

Fatih Mehmet AVŞAR<sup>1</sup>  
İ. Hakan ÖZEL<sup>1</sup>  
Mahmut BAYKAN<sup>2</sup>  
Oral SAYGUN<sup>1</sup>  
Mustafa ŞAHİN<sup>3</sup>  
Süleyman HENGİRMEN<sup>1</sup>  
Ömür ERTUĞRUL<sup>2</sup>  
Mehmet GÜRBİLEK<sup>4</sup>  
S. Neşe AYDIN<sup>1</sup>

## Effects of Oral L-Glutamine, Insulin and Laxative on Bacterial Translocation in Acute Pancreatitis

Received: January 02, 2001

**Abstract: Aim:** To investigate the effects of oral L-glutamine, insulin and laxative on the bacterial population of intestinal lumen and on bacterial translocation in acute pancreatitis.

**Materials and Methods:** Forty Sprague-Dawley adult male rats were divided into 4 groups. Experimental pancreatitis was induced by ligating the main biliopancreatic duct. All groups were given the standard rat diet and tap water. In addition, Group II was given 15 mg/kg/day of L-glutamine via a catheter enterally, Group III was given 3 ml of laxative via a catheter enterally, and Group IV was given 3 IU/kg/day of NPH insulin via a catheter enterally. The rats were sacrificed 96 hours after the induction of pancreatitis. Blood samples for biochemical analyses and blood culture, and culture samples from mesenteric lymph nodes, liver and spleen and from cecal content were taken. Aerobic and anaerobic cultures were prepared.

**Findings:** Amylase levels in all the groups after the procedure increased significantly. Bacterial translocation was observed in 6 rats in the control group, in 5 rats in the insulin group, in 3 rats in the laxative group and in 2 rats in the L-glutamine group. A significant decrease in the number of luminal bacteria was observed in the laxative and L-glutamine groups.

**Conclusion:** L-glutamine, administered in enteral solutions in rats with induced acute pancreatitis, may reduce septic complications by decreasing the bacterial translocation rate. Similar effects are also produced by laxatives through reduction in the luminal bacterial population.

**Key Words:** Acute pancreatitis, Bacterial translocation, Sepsis, L-glutamine, Laxative, Oral insulin

<sup>1</sup>Department of First General Surgery, Ankara Numune Training and Research Hospital, Ankara, Departments of <sup>2</sup>Microbiology, <sup>3</sup>General Surgery and <sup>4</sup>Biochemistry, Faculty of Medicine, Selcuk University, Konya - TURKEY

### Introduction

Acute pancreatitis is a serious clinical condition characterized by activation of digestive enzymes secreted by the pancreas, and it involves autodigestion of the pancreas. Several factors play a role in the development of this picture(1-3). Complications that may arise during the course of the disease may cause this picture to become even more serious, and septic complications are among the major complications (1). Several experimental studies have shown that the source of the sepsis is the bacteria that exist in the intestinal lumen (4-7). As a result of the inflammation in acute pancreatitis, paralysis of the intestine develops and bacterial overgrowth takes place. In addition, the immune system is suppressed. Due to such pathological changes, bacteria pass into mesenteric lymph nodes, into the spleen, into the liver and into the blood circulation by bacterial translocation,

and infect pancreatic tissue that was previously inflamed, edematous and even necrotic (4). It is also argued that intestinal bacteria may directly reach the pancreas transmurally (6).

The integrity of intestinal mucosa is an important barrier for bacterial translocation. It is known that preservation of the mucosal barrier or strengthening it in the course of acute pancreatitis will prevent bacterial translocation. L-glutamine is an important nutritional source for enterocytes and has a trophic effect on intestinal mucosa. Insulin is used nasally and orally in the treatment of type 1 diabetes and some auto-immune diseases. Laxatives reduce bacterial population in the colon by removing fecal mass. In this study, we investigated the effects of oral L-glutamine, insulin and laxative on the bacterial translocation and intestinal bacterial population in acute pancreatitis.

## Materials and Methods

This study was performed in Selcuk University Experimental Research Center, upon permission of Selcuk University Ethics Committee and upon approval of the Executive Board of the Experimental Research Center. The Turkish Law of Animal Rights was taken into account in this study.

