Early enteral nutrition improves intestinal immune barrier in a rat model of severe acute pancreatitis

Lan Peng · Li-Guo Wu · Bo Li · Jun Zhao · Li-Ming Wen

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Abstract

Background The aim of the present study was to investigate the role of early enteral nutrition (EEN) in the intestinal immune barrier in severe acute pancreatitis (SAP), and to explore its potential mechanisms.

Methods Sixty rats were randomly assigned to three groups: sham-operated group (SO group, n = 20), SAP group receiving EEN (SAP + EEN group, n = 20), and SAP group receiving total parental nutrition (SAP + TPN group, n = 20). SAP was induced by infusion of sodium taurocholate. Rats were killed 5 days after nutritional support. The pathological damage of the intestine was determined using HE staining. The expression of MAdCAM-1, CD4+, and CD8+ in Peyer’s lymph nodes of the distal ilium was examined by immunohistochemistry. Serum levels of endotoxin and bacterial translocation were determined.

Results The survival rate in the SAP + TPN (50%) and SAP + EEN (75%) groups was significantly lower than in the SO group (100%) (P < 0.05). The survival rate in the SAP + EEN group was significantly higher than in the SAP + TPN group (P < 0.05). The expression of MAdCAM-1, CD4+ and CD8+ in the intestine was decreased in SAP rats. EEN significantly increased the expression of MAdCAM-1, CD4+ and CD8+ compared with TPN, accompanied by a decrease in the serum levels of endotoxin and bacterial translocation.

Conclusions Early enteral nutrition improves intestinal immune barrier, thus reducing bacterial and endotoxin translocation and improving the survival rate in SAP rats.

Keywords Bacterial translocation · CD4+ and CD8+ · Early enteral nutrition · Endotoxin · MAdCAM-1 · Several acute pancreatitis

Introduction

Acute pancreatitis (AP) is a common acute abdominal disease with a rapid onset. AP can be classified as edematous (mild) and necrotizing (severe) AP [1]. Mild AP representing in approximately 80% of patients, is commonly associated with a low mortality rate [2]. In contrast, severe AP (SAP), representing approximately 20–30% of AP patients, is associated with 36–50% mortality rate [3]. Secondary infection of the pancreas is regarded as the main contributor to the high mortality rate of SAP [4]. SAP is susceptible to intestinal barrier functional disturbance (IBFD) [5], leading to translocation of bacteria and endotoxin into the systemic circulation, and eventually secondary infection, systemic inflammation response syndrome (SIRS), and multiple organ dysfunction syndrome [6, 7]. Therefore, it is important to develop effective treatment strategies to prevent IBFD caused by SAP [8].

Several factors such as ischemic reperfusion injury, apoptosis, and excessive release of inflammatory cytokines have been found to contribute to IBFD in AP [9]. In addition, a reduction in the immune function of the intestine has been found in the early SAP, characterized by a decrease in the CD4+ and CD8+ T lymphocytes in the intestine and an increase in the plasma concentration of endotoxin [10],
suggesting that intestinal immunosuppression may be the main cause of bacterial and endotoxin translocation in SAP. Several studies have shown that supplementation with arginine and glutamine improved the number of T lymphocytes in the intestine in the animal model of SAP, accompanied by a decrease in bacterial translocation [10, 11]. Therefore, restoring intestinal immune function may represent a promising treatment to prevent bacterial translocation, thus reducing infectious complications in SAP [12].

Since inflammatory response and infection in AP is an energy consuming process, nutrition support is necessary for AP patients who are unable to ingest sufficient calories from oral diet due to intestinal dysfunction. Total parenteral nutrition (TPN) is recommended for patients with intestinal dysfunction, especially critically ill patients. However, several studies have shown that TPN is associated with disrupted gut-barrier function, comprised immune system, and increased bacterial translocation [13–15]. In contrast, increasing evidence has shown that enteral nutrition (EN) improves overall patient outcome compared with TPN [16–19]. Furthermore, early EN (EEN) has been reported to be associated with reduced complications and improved clinical outcomes in AP patients [20, 21]. Sun et al. reported that ENN increased the number of T lymphocytes in AP patients, thus leading to a reduction in the incidences of multiple organ dysfunction syndrome, systemic inflammatory response syndrome, and pancreatic infection [22]. However, it remains to be determined how EEN improved the intestinal immune barrier in SAP.

