Effects of early enteral nutrition + alanyl-glutamine on intestinal flora, inflammation and oxidative response in patients with SAP

Hai-Long Zhu, Jun Wang, Yi Zhang, Yi Liang, Juan Wu
Department of Critical Care Medicine, the Second People’s Hospital of Deyang City Sichuan Province, Deyang, Sichuan Province, 618000

ARTICLE INFO

Objective: To investigate the effects of early enteral nutrition + alanyl-glutamine on intestinal flora, inflammation and oxidative response in patients with severe acute pancreatitis (SAP).

Methods: A total of 90 patients with SAP who were treated in the hospital between February 2013 and July 2017 were divided into normal EEN group and enhanced EEN group according to different nutritional modes. Normal EEN group received early EEN intervention, and enhanced EEN group received alanyl-glutamine intervention based on early EEN. The differences in the flora distribution in intestinal excreta as well as the contents of inflammatory factors and oxidative stress indexes in serum were compared between the two groups.

Results: Before intervention, the differences in the flora distribution in intestinal excreta as well as the contents of inflammatory factors and oxidative stress indexes in serum were not statistically significant between the two groups of patients. After intervention, the bifidobacterium and lactobacillus count in intestinal excreta of enhanced EEN group were higher than those of normal EEN group whereas the enterococcus and escherichia coli count were lower than those of normal EEN group; serum inflammatory cytokines IL-1β, IL-17 and IL-23 contents were lower than those of normal EEN group; serum oxidative stress indexes MPO and XO contents were lower than those of normal EEN group while SOD and CAT contents were higher than those of normal EEN group.

Conclusion: EEN combined with alanyl-glutamine therapy can effectively balance the intestinal flora and inhibit the systemic inflammatory response and oxidative stress response in patients with SAP.

1. Introduction

Severe acute pancreatitis (SAP) is a serious condition caused by poor control of acute pancreatitis, and the autodigestion of trypsin plays a central role in the development of disease [1,2]. SAP is dangerous and with many complications, its incidence is about 10%-20% of all acute pancreatitis, and the early mortality rate is as high as 10%-30%. Nutrition support is an important part of the SAP treatment, and the current studies have pointed out that early enteral nutrition (EEN) can promote the intestinal function recovery and enhance the body’s immunity, and is advantageous to optimizing the patients’ treatment outcome [3,4]. Conventional EEN mainly provides the necessary heat for patients, supplies carbohydrate, protein and fat according to a certain proportion and avoids the severe malnutrition under consumption state, but some scholars suggest increasing the contents of some specific amino acids in order to protect intestinal function and promote disease rehabilitation. Alanyl-glutamine can be decomposed into alanine and glutamine in the body, and glutamine belongs to the nonessential amino acid of human body, belongs to the essential amino acid under stress and participates in multiple processes such as maintaining intestinal function under physiological state [5-7]. In this study, alanyl-glutamine combined with early EEN was used to for the nutritional intervention of patients with SAP, and its effect on the disease recovery was explored in order to provide a reference for the selection of follow-up nutrition intervention methods.

2. Information and methods

2.1 Case information

A total of 90 patients with SAP who were treated in the hospital between February 2013 and July 2017 were divided into normal EEN group (n=45) and enhanced EEN group (n=45) according to different nutritional modes. There were 24 males and 21 females in...
the normal EEN group, and they were 37-71 years old; there were 23
males and 22 females in the enhanced EEN group, and they were 40-72
years old. There was no statistically significant difference in the
gender and age distribution between the two groups (\(P>0.05\)), and
the follow-up study was approved by the hospital ethics committee.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) conforming to the diagnostic criteria for
SAP; (2) with clear history of overeating and drinking; (3) without
history of SAP; (4) without history of gastrointestinal surgery; (5)
with the family’s informed consent. Exclusion criteria: (1) combined
with gastrointestinal malignant tumor disease; (2) combined with
serious infectious diseases of other tissue organs; (3) with severe
malnutrition before admission.

2.3 Intervention

Both groups accepted oxygen uptake, anti-infection, pancreatic
digestive enzyme secretion inhibition, gastrointestinal decompression
and other routine therapies. Normal EEN group received normal
EEN intervention, specifically as follows: inserting nasal jejunal
feeding tube under endoscopic monitoring, providing enteral nutrient
solution, supplying rice soup at first and then gradually changing to
Intact Protein formula, which reached 30 kcal/(kg-d) within 72 h.
Enhanced EEN group received routine EEN and adjuvant alanyl-
glutamine injection intervention, which was as follows: alanyl-
glutamine, by intravenous drip, 0.4 g/kg, 1 time/d for consecutive 7
d.

