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Effects of glutamine-supplemented enteral or parenteral nutrition on apoptosis of intestinal mucosal cells in rats with severe acute pancreatitis

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Abstract. – BACKGROUND: Hhigh mortality of the severe acute pancreatitis (SAP) is caused by the damage of intestinal mucosal barrier. Glutamine (Gln) has been used to protect the intestinal mucosal barrier in the treatments of many severe diseases.

AIM: To explore the impact of glutamine on the apoptosis of intestinal mucosa and Bax expression in rats with SAP.

MATERIALS AND METHODS: Eighty male SD rats were randomly divided into five groups: sham-operated group, SAP + parenteral nutrition (PN) group, SAP + enteral nutrition (EN) group, SAP + EN + Gln group, and SAP + PN + Gln group. Rats were sacrificed 4 days and 7 days after nutritional support. The intestinal epithelial apoptosis and the expression of Bax were examined by TUNEL assay and immunohistochemical staining, respectively.

RESULTS: The apoptotic index (AI) of SAP groups was higher than that of sham-operated group. After 7 days treatment, the AI of SAP + EN + Gln group was significantly lower than that of the SAP + EN or SAP + PN group. In addition, the AI of the SAP groups after 7 days treatment was significantly lower than that of the same groups at 4 days after treatment. Furthermore, the Bax expression of SAP + EN + Gln group was significantly lower than that in the SAP + EN or SAP + PN group. However, no significant differences were observed between SAP + EN + Gln group and SAP + PN + Gln group in AI and Bax expression.

CONCLUSIONS: Combination of Gln and parenteral nutrition or enteral nutrition inhibits the apoptosis of intestinal epithelial cells and maintains the integrity of the intestinal mucosal barrier.

Key Words:
Bax, Cell apoptosis, Glutamine, Pancreatitis, Rats.

Introduction

Severe acute pancreatitis (SAP) is one of the most common lethal diseases. In contrast to mild acute pancreatitis, which has a mortality rate of less than 1%, mortality of SAP can increase up to 20%-30%[1]. The high death rate of the SAP is usually considered as the result of secondary infection of pancreatic/peripancreatic necrosis[2]. This secondary infection is often caused by the translocation of bacteria and endotoxins from the damaged intestinal mucosal barrier to the systemic circulation[3]. Therefore, it is important to explore effective methods to prevent intestinal mucosal barrier dysfunction caused by SAP[4,5].

Glutamine (Gln) is the most abundant amino acid in blood and the small intestine is the principal organ of glucose utilization, extracting 20% to 30% of circulating glutamine[6]. Recent studies have demonstrated that Gln is not only an important fuel and energy to synthesize substrate for the rapid development of intestinal mucosa[7], but also a crucial regulator to maintain the intestinal mucosal structure[8], intestinal barrier, immune function and microecological environment[9]. Thus, Gln supplementation has been widely used to protect the intestinal mucosal barrier for treatments of many diseases, such as severe burn[10], biliary obstruction[11], liver transplantation[12], and acute myocardial infarction[13].

In this study, we aimed to explore the efficacy of enteral nutrition (EN) or parenteral nutrition (PN) supplemented with Gln in reducing intestinal mucosal barrier dysfunction caused by SAP. The stability of intestinal epithelium depends on

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the balance between the epithelial cell proliferation and apoptosis[14]. Therefore, apoptosis index and expression of Bax among different groups were compared to explain the underlying mechanisms of the gut protective effects of Gln.

Materials and Methods

Materials

EN solution included 95% hydrolyzed whey proteins (GRANDE Company, USA), medium-chain fatty acids (Shanghai Shiliao Co., Ltd), mixed vitamins, minerals (Institute of Nutrition in Academy of Military Medical Science). The ratio of energy produced by carbohydrates, protein and fat is 49:17:34. PN solution composed of 500 g/L glucose, 85 g/L compound amino acids, and 200 g/L fat emulsion, addamel and water-soluble vitamins [insulin (IU): sugar (g) = 1:4.0]. The PN solution and EN solution can provide 100 kca1 per 100 ml. The ratio of energy produced by carbohydrates, protein and fat is 55:17:28.

