 140 mmol/L, potassium 4.0 mmol/L, chlorine 118.6 mmol/L, and sodium bicarbonate without replacement fluid, 6-12 h per day. The CBP + ulinastatin group was given ulinastatin in addition to the treatment in the CBP group, morning and evening administration, for one-hour at 20000 U/kg plus 50 mL normal saline by microinfusion pump once for 7 d [6]. Ulinastatin was purchased from Guangdong Tianpu Biochemical Pharmaceutical Co., Ltd.

Outcome measures

The primary outcomes in this study were measures of myeloid cell-1 (sTREM-1), procalcitonin (PCT), APACHE II score, and survival rate. The secondary outcomes were levels of C-reactive protein (CRP), cardiac troponin I (cTnI), creatine kinase-MB (CK-MB), cluster of differentiation 8-positive (CD8\(^+\)), the ratio of the cluster of differentiation 4-positive to cluster of differentiation 8-positive (CD4\(^+\)/CD8\(^+\) ratio), total effective rate and incidence of adverse reactions.

Measurement of sTREM-1, CRP and PCT levels: Before and after treatment, 5 mL of fasting venous blood was drawn from the patients in the morning, and stored in a disposable vacuum blood collection tube (anticoagulant-free). The samples were let stand at 20°C-25°C for 60 min for coagulation and centrifuged at 10,000 g for 10 min to collect the serum, and then serum was stored at -80°C. The levels of sTREM-1, CRP, and PCT were measured by enzyme-linked immunosorbent assay (ELISA). sTREM-1 ELISA test kit was purchased from AmyJet Scientific Co., Ltd.; CRP ELISA test kit was purchased from Shenzhen Haodihuatuo Biotechnology Co., Ltd.; PCT ELISA test kit was purchased from Wuhan Fine Biotech Co., Ltd.

Measurement of APACHE II scores, cTnI, and CK-MB levels: Before and after treatment, the Acute Physiology and Chronic Health Evaluation (APACHE II) scores were calculated from aspects mainly including age, chronic health and acute physiology, with scores ranging from 0-71 points, and higher scores indicating more severe disease and worse prognosis. Before and after treatment, the cTnI levels of the patients were measured by enzyme-labeled immunoassay assay (Beijing Solai Bao Technology Co., Ltd.), and the CK-MB levels were measured by enzymatic method (Shanghai Genmed Pharmaceutical Technology Co., Ltd.).

Measurement of CD8\(^+\) level and CD4\(^+\)/CD8\(^+\) ratio: Before and after treatment, 200 μL anticoagulated blood was collected from the patients, and divided into two tubes. After removing the red blood cells, one tube was mixed with 20 μL CD4\(^+\) monoclonal antibodies, and the other tube was mixed with 20 μL CD8\(^+\) monoclonal antibodies. After incubation at room temperature for 30 min, both samples were centrifuged at 10,000 g for 5 min. Then the supernatant was discarded. The samples were washed once, fixed and resuspended by phosphate-buffered saline (PBS) to adjust the cell concentration to 1×10\(^5\)-1×10\(^6\)/mL. Finally, the samples were detected by flow cytometry (BectonDickinson, USA). CD8\(^+\) lymphocyte per-
Table 1. Comparison of sTREM-1, CRP and PCT levels before and after treatment (X ± sd)

<table>
<thead>
<tr>
<th>Group</th>
<th>CBP group (n=50)</th>
<th>CBP + ulinastatin group (n=50)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTREM-1 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>148.55±34.18</td>
<td>148.61±34.23</td>
<td>0.009</td>
<td>0.993</td>
</tr>
<tr>
<td>After treatment</td>
<td>110.41±28.36***</td>
<td>82.94±24.72***</td>
<td>5.163</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>75.15±18.37</td>
<td>75.31±18.42</td>
<td>0.043</td>
<td>0.965</td>
</tr>
<tr>
<td>After treatment</td>
<td>33.67±11.16***</td>
<td>18.23±5.82***</td>
<td>8.674</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>4.06±1.24</td>
<td>4.14±1.27</td>
<td>0.319</td>
<td>0.751</td>
</tr>
<tr>
<td>After treatment</td>
<td>2.35±0.71***</td>
<td>1.13±0.32***</td>
<td>11.081</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ***P<0.001 as compared with that before treatment. sTREM-1: Serum soluble triggering receptor expressed on myeloid cell-1; CRP: C-reactive protein; PCT: Procalcitonin; CBP: Continuous blood purification.