A total of 40 Sprague-Dawley adult male rats were included in the study. Their weights were 230-270 g (mean:  $248 \pm 14$  g). The rats were divided equally into 4 groups. All the rats were fed with standard rat pellets until the eve of the experiment, and then they were fasted. They were anesthetized with 5 mg/kg Ketamine HCl, and 2 cc blood samples were taken by cardiac puncture in order to determine plasma amylase levels. Hair on the abdominal wall was shaved off, and the abdominal wall was cleaned with Betadine<sup>®</sup> solution, after which the abdomen was entered by a midline incision under sterile conditions. The main biliopancreatic duct was isolated and ligated with 3/0 silk from the point just before the duodenum. Abdominal layers were closed by a continuous suture using 3/0 silk. After being placed into their cages, the rats were kept under controlled humidity and temperature conditions.

The groups and their respective nutrition were as follows:

- Group I: Control group; standard rat pellets and tap water were given
- Group II: L-glutamine group; standard rat pellets and tap water + 15 mg/kg/day L-glutamine (L-glutamine, GNC) via a catheter enterally.
- Group III: Laxative group; standard rat pellets and tap water + fleet enema (Sodium Phosphate, Fleet Enema; C.B. Fleet Company, USA) via a catheter enterally.
- Group IV: Insulin group; standard rat pellets and tap water + 3 IU/kg/day insulin (Humuline N; Lily) via a catheter enterally.

The rats were re-anesthetized with 5 mg/kg Ketamine HCl at the postoperative 96th hour. A 4 cc blood sample was taken by cardiac puncture. Of this, 2 cc was preserved for blood culture, and the remaining 2 cc for the determination of blood plasma amylase levels. Tissue samples from the mesenteric lymph nodes, the spleen and the liver were taken under sterile conditions. The culture

inoculation was performed immediately after collecting samples and amylase levels were measured with the enzymatic colorimetric method (Olympus AU 5200) in one hour.

**Aerobic cultures:** Tissue samples were crushed in 1 cc sterile broth using a sterile pestle in a porcelain mortar. Samples taken from this suspension by using a loop were inoculated in both blood agar (Oxoid) and EMB agar (Oxoid) mediums. After completing all the tissue culture procedures, the cecum was opened and cecal contents were removed with a sterile spatula of 0.1 ml capacity. This was mixed with 1 ml of saline solution of 0.9%, and using another sterile spatula, samples were taken from the prepared material and were inoculated in culture mediums of the same type. After incubation at 36°C for 48 hours, the culture mediums were evaluated. Isolated microorganisms were classified by Gram staining and microscopic identification.

**Anaerobic Cultures:** Tissue samples were placed on a thiogluconate culture medium (Biolife) without delay and were incubated at 36°C for 1 week in a jar in which anaerobic conditions were maintained by a GasPac Kit (Becton Dickinson).

**Blood Culture:** The 2 cc blood sample taken under sterile conditions was inoculated in a diphasic blood culture (Diomed) medium containing tryptic soy agar and broth, and incubated at 36°C for 2 weeks.

The cultures were first evaluated macroscopically, and then by preparing Gram stained preparations and by examination of passages made into the blood agar medium. Colony counts were performed in cecum cultures and the results were multiplied by 1000 to find out the number of colonies in one gram of feces.

Statistical analyses were performed according to the Kruskal-Wallis test and the Mann-Whitney U test. Values of  $p < 0.05$  were considered significant.

## Results

All the rats survived the experimental period. In all the groups, amylase levels were found to be higher than the levels before the experiment ( $p < 0.05$ ) (Table 1). This implies that the model we applied actually produced successful pancreatitis. Amylase levels in the laxative group after the experiment were found to be significantly lower than those in the control group ( $p = 0.02$ ). There

were not any significant differences among the other groups ( $p>0.05$ ).

Results of aerobic cultures prepared from mesenteric lymph nodes, the liver, the spleen and the cecum are given in Table 2. No growth was observed in the anaerobic cultures.