Animals and Groups

All animal studies have been approved by China Ethics Committee and performed in accordance with the ethical standards. Eighty 6- to 7-week-old Sprague-Dawley (SD) male rats (200 ± 10 g) were provided by the Experimental Animal Center in the Second Military Medical University. After one-week adaption during which food and water were available ad libitum, rats were randomly divided into five groups: sham-operated group, SAP + PN group, SAP + EN group, SAP + EN + Gln group, and SAP + PN + Gln group. Rats in different groups were sacrificed at 4 and 7 days after Gln treatment. In the sham-operated group, laparotomy was performed, and the pancreas was taken out and washed with normal saline followed by wound closure, and rats were intragastrically given EN solution. In the SAP + EN group and SAP + EN + Gln group, normal saline was used to flush intestine at 6 h after regaining consciousness and rats received intragastrical injection of EN solution at 12 h after regaining consciousness. The rate was increased from 1.0 ml/h at beginning to 2.0 ml/h (or 50 ml/d) at 36 h followed by maintained injection. In the PN group, the rate was increased from 1.0 ml/h to 2.0 ml/h (or 50 ml/d) within 36 h. In the SAP + EN + Gln group, Gln was added to the EN solution; in the SAP + PN + Gln group, Dipeptiven was added to the PN solution. At 1-7 days after the modeling, rats of all groups except the sham-operated group were daily offered isonitrogenous (2.5 g N) and isocaloric (1046.5 kJ) nutrient solution per kilogram (kg) of body weight.

In the SAP + EN + Gln group, rats were also treated with alanyl-glutamine dipeptide (0.4 g/kg body weight, Shanghai Xuxin Chemical Company; Purity: 98%) in which the glutamine was 78 ± 4.2 g. In the SAP + PN + Gln group, Dipeptiven (L-alanyl-L-glutamine; Huarui Pharmaceutical Co., Ltd.) was added to the PN solution at a ratio of 2 ml/kg body weight (0.4 g/kg body weight/d; ≈77.2 ± 6.4 g of Gln). There was no difference in the glutamine content between the SAP + EN + Gln group and SAP + PN + Gln group.

Animal Model and Treatments

Before introduction of SAP, rats received food deprivation for 12 h. Then, rats were intraperitoneally anesthetized with 2.5% pentothal sodium (0.1 ml/100 g, Shanghai Xinya Pharmaceutical Co., Ltd.). After shaving and sterilization, laparotomy was performed, and 3.8% sodium taurocholate: 1 ml/kg (Sigma Co., St. Louis, MO, USA) was slowly injected at the tail of the pancreas to induce SAP[15]. In the SAP + PN group and SAP + PN + Gln group, PN solution was administered via the external jugular vein. In the SAP + EN group and SAP + EN + Gln group, EN solution was administered via the stomach and duodenum.

Pathological Examination of Pancreatic Tissue

At 4th and 7th days after treatment, rats in each group were sacrificed, and then pancreatic tissues were routinely stained by hematoxylin-eosin and then observed under an optical microscope.

TUNEL Assay of Small Intestinal Apoptosis

After the rats were sacrificed, 1 cm intestinal tissues were collected and made into paraffin slices. The paraffin slices of the small intestine were dewaxed by xylene, hydrated by graded ethanol, and stained using TUNEL assay kit (Roche, Applied Science, Basel, Switzerland) according to the instructions. The apoptotic index was calculated under the microscopy. Specifically, five fields were selected and the number of apoptotic cells in the 100 epithelial cells was calculated. Apoptotic index = the number of apoptotic cells/the number of total cells × 100%.

Immunohistochemistry Assay of Bax Expression

The paraffin slices of the small intestine were obtained as described above. After dewaxed and hydrated, the intestinal slices were digested for 20 min by trypsinase and then stained by specific antibodies according to the instruction of the kit (Maixin Boitech Co., Ltd, Fuhzov, China). Bax was localized in the cytoplasm. Four fields were selected under a high power microscope and the number of Bax positive cells and total cells were counted. The positive rate = the number of Bax positive cells/the number of total cells × 100%.

Statistical Analysis

Data were shown as mean ± standard deviation. Statistical difference between groups was analyzed by Analysis of variance using SPSS11.5 software (SPSS Inc., Chicago, IL, USA). p-values < 0.05 were considered to be statistically significant.

