PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 February 21; 13(7): 1123-1128
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effect of parenteral and early intrajejunal nutrition on pancreatic digestive enzyme synthesis, storage and discharge in dog models of acute pancreatitis

Huan-Long Qin, Zhen-Dong Su, Lei-Guang Hu, Zai-Xian Ding, Qing-Tian Lin

Huan-Long Qin, Zhen-Dong Su, Lei-Guang Hu, Zai-Xian Ding, Qing-Tian Lin, Institute of Parenteral Nutrition and Enteral Nutrition, Department of Surgery, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Yishan Rd. 600, Shanghai 200233, China
Supported by grant from the Morning Star Fund of Shanghai, China, No. 99QB14010
Correspondence to: Dr. Huan-Long Qin, Institute of Parenteral Nutrition and Enteral Nutrition, Department of Surgery, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Yishan Rd. 600, Shanghai 200233, China. hlqin@sjtu.edu.cn
Telephone: +86-21-64942226 Fax: +86-21-64368920
Received: 2006-10-17 Accepted: 2007-01-04

Abstract

AIM: To study the effect of early intrajejunal nutrition on enzyme-protein synthesis and secretion during acute pancreatitis.

METHODS: Fifteen dogs were randomly divided into parenteral nutrition (n = 7) and early intrajejunal nutrition groups (n = 8). An acute pancreatitis model was induced by injecting 5% sodium taurocholate and trypsin into the pancreas via the pancreatic duct. Intrajejunal nutrition was delivered with a catheter via a jejunostomy tube after the model was established for 24 h. On d 1 and 7 and at the beginning of nutritional support, radioactive tracing and electron microscopes were used to evaluate the enzyme-protein synthesis in acinar cells, the subcellular fractionation and the change in zymogen granules after 1.85 × 10^6 Bq L-^{3}H phenylalanine was infused at 30, 60, 120, and 180 min.

RESULTS: The ^{3}H radioactivity in pancreatic acinar cells reached its peak level at 60 min, and the contents in the early intrajejunal nutrition group were higher than those in the parenteral nutrition group, which were then decreased. The mean number and area of zymogen granules did not show any significant statistical difference in both groups on d 1 or on d 7 (P > 0.05).

CONCLUSION: Early intrajejunal nutrition might be effective in dogs with acute pancreatitis.

Key words: Parenteral nutrition; Enteral nutrition; Digestive enzyme; Acute pancreatitis

Qin HL, Su ZD, Hu LG, Ding ZX, Lin QT. Effect of parenteral and early intrajejunal nutrition on pancreatic digestive enzyme synthesis, storage and discharge in dog models of acute pancreatitis. *World J Gastroenterol* 2007; 13(7): 1123-1128

http://www.wjgnet.com/1007-9327/13/1123.asp

INTRODUCTION

Beneficial effects of total enteral nutrition (TEN) have been noted in a number of diseases, such as burn, trauma, and sepsis. In comparison with parenteral nutrition (PN), TEN can reduce nosocomial infection, multiple organ failure (MOF), and the length of hospitalization[1-4]. Early enteral nutritional (EEN) support in patients with acute pancreatitis (AP) has been evaluated by some authors who reported that it can moderate the acute phase response and improve disease severity and clinical outcome[5-10]. However, the commonly encountered problems of gastric atony and outlet obstruction have limited the successful delivery of enteral nutrition to patients with severe acute pancreatitis. In addition, many surgeons believe that EEN may lead to recurrence of symptoms and delayed complications, because EEN may increase the release of digestive enzymes and lysosomal hydrolases. This action of digestive enzymes and lysosomal hydrolases may be important in the development of acute pancreatitis, as lysosomal enzymes such as cathepsin B, are known to be capable of activating trypsinogen and intracellular digestive enzymes that may trigger the autodigestive phenomenon of the pancreas[11]. However, these problems may be overcome if enteral nutrition is delivered to the jejunum as distal as possible from Treitz's ligament, thereby avoiding stimulation of the cephalic and gastric phase. Therefore, it is necessary to investigate the effect of early intrajejunal nutrition (EIN) on pancreatic acinar cell uptake of ^{3}H phenylalanine, digestive enzyme synthesis, storage and discharge in dogs with AP.

