Early intrajejunal nutrition: bacterial translocation and gut barrier function of severe acute pancreatitis in dogs

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Objective: To evaluate the effect of early intrajejunal nutrition in attenuating bacterial and /or endotoxin translocation and improving gut barrier function of severe acute pancreatitis (SAP) in dogs.

Methods: 15 dogs were divided into parenteral nutrition (PN) group(7 dogs) and early intrajejunal nutrition (EIN) group(8). EIN was delivered nutrients via a needle jejunostomy catheter feeding at 48 h after operation. SAP model was induced by injecting 1 ml/kg of combined solution of 5% sodium taurocholate and 8000 - 10000 BAEE units trypsin/ml into the pancreas via the pancreatic duct. Systemic blood samples were obtained before and 1, 3, 5, 7 d following SAP, and cultured by aerobic as well as anaerobic bacterial growth. Systemic plasma and portal vein endotoxin levels were quantified by the chromogenic limulus amebocyte lysate (LAL) technique. Portal vein blood and specimens of tissue from the mesenteriolum and mesocolon lymph nodes, lung, pulmonary portal lymph nodes, pancreatitis tissue and periopancreas tissue were adopted before the experiment was finished. Aliquots of the homogenata were cultured as blood mentioned above to determine the magnitude of the bacteria. DNA, protein and the villi, the thickness of mucosa, and the whole bowel wall of the ileum and transverse colon were measured.

Results: The study showed that the levels of systemic plasma endotoxin and the magnitude of bacterial translocation to the portal and systemic blood and distant organ were reduced significantly in the EIN group as compared with the TPN group. The contents of protein and DNA; the height of villi, the thickness of mucosa and whole bowel wall of the ileum and transverse colon in the EIN group were higher than those in the PN group.

Conclusion: Our results suggested that EIN is safe and effective to be adopted by intrajejunal delivery of nutrients in SAP, decreases the occurrence of gut bacterial translocation, and improves the gut barrier function.

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Key words: early intrajejunal nutrition; parenteral nutrition; severe acute pancreatitis; bacterial translocation; barrier function

Introduction

Infectious complications account for more than 80% of mortality in human severe acute pancreatitis(SAP) .[1] Although the pathogenesis of infection in SAP is unclear, it is known that the microorganisms that cause pancreatic infection and sepsis in patients with SAP are generally common enteric bacteria. This suggests that these bacteria may originate from the gastrointestinal tract. In fact, recent studies on animals in SAP have demonstrated that there is a loss of gut mucosal integrity with subsequent bacterial and /or endotoxin translocation(BET) from the gut lumen to other organs.[2] Extensive studies have demonstrated some major mechanisms that promote BET and secondary infections. These are disruption of the indigenous intestinal microflora, damage to the intestinal mucosa causing increased permeability, and impairment of host immunity. Further, compared with total parenteral nutrition (TPN), enteral nutrition (EN) attenuates cecal bacterial overgrowth, maintains gut mucosal integrity and permeability, and maintains immune responsiveness of the host.[3,4] Since BET is thought to be deleterious to the host, the route of nutrition can modulate BET. Therefore the purpose of this study

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Table 1. Changes of plasma endotoxin($\bar{x} \pm s$, EU/L)

Groups	P0	P1	P3	P5	P7
PN	0.56 ± 0.13	1.88 ± 0.47*	2.30 ± 0.84*	3.61 ± 0.92*	2.90 ± 0.64*
EIN	0.56 ± 0.22	1.99 ± 0.68*	2.52 ± 0.91*	2.43 ± 0.65*#	2.08 ± 0.51*#

* $P < 0.05$ vs P0; # $P < 0.01$ vs PN group.

Table 2. The content of blood and tissues (10^5CFU/ml or/g)

	PN group	EIN group
Systemic blood	9.45 ± 2.54	4.04 ± 0.12*
Portal vein blood	14.29 ± 2.65	1.23 ± 0.30*
Lung tissue	22.34 ± 10.02	8.09 ± 3.09*
PPLN	28.91 ± 13.27	9.88 ± 5.38*
Pancreas	55.37 ± 33.72	28.23 ± 22.17*
MLN	41.45 ± 21.17	8.54 ± 3.89*

* $P < 0.01$ vs PN group; PPLN: pulmonary portal lymph nodes; MLN: mesocolon lymph nodes

was to compare the effect of TPN and EIN in attenuating gut bacterial translocation and improving gut barrier function of severe acute pancreatitis in dogs. [5]

Methods

Animal model

15 dogs weighing 10–12 kg were allowed ad libitum intake of water. After 12–14 hours fasting, all dogs were induced anesthesia by intramuscular injection ketamine 10 ml/kg, and then were dauernarcosis by intravenous injection of sodium pentobarbital 30 mg/kg. SAP model was induced by injecting 1 mg/kg of combined solution of 5% sodium taurocholate and 8000–10000 BAEF units trypin/ml into the pancreas via the pancreatic duct with a pressure of 30 mmHg during abdominal operation. Ten minutes later, the catheter was out of the pancreatic duct, and the duodenum was closed. A needle catheter via jejunostomy was placed far away the Treitz's ligament 40–50 cm. The neck and interscapular region of the dogs were shaved and prepared in a sterile manner for catheterization. A silastic catheter (1.0 mm inner diameter, 1.5 mm outer diameter) was inserted through the external jugular vein to reach the superior vena cava. The catheter was tunneled subcutaneously to the midscapular region.