Results

Pathological Changes in the Pancreatic Tissues

HE staining results showed that the tissue structure of pancreatic acinus and pancreatic islet were clear and regularly arranged in the sham-operated rats, and cells had normal morphology without obvious hemorrhage and necrosis. In the SAP + PN group, spotty hemorrhage was found on the pancreatic surface and fat surrounding the pancreas, bloody ascites was also noted, saponification spots were observed in the pancreas and omentum at 4 days after treatment. Under a light microscope, there were numerous scattered acinar necrosis where inflammatory cell infiltration were also detected. However, patchy necrosis was macroscopically found in the pancreas, saponification spots were observed in the tissues surrounding the pancreas and omentum, accompanied by a large amount of bloody ascites at 7th days after treatment. Furthermore, pancreatic parenchymal necrosis and inflammatory cell infiltration were more serious compared with 4-day treatment under a light microscope. These pathological changes were alleviated by SAP + EN + Gln, SAP + PN + Gln, and SAP + EN treatments at both time points, among which the most evident effect was observed in the SAP + EN + Gln group (Figure 1).

Apoptosis of Intestinal Epithelial Cells

The *in situ* apoptosis detection results showed that compared with the sham-operated group, rats with SAP had more apoptotic intestinal epithelial cells, especially the SAP + PN group ($p < 0.01$). Although the rats of the SAP + EN + Gln

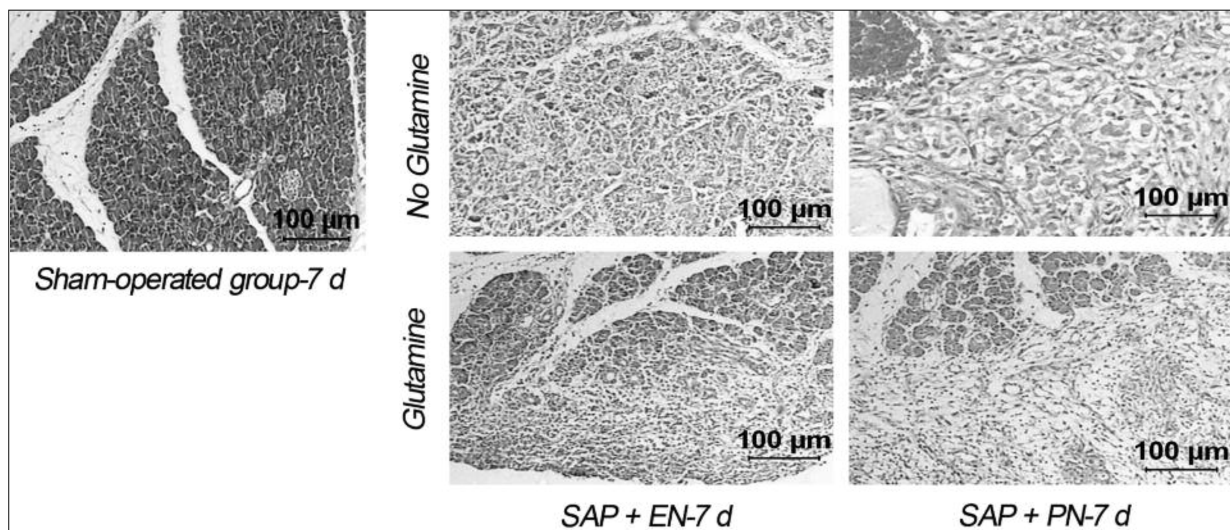

Figure 1. Hematoxylin-Eosin staining of pancreatic tissues in different groups. The tissue structure of pancreatic acinus and pancreatic islet were clear and regularly arranged in the sham-operated rats, and cells had normal morphology without obvious hemorrhage and necrosis. Pancreatic necrosis, peripancreatic and omental punctate saponification spots, and a large amount of bloody ascites were found in SAP + PN-7 d group. There were fewer pathological changes in the groups of SAP + EN + Gln, SAP + PN + Gln, and SAP + EN at both time points than that in group SAP + PN, and this effect was the most obvious in the group SAP + EN + Gln.

group had fewer apoptotic cells than SAP + PN + Gln group, there was no significant difference. Statistical analysis of apoptotic index further proved the above results (Figure 2). Furthermore, the apoptotic index of SAP + EN, SAP + EN + Gln and SAP + PN + Gln groups at 7 days after treatment were significantly lower than that in the each group at 4 days after treatment. Apoptotic index of SAP + EN + Gln was significantly lower than that in the SAP + EN group only at 7 days after treatment, but not at 4 days after treatment (Table I).