Table 1. Amylase levels before and after the experiment.

Amylase Level	Group I	Group II	Group III	Group IV
Before the Experiment	16.3 ± 2.2	18.5 ± 3.3	20.0 ± 4.2	17.8 ± 2.3
After the Experiment	180.7 ± 32.4	158.4 ± 33.2	125.7 ± 24.3	148.9 ± 33.7

Table 2. Results of aerobic cultures.

Groups	MLN	Liver	Spleen	Blood	Cecum
I	6	3	1	1	2.3 x 10 <sup>5</sup>
II	2	1	0	0	2.1 x 10 <sup>4</sup>
III	3	1	1	1	2.2 x 10 <sup>3</sup>
IV	5	2	1	1	2.4 x 10 <sup>5</sup>

Bacterial colony growth was observed in a total of 11 cultures in 6 rats in the control group, in a total of 3 cultures in 2 rats in the L-glutamine group, in a total of 6 cultures in 3 rats in the laxative group, and in a total of 8 cultures in 5 rats in the insulin group. In the bacteria count performed in the cecal culture, the colony count of the laxative group was found to be significantly lower than those of the other groups ( $p<0.05$ ). Moreover, the bacterial count in the L-glutamine group was significantly lower than those of the control and insulin groups ( $p<0.05$ ).

Microorganisms grown in the cultures were in the following order of frequency: *E. coli* in 10 subjects, *Staphylococcus* in 3 subjects, *Klebsiella* in 3 subjects and *Enterobacteria* in 2 subjects.

## Discussion

Acute pancreatitis is an enzymatic inflammation. It is rather difficult to determine the course of the disease at the beginning, but the criteria provided by Ranson and

Imrie are quite useful in the estimation of the prognosis (1,2). Treatment modalities applied are not very effective on the course of the disease, and no specific method or drug has yet been identified in the treatment of acute pancreatitis (2,3). While endogenous factors dominate the course of the disease, the occurrence of septic complications makes the general picture more grave. It has been reported that intestinal flora is the source of the bacteria causing sepsis and that such bacteria reach the pancreas directly by bacterial translocation and/or transmurally (4-8).

Three basic mechanisms have been identified in the development of bacterial translocation. These are immune suppression, bacterial overgrowth and destruction of mucosal barriers in the intestines (4-8). All three mechanisms play a role in bacterial translocation developing in acute pancreatitis. Inflammation developing within the abdominal cavity causes stasis in the intestines, and bacteria overgrow easily and rapidly in this stasis environment. In addition, the mucosal barrier deteriorates when blood circulation of intestine is impaired due to inflammation and edema(4). Furthermore, pathophysiological disorders occurring in acute pancreatitis cause immune suppression (5,7,8). It has been claimed that restoration of pathophysiological disorders that facilitate bacterial translocation in acute pancreatitis may prevent bacterial translocation and may reduce septic complications (7,8).

Several experimental studies have shown that, in intestines which are damaged by various causes, L-glutamine increases intestinal adaptation, prevents atrophy, reduces the permeability of the intestines, maintains the integrity of the intestines, and prevents bacterial translocation (9-14). L-glutamine is the most important nutritional source of enterocytes (15-17). It has been shown that the addition of this amino acid to enteral or parenteral nutritional solutions increases both the protein amount in the plasma and the protein synthesis in the muscle tissue (14).

It is obvious that all the mentioned effects of L-glutamine will restore the impaired defense systems of the intestine. We observed that bacterial translocation in rats fed L-glutamine was less than in all the other groups, and that the number of intraluminal bacteria was lower than in the control and the insulin groups. Several theories have been put forward in an attempt to explain this positive effect of L-glutamine. Kartal et al.(18) have

proposed that L-glutamine extends the length of villi in defunctioned intestinal loops, and increases the number of goblet cells, and that such effects are greater than those provided by growth hormones. Burke et al. (19) have stated that L-glutamine increases the regeneration of lymphatic tissue, and that it shows an immune modulating effect. Wischmeyer et al. (11) claim that L-glutamine shows a preventive effect against all the damage occurring in the intestines, and that this effect depends on the increase of heat shock protein (HSP). Alverdy et al. (20) have proposed that L-glutamine increases secretory IgA in intestines and impedes retention of bacteria into mucosa, thus improving the intestinal immune system. We are of the opinion that L-glutamine is effective against bacterial translocation by strengthening mucosal and immune barriers.