MATERIALS AND METHODS

Materials

^{3}H phenylalanine (5mCi/mL) was obtained from Amersham. CBZ-arginine-napthalamide , thymus DNA

and RNA-napthalamide, cytochrome c, 3- (N-morphplino) propanesulfonic acid (MOPS), phenylmethyl- sulfonyl fluoride (PMSF), and Triton X-100 were from Sigma Chemical. All other commercially available reagents were of the highest purity.

Animal model

Twenty-two dogs weighing 18-22 kg had free access to water. After fasting for 12-14 h, all dogs were anesthetized by intramuscular injection of ketamine (10 mL/kg) and intravenous injection of sodium pentobarbital (30 mg/kg). Under sterile conditions, middle laparotomy and duodenotomy were performed. An AP model was induced by injecting 1mg/kg of a combined solution of 5% sodium taurocholate and trypsin 8000-10000 BAEF units/mL into the pancreatic duct at a pressure of 30 cm H_2O. The common biliary duct was clamped. A catheter was placed at 30 cm distal to Treitz's ligament *via* jejunostomy. After the AP model was established, the duodenum and abdomen were closed. The neck regions of dogs were shaved and prepared in a sterile manner for catheterization. A silastic catheter (1.0 mm in inner diameter, 1.5 mm in outer diameter) was inserted through the external jugular vein to reach the superior vena cava and connected to the infusion solution. Fifteen dogs with AP survived after 7 d, and the death rate was 32% (7/22). The study was approved by our Institutional Animal Committee.

Experimental groups and nutritional solution preparation

Fifteen dogs with AP were randomly divided into PN group (n = 7) and EIN group (n = 8). The two groups were isocaloric and isonitrogenous. PN solutions consisted of 7% Vamin (SSPC, 9.4 g/1000 mL), 20% intralipid (SSPC), and 50% glucose (GS). Non-protein calorie was 50 kC (209.2 kJ/kg) and nitrogen was 0.3 g/kg.d. The total volume of solution infused was 70 mL/kg.d. The energy index supported with glucose and fat emulsion was 1:1. Multivitamins and electrolytes were also included in TPN solutions. The 0.9% saline solution was infused at 250 mL/kg during operation and postoperatively for 8 h, thereafter at 125 mL/kg. The nutrient solution was infused at a constant infusion rate by a pump (100-120 mL/h).

The EIN solution was Nutrison (Nutricia). The jejunum was infused through a jejunostomy catheter with 250 mL Nutrison and 500 mL 0.9% saline at 24 h after AP was induced, 500 mL Nutrison and 250mL 0.9% saline were infused after 48 h and continued for 7 d. The infusion rate was controlled by microcomputer-pump (Nutricia). During the EIN support period, the insufficient amount of calorie and nitrogen was supplemented by partial parenteral nutrition[4] (Table 1).

Table 1 Calories, nitrogen and liquid supplemented between two groups

Group	20% Intralipid (mL/kcal)	50% glucose (mL/kcal)	Vamin (9.4g/L) (mL/g)	0.9% saline (mL)	Nutrison (1 kal/mL)
PN group (1-7 d)	227/500	500/500	640/6.0	2500	0
EIN group (1 d)	170/375	187.5/375	468/4.4	1750	250 mL (1.6 g) + NS500 mL
EIN group (2-7 d)	113.6/250	125/250	298/2.8	1750	500 mL (3.2 g) + NS 250 mL

NS: natural saline.

