Effect of glutamine nutrition support on the intestinal mucosal barrier function and inflammatory response in patients with severe acute pancreatitis

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ABSTRACT

Objective: To study the effect of glutamine nutrition support on the intestinal mucosal barrier function and inflammatory response in patients with severe acute pancreatitis. Methods: Patients with severe acute pancreatitis who were treated in Pengzhou People’s Hospital between May 2014 and November 2016 were selected as the research subjects and randomly divided into two groups, control group received conventional symptomatic treatment and conventional enteral nutrition intervention, and Gln group received conventional symptomatic treatment and glutamine enteral nutrition intervention. The contents of intestinal mucosal barrier damage markers and inflammatory mediators in serum as well as the expression of inflammatory signaling molecules in peripheral blood were detected before and after treatment; the number of intestinal flora was detected after treatment. Results: After treatment, LPS, DAO, HBD2, TNF-α, sTREM-1, IL-1β and IL-6 levels in serum as well as TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of both groups of patients were significantly lower than those before treatment, LPS, DAO, HBD2, TNF-α, sTREM-1, IL-1β and IL-6 levels in serum as well as TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of Gln group after treatment were significantly lower than those of control group, and the number of lactobacillus, bifidobacterium and bacteroides were significantly higher than those of control group while the number of escherichia coli and enterococcus were significantly lower than those of control group. Conclusion: Glutamine nutrition support for severe acute pancreatitis can reduce the intestinal mucosal barrier function injury and inhibit the inflammatory response activation.

1. Introduction

Acute pancreatitis is the clinical common acute abdominal pain, it can be divided into mild acute pancreatitis and severe acute pancreatitis according to the condition, the former is mild and can obtain exact curative effect after symptomatic treatment, the latter is severe and associated with systemic inflammatory response activation, intestinal flora translocation and other changes, quite a number of patients will develop multiple organ dysfunction syndrome (MODS), and the treatment is relatively difficult[1]. Activation of the inflammatory response in the patients with acute severe pancreatitis is closely related to the intestinal mucosal barrier function injury and intestinal flora translocation to blood circulation, and the enteral nutrition intervention on the basis of conventional symptomatic treatment can effectively protect the intestinal mucosa and inhibit intestinal pathogenic flora overgrowth[3]. Glutamine is an important free amino acid in the body that is involved in the maintenance of mucosal barrier function and the regulation of immune response, and the course of severe acute pancreatitis will massively deplete glutamine and cause the relative insufficiency of glutamine in the body, so supplementing exogenous glutamine is necessary[4]. In the following study, the effect of glutamine nutrition support on the intestinal mucosal barrier function and inflammatory response in patients with severe acute pancreatitis was analyzed.
2. Research subjects and research methods

2.1 Research subjects

Patients with severe acute pancreatitis who were treated in Pengzhou People’s Hospital between May 2014 and November 2016 were selected, all the patients were in accordance with the diagnostic criteria for acute severe pancreatitis, serum amylase content at admission was more than 3 times of normal value and abdominal CT indicated that there was acute pancreatitis accompanied by peripancreatic extensive effusion or necrosis. A total of 68 cases were enrolled and divided into Gln group and control group according to the random number table method, 34 cases in each group. Gln group included 22 male cases and 12 female cases, they were 34-49 years old, and the APACHE-II score was (11.98±1.42) points; control group included 21 male cases and 13 female cases, they were 32-50 years old, and the APACHE-II score was (12.08±1.36) points. There were no statistically significant differences in the general information between the two groups (P>0.05).

2.2 Pancreatitis therapy

After admission, both groups of patients received intravenous drip of ulinastatin 200 000 U in 500 mL of 5% glucose injection, 2 times/d, continuous micro pump injection of 0.25 mg/h somatostatin, intravenous drip of ceftazidime 2 g in 100 mL saline, 1 time/12 h as well as intravenous drip of Ertapenem 1 g in 100 mL saline, 1 time/d. On the basis of the conventional symptomatic treatment, the control group received conventional enteral nutrition intervention, and the method was as follows: after test-meal by warm water confirmed that there was no adverse reaction, elemental type of enteral nutrition was provided, 125 mL/time and 1 time/d, on day 1, 125 mL/time and 3 times/d on day 2, 250 mL/time and 3 times/d on day 3, 250 mL/time and 4 times/d from day 4, and adding powder 25 g per 125 mL. Based on conventional symptomatic treatment and enteral nutrition support, glutamine granules 2.5 g was added per 125 mL of nutrient solution for Gln group.