It has been shown that the cause of septic complications in acute pancreatitis is the bacteria in the intestinal lumen and overgrowth of such bacteria (5-7). Several studies have proven that removal of these bacteria from intestinal lumen and decreasing their number will reduce bacterial translocation and septic complications (7-9, 21). We also observed that the bacterial count in the lumen in the laxative group was lower than those in the other groups, and that the bacterial translocation rate was lower than those of the control and the insulin groups. We think that laxative prevents bacterial overgrowth by eliminating stasis in the intestines, and that it reduces the number of the bacteria by removing fecal mass. Thus, bacterial overgrowth causing the basis for bacterial translocation is eliminated.

Several studies have reported that epidermal growth factor (EGF), and insulin-like growth factor (IGF) I and II increase intestinal adaptation, and they help improvement

of intestinal functions, in a manner similar to L-glutamine (22-24). We, inquiring whether real insulin could substitute IGF I and II, investigated the effects of insulin on bacterial translocation in acute pancreatitis. It has been stated that insulin may be administered by nasal, mucosal and oral ways in type I diabetics and in various auto-immune diseases (25-30). We therefore applied insulin orally; however, we found that the luminal bacteria count and bacterial translocation rate were similar to those found in the control group. Insulin has a peptide structure and thus, we think that it has been digested in denatured form by the effect of the gastric acid, and for that reason it has failed to have any positive effects. It has also been shown in some studies that orally administered insulin adjusts the proliferation of T lymphocytes as well as cytokin secretion, thus protecting beta cells in the pancreas (25, 29-31). We can therefore claim in light of these findings that orally given insulin had no effects on bacterial translocation in our model.

In conclusion, we think that L-glutamine, when administered in enteral nutritional solution to subjects with acute pancreatitis, will reduce bacterial translocation and septic complications. Similar positive effects of laxatives may be achieved through their combination with L-glutamine. Thus, precautions may be obtained against septic complications that adversely affect prognosis in acute pancreatitis. Insulin had no effects in the model we applied. This can be investigated by other models in which insulin is protected against gastric acid and digestive enzymes.

*Correspondence author:*

*Fatih Mehmet AVŞAR*

*Meşrutiyet Cad. 28/6*

*06640, Kocatepe, Ankara - TURKEY*

## References

1. Reber HA. Acute pancreatitis. In: Seymour I, Schwartz. Ed. Principles of Surgery. 6<sup>th</sup> ed. New York: McGraw-Hill;1408-1413, 1994.
2. Steer ML. Acute pancreatitis. In: Yamada T. Ed. Textbook of Gastroenterology. Philadelphia: JB Lippincott; 1859-1873, 1991.
3. Yeo CI, Cameron JL. The pancreas. In: Sabiston DC, Lyerly HK. Eds. Textbook of Surgery. Philadelphia: WB Saunders; 1152-1186, 1997.
4. Andersson R, Wang X, Ihse I. The influence of abdominal sepsis on acute pancreatitis in rats: a study on mortality, permeability, arterial pressure and intestinal blood flow. *Pancreas*. 11:363-373, 1995.
5. Runkel NS, Moody FG, Smith GS, et al. The role of the gut in the development of sepsis in acute pancreatitis. *J Surg Res*. 51:18-23, 1991.
6. Torpille E, Nystrom PO, Franzen L, Ihse I. Bacterial translocation during acute pancreatitis in rats. *Eur J Surg*. 159:109-113, 1993.
7. Şahin M, Yol S, Çiftçi E, Baykan M, Özer Ş, Aköz M, Yılmaz O, Kuru C. Does large bowel enema reduce septic complications in acute pancreatitis? *Am J Surg*. 176:331-334, 1998.
8. Medich DS, Lee TK, Melhem MF, et al. Pathogenesis of pancreatic sepsis. *Am J Surg*. 165:46-50, 1993.

