Original article

Effect of ecoimmunonutrition supports on maintenance of integrity of intestinal mucosal barrier in severe acute pancreatitis in dogs

XU Gui-fang, LU Zheng, GAO Jun, LI Zhao-shen and GONG Yan-fang

Keywords: *pancreatitis; parenteral nutrition; elemental enteral nutrition; ecoimmunonutrition enteral nutrition; intestinal mucosal damage; bacterial translocation*

Background One of the major causes of death in severe acute pancreatitis (SAP) is severe infection owing to bacterial translocation. Some clinical studies suggested that ecoimmunonutrition (EIN) as a new strategy had better treatment effect on SAP patients. But the experiment studies on the precise mechanism of the effect of EIN were less reported. In this study, we mainly investigated the effects of EIN on bacterial translocation in SAP model of dogs.

Methods SAP was induced by retrograde infusion of 5% sodium taurocholate into the pancreatic duct in healthy hybrid dogs. The SAP dogs were supported with either parenteral nutrition (PN) or elemental enteral nutrition (EEN) or EIN. The levels of serum amylase, serum aminotransferase and plasma endotoxin were detected before and after pancreatitis induction. On the 7th day after nutrition supports, peritoneal fluid, mesenteric lymph nodes (MLN), liver, and pancreas were collected for bacterial culture with standard techniques to observe the incidence of bacterial translocation. Pathology changes of pancreas were analyzed by histopathologic grading and scoring of the severity of pancreas, and the degree of intestinal mucosal damage was assessed by measuring mucosal thickness, villus height, and crypt depth of ileum.

Results Compared with PN and EEN, EIN significantly decreased the levels of serum amylase, serum aminotransferase, plasma endotoxin, and the incidence of bacterial translocation. Furthermore, compared with the others, the histology scores of inflammation in pancreas and the ileum injury (ileum mocosa thickness, villus height, and crypt depth) were significantly alleviated by EIN ($P<0.05$). Moreover, concerning liver function, the serum levels of alanine aminotransferase, aspartate aminotransferase and albumin were ameliorating significantly in the EIN group.

Conclusion Our results suggested that EIN could maintain the integrity of intestinal mucosal barrier and reducing the incidence of bacterial translocation in SAP dogs. Early EIN was safe and more effective treatment for SAP dogs.

Chin Med J 2006; 119(8):656-661

Despite recent advances in intensive care management, the mortality rate of severe acute pancreatitis (SAP) remains high, and secondary infection of pancreatic necrosis is associated with particularly high mortality rates as high as 40%.[1-3] Sepsis and multiple organ failure, mainly owing to pancreatic or peripancreatic infection, have emerged as the most serious complications and now account for more than 80% of deaths.[4] Experimental studies indicated that pancreatic infection in SAP appeared to be due to translocation of bacteria from the gut to mesenteric lymph nodes (MLN), peritoneal fluid, and blood, then from these sites to the pancreas itself. Several experimental studies revealed that acute pancreatitis promotes bacterial translocation, which in turn leads to infection of the pancreas and septic

Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China (Xu GF, Lu Z, Gao J, Li ZS and Gong YF)

Correspondence to: Prof. LI Zhao-shen, Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China (Tel: 86-21-25070556. Fax: 86-21-25074635. Email: lizhaoshen111@yahoo.com.cn)

XU Gui-fang and LU Zheng contributed equally to this work.

This work was supported by a grant from the National Natural Science Foundation of China (No. 30370647).

complications.[5,6] The main mechanisms that governed the processes of bacterial translocation in SAP are probably related to the over-growth of enteric flora due to intestinal dysfunction, damage to intestinal permeability, and impairment of host immunity.[7] Furthermore, endothelial and epithelial barrier functions are important to protect against potential invasion of enteric microorganisms, and their function may also be essential to prevent the development of multiple organ dysfunction.[8,9] How to prevent bacterial translocation and how to protect the intestinal mucosa from being damaged might be a key to limiting the septic complication in SAP.

Many clinical studies pointed out that compared with parenteral nutrition (PN) and elemental enteral nutrition (EEN), ecoimmunonutrition (EIN) hastened the recovery of SAP patients, stimulated gastrointestinal motility, and alleviated the degree of systemic inflammatory response syndrome (SIRS). Howerver, the experiment studies on the precise mechanism of the effect of EEN, especially EIN, were less reported. In this study, the benefits of EIN were observed with a SPA model of dogs.