Amino acid uptake

Dogs with AP were infused with radioactive ^3H phenylalanine (1.85×10^6 Bq) at beginning of PN or EIN on d 1 and 7, respectively. The abdomen was opened twice and partial pancreas was rapidly removed at 30, 60, 120 and 180 min after ^3H phenylalanine pulse infusion. After rinsed with a cold homogenization buffer containing 5 mmol/L MOPS (pH 7.0), 250 mmol/L sucrose, 1 mmol/L $MgSO_4$, and 0.1 mmol/L PMSF and trimmed of fat, the pancreas was homogenized in this cold buffer using a Brinkman polytron. The homogenate was centrifuged at 150 × g for 15 min at 4℃ to pellet unbroken cells and the resulting supernatant was used to measure ^3H phenylalanine uptake. For this purpose, an aliquot of the supernatant was mixed with an equal volume of cold 20% trichloroacetic acid (TCA) and centrifuged at 4000 × g for 15 min. The remaining radioactive ^3H phenylalanine in the resulting supernatant was quantified using a Packard liquid scintillation counter.

Protein synthesis

The pancreas was sampled at the above fixed time points. The pancreas was rinsed in cold homogenization buffer, trimmed of fat, and divided into small fragments, which were homogenized in 8 mL of homogenization buffer using 5 full up- and down strokes of a motorized glass-Teflon homogenizer. The homogenate was centrifuged at 150 × g for 15 min at 4℃ to remove unbroken cells and debris. An aliquot was mixed with an equal volume of cold 20% TCA. After incubation on ice for 1 h to precipitate proteins, the sample was centrifuged at 4000 × g for 15 min at 4℃. The pellet was washed twice in 2 mL of 10% TCA. The final pellet was dissolved using a Packard liquid scintillation counter after addition of 10 mL of Beckman Ready-Solv.

Subcellular fractionation

The pancreas was removed, homogenized and subcellularly fractionated using the method of Tartakoff and Jamieson[12] with some modifications by DeLisle *et al*[13]. Briefly, the pancreas was divided into fragments, homogenized in 8 mL homogenization buffer by 5 full up- and -down strokes of a motorized glass-Teflon homogenizer, unbroken cells and debris were removed by centrifugation at 150 × g for 15 min at 4℃. The resulting supernatant was considered to be the entire sample for later calculation and to contain 100% of all measured components, and centrifuged at 1300 × g for 15 min at 4℃, yielding the "zymogen granule" pellet and a supernatant. The latter was harvested and centrifuged at 12000 × g for 12 min at 4℃ to obtain the "lysosome-mitochondria" pellet and a 12000 × g supernatant. This supernatant was centrifuged at 105000 × r/min for 60 min at 4℃