2.3 Serum index detection

3 mL of cubital venous blood was collected from two groups of patients before treatment and 5 days after treatment, and centrifuged to get serum, and the LPS, DAO, HBD2, TNF-α, sTREM-1, IL-1β and IL-6 levels were measured by enzyme-linked immunosorbent assay kits.

2.4 Intestinal flora number detection

Right amount of faeces was collected from both groups of patients 5 d after treatment, genomic DNA extraction kit was used to separate the genomic DNA in the faeces, fluorescence quantitative PCR kits as well as lactobacillus, bifidobacterium, bacteroides, escherichia coli and enterococcus primers were used for testing, and PCR reaction curve was referred to calculate the DNA copy number of the above intestinal flora.

2.5 Peripheral blood signaling molecule detection

Before treatment and 5 d after treatment, 3 mL of cubital venous blood was collected from two groups of patients, added in Ficoll separating medium and centrifuged, the peripheral blood mononuclear cells suspended in the middle were absorbed, the total RNA in cells was extracted for fluorescence quantitative PCR amplification, the TLR4, NF-kB, MyD88 and p38MAPK were amplified respectively, and the amplification curve was referred to calculate the mRNA expression of these molecules.

2.6 Statistical methods

SPSS 21.0 software was used to input the test data, the data between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Intestinal mucosal barrier function of two groups of patients

Before treatment and 5 days after treatment, analysis of serum intestinal mucosal barrier injury marker molecules LPS (EU/mL), DAO (μg/mL) and HBD2 (ng/mL) levels between two groups of patients was as follows: before treatment, serum LPS, DAO and HBD2 levels were not significantly different between two groups of patients (P>0.05); after treatment, serum LPS, DAO and HBD2 levels of both groups of patients were significantly lower than those before treatment (P<0.05), and serum LPS, DAO and HBD2 levels of Gln group were significantly lower than those of control group (P<0.05).

5 d after treatment, analysis of intestinal flora lactobacillus,
bifidobacterium, bacteroides, escherichia coli and enterococcus number between two groups of patients was as follows: the number of lactobacillus, bifidobacterium and bacteroides of Gln group were significantly higher than those of control group while the number of escherichia coli and enterococcus were significantly lower than those of control group. Differences in intestinal flora lactobacillus, bifidobacterium, bacteroides, escherichia coli and enterococcus number were statistically significant between two groups of patients 5 d after treatment ($P<0.05$).

### 3.2 Peripheral blood inflammatory signaling molecule expression in two groups of patients

Before treatment and 5 d after treatment, analysis of inflammatory signaling molecules TLR4, NF-$\kappa$B, MyD88 and p38MAPK expression in PBMCs between two groups of patients was as follows: before treatment, TLR4, NF-$\kappa$B, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells were not significantly different between two groups of patients ($P>0.05$); after treatment, TLR4, NF-$\kappa$B, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of both groups of patients were significantly lower than those before treatment ($P<0.05$), and TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of Gln group were significantly lower than those of control group ($P<0.05$).

### Table 1.

Serum LPS, DAO and HBD2 levels in two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>LPS</th>
<th>DAO</th>
<th>HBD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln group</td>
<td>34</td>
<td>Before</td>
<td>7.3±0.93</td>
<td>6.2±0.77</td>
<td>34.6±5.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>3.3±0.42</td>
<td>3.1±0.36</td>
<td>17.0±2.41</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>Before</td>
<td>7.2±0.87</td>
<td>6.3±0.81</td>
<td>33.6±4.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>4.5±0.56</td>
<td>4.2±0.52</td>
<td>23.4±3.45</td>
</tr>
</tbody>
</table>

1: comparison between Gln group and control group, $P<0.05$; 2: comparison between before and after treatment, $P<0.05$.