METHODS

Experimental model of SAP

Fifteen healthy hybrid dogs, weighing 15－18 kg, were obtained from the Experimental Animal Center of Second Military Medical University, China. The animals were deprived of foods but with water for 14 hours before treatment. Surgical anesthesia was induced by administration of pentobarbital (30 mg/kg) through an infusion pump. The right femoral vein was cannulated with a soft polyethylene for infusion of the nutrition. First, we operated the dogs to make stomach fistula, gall bladder fistula, jejunum fistula, and bladder fistula. Then we put tubes in duodenum, main pancreatic duct, and accessory pancreatic duct, respectively, under the condition of asepsis. On the 4th day, SAP were induced by retrograde infusion of 5% sodium taurocholate into the pancreatic duct in healthy hybrid dogs. All animals were handled in accordance with the guidelines of the Shanghai Experimental Animal Society, China.

During the operation, the dogs were supported by PN. After 24 hours of the induction of SAP, 15 dogs were randomly divided into three groups equally: PN group, EEN group and EIN group. In the PN group, the maintenance energy requirement (MER) of 500－700 kcal was injected according to the ratio of 38 kcal/kg in each dog daily, which contained 15% amino acids, 45% fat, and 40% glucose. The formula was constituted by 8.5% compound amino acids injection (Novamin), 20% fat emulsion injection (Intralipid) and 10% glucose injection in the solution (1800 － 2500 ml), which was the daily fluid replacement volume. In the EEN group, the MER and volume of fluid replacement were both equal to the PN group, in which 200 ml Pepti-2000 variant was supported by duodenum tube within the 24 hours after SAP induced followed by the dosage increasing to 400 ml/d. In the EIN group, 40 mg lactobacillus and 40 mg bifidobacterium were added and supported by the same method applied in the EEN group.

Biochemical study

Pancreatic amylase activities and liver function were determined by automated HITACHI-7150 analyzer (Japan). Blood samples were collected with pyrogen-free tubes from the 15 dogs for endotoxin measurement. Plasma endotoxin concentration was quantitated by the chromogenic limulus amebocyte lysate technique.

Bacteriologic study

Specimens from MLN, livers, and pancreas were collected, weighed and homogenized with two-fold volume of saline under strict sterile conditions. Peritoneal fluid and homogenate were inoculated on blood agar plates, incubated at 37˚C, and examined at 24 hours by a bacteriologist who was unknown about the animal groups. The results of the cultures were regarded as positive if the number of visible colonies exceeded 5. Typical colonies of bacteria were then sent for isolation and identification by the use of a VITEK automicroorganism identification system.

Histology

The animals were killed to obtain the pancreas and terminal ileum on the 7th day after the nutritional supports without pain. The samples were stained with hematoxylin and eosin and graded in a blinded manner. Histopathologic grading and scoring of severity of pancreas were using a scale from 0 to 3 for edema, inflammatory infiltrate, and parenchymal

Table 1. The changes of pancreatic fluid volume in SAP dogs (ml)

Groups	*n*	Before	1st day	3rd day	5th day	7th day
PN	5	137.6±6.37	13.6±1.36*	48.2±2.93*	73.4±5.99*	94.2±8.82*
EEN	5	139.6±7.09	13.6±1.94#	52.8±7.55#	71.4±3.67#	95.2±9.15#
EIN	5	141.2±9.15	14.6±1.02△	53.4±8.19△	74.2±3.54△	92.8±7.63△

*P<0.01, vs before pancreatitis induction in PN group; #P<0.01, vs before pancreatitis induction in EEN group; △P<0.01, vs before pancreatitis induction in EIN group. SAP: severe acute pancreatitis. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonutrition.

necrosis, according to Rongione.[10] The degree of intestinal mucosal damage was assessed by measuring mucosal thickness, villus height, and crypt depth of ileum. Histological evaluations were performed by a single blinded investigator.

Statistical analysis

Descriptive data of continuous variables were expressed as mean ± standard deviation (SD). Bacterial-positive rates were compared using the χ^2 test (Fisher's exact probability). The groups of values were compared by means of nonpaired *t* test. Analysis was completed by the statistical software SPSS 11.0, and P <0.05 was considered statistically significant difference.