Results

Comparison of general data

In the CBP group, there were 26 males and 24 females, aged between 5 months and 11 years old (mean, 5.4±5.1 years), and weighing between 6.5 and 36.3 kg (mean, 20.3±14.2 kg). In the CBP + ulinastatin group, there were 30 males and 20 females, aged between 4 and 12 years old (mean, 5.1±5.8 years), and weighing between 6.0 and 35.5 kg (mean, 19.7±13.4 kg). There was no significant difference in the general data between the two groups, and the groups were comparable.

Comparison of sTREM-1, CRP and PCT levels before and after treatment

As shown in Table 1, there was no significant difference in sTREM-1, CRP and PCT levels before treatment between the two groups (P>0.05), while after treatment, sTREM-1, CRP and PCT levels all significantly decreased in both groups (P<0.001). After treatment, sTREM-1, CRP and PCT levels were significantly lower in the CBP + ulinastatin group than those in the CBP group (P<0.001).

Comparison of APACHE II score, cTnI, and CK-MB levels before and after treatment

As shown in Table 2, there was no significant difference in APACHE II score, cTnI, and CK-MB levels before treatment between the two groups (P>0.05), while after treatment, APACHE II score, cTnI, and CK-MB levels all significantly decreased in both groups (P<0.001). After treatment, APACHE II score, cTnI, and CK-MB levels in the CBP + ulinastatin group were
Table 2. Comparison of APACHE II score, cTnI and CK-MB levels before and after treatment (X ± sd)

<table>
<thead>
<tr>
<th>Group</th>
<th>CBP group (n=50)</th>
<th>CBP + ulinastatin group (n=50)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II score (points)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>23.21±7.16</td>
<td>23.36±7.21</td>
<td>0.104</td>
<td>0.917</td>
</tr>
<tr>
<td>After treatment</td>
<td>17.57±5.31***</td>
<td>12.04±3.37***</td>
<td>6.218</td>
<td>0.001</td>
</tr>
<tr>
<td>cTnI (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>1.68±0.51</td>
<td>1.71±0.53</td>
<td>0.288</td>
<td>0.774</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.97±0.30***</td>
<td>0.63±0.16***</td>
<td>7.071</td>
<td>0.001</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>60.48±15.34</td>
<td>60.52±15.37</td>
<td>0.013</td>
<td>0.990</td>
</tr>
<tr>
<td>After treatment</td>
<td>35.91±10.27***</td>
<td>27.16±7.68***</td>
<td>4.825</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ***P<0.001 as compared with that before treatment. APACHE II: Acute physiology and chronic health evaluation; cTnI: Cardiac troponin I; CK-MB: Creatine kinase-MB; CBP: Continuous blood purification.

Table 3. Comparison of CD8<sup>+</sup> level and CD4<sup>+</sup>/CD8<sup>+</sup> ratio before and after treatment (X ± sd)

<table>
<thead>
<tr>
<th>Group</th>
<th>CBP group (n=50)</th>
<th>CBP + ulinastatin group (n=50)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>16.11±3.16</td>
<td>16.02±3.11</td>
<td>0.144</td>
<td>0.886</td>
</tr>
<tr>
<td>After treatment</td>
<td>20.34±5.61***</td>
<td>24.82±7.13***</td>
<td>3.492</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;/CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>0.96±0.21</td>
<td>0.95±0.20</td>
<td>0.244</td>
<td>0.808</td>
</tr>
<tr>
<td>After treatment</td>
<td>1.19±0.35***</td>
<td>1.68±0.54***</td>
<td>5.384</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ***P<0.001 as compared with that before treatment. CBP: Continuous blood purification; CD: cluster of differentiation.