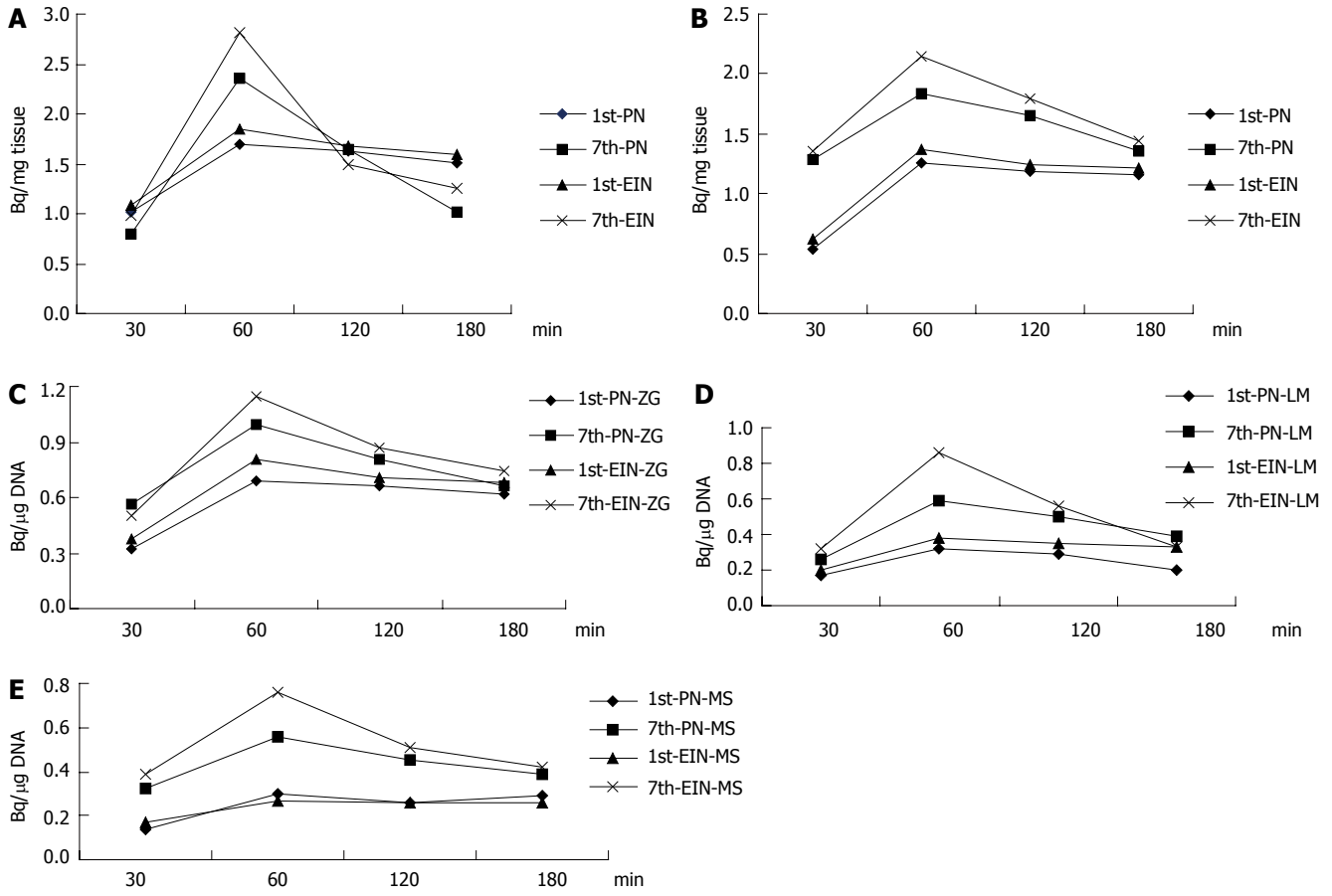

Figure 1 Change of ^{3}H radioactivity in acinar cell uptake (**A**), enzyme-protein synthesis (**B**), zymogen granules (**C**), lysosomal mitochondria (**D**), and microsomal (**E**) at different time points in two groups.

to produce a "microsomal" pellet and postmicrosomal or soluble fraction. The pellets described above were individually resuspended in 2 mL of homogenization buffer prior to measurement of marker enzymes and DNA. To measure the content of newly synthesized protein in each fraction, an aliquot of the sample or the resuspended pellet was mixed with an equal volume of 20% TCA, incubated for 1 h at 4℃ and centrifuged at 4000 × *g*. The pellet was washed as described above prior to measurement of TCA-precipitable radioactivity.

Electron microscopy

The fixation procedure used for conventional thin-section electron microscopy involved incubation with OsO4 alone (1% or 2% in phosphate buffer) at 0℃ for 30 min. After fixation, the sample was washed extensively in Veronal acetate buffer (90 mmol/L, ph 6.0), stained by incubation at 0℃ for 60 min in uranyl-magnesium acetate (0.5%) in the same buffer, washed again, dehydrated and embedded. Thin sections were doubly stained with uranyl acetate and lead nitrate, and examined under Philip EM 400 electron microscope.

A systematic randomized protocol was used to select tissue areas for morphometric analysis[14]. In each grid, 10 pictures were taken by one operator at a magnification of 150 for a total of ten negatives per sample. Two hundred and thirty pictures (PN group, n = 110; EIN group, n = 120) were printed and observed by one operator with a semi automated method using a digitizing tablet and pen, and a PC with dedicated software (Image Measure, Microscince, USA). The interstitial and vascular space, nonexocrine cells, exocrine cell nuclei, and acinar lumen space were not considered. The cross-sectional cytoplasmic area (mm^2) of exocrine cells and mature exocrine enzyme granules was directly measured on prints. In each group of samples, the mean zymogen granular number and area were obtained.