2.4 Observation indexes

Before and after intervention, two groups of patients were given
enema, and the intestinal excreta was collected for bacterial culture,
viable plate count was used to calculate the bacteria count of
bifidobacterium, lactobacillus, enterococcus and e. coli. Cubital
venous blood serum was collected from two groups of patients
over the same period and centrifuged to separate the upper serum,
ELISA was used to detect the levels of inflammatory factors and
oxidative stress indexes, inflammatory factors included interleukin-
1 \( \beta \) (IL-1 \( \beta \)), interleukin-17 (IL-17) and interleukin-23 (IL-23), and
oxidative stress indexes included myeloperoxidase (MPO), xanthine
oxidase (XO), superoxide dismutase (SOD) and catalase (CAT).

2.5 Statistical processing

Intestinal flora count, inflammatory factor levels and oxidative
stress index levels were input in SPSS 26.0, \( t \) test was used to calculate the \( P \) value and \( P<0.05 \) indicated that the differences were
significant statistically.

3. Results

3.1 Intestinal flora count

Comparison of bifidobacterium, lactobacillus, enterococcus
and escherichia coli count in intestinal excreta between the two
groups was as follows: before intervention, the differences in
bifidobacterium, lactobacillus, enterococcus and escherichia coli
count in intestinal excreta were not statistically significant
between the two groups of patients (\(P>0.05\)). After intervention,
the bifidobacterium and lactobacillus count in intestinal excreta of
both groups were higher than those before intervention whereas
the enterococcus and escherichia coli count were lower than those
before intervention; the bifidobacterium and lactobacillus count in
intestinal excreta of enhanced EEN group were higher than those of
normal EEN group whereas the enterococcus and escherichia coli
count were lower than those of normal EEN group (\(P<0.05\), shown
in Table 1.

3.2 Inflammatory factor levels

Comparison of serum inflammatory factors IL-1 \( \beta \), IL-17 and IL-
23 levels between the two groups was as follows: before intervention,
the differences in serum IL-1 \( \beta \), IL-17 and IL-23 levels were not
statistically significant between the two groups of patients (\(P>
0.05\)). After intervention, serum IL-1 \( \beta \), IL-17 and IL-23 contents
of both groups were lower than those before intervention; serum IL-
1 \( \beta \), IL-17 and IL-23 contents of enhanced EEN group were lower
than those of normal EEN group (\(P<0.05\), shown in Table 2.

3.3 Oxidative stress index levels

Comparison of serum oxidative stress indexes MPO, XO, SOD
and CAT levels between the two groups was as follows: before
intervention, the differences in serum MPO, XO, SOD and CAT
levels were not statistically significant between the two groups of
patients (\(P>0.05\)). After intervention, serum MPO and XO contents
of both groups were lower than those before intervention while
SOD and CAT contents were higher than those before intervention;

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Before intervention</th>
<th>After intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal EEN group</td>
<td>45</td>
<td>3.4±0.37</td>
<td>4.3±0.48</td>
<td>4.5±0.49</td>
<td>5.2±0.58</td>
<td>7.1±0.76</td>
<td>6.0±0.64</td>
<td>10.2±1.63</td>
<td>8.1±0.89</td>
</tr>
<tr>
<td>Enhanced EEN group</td>
<td>45</td>
<td>3.3±0.35</td>
<td>5.9±0.64</td>
<td>4.5±0.48</td>
<td>6.9±0.74</td>
<td>7.0±0.73</td>
<td>5.1±0.52</td>
<td>10.3±1.74</td>
<td>5.0±0.53</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>0.39</td>
<td>7.93</td>
<td>0.273</td>
<td>8.36</td>
<td>0.049</td>
<td>9.103</td>
<td>0.062</td>
<td>15.275</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1.

Comparison of flora count in intestinal excreta before and after treatment (ln/g).

Note: compared with same group before intervention, \( P<0.05 \).
serum MPO and XO contents of enhanced EEN group were lower than those of normal EEN group while SOD and CAT contents were higher than those of normal EEN group ($P<0.05$), shown in Table 3.

### 4. Discussion

The SAP involves multiple organs, which leads to the increase of protein decomposition and fat mobilization, the aggravation of gluconeogenesis and so on, and ultimately results in hypoalbuninemia, malnutrition, intestinal mucosal atrophy and intestinal mucosal barrier dysfunction. Glutamine is a non-essential amino acid in the physiological state, which can supply energy for intestinal mucosa cells, promote their proliferation and reduce the intestinal flora/toxin translocation. When SAP occurs, the intestinal mucosal epithelial cells utilize more glutamine, which causes the lack of endogenous glutamine, aggravates gut-derived infection and is one of the important causes of sustained aggravation of SAP patients[10,11]. In this study, alanyl-glutamine was added in the early EEN, and its optimizing effect on the patient's condition was discussed from three aspects: intestinal flora, inflammation and oxidative reaction.