Effect of ulinastatin plus continuous blood purification on sepsis in children

As shown in Table 2, the total effective rate was significantly higher in the CBP + ulinastatin group than that in the CBP after treatment (P<0.01).

Comparison of incidence of adverse reactions

As shown in Table 5, the incidence of adverse reactions after treatment in the CBP + ulinastatin group was lower than that in the CBP group, but the difference was not statistically significant between both groups (P>0.05).

28-day survival rate of patients with sepsis

As shown in Figure 3, the 28-day cumulative survival rate was 90.00% (45/50) in the CBP + ulinastatin group, which was significantly higher than 74.00% (37/50) in the CBP group (χ<sup>2</sup>=4.336, P=0.037).

Discussion

Sepsis easily develops into severe sepsis. So far, the pathogenesis of this disease has gradually been identified in pediatric clinics, it mostly affects cardiac function and results in multiple organ dysfunction syndromes. Early diagnosis and treatment of sepsis is of great significance for reduction of mortality in patients [7]. The pathomechanisms of pediatric sepsis are complex, but current studies have shown that it is associated with a combination of factors, including an excessive inflammatory response, impaired endothelial function, oxidative stress, and activation of the coagulation system [8, 9]. The key to reduce the mortality of children with sepsis is early detection and timely correction of the imbalance between oxygen supply and demand, which can effectively remove various toxic substances and inflammatory mediators in patients [10]. The working principles of CBP mainly includes convection, diffusion, and adsorption, which is a method of continuous and slow removal of solutes and fluid, and can effectively promote the
Effect of ulinastatin plus continuous blood purification on sepsis in children

Immune response of the patients [11]. Ulinastatin is an endogenous anti-inflammatory substance found in human body which can scavenge oxygen free radicals [12]. In this study, we explored the strategies to improve the therapeutic effect of sepsis, in an effort to provide a new direction for clinical treatment of sepsis in children.

STREM-1 plays an important role in the initiation of sepsis. During infection of various pathogens, the level of sTREM-1 is significantly increased, and it is closely related to the degree of stress and intensity of the inflammatory response [13]. CRP can be used to evaluate the degree of infection in children with sepsis. As a non-specific factor, it can be detected in the early stages of inflammatory infection and bacteremia, which is of great significance for observation of therapeutic response. Relevant studies have shown that PCT, mainly secreted by thyroid C cells, increases in children with sepsis, and rises dramatically in the serum shortly after infection, which is of great value for the diagnosis of bacterial infections [14]. In our study, it was shown that ulinastatin combined with CBP can significantly reduce sTREM-1, CRP and PCT levels in patients. One study of Zuccari et al. has shown that CBP can effectively remove inflammatory mediators and toxic metabolites in children with sepsis, which is consistent with the results of our study.

Figure 1. Flow cytometric analysis of CD4+. A: CBP group before treatment; B: CBP + ulinastatin group before treatment; C: CBP group after treatment; D: CBP + ulinastatin group after treatment. CBP: Continuous blood purification; CD: cluster of differentiation.
Effect of ulinastatin plus continuous blood purification on sepsis in children

Figure 2. Flow cytometric analysis of CD8+. A: CBP group before treatment; B: CBP + ulinastatin group before treatment; C: CBP group after treatment; D: CBP + ulinastatin group after treatment. CBP: Continuous blood purification; CD: cluster of differentiation.

