Enteral Nutrition Prevents Bacterial Translocation but Does Not Improve Survival During Acute Pancreatitis

Joji Kotani, MD; Makoto Usami, MD; Hideaki Nomura, MD; Atsunori Iso, MD; Hiroshi Kasahara, MD; Yoshikazu Kuroda, MD; Harumasa Oyanagi, MD; Yoichi Saitoh, MD

Objective: To evaluate the effect of enteral nutrition (EN) in attenuating bacterial and/or endotoxin translocation, maintaining immune responsiveness, and improving outcome in early acute pancreatitis (AP) in Wistar male rats.

Design: Acute pancreatitis was induced in rats receiving total parenteral nutrition (TPN) (AP/TPN group) (n=34) and EN (AP/EN group) (n=35) by pressure injection of 1% deoxycholate into the biliopancreatic duct (0.6 mg/kg of body weight). Rats in the sham/TPN and sham/EN groups (n=10 each) underwent laparotomy without induction of AP. Catheters for TPN and EN were placed into the external jugular vein and jejunum, respectively. Rats were infused with Ringer lactate solution for 48 hours followed by TPN in the AP/TPN and sham/TPN groups, and EN in the AP/EN and sham/EN groups until day 7. The fluid volume and energy (calories) intake were similar in all groups.

Setting: Medical school research laboratory.

Main Outcome Measures: Survival, blood endotoxin level, villus height, 5-bromo-2'-deoxyuridine (BrdU) uptake in the jejunum and ileum, bacterial culture of mesenteric lymph nodes, and CD4/CD8 ratio of T cells in mesenteric lymph nodes, spleen, and peripheral blood.

Results: There was no difference in survival and pancreatic healing between the AP/TPN and AP/EN groups. Colony-forming units of the mesenteric lymph nodes and the endotoxin level were significantly lower in the AP/EN group than in the AP/TPN group (P<.05). Villus height and BrdU intake was significantly higher in the AP/EN group than in the AP/TPN group (P<.05). The CD4/CD8 ratio of T cells in spleen and peripheral blood was higher in the AP/EN group than in the AP/TPN group (P<.05), whereas there was no difference in mesenteric lymph nodes.

Conclusions: Jejunal administration of EN is well tolerated in early AP, maintains immune responsiveness and gut integrity, and reduces bacterial and/or endotoxin translocation. However, compared with TPN, EN does not improve outcome. These results suggest that factors other than bacterial and/or endotoxin translocation may be responsible for mortality in this rat model of early AP. However, additional studies of both early bacterial and/or endotoxin translocation and late assessment of outcome are indicated.


Infectious complications account for more than 80% of mortality in human acute pancreatitis (AP).1,2 Although the pathogenesis of infection in AP is unclear, it is known that the microorganisms that cause pancreatic infection and sepsis in patients with AP are generally common enteric bacteria.2,3 This suggests that these bacteria may originate from the gastrointestinal tract. In fact, recent work on animals in AP has 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.3,4 However, few reports have demonstrated that BET per se has deleterious consequences for the host in experimental models of AP.

Extensive studies have demonstrated 3 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.6-10 Furthermore, compared with total parenteral nutrition (TPN), enteral nutrition (EN) attenuates cecal bacterial overgrowth,11 maintains gut mucosal integrity12 and permeability,13 and maintains immune responsiveness of the host.8,14,15 Since BET is thought to be deleterious to the host and the route of nutrition can modulate BET, the purpose of this study was to compare the effect of TPN and EN on gut and immune system integrity and outcome in AP in Wistar male rats.
MATERIALS AND METHODS

ANIMALS

The experiments conducted were approved by the Kobe (Japan) University of Medicine Animal Care and Use Committee. All animals were maintained according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals and handled under the Guideline for Animal Experiments of the Kobe University School of Medicine. Animals were anesthetized with ether (Wako Pure Chemical Industries Ltd, Osaka, Japan). Laparotomy was performed though a midline incision, and the abdomen was closed in 2 layers. All procedures were performed using aseptic techniques.

EXPERIMENTAL GROUPS

Eighty-nine adult male Wistar rats weighing 230 to 250 g were studied in 4 groups: 34 rats underwent laparotomy with induction of AP and received TPN (AP/TPN group) and 35 rats underwent laparotomy with induction of AP and received EN (AP/EN group). The remaining 20 rats underwent laparotomy without induction of AP and received TPN (sham/TPN group; n=10) or EN (sham/EN group; n=10).