RESULTS

Pancreatic fluid volume and pancreatic amylase

Pancreatic fluid volume was significantly decreased at 24 hours after pancreatitis induction in all three pancreatitis groups (P <0.01 vs before pancreatitis induction, Table 1), while no significant difference at any time point among the three groups after pancreatitis induction was found.

The marker for pancreatic amylase was significantly increased on day 1 after pancreatitis induction and reached the peak level on day 3. The amylase level in the EIN group was lower than that in the PN and EEN group, but this decrease was not significant. However, there was a significant difference in the amylase level on days 5 and 7 between the EIN and the other two groups (P <0.05, Fig. 1).

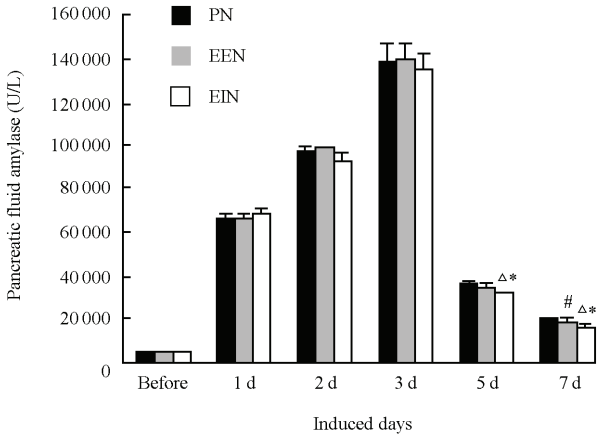

Fig. 1. Changes of the pancreatic fluid amylase levels in different nutritional support groups. There was no significant difference at any time point among the three groups before pancreatitis induction. The marker for pancreatic fluid amylase was significantly increased on the first day after pancreatitis induction and reached the peak level on the 3rd day. *P<0.05, EIN group vs EEN group; #P<0.05, EEN group vs PN group; △P<0.01, EIN group vs PN group. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonutrition.

Histology

Edema, inflammatory infiltrate, necrosis, and total histology scores in pancreas tissue were lower in the EIN group compared with the other two groups, and the differences were significant (Fig. 2). The morphologic studies demonstrated that the ileum mocosa thickness, villus height, and crypt depth were decreased in all SAP dogs. However, the levels of EIN group were less decreased than the other two groups (Fig. 3).

Plasma endotoxin concentration

Compared with before pancreatitis induced, plasma endotoxin levels were significantly increased in all the three pancreatitis groups after pancreatitis induced (P <0.05). However, in contrast to both of PN group and EEN group, endotoxin levels in the EIN group were significantly decreased (P<0.05) on days 5 and 7 (Table 2).

Microbiology

Bacterial cultures using the samples from ascites, MLN, pancreas, and liver in all SAP dogs were almost positive (Table 3). However, in the EIN group, the bacteria positive only occurred in the samples isolated from MLN and pancreas of few dogs. The bacteriologic spectrum was showed that the most bacteria isolated from dogs belonged into three groups, *Escherichia coli (E.coli), Enterococ-cus,* and *Bacillus proteus*, which belonged to enteric flora.

Table 2. The changes of plasma endotoxin levels in SAP dogs (EU/L)

Groups	*n*	Before	1st day	3rd day	5th day	7th day
PN	5	0.52±0.20	1.77±0.19	2.64±0.16	2.51±0.09	2.29±0.12
EEN	5	0.51±0.05	1.79±0.12	2.41±0.11$^{\#}$	2.29±0.16$^{\#}$	2.07±0.07$^{\#}$
EIN	5	0.53±0.03	1.74±0.03	2.23±0.08$^{\triangle *}$	2.06±0.06$^{\triangle *}$	1.84±0.13$^{\triangle *}$

$^{*}P$<0.05, EIN group vs EEN group; $^{\#}P$<0.05, EEN group vs PN group; $^{\triangle}P$<0.01, EIN group vs PN group. SAP: severe acute pancreatitis. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonutrition.