Experimental groups and nutritional solution

Dogs were allocated randomly into two groups: PN group (7 dogs) and EIN group (8). The two groups were isonitrogenous and isocaloric. The PN solutions were consisted of 7% vamin (SSPC, 9.4 g/1000ml), 20% intralipid (SSPC), and 50% glucose. Non-protein calories was 50kC(209.2 kJ/kg) and nitrogen was 0.3g · kg^{-1} · d^{-1}. The total volume of solution infused was 70 ml. kg^{-1} · d^{-1} Energy index was supported with glucose and fat emulsion was 1: 1. Multivitamins and electrolytes were also included in TPN solutions. The liquid was infused by 250 ml/kg during the operation and 8 h after operation and then infused with 125±25 ml/kg. The nutrient solution was infused at a constant infusion rate by a pump (10–15 ml/h).

The EIN solution was Nutrison(Nutricia). 24h after SAP, the jejunum through jejunostomy catheter was infused 250 ml normal saline (NS); 48 h after SAP, 250 ml Nutrison and 500 ml NS were infused; at the third day, 500ml Nutrison and 500 ml NS were infused and the duration was 4 days. The infusion rate was controlled by microcomputer-pump (Nutricia).

General observations

Central vein pressure (CVP), pulse, respiration, artery blood gas, WBC, and 24-hour urine volume were determined every day. Systemic blood sample before and 1, 3, 5, 7 d following SAP and the portal vein blood before the experiment finished were adopted. Systemic plasma endotoxin was quantified by the chromogenic limulus amebocyte lysate(LAL) technique. The bacterial volumes in the systemic blood and portal vein were tested by aerobic as well as anaerobic bacterial

Table 3. Ileum and transverse colon protein and DNA ($\bar{x} \pm s$, mg/g)

Groups	Ileum		Transverse colon	
	Protein	DNA	Protein	DNA
PN	141.62 ± 37.90	10.20 ± 3.48	183.67 ± 65.33	17.82 ± 3.68
EIN	161.79 ± 36.75*	16.79 ± 2.14*	222.44 ± 78.21*	29.92 ± 4.21*

* $P < 0.01$ vs PN group.

Table 4. Pathological changes in intestinal and transverse colon ($\bar{x} \pm s$, μm)

Groups	Ileum			Transverse colon		
	Total layer	Mucosa	Villi	Total layer	Mucosa	Villi
PN	605.77 ± 57.63	519.60 ± 69.31	322.57 ± 63.09	1034.27 ± 243.66	934.54 ± 173.22	677.42 ± 147.57
EIN	715.26 ± 87.43*	674.55 ± 80.02*	471.50 ± 76.71*	1227.63 ± 167.76	1179.33 ± 181.34	803.29 ± 108.43*

P <0.01 vs PN group.

culture.

Gut barrier function measurement

When the experiment was finished, the dogs were placed on a sterile field, the chest and the ventral abdomen were cleaned with 70% isopropyl alcohol and opened with sterile instruments. Lung tissue, pulmonary portal lymph nodes and mesenteric lymph nodes (MLNs), pancreatitic tissue and periopancreatic necrosis tissue were isolated, weighed and put into sterilized glass tissue grinders. After manual grinding, 200 μl of the homogenate was plated onto general agar plates containing 5% sheep's blood. Aliquots of the homogenata were incubated as the blood mentioned above. Following of excision several tissues, 20 cm segments of the ileum and transverse colon were then rapidly excised, and 10 cm segments were opened along the antimensentric border, and the mucosa was blotted with tissue paper and then weighed. All samples were immediately frozen at −70℃. The mucosae of the ileum and transverse colon segments were scraped off from the specimens with a glass slide and homogenized in a blender at 30,000 rpm for 30 seconds. The homogenates were centrifuged for 15 minutes at 3000 g and the supernatants assayed spectrophotometrically for protein, DNA.[5] Values were expressed per gram of gut tissue. Another 10 cm segments of the ileum and transverse colon were cut, pinned flat onto wax, and fixed in Boin's solution. Tissues were subsequently embedded in paraffin, and 5 μm sections were taken and stained with hematoxylin and eosin. All slides were coded and morphologically interpreted in a "blind" fashion. The villi, the thickness of mucosa, and the whole bowel wall of the ileum and transverse colon were determined.