Statistical analysis

The data were collected by two blinded observers and presented as mean ± SE for multiple determinations. The statistical significance of observed changes was evaluated by t test using SPSS 10.0 statistical-software. $P < 0.05$ was considered statistically significant.

RESULTS

Pancreatic exocrine secretion stimulation test (PESST) on the first day

Acinar cell uptake of ^{3}H phenylalanine: The pancreatic acinar cell uptake of ^{3}H phenylalanine was evaluated at 60 min after the start of pulse infusion, and ^{3}H radioactivity in the EIN group was higher than that in PN group ($P < 0.05$), and then gradually decreased. There was no statistical difference between the two groups (Figure 1A).

Enzyme-protein synthesis: The maximal values for

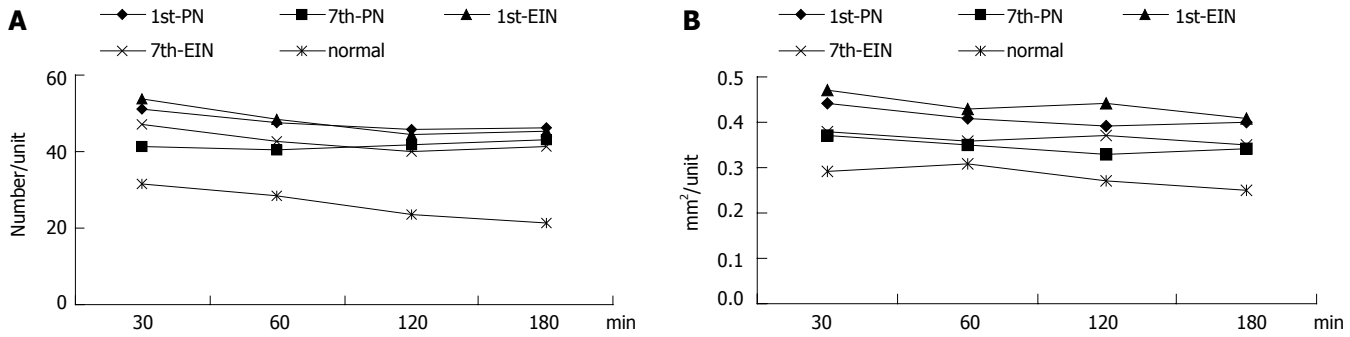

Figure 2 Change of MZGA in EIN group (**A**) and PN group (**B**) at different time points.

both groups were similar in acinar cell uptake of ^{3}H phenylalanine. The incorporation of ^{3}H-phenylalanine into enzyme protein was maximal at 60 min in both groups, and then gradually decreased. There was no difference between the two groups at 180 min (Figure 1B).

Intracellular transport of newly synthesized protein: The peak level of ^{3}H radioactivity was reached in the three subcellular fractionations at 60 min, and ^{3}H radioactivity in zymogen granules in the EIN group was higher than that in the PN group at 60min ($P < 0.05$). ^{3}H radioactivity in lysosomal-mitochondria and the microsomal subcellular fractionation did not reach statistical difference at the fixed time between the two groups, and then gradually decreased, the changes did not reach statistical difference (Figure 1C-E).

Electron microscopy

There was no difference in the submicroscopic cytology of acinar cells between the two groups. In particular, the fine structure of mature secretory granules was consistently similar. The electron density of membrane-bound mature exocrine granules had no change in this study in both PESST determinations. However, the mean zymogen granule number (MZGN) and mean zymogen granule area (MZGA) were not apparently changed on d 1 and 7 in either group, and the change in their number and unit area at different time points did not reach statistical difference (Figure 2A and B).