Glutamine is the main energy source of the small intestine and also helps maintain the intestinal immune function. In the condition of severe stress and catabolism, the utilization of glutamine increases and the endogenous glutamine is relatively insufficient, which decreases intestinal cell proliferation rate and inhibits intestinal cell differentiation and mucosal cell renewal, and then results in intestinal flora translocation and immune dysfunction. The intestinal bacterial translocation, the inhibition of dominant bacterial community growth and the massive reproduction of conditioned pathogen are the important reasons for the occurrence of SAP, and the specific length of intestinal translocation is consistent with the patient’s condition. Bifidobacterium and lactobacillus are dominant in human body in physiological status, which can promote the decomposition and function of intestinal contents, and promote the normal operation of intestinal function; enterococcus and escherichia coli belong to the conditioned pathogens, and their reproductive rate is in a dynamic equilibrium with that of bifidobacterium and lactobacillus in the non-disease state[12,13]. In the case of SAP, the conditioned pathogen massively multiplies while dominant bacterial community is inhibited, which leads to the occurrence of intestinal infection and the aggravation of SAP. In this study, the counting of above four kinds of bacteria in intestinal excreta of patients with SAP was detected, and it was found that compared with those before intervention, the dominant bacterial community bifidobacterium and lactobacillus count in intestinal excreta of both groups increased while the conditioned pathogens enterococcus and escherichia coli count decreased after intervention; compared with those of normal EEN group, the bifidobacterium and lactobacillus count in intestinal excreta of enhanced EEN group were higher whereas the enterococcus and escherichia coli count were lower, it confirms that alanyl-glutamine combined with early nutritional intervention can further equalize the intestinal flora distribution in patients with SAP and this is the visual symbol that the patients’ condition is optimized. Severe systemic inflammatory response is a sign of SAP, and also the core cause of the important tissue organ damage, a variety of inflammatory factors are involved in it, and their contents can objectively reflect the SAP illness and outcome. IL-1$\beta$ is a typical pro-inflammatory factor, which is produced early after trypsin digests the pancreas, is released into the blood and participates in subsequent inflammatory cascades[14,15]. IL-17 is secreted by Th17 cells, which can induce epithelial cells and endothelial cells to synthesize and secrete IL-6, IL-8 and other pro-inflammatory factors, and amplify inflammatory response[13]. IL-23 is secreted by activated DC cells, it is an important member of the IL-12 family, its secretion increases rapidly after the occurrence of infection, inflammation and so on, it can adjust the Th1 cell function and participate in the immune response and inflammatory response, and it is found that IL-23 content significantly increases in patients with acute pancreatitis[16]. The results of this study showed that compared with those before intervention, serum inflammatory factors IL-1$\beta$, IL-17 and IL-23 contents of both groups decreased after intervention;
are eventually in the lower expression state of SOD and CAT is limited and their consumption increases, so they stress reaction amplification, but when SAP persists, the synthesis of oxygen free radicals and oxidative metabolites and avoid oxidative increase reactively in order to neutralize the massively generated early stage of oxidative stress response, antioxidants SOD and CAT increase of systemic inflammatory response in patients typical oxidative metabolites, and their synthesis increases with the disease, and the specific manifestations are the increased synthesis with SAP, the oxidative stress is throughout the whole course of the intervention, it confirms that the early EEN + alanyl-glutamine IL-17 and IL-23 contents of enhanced EEN group were lower after further compared with those of normal EEN group, serum IL-1β , IL-17 and IL-23 contents of enhanced EEN group were lower after the intervention, it confirms that the early EEN + alanyl-glutamine can effectively inhibit the systemic inflammatory response in patients with SAP, and this is directly related to the effects of glutamine on optimizing intestinal function and reducing gut-derived infection.

Corresponding to the systemic infectious symptoms in patients with SAP, the oxidative stress is throughout the whole course of disease, and the specific manifestations are the increased synthesis of oxygen free radicals, the accumulation of oxidative metabolites and excessive consumption of antioxidant factors. MPO and XO are typical oxidative metabolites, and their synthesis increases with the increase of systemic inflammatory response in patients[17,18]. In the early stage of oxidative stress response, antioxidants SOD and CAT reactively in order to neutralize the massively generated oxygen free radicals and oxidative metabolites and avoid oxidative stress reaction amplification, but when SAP persists, the synthesis of SOD and CAT is limited and their consumption increases, so they are eventually in the lower expression state[19,20]. The study results showed that compared with those before intervention, serum MPO and XO contents of both groups decreased while SOD and CAT contents increased after intervention; further compared with those of EEN group, serum MPO and XO contents of enhanced EEN group were lower while SOD and CAT contents were higher, confirming that the alanyl-glutamine based on early EEN can effectively inhibit the systemic oxidative stress response in patients with SAP.

Early EEN combined with alanyl-glutamine intervention can effectively equalize the intestinal flora and inhibit the systemic inflammation and oxidative stress in patients with SAP, it helps to improve SAP condition, and it is worth popularization and application in clinical practice in the future.

References