Bax Protein Expression in Intestinal Mucosal Cells

There was apparent Bax expression in the intestinal mucosal cells of SAP rats no matter 4 or 7 days after treatment, compared with the sham-operated group ($p < 0.01$). SAP + PN treatment induced the most Bax expression and SAP + EN + Gln group triggered the least Bax expression, which was significantly different ($p < 0.05$). The Bax expression of SAP + EN + Gln group was significantly lower than that of SAP + EN group at both time points ($p < 0.05$). Although the Bax expression rate of the SAP + EN + Gln group was lower than that in the SAP + PN + Gln group, no significant difference was observed, which was in accordance with the AI change of these two groups (Table II).

Discussion

Intestinal epithelial cell layer is the primary defensive barrier to resist the invasion of harmful micro-organisms and potential pathogens in the intestine into the peripheral organs. The dysfunction of intestinal barrier caused by various reasons in patients with SAP is the main reason of systemic secondary infection and occurrence of multiple organ dysfunction syndrome[16], which directly result in the increased mortality. How to maintain the integrity of the intestinal barrier is a very critical and difficult clinical problem. Many studies have shown that loss of enteral nutrition results in intestinal epithelial barrier dysfunction in a mouse model[17] and it is also safe and conducive to maintain the function of the intestinal barrier of the SAP patients by giving EN to the jejunum in the early phase of SAP[18]. According to nutritional needs and metabolic characteristics of SAP, we developed EN solution suitable for SAP. In addition, Gln was also supplemented into EN, which has been demonstrated to be effective for regulating the balance between pro-inflammation and anti-inflammation in SAP rats[19]. However, the detail apoptosis mechanism of intestinal epithelial cells has not been investigated. Thus, we tried to explore the role of Gln in the apoptosis of intestinal mucosa and apoptotic gene expression in SAP with different nutritional ways.