INDUCTION OF AP

Acute pancreatitis was induced in the AP/TPN and AP/EN groups as follows. A microaneurysm clip was placed on the biliary duct close to the liver. An incision was made, and a sterilized polyethylene catheter (inner diameter, 0.28 mm; outer diameter, 0.38 mm; Becton Dickinson and Co, Franklin Lakes, NJ) was inserted into the common biliopancreatic duct, and fixed in all animals. The end of the catheter was taken above and below the incision by ligation with 3-0 silk suture. Then, 0.6 mL/kg of 1% deoxycholate (Wako Pure Chemical Industries Ltd, Osaka, Japan) was injected into the common biliopancreatic duct, introduced into the duodenum through the ampulla of Vater, and then the end of the catheter was taken to the jejunum via a gastrostomy for EN using standard techniques. The feeding catheters were tunneled subcutaneously and brought out in the suprascapular region. The catheters were passed through a stainless protective coil and attached to a swivel apparatus allowing mobility. Sterile polyethylene tubing was connected from the swivel apparatus through a microtube pump (Tokyo Rikakikai Co, Tokyo) to the sterile nutritional source.

NUTRITIONAL SUPPORT

All animals were placed in metabolic cages, and infused intravenously with Ringer lactate solution at the rate of 400 mL/kg per day for the first 24 hours, and then 300 mL/kg for the next 24 hours. Then, the intravenously fed animals (AP/TPN and sham/TPN groups) or intrajejunally fed animals (AP/EN and sham/EN groups) were infused with a commercially available TPN solution (TAB; Otsuka Pharmacy Co, Osaka, Japan) via the TPN tube or with a commercially available EN solution (Enterued; Terumo Co, Tokyo) via the EN tube, respectively, at the rate of 264 mL/kg per day by day 7.

The TPN solution (glucose, 111.1 g/L; xylitol, 27.8 g/L; fructose, 55.6 g/L; amino acids, 33.3 g/L) was diluted to 1.2 on day 3 or 1.13 on day 4 with water. The EN solution (dextrin, 161.3 g/L; protein hydrolysate, 41.2 g/L; fat, 11.2 g/L; pH 6.5-7.5; osmolar pressure, 510-550 mOsm/kg) was diluted similarly with isotonic sodium chloride solution. On days 5 to 7, undiluted solutions were given for both TPN and EN. The administered energy intake per day was 502 kJ/kg (120 kcal/kg) on day 3, 753 kJ/kg (180 kcal/kg) on day 4, and 1004 kJ/kg (240 kcal/kg) on days 5 to 7 in all animals.

LABORATORY TESTS

All animals were reanesthetized with ether inhalation on day 7. After careful skin disinfection and draping, the abdominal cavity was opened through a wide midline incision, and the abdomen was closed in 2 layers. All procedures were performed using aseptic techniques.

RESULTS

SURVIVAL

The survival rate was 85% (29/34) for the AP/TPN group and 74% (26/35) for the AP/EN group (Figure 1). There was no statistically significant difference in survival between these 2 groups. No rats died in the sham groups.

PANCREATIC PATHOLOGY

At the killing of the rats on day 7, the pancreas in the AP/TPN and AP/EN groups appeared slightly enlarged and swollen without visible areas of parenchymal or fat necrosis. No ascites were found in any rats in either group. Histological examination revealed a mixed finding of pancreatic collapse and healing fibrosis with mononuclear leukocytic infiltration of the stroma in both the AP/TPN group and the AP/EN group (Figure 2). There was no difference in pancreatic healing between these 2 groups. No ascites were found at the time the rats were killed, and the pancreas was grossly and histologically normal in the 2 sham groups.

TOTAL PROTEIN, ALBUMIN, AMYLASE, AND LIPASE LEVELS

Serum amylase and lipase levels were significantly lower in the AP/EN group than in the AP/TPN group (Table 1). There was no difference in serum total protein and albumin levels between the 2 AP groups, although they tended to be lower in the AP/EN group compared with the AP/TPN group.

©1999 American Medical Association. All rights reserved.
The pancreas was excised, fixed in 10% formalin, and stained with hematoxylin-eosin for histological evaluation. A 5-cm segment of the ileum, beginning 5 cm before the ileocecal junction, was taken, fixed in 70% ethanola1940 (Gibco, Ground Island, NY), and the heparinized peritoneally 1 hour before the AP-induced rats were humanely killed. At the time the animals were killed, a 5-cm segment of jejunum, beginning 5 cm beyond the pyloroduodenal junction, was taken, fixed in 70% ethanol, stained with rabbit anti-BrdU antibody (Becton Dickinson and Co, Sandy, Utah) followed by peroxidase conjugate anti-rabbit IgG (Dako Co, Carpinteria, Calif). The number of BrdU-positive epithelial cells from the bottom of the crypt to the top of the villus was measured in 200 villi in each sample, and the mean value was evaluated as BrdU uptake per crypt in the jejunum of each animal.