DISCUSSION

Traditionally, enteral nutrition is implemented after a 2-3 wk period of parenteral nutritional support[15]. This clinical strategy has been developed to provide a sufficient time period for the pancreas to rest and rehabilitate. The concept of pancreatic rest stems from the belief that stimulation of pancreatic exocrine function in patients with acute pancreatitis releases large quantities of proteolytic enzymes which result in autodigestion of the pancreas and peripancreatic tissues, causing deterioration in the patient's condition. The presence of food in the stomach and duodenum elicits gastropancreatic and duodenopancreatic reflexes that result in the stimulation of pancreatic exocrine secretion. However, these effects are insignificant when nutrients are delivered directly into the jejunum[16-25]. Kaushik *et al*[26] reported that enteral feeding can be given without stimulating pancreatic trypsin secretion provided it is delivered into the mid-distal jejunum, because the avoidance of trypsin stimulation may optimize enteral feeding in patients with acute pancreatitis.

It is known that digestive proteins and lysosomal enzymes are synthesized by polyribosomes binding to the cytoplasmic surface of endoplasmic reticulum (ER) membrane, and then transferred to the lumen of ER cisternae[27]. They are transported together to the Golgi, and normally separated from each other in the Golgi complex and condensing vacuole (CV) stage of intracellular transport. Normally, digestive enzymes and lysosomal hydrolases, which are synthesized in ribosomes attaching to the rough-surfaced endoplasmic reticulum and migrating to the Golgi complex and CV, are separated from each other by a complex sorting mechanism. Eventually, they are targeted for inclusion in distinct organelles: zymogen granules and lysosomes. In order to evaluate the effect of EIN on pancreatic acinar cell uptake of ^{3}H-phenylalanine and incorporation into zymogen protein in acute pancreatitic dogs, morphological changes were observed by determining the process of amino acid uptake, enzyme-protein synthesis, intracellular transport, and discharge of newly synthesized proteins from the pancreas within 3 h after beginning of PN and EIN on the first day. The results showed that ^{3}H phenylalanine radioactivity due to amino acid uptake and the extent of incorporation into newly synthesized proteins reached its peak level at 60 min in both groups. The parameters in the EIN group were higher than those in the PN group ($P < 0.05$), and then gradually decreased. To further analyze the effect of EIN on uptake of amino acids and enzyme-protein synthesis in subcellular fractions on the first day, we used differential and density-gradient centrifugation to determine the ^{3}H radioactivity in zymogen granules, lysosome-mitochondria and microsomal subcellular fractionation. In this study, the maximal values were obtained at 60 min in each of these fractions in lysosomes (cathepsin B), mitochondria (cytochrome oxidase) and zymogen granules (amylase), and then gradually decreased. These results suggest that the EIN alters neither amino acid uptake nor the extent of incorporation of ^{3}H radioactivity into newly synthesized proteins.

To study the possibility of recurrent pancreatitis,

PESST was performed to determine whether EIN increases ^{3}H radioactivity in lysosomes, mitochondria, and zymogen granules during AP. The results showed that the ^{3}H radioactivity in amino acid uptake, enzyme-protein synthesis, and subcellular fractionation reached its peak level at 60 min in both groups, which was higher in the EIN group than in the PN group ($P < 0.05$), and then gradually decreased. The content of ^{3}H in zymogen granules, lysosome-mitochondria and microsomal subcellular fractionation were consistent with the changes of amino acid uptake. The peak level of radioactivity on d 7 was higher than that on d 1. The tissue level of pulse-labeled proteins declined as expected due to secretion of labeled digestive zymogens into the duodenum via the pancreatic duct system. These results suggest that 7 d continuous intrajejunal nutrition can neither over-stimulate acinar cell uptake of ^{3}H nor enhance enzyme-protein synthesis and release. Cell fractionation studies indicated that intracellular transport of granules was not affected by EIN stimulation. Indeed, disappearance of pulse-labeled proteins from 10 500 × *g* pellet (total microsomal fraction enriched in rough endoplasmic reticulum elements and expected to contain Golgi elements as well) and their appearance in the 1300 × *g* pellet (enriched in zymogen granules and presumably condensing vacuoles) were not significantly different in EIN stimulation.