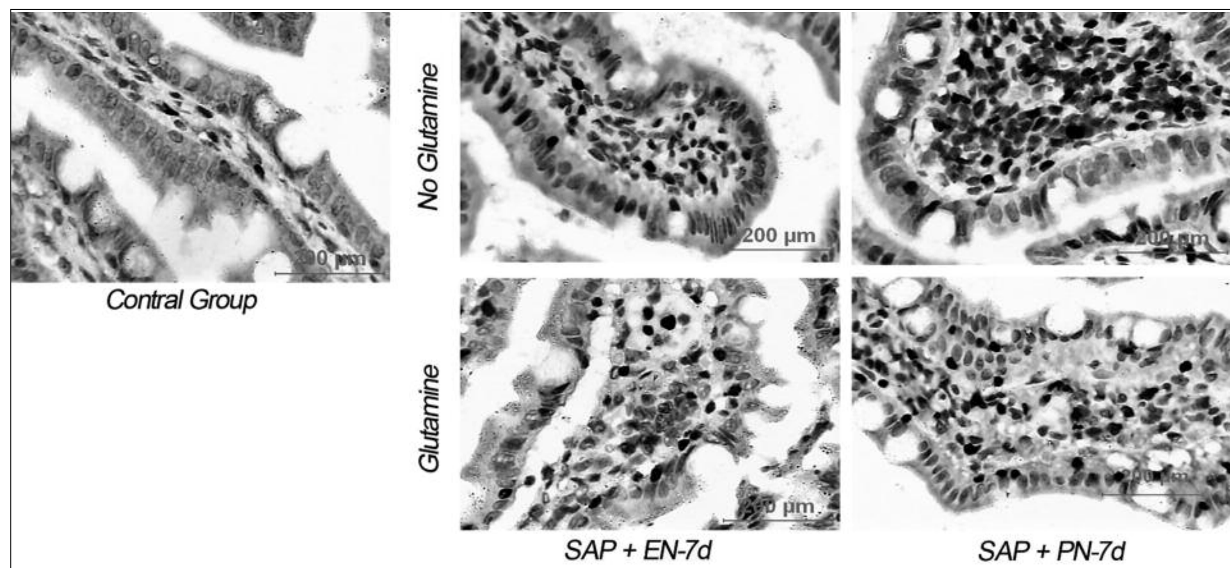

Figure 2. Intestinal epithelial apoptosis of rats in different groups (TUNEL, ×400). Apoptotic intestinal cells were stained dark brown. A few apoptotic intestinal cells were found in the control-7 d group, while there were many apoptosis in the intestinal epithelial cells of SAP+PN-7 d group. However, fewer apoptotic cells were observed in SAP + EN- 7 d group, SAP + EN + Gln- 7 d group, and SAP + PN + Gln- 7 d group compared with the SAP + PN group.

Table I. The results of intestinal epithelial AI ($\bar{x} \pm s$).

Group	n	4d	7d
Sham-operated group	8	21.0 ± 4.43	21.62 ± 3.9
SAP+PN	8	63.67 ± 6.65*	64.7 ± 6.9*
SAP+EN	8	53.5 ± 7.54$^{*,\&}$	42.5 ± 8.5$^{*,\#,§,\&}$
SAP+EN+Gln	8	48.5 ± 6.55$^{*,\&}$	33.2 ± 6.2$^{*,§,\&}$
SAP+PN+Gln	8	52.51 ± 5.64$^{*,\&}$	36.7 ± 8.5$^{*,§,\&}$

*$p < 0.01$, compared with sham-operated group; $^{\#}p < 0.05$, compared with the SAP + EN + Gln; $^{§}p < 0.05$, compared wih groups with 4 days of feeding; $^{\&}p < 0.01$, compared with SAP + PN group.

Several studies have found that the separation of villus epithelium is one of the early morphological changes of small intestinal mucosa injury[20]. Approximately 80% of the separated epithelial cells have the apoptotic morphological characteristic, namely chromatin condensation and nuclear fragmentation. SAP induced apoptosis can cause the small intestine mucosa defects, destroy the connection between the cells, bare the interstitial substance and form a temporary "bare area" before the repair of mucosa[21]. This "bare area" is conducive to the translocation of bacteria and endotoxin, which can cause enterogenous secondary infection and then systemic inflammatory response syndrome. So, apoptosis in SAP is very important for the early dysfunction of intestinal barrier. Although apoptosis is a protective mechanism, various stimuli could induce excessive apoptosis and block the repair and regeneration of the intestinal epithelial cells, resulting in the intestinal barrier dysfunction[22-24]. As expected, we found that the apoptotic index of the rats in SAP groups were significantly higher than that of the sham-operated group. However, the apoptotic index of SAP + EN group was significantly lower than that of the SAP + PN group at both time points, which demonstrated that EN was better than PN to protect intestinal epithelial cells from apoptosis. These findings were in consistent with our previous study[19]. In addition, Gln-supplemented EN or PN treatment showed superior effect on the reducing apoptotic index compared with the EN or PN alone 7 days after treatment. The probable mechanisms of Gln to reduce the apoptosis of intestinal epithelial cells are as follows: (1) directly or indirectly affecting the intracellular signaling mediators, such as cAMP and Ca^{2+} to increase the resistance of tight junction and change its selectivity to noxious passage[25]; (2) protecting endothelial cells from oxygen free radicals induced damage; (3) changing the ratio of insulin/pancreatic glucagon, and enhancing glutahione synthesis and antioxidant capacity of intestinal cells; (4) limiting the production of cytokines and infiltration of polymorphonuclear leukocytes (PMNS), which reduces chemotactic gradient migration of PMNS and maintains the intestinal mucosal structure and its function[26]. Amazingly, no significant difference was observed between SAP + EN + Gln group and SAP + PN + Gln group. This result may be explained by the following reason: When Gln was applied in a PN way, Gln directly entered into the blood, where it could be metabolized quickly and hence exerts protective action. However, Gln was not so stable in the enteral nutrition liquid and likely to be digested by the gastric acid. As a result, although EN was

Table II. Change of Bax protein expression in intestinal mucosal cells ($\bar{x} \pm s$, %).