**BrdU UPTAKE BY EPITHELIUM IN JEJUNUM**

A total of 20 mg/kg of 5-bromo-2′-deoxyuridine (BrdU) (Sigma Chemical Co, St Louis, Mo) was injected intraperitoneally 1 hour before the AP-fed rats were humanely killed. At the time the animals were killed, a 5-cm segment of the jejunum, beginning 5 cm beyond the pyloroduodenal junction, was taken, fixed in 70% ethanol, stained with rabbit anti-BrdU antibody (Becton Dickinson and Co, Sandy, Utah) followed by peroxidase conjugate anti-rabbit IgG (Dako Co, Carpinteria, Calif). The number of BrdU-positive epithelial cells from the bottom of the crypt to the top of the villus was measured in 200 villi in each sample, and the mean value was evaluated as BrdU uptake per crypt in the jejunum of each animal.

**ILEUM VILLUS HEIGHT AND JEJUNUM**

**BrdU INTAKE**

Ileum villus height was significantly higher in the EN groups compared with the TPN groups for both AP and sham rats (Table 1). Furthermore, villus height was not different between AP and sham rats in either EN or TPN group. Jejunum BrdU intake, an index of enterocyte proliferation, was significantly higher in the AP/EN group than in the AP/TPN group.

**CD4/CD8 RATIO**

In both sham and AP rats, the CD4/CD8 ratio of T cells of spleen cells was significantly higher in the EN groups than in the TPN groups (Table 1). Although the CD4/CD8 ratio was lower in the AP/TPN group than in the sham/TPN group, there was no significant difference between AP/EN and sham/EN groups. Similar results for this ratio were seen in MLN and peripheral blood, except for MLN of rats in the AP/TPN group, which had a mean ratio as high as that in MLN of the AP/EN group.

**MAGNITUDE OF BACTERIAL TRANSLOCATION IN MLN**

Intravenous feeding induced bacterial translocation to MLN in the sham/TPN group, whereas no positive culture was found in the sham/EN group (Figure 3, top). Furthermore, AP induced a significantly greater bacterial translocation to MLN. However, the magnitude was
significantly lower in the AP/EN group than in the AP/TPN group, indicating that enteral feeding prevented AP-induced bacterial translocation compared with intravenous feeding.

**BACTERIAL SPECIES ISOLATED FROM MLN**

Species of bacteria isolated from MLN were not different between the AP/TPN and AP/EN groups. Bacterial species were all common enteric bacteria and mostly gram-negative bacteria (Table 2).

**PLASMA ENDOTOXIN LEVEL**

The plasma endotoxin level was significantly higher in the AP/TPN group than in the AP/EN group, in which it was as low as both sham groups (Figure 3, bottom). In other words, EN prevented AP-induced endotoxin translocation from the gut to the systemic circulation.

**COMMENT**

Recent work in animal models has demonstrated that there is a loss of gut mucosal integrity with subsequent BET from the gut lumen to other organs in AP. Likewise, in this study, the overall incidence of AP-induced translocation of enteric bacteria to the MLN was 100% in both AP groups, and blood endotoxin levels were significantly higher in the AP/TPN group than in the control rats (sham groups). However, few reports have demonstrated the contribution of BET to deleterious consequences for the host in experimental AP. Isaji et al reported experimental data showing the effect of oral antibiotics on the prognosis and incidence of infections in diet-induced hemorrhagic AP of mice. The data suggested that reduction of the intestinal flora inhibits secondary infection caused by bacterial translocation and improves survival. However, as the authors pointed out, there is the pos-

![Figure 1. Kaplan-Meier plots of overall survival in rats after onset of acute pancreatitis (AP) followed by total parenteral nutrition (TPN) (AP/TPN group) or enteral nutrition (EN) (AP/EN group), and sham operation followed by TPN (sham/TPN group) or EN (sham/EN group).](image1)

![Figure 2. Histological findings of pancreas in a rat induced with acute pancreatitis (AP) followed by total parenteral nutrition (left) and a rat induced with AP followed by enteral nutrition (right) on day 7 after AP induction (hematoxylin-eosin, magnification ×200).](image2)
sibility that oral antibiotics might improve the survival by diminishing the severity of injury to the pancreas as a result of decreased absorption of ethionine rather than reduced translocation of bacteria from the gut.