dy [15]. Impaired immune function can lead to invasion of pathogenic microorganisms, worsening of the disease, and seriously impacting the children. T lymphocytes can reflect the changes of a cells’ immune function [16]. Clinical studies have found that CBP can directly inhibit immunosuppression and delay disease development in children by reducing secretion of inflammatory mediators, and promoting filtration and adsorption of immunosuppressive cells [17]. The results of our study pointed out that the use of ulinastatin combined with CBP in children can improve immune function. Relevant studies have shown that abnormally elevated cTnl and CK-MB levels in patients with sepsis indicate higher mortality [18]. APACHE II score is an index for assessment of disease severity and prognosis in critically ill patients. In this study, ulinastatin combined with CBP reduced APACHE II score and improved cardiac function in children, which was consistent with above studies.

Ulinastatin can protect vital organs of the body, regulate and enhance immune function of the patients, which can be used in treating a variety of diseases to exert a good effect on the body [19]. In a study by Xie et al. 2000 U/
kg/d ulinastatin was added into 20 mL of 0.9% sodium chloride injection for the treatment of sepsis in children, given for 1 h, 3 times a day for 5 d, which was the same as the dose used in our study [20]. We found that ulinastatin combined with CBP has significant therapeutic effects. In clinical studies, it was found that ulinastatin can stabilize the cell membrane and inhibit the release of inflammatory mediators, thus suppressing various hydrolytic enzyme activities and protecting vital organs, which has been widely used in the treatment of systemic inflammatory response syndrome [15]. The results of our study showed that ulinastatin combined with CBP can effectively improve survival rate of children. Relevant studies have shown that CBP can improve tissue oxygenation and cardiopulmonary function, maintain a stable internal environment, restore monocyte function, accelerate the recovery of endothelial cell function, and reduce abnormal apoptosis of lymphocytes, which can greatly improve the success rate of rescue in patients with sepsis and is consistent with the results in this study [21].

However, there are certain limitations to this study. The sample size is small and the time of observation is short. Therefore, further study with larger sample size and observation over an extended period of time are needed to verify our experimental conclusion.

In conclusion, ulinastatin combined with CBP has a significant therapeutic effect on sepsis in children. It can reduce the inflammatory response, effectively decrease sTREM-1 and PCT levels, improve immune function, and protect cardiac function in patients, which is feasible in clinical practice.

Disclosure of conflict of interest
None.

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References
[1] Sankar J, Dhochak N, Kumar K, Singh M, Sankar MJ and Lodha R. Comparison of international pediatric sepsis consensus conference

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**Table 4. Comparison of treatment effective rate between the two groups (n (%))**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Markedly effective</th>
<th>Effective</th>
<th>Ineffective</th>
<th>Total effective rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBP group</td>
<td>50</td>
<td>19 (38.00)</td>
<td>15 (30.00)</td>
<td>16 (32.00)</td>
<td>34 (68.00)</td>
</tr>
<tr>
<td>CBP + ulinastatin group</td>
<td>50</td>
<td>32 (64.00)</td>
<td>14 (28.00)</td>
<td>4 (8.00)</td>
<td>46 (92.00)</td>
</tr>
</tbody>
</table>

χ²: 9.000  P: 0.003

**Table 5. Comparison of incidence of adverse reactions in the two groups (n (%))**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Diarrhea</th>
<th>Vomiting</th>
<th>Rash</th>
<th>Incidence of adverse reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBP group</td>
<td>50</td>
<td>4 (8.00)</td>
<td>2 (4.00)</td>
<td>3 (6.00)</td>
<td>9 (18.00)</td>
</tr>
<tr>
<td>CBP + ulinastatin group</td>
<td>50</td>
<td>2 (4.00)</td>
<td>3 (6.00)</td>
<td>2 (4.00)</td>
<td>7 (14.00)</td>
</tr>
</tbody>
</table>

χ²: 0.709  P: 0.340

χ²: 0.211  Ρ: 0.646

χ²: 0.211  P: 0.646

χ²: 0.298  P: 0.585

Note: CBP: Continuous blood purification.