Group	n	4d	7d
Sham-operated	8	10.14 ± 1.06	8.27 ± 1.06
SAP + PN	8	52.43 ± 4.02*,§	48.31 ± 5.13*,§
SAP + EN	8	50.01 ± 3.17$^{*,\#,§}$	40.52 ± 4.32$^{*,\#,§}$
SAP + EN + Gln	8	42.07 ± 2.39$^{*,\#}$	31.72 ± 3.33$^{*,\#}$
SAP + PN + Gln	8	44.2 ± 2.67$^{*,\#}$	33.45 ± 3.87$^{*,\#}$

*$p < 0.01$, compared with sham-operated group; $^{\#}p < 0.05$, compared with the SAP + EN + Gln; $^{§}p < 0.05$, compared wih groups with 4 days of feeding; $^{\&}p < 0.01$, compared with SAP + PN group.

more effective than PN to resist intestinal cell apoptosis, EN plus Gln was not more significantly to protect the intestinal epithelial cells from apoptosis than the PN plus Gln.

There are many genes involved in the regulation of apoptosis and Bax is one of them. With the similar structure with Bcl-2, Bax binds to the Bcl-2, inactivates it, and then promotes apoptosis[27]. The apoptosis-inducing gene Bax is found to be highly expressed when the intestine epithelial cells migrate into the tip villi[28]. As expected, the expression of Bax was also significantly increased in all SAP groups. Compared with the SAP + PN group, Bax expression was significantly reduced after SAP + EN, SAP + EN + Gln, SAP + PN + Gln treatments, with the most obvious change in the SAP + EN + Gln group.

Conclusions

Combination of Gln and different nutritional ways may reduce the excessive apoptosis of intestinal epithelial cells and hence protect the intestinal barrier in rats with SAP. Our study would provide some clues to the clinical treatment of SAP. However, the clinical treatment of SAP is very complicated and there are still many controversies about the time point of EN treatment and the corresponding preparation. The application of Gln also confronts many unresolved issues, such as the method of administration, and the combination with antibiotics. Systematic application of Gln in the clinical treatment of SAP still needs to be further studied.

Conflict of Interest

The Authors have no conflict of interest to declare

References

1) Ioannidis O, Lavrentieva A, Botsios D. Nutrition support in acute pancreatitis. JOP 2008; 9: 375-390.
2) Zaheer A, Singh VK, Qureshi RO, Fishman EK. The revised atlanta classification for acute pancreatitis: updates in imaging terminology and guidelines. Abdom Imaging 2013; 38: 125-136.
3) Shi C, Zhao X, Lagergren A, Sigvardsson M, Wang X, Andersson R. Immune status and inflammatory response differ locally and systemically in severe acute pancreatitis. Scand J Gastroenterol 2006; 41: 472-480.
4) Wang YZ, Wang SW, Zhang YC, Sun ZJ. Protective effect of exogenous igf-i on the intestinal mucosal barrier in rats with severe acute pancreatitis. World 2012; 3: 213-220.
5) Jha RK, Yong M, Chen S. The protective effect of resveratrol on the intestinal mucosal barrier in rats with severe acute pancreatitis. Med Sci Monitor 2008; 14: BR14-19.
6) Mc Anena O, Moore F, Moore E, Jones T, Parsons P. Selective uptake of glutamine in the gastrointestinal tract: Confirmation in a human study. Br J Surg 1991; 78: 480-482.
7) Shan Y, Shan A, Li J, Zhou C. Dietary supplementation of arginine and glutamine enhances the growth and intestinal mucosa development of weaned piglets. Livestock Sci 2012; 150: 369-373.
8) dos Santos RdGC, Viana ML, Generoso SV, Arantes RE, Correia MITD, Cardoso VN. Glutamine supplementation decreases intestinal permeability and preserves gut mucosa integrity in an experimental mouse model. JPEN J Parenter Enteral Nutr 2010; 34: 408-413.
9) Ewaschuk JB, Murdoch GK, Johnson IR, Madsen KL, Field CJ. Glutamine supplementation improves intestinal barrier function in a weaned piglet model of escherichia coli infection. Br J Nutr 2011; 106: 870-877.
10) Peng X, Yan H, You Z, Wang P, Wang S. Effects of enteral supplementation with glutamine granules on intestinal mucosal barrier function in severe burned patients. Burns 2004; 30: 135-139.
11) White J, Hoper M, Parks R, Clements W, Diamond T. Glutamine improves intestinal barrier function in experimental biliary obstruction. Eur Surg Res 2006; 37: 342-347.
12) Li Y, Chen Y, Zhang J, Zhu J-F, Liu Z-J, Liang S-Y, Sun K, Liao W-Y, Gong J-P. Protective effect of glutamine-enriched early enteral nutrition on intestinal mucosal barrier injury after liver transplantation in rats. Am J Surg 2010; 199: 35-42.
13) Mao Y, Wang SQ, Mao XB , Zeng QT, Li YS. Intestinal barrier function in patients with acute myocardial infarction and the therapeutic effect of glutamine. Int J Cardiol 2011; 146: 432-433.
14) Ban K, Kozar RA. Glutamine protects against apoptosis via downregulation of sp3 in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 2010; 299: G1344-G1353.
15) Peng JS, Liu ZH, Li CJ, Wu XB, Diad DC, Du YP. Development of a real-time pcr method for the detection of bacterial colonization in rat models of severe acute pancreatitis. Chin Med J (Engl) 2010; 123: 326-331.
16) Skucaite N, Peciuliene V, Vitkauskiene A, Machiulskiene V. Susceptibility of endodontic pathogens to antibiotics in patients with symptomatic apical periodontitis. J End 2010; 36: 1611-1616.
17) Feng Y, Ralls MW, Xiao W, Miyasaka E, Herman RS, Teitelbaum DH. Loss of enteral nutrition in a mouse model results in intestinal epithelial barrier dysfunction. Ann NY Acad Sci 2012; 1258: 71-77.