Table 3. The results of bacterial culture from different organs

Groups	*n*	MLN	Pancreas	Livers	Peritoneal fluid	Incidence of translocation (%)
PN	5	5	4	4	3	80
EEN	5	4	2	1	2	45$^{\#}$
EIN	5	1	1	0	0	10$^{\triangle *}$

* P<0.05, EIN group vs EEN group; $^{\#}P$<0.05, EEN group vs PN group; $^{\triangle}P$<0.01, EIN group vs PN group. MLN: mesenteric lymph nodes. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonutrition.

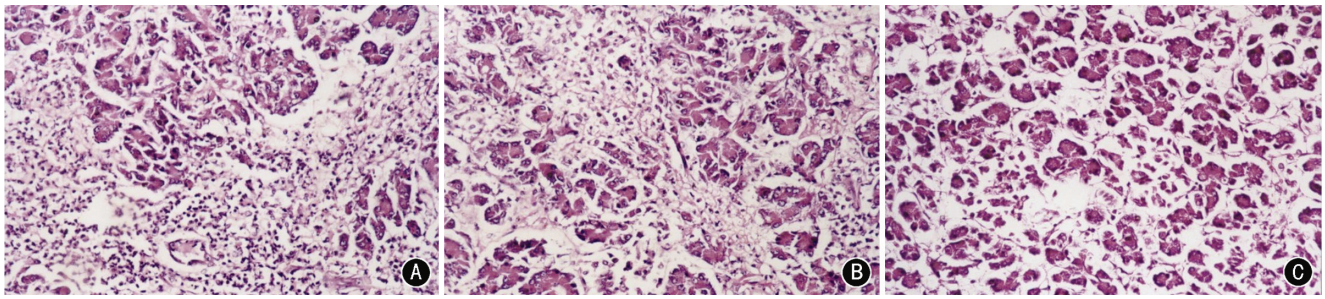

Fig. 2. Pancreatitic sections from SAP dogs. **A:** PN group; **B:** EEN group; **C:** EIN group. EIN group alleviated inflammatory reaction of the pancreatitis (HE stain, original magnification×200). SAP: severe acute pancreatitis. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonutrition.

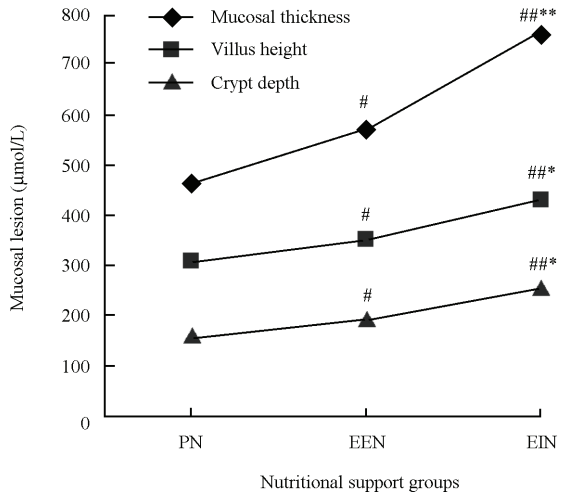

Fig. 3. Changes of mucosal thickness, villus height, and crypt depth of ileum in different nutritional support groups. *P<0.05 and **P<0.01, EIN group vs EEN group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01, EEN group vs PN group. PN: parenteral nutrition. EEN: elemental enteral nutrition. EIN: ecoimmunonu- trition.

Changes of liver function

In the three pancreatitis groups, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased significantly (P<0.05, vs before pancreatitis induction). However, compared with the PN group and the EEN group, EIN group had significantly decreased levels of ALT and AST (P<0.05) on days 5 and 7. The levels of serum albumin were decreased significantly (P<0.05, vs before pancreatitis induction). There were significant differences in the levels of serum albumin on days 5 and 7 between the EIN and the other two groups (P<0.05).

DISCUSSION

The morbidity and mortality of SAP still remain high. Infection of pancreatic necrosis is one of the major causes of death in acute pancreatitis.[11,12] SAP is characterized by various degrees of necrosis of pancreatic parenchyma together with local and systemic complications, such as SIRS and multiple organ failure (MOF). These latter forms of the disease represent a typical hypermetabolic septic model with increased resting energy requirements, and considerable protein catabolism, which leads to

severe malnutrition. As a result, nutritional support in acute pancreatitis should be one of the main therapeutic aims, and nutritional management should depend on the underlying pancreatic disease form.

