Effect of ecological immune enteral nutrition intervention on intestinal barrier function and systemic inflammatory response in rat models with severe pancreatitis

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ARTICLE INFO ABSTRACT

Objective: To study the effect of ecological immune enteral nutrition intervention on intestinal barrier function and systemic inflammatory response in rat models with severe pancreatitis. 

Methods: SD male rats were taken and divided into control group, pancreatitis group and intervention group, and after severe pancreatitis models were built, intervention group received ecological immune enteral nutrition intervention. Serum endotoxin contents, intestinal mucosal mechanical barrier and biological barrier function, pancreas and liver injury degree and inflammation degree of three groups were detected. Results: (1) intestinal barrier function: serum endotoxin content and the number of intestinal Escherichia coli of pancreatitis group were higher than those of control group, and intestinal mucosal thickness, villus height and the number of intestinal Bifidobacterium and Lactobacillus were lower than those of control group; serum endotoxin content and the number of intestinal Escherichia coli of intervention group were lower than those of pancreatitis group, and intestinal mucosal thickness, villus height and the number of intestinal Bifidobacterium and Lactobacillus were higher than those of pancreatitis group; (2) systemic inflammation: serum amylase, aspartate aminotransferase, TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents of pancreatitis group were higher than those of control group, and serum amylase, aspartate aminotransferase, TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents of intervention group were lower than those of pancreatitis group.

Conclusion: Ecological immune enteral nutrition intervention can improve the intestinal barrier function, alleviate systemic inflammation degree and reduce pancreas and liver injury degree in rats with severe pancreatitis.

1. Introduction

Severe acute pancreatitis (SAP) is a more critical acute abdomen of general surgery, disease progress is rapid, there are more complications, and severe cases may develop into multiple organ failure on the basis of systemic inflammatory response[1]. In the pathological process of severe acute pancreatitis, intestinal mucosal barrier function injury is closely related to the occurrence of systemic inflammatory response and multiple-organ dysfunction. Intestinal tract is the storage site of endotoxin and bacteria, in cases of mucosal barrier function injury, endotoxin is largely released into blood, and flora ectopia reaches other visceral organs of the body, thus causing systemic inflammation activation and multiple organ function injury[2]. Enteral nutrition support is an important component of SAP integrated treatment program, and its goal is to improve intestinal mucosal barrier function and inhibit the pathological process of pancreatitis through enteral nutrition intervention. Ecological immune enteral nutrition is a newly developed method of enteral nutrition in recent years that is helpful to regulate intestinal immune barrier function and flora balance[3]. In the following research, the effect of ecological immune enteral nutrition intervention on intestinal barrier function and systemic inflammatory response in rat models with severe pancreatitis was analyzed.
2. Subjects and methods

2.1. Experimental materials

SD male rats were provided by the university animal center, body weight 200–260 g; ELISA was from Bio-Rad Company, automatic biochemical analyzer was from HITACHI Company and PCR apparatus was from Biometra Company; Fresubin and supportan were from Sino-Swed Pharmaceutical Corp. Ltd., and triple viable preparation (bifico) was from Shanghai Pharmaceutical Group Co., Ltd.

2.2. Experimental methods

2.2.1. Pancreatitis model building methods

Pancreatitis group and intervention group built pancreatitis models according to the following methods: performing intraperitoneal anesthesia after 12 h of fasting, making median abdominal incision, entering the abdominal cavity, looking for duodenum, isolating pancreatobiliary duct and clipping it with artery clip; retrograde inserting polyethylene catheter through vater’s papilla, depth about 0.5 cm, injecting 5% sodium taurocholate with micro-pump through catheter, dosage 1.5 mL/kg and speed 10 mL/h; observing pancreatic morphology, removing catheter and artery clip when pancreas showed edema and hyperemia visible to the naked eye, and suturing local duodenum incision. In addition, silicone tube with inner diameter of 1.0 mm was inserted via gastrostomy, placed on superior segmental jejunum, fixed in the stomach and then lead out via subcutaneous tunnel, and opening was in neck skin.

2.2.2. Enteral nutrition methods

Pancreatitis group received infusion of normal saline by jejunum tube, and intervention group configured nutrient solution of Fresubin, supportan and triple viable preparation by jejunum tube, the solution was infused by jejunum tube, initial concentration was 50%, 2.5 mL warm mixed preparation was infused, then volume was gradually increased to 50 mL in 24 h, and then 5 mL was infused each time, 1 time every 2 h and total 8 times.

2.2.3. Blood index detecting methods

12, 24, 48 and 72 h after treatment, the way of orbital blood was used to get serum specimens, immunoturbidimetry was used to detect amylase and AST contents, and enzyme-linked immunosorbent assay was used to detect TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents.