### Table 2.

Comparison of intestinal flora number between two groups of patients after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
<th>Bacteroides</th>
<th>Escherichia coli</th>
<th>Enterococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln group</td>
<td>34</td>
<td>6.5±0.67</td>
<td>4.0±0.51</td>
<td>0.7±0.09</td>
<td>1.7±0.22</td>
<td>0.9±0.11</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>4.3±0.58</td>
<td>3.4±0.46</td>
<td>0.5±0.07</td>
<td>2.9±0.35</td>
<td>1.6±0.24</td>
</tr>
<tr>
<td>$T$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 3.

Peripheral blood inflammatory signaling molecule expression in two groups of patients and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TLR4</th>
<th>NF-kB</th>
<th>MyD88</th>
<th>p38MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln group</td>
<td>34</td>
<td>Before</td>
<td>1.0±0.13</td>
<td>1.0±0.14</td>
<td>0.9±0.12</td>
<td>1.0±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>0.4±0.08</td>
<td>0.3±0.06</td>
<td>0.4±0.06</td>
<td>0.3±0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>Before</td>
<td>1.0±0.12</td>
<td>1.0±0.17</td>
<td>1.0±0.16</td>
<td>1.0±0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>0.7±0.09</td>
<td>0.6±0.08</td>
<td>0.6±0.08</td>
<td>0.6±0.09</td>
</tr>
</tbody>
</table>

1: comparison between Gln group and control group, $P<0.05$; 2: comparison between before and after treatment, $P<0.05$.

### Table 4.

Serum TNF-α, sTREM-1, IL-1β and IL-6 levels before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TNF-α</th>
<th>sTREM-1</th>
<th>IL-1β</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln group</td>
<td>34</td>
<td>Before</td>
<td>17.5±2.34</td>
<td>0.6±0.09</td>
<td>22.3±3.56</td>
<td>39.1±5.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>6.7±3.94</td>
<td>0.33±0.07</td>
<td>10.5±2.35</td>
<td>23.4±3.46</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>Before</td>
<td>17.1±2.62</td>
<td>0.6±0.09</td>
<td>22.4±2.93</td>
<td>39.8±5.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>9.5±1.25</td>
<td>0.49±0.07</td>
<td>14.7±1.75</td>
<td>35.3±4.67</td>
</tr>
</tbody>
</table>

1: comparison between Gln group and control group, $P<0.05$; 2: comparison between before and after treatment, $P<0.05$. 

In peripheral blood mononuclear cells of both groups of patients were significantly lower than those before treatment ($P<0.05$), and TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of Gln group were significantly lower than those of control group ($P<0.05$).
4. Discussion

Severe acute pancreatitis is critically ill and has more complications. Trypsin autodigestion of local tissue can cause acute chemical inflammation, the damage of continuous fasting, gastrointestinal decompression and other factors to the intestinal mucosal barrier function will further cause intestinal flora translocation and promote endotoxin release into the blood, and the systemic inflammatory response syndrome easily occur in severe acute pancreatitis patients and cause several viscera function damage, which greatly increases the occurrence risk of MODS[5]. In recent years, the concept of fasting and total parenteral nutrition therapy for severe acute pancreatitis has been gradually abandoned, and early enteral nutrition support is considered to be able to effectively protect the intestinal mucosal barrier function, reduce the flora translocation and suppress the activation of systemic inflammatory response[6]. Glutamine is one of necessary amino acids in the body, which can be involved in intestinal mucosal epithelial cell proliferation and function maintenance as energy supply material, and can also participate in immune cell differentiation and maturation as well as immune response regulation[7]. The hypermetabolism in patients with severe acute pancreatitis can massively consume glutamine and cause glutamine insufficiency in the body, which needs to be supplemented in time by means of exogenous intake. Study has shown that the glutamine therapy on the basis of conventional enteral nutrition support can improve the condition of severe acute pancreatitis[8], but there is no clear report about the effects of glutamine on intestinal mucosal barrier function.