Contamination of pancreatic necrosis and consequent sepsis is the main causes of late death in SAP.[12] The organisms responsible for secondary pancreatic infection are usually gram negative bacteria of the same type that colonize the gastrointestinal tract. This suggests gut barrier dysfunction, increased intestinal permeability, and subsequent bacterial translocation through the gut wall. Intestinal permeability changes were proven to occur in acute pancreatitis and were directly related to the severity of the disease. A lot of clinical studies have proved that total PN has failed to show any clinical benefits for the patients, as it cannot protect the gut mucosa.[13]

On the contrary, enteral feeding repairs the mucosal damage of fasting and given very early; it preserves epithelial integrity, and bacterial ecology, thereby helping to maintain gut barrier function. It was recently reported that EIN including probiotics and fibre as a new kind of enteral nutrition, which can reduce the rate of infectious complications both in acute pancreatitis and in patients undergoing major abdominal surgery.[14-16] Olah et al[14] published the first randomized study which attempted to identify the role of lactobacillus as a supplement to enteral feeding. They concluded that supplementary *Lactobacillus plantarum* 299 is effective in reducing both of pancreatic sepsis and the number of surgical interventions. Although many studies pointed out that EEN and EIN have many effects on SAP: (1) stimulation of the production of anti-inflammatory cytokines, especially interleukin-10, at the level of the intestinal mucosa,[17] (2) stimulation of gastrointestinal motility,[18] and (3) competitive inhibition of opportunistic pathogens, the experimental studies on the precise mechanism of the effect of EEN, especially EIN，in SAP model of big animals were insufficient.

In this study, with the SAP model of dogs established, many parameters were detected, such as the function of pancreatic exocrine secretion and live, the bacterial culture for the samples from the celiac organs, the endotoxin level in the sera, and the changes of mucosal thickness, villus height, and crypt depth of ileum. The benefits of EIN were observed through many experimental determination compared with PN and EEN. We found that pancreatic fluid volume was decreased after pancreatitis induction, but no significant differences among the three groups was found at any time. This data showed that pancreatic exocrine secretion was significantly injured in SAP dog model. It was observed that EIN did not stimulate pancreatic exocrine secretion as well as PN and EEN. However, pancreatic amylase, edema, inflammatory infiltrate, necrosis, and total pancreas tissues' histology scores were lower in the EIN group, compared with PN and EEN groups. Therefore, early use of EIN is safe and might be effectively alleviate the inflammation in the pancreatic tissue of dogs.

Furthermore, the samples of peritoneal fluis, MLN, pancreas, livers from the SAP dogs in all groups were found positive bacterial culture, but the positive incidence of PN, EEN and EIN groups were 80%, 45% and 10%, respectively. The major species of the detected bacteria were *E.coli*, *Enterococ-cus*, and *Bacillus proteus*, which belonged to enteric flora. Meanwhile, it was detected that plasma endotoxin levels were significantly increased in all SAP dogs compared with before panceatitis induction. However, endotoxin levels in the EIN group were significantly decreased ($P<0.05$) on days 5 and 7 compared with PN and EEN groups. Endotoxin, the lipopoly-saccharide (LPS) present in the gram-negative bacterial wall, plays a key role in the initiation of septic shock caused by gram-negative bacteria and has been given considerable attention as a major contributor to pancreatitis associated multisystemic injury.

Vasilescu et al[19] found that early endotoxin translocation from the gut lumen in the intestinal wall and consequent access of gut-derived endotoxin to the MLN, liver, and lung. Additionally, the morphologic studies in our study found that the ileum mocosa thickness, villus height, and crypt depth were decreased in all experimental SAP dogs, while the levels of EIN group were less decreased than the other two groups. Our data indicated that EIN can maintain the epithelial integrity and mucosal barrier, supple probiotics, decrease the bacterial translocation and endotoxin translocation, inhibit the

enteric bacteria, and reduce the pancreatic infections. Acute pancreatitis can result in remote tissue injury, and liver injury is an important prognostic indicator during acute pancreatitis.[20] We found that EIN also improved the function of livers in SAP dogs. The mechanism might be related to the reduction of celiac organs infection in EIN group, since EIN can decrease the bacterial translocation and endotoxin translocation.