2.2.4. Mechanical barrier function detecting methods

Small intestinal segments 10cm from pylorus with length of about 2.0 cm were taken, fixed with formaldehyde and made into paraffin sections that were observed under microscope after HE staining, and the villus height of 25 small intestinal villi and corresponding small intestinal mucosal thicknesses were detected.

2.2.5. Biological barrier function detecting methods

Specific PCR products of Bifidobacterium, Lactobacillus and Escherichia coli genome DNA were designed, stool specimens were taken, genome DNA extraction kits were used to extract DNA, and PCR amplification was used to analyze the copy number of different floras.

2.2.6. Statistical methods

SPSS22.0 software was used to input data, comparison of data among three groups and comparison of data at various points in time within group were by variance analysis, and differences were considered to be statistically significant at a level of P<0.05.

3. Results

3.1. Serum endotoxin contents

12, 24, 48 and 72 h after treatment, serum endotoxin contents of pancreatitis group and intervention group showed increasing trend, and specific trend was shown in Figure 1. Overall increasing trend of serum endotoxin of intervention group was weaker than that of pancreatitis group; at various points in time, serum endotoxin contents of pancreatitis group were higher than those of control group, and serum endotoxin contents of intervention group were lower than those of pancreatitis group.

![Figure 1. Dynamic trend of serum endotoxin contents of three groups.](image)

3.2. Intestinal mucosal mechanical barrier and biological barrier function

Analysis of intestinal mucosal mechanical barrier of three groups was as follows: intestinal mucosal thickness and villus height of pancreatitis group were lower than those of control group, and intestinal mucosal thickness and villus height of intervention group were higher than those of pancreatitis group; analysis of intestinal mucosal biological barrier was as follows: the number of intestinal Escherichia coli of pancreatitis group was significantly more than that of control group, and the number of Bifidobacterium and Lactobacillus was significantly less than that of control group; the number of intestinal Escherichia coli of intervention group was significantly less than that of pancreatitis group, and the number of Bifidobacterium and Lactobacillus was significantly more than that of pancreatitis group (Table 1).

![Figure 1. Dynamic trend of serum endotoxin contents of three groups.](image)
Comparison of serum inflammatory mediator contents of three groups.

Table 2 was significantly more than that of control group, and the number of intestinal mucosal thickness and villus height were lower than those of control group. The content of pancreatitis group was higher than that of control group, and the function in pancreatitis model rats showed that serum endotoxin content and intestinal barrier function injury is related to the development of SAP and the occurrence of SIRS and MODS[5]. Intestinal tract is not only an important nutrient digestion and absorption site, but also the largest repository of bacteria and endotoxin in the body[6]. Intestinal flora and endotoxin ectopia is the initial factor of multiple organ failure. Intestinal mucosa has barrier function that is mainly composed of mechanical barrier, biological barrier, immune barrier, and so on, and the integrity of its barrier function plays an important role in resisting intestinal flora imbalance and ectopia as well as reducing endotoxin in bloodstream[7].

In the research, detection of endotoxin content and intestinal barrier function in pancreatitis model rats showed that serum endotoxin content of pancreatitis group was higher than that of control group, intestinal mucosal thickness and villus height were lower than those of control group, the number of intestinal Escherichia coli was significantly more than that of control group, and the number of Bifidobacterium and Lactobacillus was significantly less than that of control group. Bifidobacterium and Lactobacillus are intestinal probiotics that can form protective pellicle, participate in the composition of intestinal biological barrier, and also have inhibitory effect on pathogen-Escherichia coli[8]. Based on above analysis results, it could show that in the occurrence and development process of severe pancreatitis, intestinal mucosal barrier function would be injured, thereby causing the release of endotoxin into blood and the injury of intestinal barrier function. Based on above understanding, in pathological process of severe pancreatitis, effective methods of intervention and support should be given to protect intestinal mucosal barrier function, reduce the release of endotoxin into blood, regulate intestinal flora balance and inhibit flora ectopia, thereby protecting the function of various visceral organs of the body.

Enteral nutrition support is the routine intervention for clinical treatment of severe pancreatitis, and choosing ideal enteral nutrient solution is helpful to improve the treatment effect of

Figure 2. Comparison of serum amylase and AST contents of three groups.

Table 2 Comparison of serum inflammatory mediator contents of three groups.

3.3. Serum amylase and AST contents

72 h after treatment, analysis of serum amylase and AST contents of three groups was shown in Figure 2. Serum amylase and AST contents of pancreatitis group were higher than those of control group, and serum amylase and AST contents of intervention group were lower than those of pancreatitis group.