It is known that stored zymogens granules and lysosomal enzymes could play a key role in the development of pancreatitis and may, in fact, explain the intrapancreatic activation of digestive enzymes occurring in the course of many forms of pancreatitis[28]. Secretory proteins, on the other hand, progressively increase their concentration within the dilated Golgi cisternal rims and/or CV. The CV ultimately matures to ZG which is transported to the luminal plasmalemma and releases its contents by exocytosis. The large vacuoles containing both secretory and lysosomal enzymes in acute pancreatitis suggest that AP stimulates the process of CV maturation. Therefore, theoretically, EIN might increase intracellular transport and expand the CV compartment (formation of large vacuoles). To quantitatively assess the effect of EIN on the secretory granule cell compartment, ultrastructural morphometrical study of the pancreas under electron microscope was designed. The results showed that more ZG was accumulated in the interstitial space. The number and area of ZG were higher than those in normal. However, no gross difference in acinar cell ultrastructure was observed. These findings did not reach statistical difference between the two groups on d 1 and 7 during the nutritional support period, suggesting that EIN does not promote pancreatic acinar cell enzyme protein synthesis and release. The precise mechanism of EIN-stimulating acinar cells is unclear. However, some results indicate that receptor-triggered events of transmembrane signaling (rise of cytoplasmic Ca^{2+}) and the responsiveness and sensitivity to gastrointestinal hormones in pancreatic acinar cells are significantly inhibited[29].

In conclusion, early intrajejunal nutrition might be effective in dogs with acute pancreatitis. However, further study is needed to evaluate the effects of EIN and PN in patients with AP.

REFERENCES

1 **MacFie J**. Enteral versus parenteral nutrition. *Br J Surg* 2000; **87**: 1121-1122
2 **Neoptolemos JP**, Raraty M, Finch M, Sutton R. Acute pancreatitis: the substantial human and financial costs. *Gut* 1998; **42**: 886-891
3 **Al-Omran M**, Groof A, Wilke D. Enteral versus parenteral nutrition for acute pancreatitis. *Cochrane Database Syst Rev* 2003; (1): CD002837
4 **Qin HL**, Su ZD, Hu LG, Ding ZX, Lin QT. Effect of early intrajejunal nutrition on pancreatic pathological features and gut barrier function in dogs with acute pancreatitis. *Clin Nutr* 2002; **21**: 469-473
5 **Saluja A**, Saito I, Saluja M, Houlihan MJ, Powers RE, Meldolesi J, Steer M. In vivo rat pancreatic acinar cell function during supramaximal stimulation with caerulein. *Am J Physiol* 1985; **249**: G702-G710
6 **Abou-Assi S**, Craig K, O'Keefe SJ. Hypocaloric jejunal feeding is better than total parenteral nutrition in acute pancreatitis: results of a randomized comparative study. *Am J Gastroenterol* 2002; **97**: 2255-2262
7 **Oláh A**, Pardavi G, Belágyi T, Nagy A, Issekutz A, Mohamed GE. Early nasojejunal feeding in acute pancreatitis is associated with a lower complication rate. *Nutrition* 2002; **18**: 259-262
8 **Chen QP**. Enteral nutrition and acute pancreatitis. *World J Gastroenterol* 2001; **7**: 185-192
9 **Hallay J**, Kovács G, Szatmári K, Bakó A, Szentkereszty Z, Lakos G, Sipka S, Sápy P. Early jejunal nutrition and changes in the immunological parameters of patients with acute pancreatitis. *Hepatogastroenterology* 2001; **48**: 1488-1492
10 **McGregor CS**, Marshall JC. Enteral feeding in acute pancreatitis: just do it. *Curr Opin Crit Care* 2001; **7**: 89-91
11 **Sanabria A**. Randomized controlled trial of the effect of early enteral nutrition on markers of the inflammatory response in predicted severe acute pancreatitis. *Br J Surg* 2001; **88**: 728
12 **Tartakoff AM**, Jamieson JD. Subcellular fractionation of the pancreas. *Methods Enzymol* 1974; **31**: 41-59
13 **De Lisle RC**, Schulz I, Tyrakowski T, Haase W, Hopfer U. Isolation of stable pancreatic zymogen granules. *Am J Physiol* 1984; **246**: G411-G418
14 **Falconi M**, Caldiron E, Zancanaro C, Benati D, Talamini G, Bassi C, Pederzoli P. In vivo octreotide administration acutely reduces exocrine granule size in the human pancreas. *Pancreatology* 2001; **1**: 30-35
15 **Oláh A**, Belágyi 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
16 **Takács T**, Hajnal F, Németh J, Lonovics J, Pap A. Stimulated gastrointestinal hormone release and gallbladder contraction during continuous jejunal feeding in patients with pancreatic pseudocyst is inhibited by octreotide. *Int J Pancreatol* 2000; **28**: 215-220
17 **Eckerwall G**, Andersson R. Early enteral nutrition in severe acute pancreatitis: a way of providing nutrients, gut barrier protection, immunomodulation, or all of them? *Scand J Gastroenterol* 2001; **36**: 449-458
18 **Eatock FC**, Brombacher GD, Steven A, Imrie CW, McKay CJ, Carter R. Nasogastric feeding in severe acute pancreatitis may be practical and safe. *Int J Pancreatol* 2000; **28**: 23-29
19 **Lehocky P**, Sarr MG. Early enteral feeding in severe acute pancreatitis: can it prevent secondary pancreatic (super) infection? *Dig Surg* 2000; **17**: 571-577
20 **Erstad BL**. Enteral nutrition support in acute pancreatitis. *Ann Pharmacother* 2000; **34**: 514-521
21 **Duerksen DR**, Bector S, Yaffe C, Parry DM. Does jejunal