In the present study, all bacterial species isolated from MLN were common enteric bacteria, suggesting a process whereby intestinal microflora relocated to this site. In addition, the lower magnitude of recovery of bacteria from the MLN in the AP/EN group compared with the AP/TPN group on day 7 suggests that enteral feeding (1) induced more rapid clearance of bacteria in MLN, (2) prevented continuing translocation from the intestine to MLN, and/or (3) did not permit a persistent infection in MLN following temporary translocation from the intestine to MLN.\textsuperscript{10} Despite the significantly lower magnitude of bacterial translocation and significantly lower blood endotoxin levels on day 7 in the AP/EN group than in the AP/TPN group, the survival rate was not different between these 2 groups. These results suggest that the presence of live bacteria in MLN does not play a decisive role for a deleterious outcome at least up to day 7 in this AP rat model. However, it is still possible that BET early after onset of AP may be responsible for the similar mortality in both groups because it is assumed that BET occurred to the same extent in both groups before EN or TPN was initiated. Furthermore, it is also possible that BET may influence the subsequent outcome after day 7. In fact, in human AP, the development of a bacterial infection has emerged as a major determinant affecting survival in patients with necrotizing pancreatitis surviving longer than 1 week,\textsuperscript{3} although bacterial contamination in pancreatic necrosis could be detected as early as the first week.\textsuperscript{3}

In humans, the most severe complication of AP is pancreatic infection.\textsuperscript{3} The finding that the microorganisms causing pancreatic infection are common enteric bacteria implies that the bacterial translocation from intestinal tract to pancreas may play a role in the pathogenesis of pancreatitis-induced sepsis. However, consistent pancreatic infection is rarely observed in experimental AP models in animals.\textsuperscript{4,5,18,20} This was confirmed in the pres-
ent study in that no organisms were found in the pancreas of rats in the AP/TPN or AP/EN groups on day 7 (data not shown). Because pancreatic infection is related to the extent of pancreatic necrosis in human and animal AP, one explanation for the lack of infection in this model of AP is that pancreatic necrosis was seen only after 24 hours (data not shown), but not seen on day 7 in either of the AP groups. One may thus expect pancreatitis with necrosis in a more severe and/or sustained model of pancreatitis.

Enteral feedings reportedly have several noteworthy advantages over TPN other than for the prevention of BET. In a number of disease states, such as AP, burns, trauma, and sepsis, EN decreases risk of nosocomial infection, multiple-organ failure, and length of hospitalization, and lowers cost and improves survival in patients with sepsis and gut injury, compared with TPN. These advantages of EN might be due to restoration of defective mucus barrier function, attenuation of both the counterregulatory hormone and enhanced proinflammatory cytokine responses, down-regulation of coagulation, and normalization of the tumor necrosis factor receptor systems. Recent evidence in both animal and human studies suggests that elemental or semi-elemental small peptide formulas are well tolerated, and efficiently absorbed in the gut lumen with little or no pancreatic enzyme secretion. Previous studies have shown that jejunal feeding results in negligible increases in pancreatic enzyme, bicarbonate, and volume output from the pancreas.

In summary, jejunal administration of EN was well tolerated in early AP, maintained immune responsiveness and gut integrity, and reduced BET. However, compared with TPN, EN did not improve outcome. These results suggest that BET in MLN is not associated with deleterious consequences for the host during the early period of experimental AP in rats. Finally, factors other than BET may be responsible for mortality in the rat AP model.

This study was supported by a research grant from Otsuka Pharmaceutical Factory Inc, Naruto, Tokushima, Japan, and Alpha Therapeutic Corporation, Los Angeles, Calif.


We thank Steve E. Calvano, PhD, and Edward Lin, DO, for help with preparation of the manuscript. We acknowledge the help of Syohiro Kinoshita, BS, Hironori Yokoyama, PhD, Yoshiyuki Shinagawa, BS, Seiichiro Kano, BS, and Keiichi Okamoto, BS, in the development of this project.

Reprints: Joji Kotani, MD, Department of Surgery, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, Surgical Science, CAB, Room 7076, 125 Patterson St, New Brunswick, NJ 08903-0019 (e-mail: kotani4@lbm.net).