In the present study, we studied the effect of EEN versus TPN on the intestinal immune barrier function in rats with SAP induced by intrapancreatic infusion of sodium taurocholate. The purpose of this study was to study the role of EEN in the intestinal immune barrier in SAP, and to explore its potential mechanisms.

Methods

Animals

Animal experimental protocols were approved by the Committee for Animal Experiment at Luzhou Medical College. Adult female Sprague–Dawley rats (3–4 months old, weighing 180–250 g) were used in this study. Animals were housed in a clear lab at room temperature (25°C) with 60% humidity and a 12 h light/dark cycle. Animals were fed standard rat chow and water ad libitum.

Sixty rats were randomly assigned to three groups: sham-operated group (SO group, n = 20), SAP group receiving early enteral nutrition (SAP + EEN group, n = 20), and SAP group receiving total parental nutrition (SAP + TPN group, n = 20).

Materials

Sodium taurocholate was purchased from Sigma (St. Louis, MO, USA). Polyclonal goat anti-rat MAdCAM-1 (mucosal adhesion cell adhesion molecule 1) antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies against CD4^+ and CD8^+ were purchased from Maixin Company (Fuzhou, China). EEN and TPN fluids were obtained from Huarui Company (China).

Animal model of SAP and nutrition support

Severe acute pancreatitis was induced in rats by infusion of 3.8% of sodium taurocholate as previously described by Wang et al. [23]. Briefly, rats were deprived of food for 12 h and fed water ad libitum. Animals were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (4 mg/100 g body weight). Animals were placed in a prone position, and a 2–3 cm midline incision was made in the upper abdomen after antisepsis. After exposure of the pancreas, 3.8% sodium taurocholate was slowly injected from the tail of the pancreas after the pancreatic duct was catheterized with a needle. Approximately 2 min after injection of sodium taurocholate, the pancreas exhibited edema, exudation, and local hemorrhage, indicating that SAP was successfully induced. Rats in the SO group received the same surgical procedure without intrapancreatic injection of sodium taurocholate. For rats in the SAP + EEN group, one end of the enteral nutrient tube (1.2 mm epidural catheter) was placed at 1.5 cm below the duodenal papilla. The other end of the tube was dragged backward through the subcutaneous tunnel and fixed on the back of the neck. For rats in the SAP + TPN group, one end of a 1.2 mm epidural catheter was inserted into the right jugular vein in craniocaudal direction. The other end of the catheter was tunneled subcutaneously and fixed on the back of the neck. After surgery, each rat was administered 2 ml saline subcutaneously to replace the fluid loss during surgery.

Rats in the SAP + EEN group received the EEN solution at 12 h after induction of SAP. The EEN solution was continuously injected via an electronic pump at a rate of 2 ml/h for 10–12 min at beginning. The amount of EEN solution was increased to and maintained at 50 ml/day. Rats in the SAP + TPN group received the TPN solution via the jugular vein at 12 h after induction of SAP. The TPN solution was continuously injected via an electronic pump at a rate of 2 ml/h, and the amount of EEN solution was maintained at 50 ml/d. Each rat in both groups received the energy of 30 Kcal/100 g per day.
Measurement of serum amylase contents

Five days after induction of SAP, rats were killed and blood samples were collected from the heart. The serum amylase contents were tested using an automated analyzer.

Pathological evaluation of ilium mucosa

Tissues (1 cm length) in the distal end of the ilium were removed, fixed in formalin for 24 h, and embedded in paraffin. Tissue sections (5 μm thick) were stained with hematoxylin and eosin, and examined under the light microscope. The pathological conditions were evaluated as follows: 0, normal mucosa; 1, loss of <1/3 of glands in the crypt; 2, loss of 1/3–2/3 of glands in the crypt; 3, complete loss of all glands in the crypt; and 4, erosion and disruption of the epithelium with obvious inflammation cell infiltration.