3.4. Serum inflammatory mediator contents

72 h after treatment, analysis of serum inflammatory mediators TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents of three groups was shown in Table 2. Serum TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents of pancreatitis group were higher than those of control group, and serum TNF-α, HMGB-1, MCP-1, IL-6 and MIF contents of intervention group were lower than those of pancreatitis group.

4. Discussion

Severe pancreatitis is a more critical clinical acute disease of digestive system, the disease progresses rapidly and will develop into systemic inflammatory response syndrome (SIRS) and multiple-organ dysfunction syndrome (MODS) in a short time, and it seriously endangers patients’ life safety[4]. Studies in recent years believe that intestinal mucosal barrier function injury is related to the development of SAP and the occurrence of SIRS and MODS[5].

<table>
<thead>
<tr>
<th>Group</th>
<th>Intestinal mucosal mechanical barrier</th>
<th>Intestinal mucosal biological barrier</th>
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<tbody>
<tr>
<td></td>
<td>Mucosal thickness (μm)</td>
<td>Villus height (μm)</td>
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<tr>
<td>Control group</td>
<td>672.32±72.52</td>
<td>39.52±4.96</td>
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<tr>
<td>Pancreatitis group</td>
<td>324.52±41.25</td>
<td>18.14±2.07</td>
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<tr>
<td>Intervention group</td>
<td>489.59±56.12</td>
<td>27.56±3.15</td>
</tr>
<tr>
<td>F</td>
<td>8.182</td>
<td>11.049</td>
</tr>
<tr>
<td>P</td>
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<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (ng/L)</th>
<th>HMGB-1 (ng/L)</th>
<th>MCP-1 (ng/L)</th>
<th>IL-6 (ng/L)</th>
<th>MIF (ng/L)</th>
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<tr>
<td>Control group</td>
<td>34.52±4.45</td>
<td>175.34±20.34</td>
<td>92.35±10.17</td>
<td>241.34±34.52</td>
<td>136.65±15.37</td>
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<td>Pancreatitis group</td>
<td>87.71±9.53</td>
<td>627.76±71.23</td>
<td>315.34±37.75</td>
<td>849.30±97.42</td>
<td>314.52±41.78</td>
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<tr>
<td>Intervention group</td>
<td>57.71±6.75</td>
<td>304.90±38.82</td>
<td>178.79±20.34</td>
<td>413.23±52.34</td>
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enteral nutrition support[9]. Ecological immune enteral nutrition is a newly developed enteral nutrition concept that is based on conventional enteral nutrition option, adds glutamine, arginine and other nutrient substances that have immunomodulatory function as well as probiotics that have flora-regulating effect, and helps to regulate intestinal flora balance and improve local immune barrier function[10]. After ecological immune enteral nutrition intervention, detection results of endotoxin content and intestinal barrier function showed that serum endotoxin content of intervention group was lower than that of pancreatitis group, intestinal mucosal thickness and villus height were higher than those of pancreatitis group, the number of intestinal Escherichia coli was significantly lower than that of pancreatitis group, and the number of Bifidobacterium and Lactobacillus was significantly higher than that of pancreatitis group, which indicated that ecological immune enteral nutrition intervention helped to improve the intestinal mucosal barrier function in rats with severe pancreatitis.

Complete barrier function of intestinal mucosa is helpful to reduce the release of intestinal endotoxin into blood and the pathogen ectopia to other visceral organs of the body, thereby inhibiting the progress of pancreatitis and protecting the function of target organs[11,12]. Analysis of maker molecule contents of pancreas injury and liver injury showed that serum amylase and AST contents of pancreatitis group were higher than those of control group, and serum amylase and AST contents of intervention group were lower than those of pancreatitis group, which indicated that ecological immune enteral nutrition intervention was helpful to reduce pancreas and liver injury. Activation of inflammatory response caused by endotoxin release and intestinal flora ectopia is an important part to cause multiple organ dysfunction of the body, and inflammatory factors directly cause injury of cells in target organ tissue. TNF-α, HMGB-1, MCP-1, IL-6 and MIF are important inflammatory factors participating in inflammatory response, and they involve processes such as inflammatory cascade amplification, inflammatory injury of tissues and cells[13-15]. In the research, analysis of serum inflammatory mediator contents showed that ecological immune enteral nutrition intervention helped to reduce inflammatory response and decrease serum inflammatory mediator contents.

Based on above discussion, it can be concluded that ecological immune enteral nutrition intervention can improve the intestinal barrier function, alleviate systemic inflammation degree and reduce pancreas and liver injury degree in rats with severe pancreatitis.

References