Statistical analysis

Bacterial translocation was analyzed using x^2. The specimen measures were expressed as $\bar{x} \pm s$ of the sample and comparisons between the two groups was made using one way analysis of variance. P value below 0.05 was considered statistically significant.

Results

General observation

PN group and EIN group did not differ in CVP, pulse, respiration, artery blood gas, WBC, and 24-hour urine volume. Their differences were not statistically significant.

Systemic endotoxin content and distal organ bacterial translocation

There was a significant increase of endotoxin in the PN group. A decrease of endotoxin was noted in the EIN group as compared with the PN group at 5, 7 day ($P<0.05$) (Table 1). The bacterial volume of the distal organs such as lung,

pulmonary portal lymph nodes and MLNs, pancreatitic tissue and periopancreatic necrosis tissue in the EIN group was lower than in the PN group (Table 2).

Changes of DNA, protein and histologic observations

The mucosal protein and DNA contents of the ileum and transverse colon in the EIN group were markedly improved as compared with the PN group($P< 0.01$) (Table 3). The bowel wall thickness, mucosal thickness, and villus height in the EIN group were significantly improved as compared with the PN group($P< 0.01$) (Table 4).

Discussion

In this study, we determined whether early intrajejunal nutrition(EIN) is safely and effectively used in SAP dogs. We found that the changes of CVP, WBC and artery gas were not significantly different between the two groups during the entire experiment. Our primary study showed that EIN did not cause pancreatitis deterioration and stimulate pancreas tissue secretion.

Kalfarentzos et al[6] reported that there were significantly fewer complications with EIN than with TPN. A similar reduction in septic complications has also been observed in other clinical situations including severe trauma and burns. Although the exact mechanism of this reduction is unknown, experimental and clinical data indicate that early enteral nutrition is capable of maintaining the integrity and function of the intestinal mucosa. On the contrary, the administration of TPN may lead to malfunction of the intestinal mucosal barrier which may promote gut origin sepsis. In the present study, all bacterial species isolated from MLNs and distant organs were common enteric bacteria, suggesting a process whereby intestinal microflora relocated to this site. Our results also suggested serum endotoxin in the EIN group was lower than that in the PN group at 5, 7 days, and the magnitude of bacterial translocation to the portal, plasma and distant organ (mesenteriolum, mesocolon lymph nodes, lung, pulmonary portal lymph nodes and pancreas) in the EIN group was lower than that in the PN group. These results suggested that enteral feeding (1) induced more rapid clearance of bacteria in MLNs and distant organs; (2) prevented continuing translocation from the intestine to MLNs and distant organs; and/or (3) did not permit a persistent infection in MLNs and distant organs following temporary translocation from the intestine to MLNs and distant organs. In fact, in human SAP, the development of a bacterial infection has emerged as a major determinant affecting survival in patients with necrotizing pancreatitis surviving longer than 1 week, although bacterial contamination in pancreatic necrosis could be detected as early as the first week.

Enteral feeding has several noteworthy advantages over TPN other than for the prevention of bacterial and endotoxin translocation(BET). In a number of disease states, such as SAP, burns, trauma, and sepsis, EN decreases risk of nosocomial infection, multiple-organ failure, and length of hospitalization, and lowers cost when compared with TPN.[7,8] These advantages of EN might be due to restoration of defective macrophage function, attenuation of both counterregulatory hormone and enhanced proinflammatory cytokine responses, down-regulation of coagulation, and normalization of the tumor necrosis factor receptor systems. Recent evidences in animal and human studies suggested that elemental or semi-elemental small peptide formulae are well tolerated, and efficiently absorbed in the gut lumen,with little or no pancreatic enzyme secretion. Furthermore, compared with TPN, early EN is maintained in increased intestinal structure, which is probably implicated in increased enterocyte proliferation, suggesting that early EN can potentially maintain gut integrity during SAP. In addition, it is frequently stated that TPN leads to mucosal atrophy and the assumption is made that this predisposes to bacterial translocation which may account for increased septic morbidity. In our study, the EIN group increased mucosal protein, DNA contents and villus height, mucosal thickness and bowel

thickness of the ileum and transverse colon. Hence, EIN can improve the gut immune system, restore normal gut structure and microflora, and aid the mucosa. In summary, jejunal administration of nutrients started at 48 h was as well-tolerated, feasible and desirable as TPN in the management of diseases asscociated with SAP. When failed to reveal any detrimental effect on the clinical or pathologic course of SAP, however, it is effective to maintain gut integrity and reduce bacterial and endotoxin translocation with EIN as compared with TPN.

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