Immunohistochemistry

Tissue sections (5 μM thick) from the distal end of the ilium were obtained from formalin-fixed and paraffin-embedded tissue blocks for immunohistochemical staining. The samples were then incubated in primary antibodies against MAdCAM-1 (polyclonal goat anti-rat MAdCAM-1 antibodies, dilution 1:500, Santa Cruz Biotechnology), CD4+ (polyclonal goat anti-rat CD4+, Maixin Company), and CD8+ (polyclonal goat anti-rat CD8+, Maixin Company) overnight at 4°C. After the primary antibody was washed off, the components of the Envision-plus (DAKO) detection system were applied, and sections were counterstained with hematoxylin. The immunostained sections were examined under the light microscope. Images were analyzed using the computerized color pathological image system and Image-Pro Plus 6.0 software. The integral optic density (IOD) value of the immunostaining was analyzed. The percentage of immunoreactivity was calculated by a ratio of the immunoreactive area to the total area in a section.

Detection of serum endotoxin

Blood samples were collected and used to detect serum endotoxin at the Department of Clinical Laboratory of Luzhou Medical College according to the manufacturer’s instruction, using limulus endotoxin detection kits.

Assessment of bacterial translocation

Under aseptic conditions, blood samples (3 ml) from the portal vein were collected and used for bacterial culture. Tissues of the pancreas, mesenteric lymph nodes, and lung were removed under aseptic conditions, and washed thoroughly with aseptic saline to remove the blood. The tissues were homogenized in aseptic saline (10 ml/g) and the homogenate (1 ml) were cultured on the agar medium for 24 h at 37 °C. The number of bacterial clones was counted, and the bacteria were identified according to the Bergey’s Manual of Determinative Bacteriology. The bacterial translocation rate (BTR) was calculated as follows: BTR = the number of bacterial positive sample/total number of samples.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 software. Quantitative data are expressed as means ± standard deviation. Levene’s tests were used to test homogeneity of variance. For data with equal variance, one-way analysis of variance (ANOVA) was used to compare the difference among groups. For data with unequal variance, Kruskal–Wallis H test was used to compare the difference among groups followed by posthoc Q tests. Categorical data were analyzed using χ² tests. Probability values less than 0.05 were considered statistically significant.

Results

Survival rate

In the SO group, no rats died. In contrast, 10 and 15 rats died in the SAP + TPN and SAP + EEN groups 5 days after induction of SAP, respectively. The 5-day survival rates were 100% in the SO group, 50% in the SAP + TPN group, and 75% in the SAP + EEN group (Table 1). The survival rate was significantly lower in the SAP + TPN and SAP + EEN groups than

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The survival rate</th>
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<tr>
<td>Groups</td>
<td>Survival n (%)</td>
</tr>
<tr>
<td>SO group</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>SAP + TPN group</td>
<td>10 (50%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAP + EEN group</td>
<td>15 (75%)&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> P < 0.01 vs. SO group
<sup>b</sup> P < 0.05 vs. SAP + TPN group

EEN early enteral nutrition, SAP severe acute pancreatitis, SO sham-operated group, TPN total parental nutrition
in the SO group ($P<0.05$, Table 1). The survival rate was significantly higher in the SAP + EEN group than in the SAP + TPN group ($P<0.05$, Table 1).

Serum amylase contents

The serum amylase content was significantly higher in the SAP + TPN and SAP + EEN groups than in the SO group ($P<0.05$, Table 2). The serum amylase content was significantly lower in the SAP + EEN group than in the SAP + TPN group ($P<0.05$, Table 2).

Pathological damage of the ilium

HE staining results showed that in the SO group, the structure of ilium was clear and regularly arranged (Fig. 1a). In the SAP + TPN group, complete loss of all glands in the crypt, hemorrhage, and inflammatory cell infiltration were observed. The structure of the ilium was disrupted (Fig. 1c). In the SAP + EEN group, the structure of the ilium was clear with loss of some glands in the crypt (Fig. 1b). The pathological score of the ilium was significantly higher in the SAP + EEN and SAP + TPN groups than in the SO group ($P<0.05$, Fig. 1d). The pathological score of the ilium in the SAP + EEN group was significantly lower than in the SAP + TPN group ($P<0.05$, Fig. 1d).