18) Olah A, Belagyi T, Issekutz A, Gamal ME, Bengmark S. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br J Surg 2002; 89: 1103-1107.
19) Han T, Li X, Cai D, Zhong Y, Chen L, Geng S, Yin S. Effect of glutamine on apoptosis of intestinal epithelial cells of severe acute pancreatitis rats receiving nutritional support in different ways. Int J Clin Exp Pathol 2013; 6: 503-509.
20) Yasuda T, Takeyama Y, Ueda T, Shinzeki M, Sawa H, Nakajima T, Kuroda Y. Breakdown of intestinal mucosa via accelerated apoptosis increases intestinal permeability in experimental severe acute pancreatitis. J Surg Res 2006; 135: 18-26.
21) Bhatia M. Apoptosis of pancreatic acinar cells in acute pancreatitis: Is it good or bad? J Cell Mol Med 2004; 8: 402-409.
22) Denning TL, Takaishi H, Crowe SE, Boldogh I, Jevnikar A, Ernst PB. Oxidative stress induces the expression of fas and fas ligand and apoptosis in murine intestinal epithelial cells. Free Radic Biol Med 2002; 33: 1641-1650.
23) Jones SA, Butler RN, Sanderson IR, Wilson JW. The effect of specific caspase inhibitors on tnf-alpha and butyrate-induced apoptosis of intestinal epithelial cells. Exp Cell Res 2004; 292: 29-39.
24) Kojima M, Iwakiri R, Wu B, Fujise T, Watanabe K, Lin T, Amemori S, Sakata H, Shimoda R, Oguzu T, Ootani A, Tsunada S, Fujimoto K. Effects of antioxidative agents on apoptosis induced by ischaemia-reperfusion in rat intestinal mucosa. Aliment Pharmacol Ther 2003; 18(Suppl 1): 139-145.
25) Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci 2013; 70: 631-659.
26) Papaconstantinou HT, Hwang KO, Rajaraman S, Hellmich MR, Townsend CM Jr., Ko TC. Glutamine deprivation induces apoptosis in intestinal epithelial cells. Surgery 1998; 124: 152-159; discussion 159-160.
27) Arii S, Teramoto K, Kawamura T. Current progress in the understanding of and therapeutic strategies for ischemia and reperfusion injury of the liver. J Hepatobiliary Pancreat Surg 2003; 10: 189-194.
28) Masuko Y. Impact of stress response genes induced by l-glutamine on warm ischemia and reperfusion injury in the rat small intestine. Hokkaido Igaku Zasshi 2002; 77: 169-183.