REFERENCES


Theors use double-spiral CT scanning? We currently have an NIH [National Institutes of Health]–funded study whereby we are comparing CT scanning, laparoscopic ultrasound, and laparoscopy for staging these patients which we are in the process of completing. We found that the diagnostic accuracy for staging vascular involvement with a state of the art, double-spiral CT is about 85% to 90%. In this study, only 40% of the patients were accurately staged with CT. So, clearly the problem here was not the value of EUS as much as the fact that the quality of CT scanning that was utilized in this study was poor. Endoscopic ultrasound is very much operator dependent. Our experience at USC [University of Southern California] has found that in about one third of the patients, the endoscopist is unable to make the determination of vascular involvement. There are 2 critical factors for resectability of pancreatic cancer: vascular involvement and metastatic disease. Very little data are present on this in this paper and correlated with accuracy of EUS. There are 2 specific questions that I would like the authors to address. What type of CT scanning technique was used in this study? How accurate was ultrasound assessment for identifying vascular involvement? Did the authors use intraoperative laparoscopy to rule out small metastatic deposits?

Richard Prinz, MD, Chicago: I also wonder about the need for preoperative biopsy in patients with resectable pancreatic lesions. My question concerns the fact that more than half of the patients in this study did not undergo operation. I would like to know the reasons why these patients were not explored, and whether patients with resectable lesions were denied pancreaticoduodenectomy?

Steven Stain, MD, Los Angeles: Did you actually compare your sensitivity of CT scan in predicting portal vein involvement?

Dr Frazier: Dr Michelassi, you asked, does ultrasound lead to an earlier diagnosis? This is one of the advantages of EUS in that it is very sensitive for detecting small tumors. That is one factor that has been shown to be an independent predictor of survival, and is one of the potential advantages of EUS over the other modalities.

The other questions pertain to cost-effectiveness. The importance of EUS in this area is by eliminating a variety of other diagnostic procedures. By going straight to the EUS examination with FNA, a number of adjuvant tests that have been done in the past, including ERCP [endoscopic retrograde cholangiopancreatography] and angiography, can be eliminated as part of the diagnostic workup and leads to a more cost-effective evaluation. The other area where it leads to cost-effectiveness is identifying patients who would not benefit from surgical therapy. We will then avoid the cost and the morbidity to the patient of unnecessary abdominal exploration.

You asked about laparoscopic ultrasound. We are just initiating that process in the evaluation of our patients with pancreatic tumors, so we do not have enough data to comment on that at this point.

Several of the discussants asked about EUS and its use for vascular invasion. Actually, this is one of the benefits of EUS. It is very accurate for detecting the relationship of the pancreatic mass to the portal and superior mesenteric veins. We had 1 patient in whom EUS overpredicted the portal vein invasion. This was out of a total of 10 patients shown to have vascular invasion. Three patients in our series had portal venous resection as part of their operation. It helps to identify preoperatively those patients who are revascularization.

Dr Aranha, you brought up the issue of preoperative biopsy in the patient with a pancreatic mass. In the past, my philosophy regarding operative biopsy of a pancreatic mass in the patient who clinically presents with carcinoma has been similar to your own. I based the decision to perform resection upon clinical criteria and suspicion for a carcinoma. Because EUS-FNA can be done with such minimal morbidity, and gives a tissue diagnosis, I have now changed my philosophy in regards to preoperative biopsy. It is also very useful in those patients who are not candidates for surgical therapy. It is a very safe way to obtain a cytologic diagnosis and then put those patients in the category of palliative care or enter them into neoadjuvant therapy protocols in the hopes of performing later resection.

You mentioned the concern of peritoneal cytology and, as Dr Thirlby mentioned, the path of the needle is transduodenal and so it does not traverse the peritoneal cavity. In theory, this should not create problems with peritoneal seeding, but again, this is something that needs further study.

You also asked, does this open the door to neoadjuvant therapy, and indeed it does. If we can identify the patients who have metastatic lymph node involvement who do not have distant disease preoperatively, it opens the door for entering those patients into neoadjuvant therapy protocols and then offer them later resective therapy.

One of the discussants brought up the use of double-phase helical CT and the majority of the CT scans in this series were standard CT. The purpose of our paper was not to compare those, but other authors have done that and have shown similar results in delineating the relationship of the tumor in relationship to the vascular structures. Helical CT, however, was not as sensitive in detecting small tumors measuring less than 3 cm. So, for the tumor size, 3 cm and under, EUS is more accurate.

Dr Prinz, you asked why half the patients were not explored. A significant number of the patients had benign disease and therefore did not receive exploration. The others had disease that was not amenable to resective therapy. They were entered into palliative care at that stage.