Expression of MAdcAM-1, CD$^{4+}$ and CD$^{8+}$ in Peyer’s lymph nodes of the distal ilium

We examined the expression of MAdcAM-1, CD$^{4+}$ and CD$^{8+}$ in Peyer’s lymph nodes of the distal ilium from each group, using immunohistochemistry (Fig. 2). Compared with the SO group, the expression levels of MAdcAM-1, CD$^{4+}$ and CD$^{8+}$ in Peyer’s lymph nodes were significantly lower in the SAP + TPN and SAP + EEN groups ($P<0.05$, Fig. 2). The expression levels of MAdcAM-1, CD$^{4+}$ and CD$^{8+}$ in Peyer’s lymph nodes were significantly higher in the SAP + EEN than in SAP + TPN groups ($P<0.05$, Fig. 2).

Serum levels of endotoxin

Compared with the SO group, serum levels of endotoxin were significantly higher in the SAP + TPN and SAP + EEN groups ($P<0.05$, Fig. 3). Serum levels of endotoxin were significantly lower in the SAP + EEN group compared with the SAP + TPN group ($P<0.05$, Fig. 3).

Table 2  Serum amylase contents in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Amylase (U/L)</th>
</tr>
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<tbody>
<tr>
<td>SO group</td>
<td>20</td>
<td>744.2 ± 40.7</td>
</tr>
<tr>
<td>SAP + TPN group</td>
<td>10</td>
<td>3278 ± 219.2a</td>
</tr>
<tr>
<td>SAP + EEN group</td>
<td>15</td>
<td>2227 ± 168.9ab</td>
</tr>
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</table>

* $P<0.01$ vs. SO group
  b $P<0.01$ vs. SAP + TPN

EEN early enteral nutrition, SAP severe acute pancreatitis, SO sham-operated group, TPN total parental nutrition

Fig. 1  The pathological examination of intestinal tissues from rats in the SO, SAP + EEN, and SAP + TPN groups. (a-c) Representative HE staining of intestinal tissues from rats in the (a) SO, (b) SAP + EEN, and (c) SAP + TPN groups. (d) The pathological scores in the SO, SAP + TPN, and SAP + EEN groups. *$P<0.05$ vs. SO group, #P $<0.05$ vs. SAP + TPN. BTR bacterial translocation rate, EEN early enteral nutrition, SAP severe acute pancreatitis, SO sham-operated group, TPN total parental nutrition
Compared with the SO group, the BTR was significantly higher in the SAP + TPN and SAP + EEN groups (P < 0.005, Table 3). The BTR was significantly lower in the SAP + EEN group compared with the SAP + TPN group (P = 0.006, Table 3).

Discussion

The gastrointestinal tract contains various immunocytes to prevent bacterial and endotoxin translocation from the gut into other organs. During SAP, the gut barrier is damaged, thus leading to bacteremia and endotoxemia that caused serious complications such as systemic inflammation response syndrome and multiple organ dysfunction syndrome. In the present study, we investigated the effect of EEN on the intestinal immune barrier function in SAP rats in comparison with TPN. We found that the expression of MAdCAM-1, CD4+ and CD8+ in Peyer’s lymph nodes of the distal ilium was decreased in SAP rats compared with controls. EEN treatment significantly increased the expression of MAdCAM-1, CD4+ and CD8+ in Peyer’s lymph nodes compared with TPN. Furthermore, we found that EEN treatment improved the survival rate of SAP rats and reduced the pathological damage of the intestine, accompanied by a decrease in the serum level of endotoxin and bacterial translocation. Our study suggests that EEN may improve the function of intestinal immune barrier, and reduced the bacterial and endotoxin translocation, thus leading to a decrease in the mortality rate in SAP rats. Our study is consistent with clinical findings that EEN treatment is associated with good clinical outcomes in AP patients.
damage of the intestine and a decrease in the serum level of endotoxin and bacterial translocation in SAP rats. These findings suggest that EEN can improve the intestinal immune barrier by increasing the number of CD4+ and CD8+ in the intestine, thus reducing bacterial and endotoxin translocation in SAP.

MAdCAM-1 is a member of the immunoglobulin superfamily that is mainly expressed in the Peyer’s and mesenteric lymph nodes of the distal ilium [27]. MAdCAM-1 interacts with its receptors such as integrins to play an important role in lymphocyte homing to the intestinal mucosa [28]. Clinical studies have shown that the level of peripheral blood lymphocytes is significantly reduced in SAP patients within 24–72 h after SAP attacks [29, 30]. A reduction in gut-associated lymphocyte homing has been reported to contribute to the reduction of peripheral blood lymphocytes [31]. In the present study, we found that the expression of MAdCAM-1 in Peyer’s lymph nodes of the distal ilium was significantly reduced in SAP rats, suggesting that MAdCAM-1-mediated immune defense in the intestine was impaired in SAP. We further found that EEN treatment significantly upregulated the expression of MAdCAM-1 in SAP rats, suggesting that EEN can improve the intestinal immune defense by increasing the expression of MAdCAM-1 in SAP.