feeding with a polymeric immune-enhancing formula increase pancreatic exocrine output as compared with TPN? A case report. *Nutrition* 2000; **16**: 47-49

22 **Sahin M**, Ozer S, Vatansev C, Aköz M, Vatansev H, Aksoy F, Dilsiz A, Yilmaz O, Karademir M, Aktan M. The impact of oral feeding on the severity of acute pancreatitis. *Am J Surg* 1999; **178**: 394-398

23 **Windsor AC**, Kanwar S, Li AG, Barnes E, Guthrie JA, Spark JI, Welsh F, Guillou PJ, Reynolds JV. Compared with parenteral nutrition, enteral feeding attenuates the acute phase response and improves disease severity in acute pancreatitis. *Gut* 1998; **42**: 431-435

24 **Lobo DN**, Memon MA, Allison SP, Rowlands BJ. Evolution of nutritional support in acute pancreatitis. *Br J Surg* 2000; **87**: 695-707

25 **Kalfarentzos F**, Kehagias J, Mead N, Kokkinis K, Gogos CA. Enteral nutrition is superior to parenteral nutrition in severe acute pancreatitis: results of a randomized prospective trial. *Br J Surg* 1997; **84**: 1665-1669

26 **Kaushik N**, Pietraszewski M, Holst JJ, O'Keefe SJ. Enteral feeding without pancreatic stimulation. *Pancreas* 2005; **31**: 353-359

27 **Rosenfeld MG**, Kreibich G, Popov D, Kato K, Sabatini DD. Biosynthesis of lysosomal hydrolases: their synthesis in bound polysomes and the role of co- and post-translational processing in determining their subcellular distribution. *J Cell Biol* 1982; **93**: 135-143

28 **Watanabe O**, Baccino FM, Steer ML, Meldolesi J. Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. *Am J Physiol* 1984; **246**: G457-G467

29 **Powers RE**, Saluja AK, Houlihan MJ, Steer ML. Aberration in stimulus-secretion coupling caused by a choline deficient 0.5% ethionine containing (CDE diet) (Abstract). *Federation Proc* 1985; **44**: 535

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Ma WH