In conclusion, EIN has beneficial effects on maintaining the integrity of intestinal mucosal barrier and reducing the incidence of bacterial translocation in SAP dogs. Early EIN is safe because it did not irritate exocrine secretion function of pancreas, and alleviated liver injury of pancreatitis. This study provided an experimental base for clinical treatment of SAP patients with EIN.

REFERENCES

1. Branum G，Galloway J, Hirchowitz W, Fendley M, Hunter J. Pancreatic necrosis: results of necrosectomy, packing, and ultimate closure over drains. Ann Surg 1998; 227:870-877.
2. Bradley EL. A fifteen-year experience with open drainage for infected pancreatic necrosis. Surg Gynecol Obstet 1993; 177:215-222.
3. Miller BJ, Henderson A, Strong RW, Fielding GA, DiMarco AM, O'Loughlin BS. Necrotizing pancreatitis: operating for life. World J Surg 1994; 8:906-911.
4. Renner IG, Savage WT 3rd, Pantoja JL, Renner VJ. Death due to acute pancreatitis: A retrospective analysis of 405 autopsy cases. Dig Dis Sci 1985; 30:1005-1018.
5. Foitzik T, Mithofer K, Ferraro MJ, Fernandez-del Castillo C, Lewandrowski KB, Rattner DW, et al. Time course of bacterial infection of the pancreas and its relation to disease severity in a rodent model of acute necrotizing pancreatitis. Ann Surg 1994; 220:193-198.
6. Kazantsev GB, Hecht DW, Rao R, Fedorak IJ, Gattuso P, Thompson K, et al. Plasmid labeling confirms bacterial translocation in pancreatitis. Am J Surg 1994; 167:201-206.
7. Foitzik T, Fernandez-del Castillo C, Ferraro MJ, Mithofer K, Rattner DW, Warshaw AL. Pathogenesis and prevention of early pancreatic infection in experimental acute necrotizing pancreatitis. Ann Surg 1995; 222:179-185.
8. Andersson R, Wang X, Sun Z, Deng X, Soltesz V, Ihse I. Effect of a platelet-activating factor antagonist on pancreatitis-associated gut barrier dysfunction in rats. Pancreas 1998; 17:107-119.
9. Osman NE, Westrom B, Karlsson B. Serosal but not mucosal endotoxin exposure increases intestinal permeability *in vitro* in the rat. Scand J Gastroenterol 1998; 33:1170-1174.
10. Rongione AJ, Kusske AM, Ashley SW, Reber HA, McFadden DW. Interleukin 10 reduces the severity of acute pancreatitis in rats. Gastroenterology 1997; 112:960-967.
11. Baron TH, Morgan DE. Acute necrotizing pancreatitis. N Engl J Med 1999; 340:1412-1417.
12. Buchler MW, Gloor B, Muller CA, Friess H, Seiler CA, Uhl W. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232:619-626.
13. Sax HC, Warner BW, Talamini MA, Hamilton FN, Bell RH Jr, Fischer JE, et al. Early total parenteral nutrition in acute pancreatitis: lack of beneficial effects. Am J Surg 1987; 153:117-124.
14. 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.
15. Rayes N, Seehofer D, Hansen S, Boucsein K, Muller AR, Serke S, et al. Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. Transplantation 2002; 74:123-127.
16. Rayes N, Hansen S, Seehofer D, Muller AR, Serke S, Bengmark S, et al. Early enteral supply of fiber and *Lactobacilli* versus conventional nutrition: a controlled trial in patients with major abdominal surgery. Nutrition 2002; 18:609-615.
17. Niers LEM, Rijkers GT, Timmerman HM, van Bleek GM, van Vden NOP, Kimpen JLL, et al. A central immunoregulatory role for IL-10 in the suppression of type 2 T helper cell cytokines by probiotic bacteria. In: Proceedings of the XXIII EAACI Congress. Amsterdam; 2004.
18. Ouwehand AC, Lagstrom H, Suomalainen T, Salminen S. Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly. Ann Nutr Metab 2002; 46:159-162.
19. Vasilescu C, Herlea V, Buttenschoen K, Beger HG. Endotoxin translocation in two models of experimental acute pancreatitis. J Cell Mol Med 2003; 7:417-424.
20. Armbruster C, Kriwanek S. Multicenter audit of death from acute pancreatitis. Br J Surg 1994;81:1697.

(Received September 26, 2005)
Edited by LIU Dong-yun