Intestinal tract is where the bacteria gather, lactobacillus, bifidobacterium, bacteroides and other probiotics are dominant under physiological conditions, and the biological barrier formed by dominant bacterial community and the mechanical barrier formed by intestinal mucosal epithelial cells are both involved in the composition of intestinal mucosal barrier and can make escherichia coli, enterococcus and other pathogenic flora inhibited[9,10]. When the intestinal mucosal barrier function is damaged, pathogenic flora multiply and transfer into blood circulation, which on the one hand, will synthesize and release LPS[11], and on the other hand, can also cause the release of DAO, HBD2 and so on from intestinal mucosal epithelial cells into the blood circulation [12,13]. In the study, analysis of the intestinal mucosal barrier injury marker molecule contents before and after treatment showed that serum LPS, DAO and HBD2 levels of both groups of patients significantly decreased after treatment, and serum LPS, DAO and HBD2 levels of Gln group after treatment were significantly lower than those of control group. This means that both conventional enteral nutrition intervention and glutamine enteral nutrition intervention can effectively protect the intestinal mucosa, and glutamine enteral nutrition is superior to the conventional enteral nutrition intervention in protecting the intestinal mucosal barrier. Further analysis of the number of intestinal flora showed that the number of lactobacillus, bifidobacterium and bacteroides of Gln group were significantly higher than those of control group while the number of escherichia coli and enterococcus were significantly lower than those of control group. It means that both conventional enteral nutrition intervention and glutamine enteral nutrition intervention can effectively regulate the intestinal flora, glutamine enteral nutrition is superior to the conventional enteral nutrition intervention in regulating intestinal flora, and it can more effectively inhibit the pathogenic flora reproduction and promote the probiotics reproduction.

Activation of systemic inflammatory response is an important pathological feature of severe acute pancreatitis, and also an important pathological link that causes the function injury of multiple organs in the whole body. The inflammatory response of the body in pathological conditions is regulated by multiple signaling pathways, and the TLR4/MyD88/NF-kB and p38MAPK pathways are closely related to the regulation of inflammatory response in the course of pancreatitis. TLR4 is a kind of pattern recognition receptor, which can recognize various pathogen pattern molecules, including the intestinal flora translocated into the blood circulation[14]; after combined with the pathogenic molecules, TLR4 can transmit the biological signal through the adaptor molecule MyD88, activate the NF-kB and then make it transferred into the nucleus to activate the expression of various inflammatory mediators[15]. p38MAPK is a member of the MAPKs family, which is activated by the action of various proinflammatory factors and mediates the cascade amplification of inflammatory reaction[16,17]. In the research, the analysis of peripheral blood inflammatory signaling molecule expression before and after the treatment showed that TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of both groups of patients significantly decreased after treatment, and TLR4, NF-kB, MyD88 and p38MAPK mRNA expression in peripheral blood mononuclear cells of Gln group after treatment were significantly lower than those of control group. This means that both conventional enteral nutrition intervention and glutamine enteral nutrition intervention can effectively restrain the signaling pathway activation in the course of severe acute pancreatitis inflammation, and glutamine enteral nutrition is superior to the conventional enteral nutrition intervention in inhibiting the activation of inflammatory signaling pathways. The activation of inflammatory signaling pathways in patients with pancreatitis can
cause the massive secretion of TNF-α, sTREM-1, IL-1β, IL-6 and other inflammatory mediators,[18,19] and further analysis of the changes in serum levels of these inflammatory mediators before and after treatment showed that serum TNF-α, sTREM-1, IL-1β and IL-6 levels of both groups of patients significantly decreased after treatment, and serum TNF-α, sTREM-1, IL-1β and IL-6 levels of Gln group after treatment were significantly lower than those of control group. This further confirms that glutamine enteral nutrition is superior to the conventional enteral nutrition intervention in inhibiting the inflammatory response in the course of severe acute pancreatitis.

Based on above discussion and analysis, it can be preliminarily concluded in the study that glutamine enteral nutrition support for severe acute pancreatitis is more effective than conventional enteral nutrition support in reducing the intestinal mucosal barrier function injury and inhibiting the activation of inflammatory response.

References