In summary, we investigated the effect of EEN versus TPN on the intestinal immune barrier in a rat model of SAP induced by infusion of sodium taurocholate. We found that EEN reduced SAP-induced death and pathological damage of the intestine. In addition, we found that EEN reduced SAP-induced death and pathological damage of the intestine. In addition, we found that EEN reduced SAP-induced death and pathological damage of the intestine. In addition, we found that EEN reduced SAP-induced death and pathological damage of the intestine. In addition, we found that EEN reduced SAP-induced death and pathological damage of the intestine.

### Table 3 Assessment of bacterial translocation

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Portal vein</th>
<th>Lung</th>
<th>Pancreas</th>
<th>Mesenteric lymph node</th>
<th>BTR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO group</td>
<td>20</td>
<td>0 % 0 %</td>
<td>0 % 0</td>
<td>0 % 0 %</td>
<td>2 % 10 %</td>
<td>2.5</td>
</tr>
<tr>
<td>SAP + TPN group</td>
<td>10</td>
<td>5 % 50 %</td>
<td>2 % 20</td>
<td>3 % 30 %</td>
<td>8 % 80 %</td>
<td>45%</td>
</tr>
<tr>
<td>SAP + EEN group</td>
<td>15</td>
<td>3 % 20 %</td>
<td>1 % 6.7</td>
<td>2 % 13.3</td>
<td>6 % 40 %</td>
<td>20%</td>
</tr>
</tbody>
</table>

* P < 0.01 vs. SO group

** BTR bacterial translocation rate, EEN early enteral nutrition, SAP severe acute pancreatitis, SO sham-operated group, TPN total parental nutrition

[20, 21]. Our findings suggest that EEN treatment may reduce serious complications in SAP patients by improving the intestinal immune barrier function.

Several clinical studies have demonstrated that EEN can produce better clinical outcomes than TPN [16–19]. Consistent with these studies, we found that EEN treatment resulted in a higher survival rate in SAP rats compared with TPN, accompanied by a decrease in the serum amylase content. Intestinal barrier dysfunction is the main reason that leads to secondary infection, SIRS, and multiple organ dysfunction syndrome and increased mortality rate in AP patients [9]. Consistent with this idea, we found that SAP rats exhibited severe pathological damage of the intestine, accompanied by a decrease in the serum level of endotoxin and bacterial translocation. In addition, Feng et al. reported that loss of enteral nutrition resulted in intestinal barrier dysfunction in mice [24]. Furthermore, EEN support has been shown to maintain the function of intestinal epithelial barrier in SAP patients [25]. Therefore, our findings that EEN improved the survival rate with reduced intestinal damage suggest that EEN may prevent damage of the intestinal barrier in AP, thus reducing the mortality rate of SAP patients.

The intestinal immune barrier plays an important role in preventing bacterial invasion and infection [26]. It has been reported that the number of CD4+ and CD8+ T lymphocytes in the intestinal mucosa were significantly decreased in early SAP (within 24 h after AP onset), suggesting that dysfunction of intestinal CD4+ and CD8+ T lymphocytes plays an important role in the development of SAP [10]. Consistent with the literature, we found that the expression of CD4+ and CD8+ was significantly decreased in SAP rats compared with controls. Furthermore, we found that the decreased in the intestinal immune function was accompanied by an increase in the serum level of endotoxin and bacterial translocation in SAP rats, suggesting that SAP impaired the intestinal immune barrier. EEN treatment resulted in a significant increase in the expression of CD4+ and CD8+ in Peyer’s lymph nodes of the distal ilium in SAP rats, accompanied by a reduction in the pathological function was accompanied by an increase in the serum level of endotoxin and bacterial translocation in SAP rats. These findings suggest that EEN can improve the intestinal immune barrier by increasing the number of CD4+ and CD8+ in the intestine, thus reducing bacterial and endotoxin translocation in SAP.
Conflict of interest None declared

References