9. Bai MX, Jiang JM, Liu YW, Wang WT, Li D, Wilmore DW. Effects of Alanyl-Glutamine on gut barrier function. *Nutrition*. 12:793-796, 1196.
10. Scheppach W, Dusel G, Kuhn T, Loges C, Karch H, Bartram HP, Richter F, Christl SU, Kasper H. Effect of L-Glutamine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. *Gut*. 38:878-885, 1996.
11. Wischmeyer PE, Musch MW, Madonna MB, Thisted R, Chang EB. Glutamine protects intestinal epithelial cells: role of inducible HSP70. *Am J Physiol*. 272:879-884, 1997.
12. Haque SMM, Chen K, Usui N, Liboshi Y, Okuyama H, Masunari A, Nezu R, Takagi Y, Okada A. Alanyl-Glutamine dipeptide-supplemented parenteral nutrition improves intestinal metabolism and prevents increased permeability in rats. *Ann Surg*. 223:334-341, 1996.
13. Chen K, Nezu R, Sando K, Haque SMM, liboshi Y, Masunari A, Yoshida H, Kamata S, Takagi Y, Okado A. Influence of Glutamine-supplemented parenteral nutrition on intestinal amino acid metabolism in rats after small bowel resection. *Jpn J Surg*. 26:618-623, 1996.
14. Li YS, Li JS, Jiang JW, Liu FN, Li N, Qin WS, Zhu H. Glycyl-Glutamine-Enriched long-term total parenteral nutrition attenuates bacterial translocation following small bowel transplantation in the pig. *J Surg Res*. 82:106-111, 1999.
15. Souba WW, Herskowitz K, Solloum RM. Gut glutamine metabolism. *JPEN*. 14:455-458, 1990.
16. Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism "in vivo" in small intestine of fed rats. *J Biol Chem*. 255:107-112, 1980.
17. Nemoto A, Krajack A, Suzuki T, Tzkeyoshi I, Hamada N, Zhang S, Zhu Y, Starzl TE. Glutamine metabolism of intestine grafts: influence of mucosal injury by prolonged preservation and transplantation. *Transplant Proc*. 28:2545-2550, 1996.
18. Kartal A, Dilsiz A, Kaymakçı A, Yol S, Duman S, Aktan M. Trophic effects of recombinant human growth hormone-ZnSO<sub>4</sub> and L-Glutamine on defunctioned small bowel in rats. *Ulusal Cerrahi Dergisi*. 13:301-307, 1997.
19. Burke DJ, Alverdy JC, Aoyo E, Moss GS. Glutamine-supplemented total parenteral nutrition improves gut immune function. *Arch Surg*. 124:1396-1400, 1989.
20. Alverdy JA, Aoyo E, Carrington PW, Burke DA. The effect of glutamine-enriched TPN on gut immune cellularity. *J Surg Res*. 52:34-38, 1992.
21. Erbil Y, Berber E, Özarmagan S, Seven R, Eminoglu L, Çaliş A, Olgaç V, Gürler N. The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. *Hepatogastroenterology*. 46:2791-2795, 1999.
22. Wiren M, Adrian TE, Arnelo U, Permert J, Staab P, Larsson J. Early gastrointestinal regulatory peptide response to intestinal resection in the rat is stimulated by enteral glutamine supplementation. *Dig Surg*. 16:197-203, 1999.
23. Helmuth MA, Shin CE, Fox JW, Erwin CR, Warner BW. Adaptation after small bowel resection is attenuated by sialoadenectomy: the role for endogenous epidermal growth factor. *Surgery*. 124:848-854, 1998.
24. Zhang GX, Lai JH, Jia TW, Wang WZ, Wang JY. Effect of epidermal growth factor on glutamine metabolic enzymes in small intestine and skeletal muscle of parenterally fed rats. *Nutrition*. 13:652-655, 1997.
25. Chaillous L, Lefevre H, Thivolet C, Boitard C, Lahlou L, Atlan-Gebner C, et al. Oral insuline administration and residual B-cell function in recent onset type 1 diabetes: a multicentre randomised controlled trial. *Lancet*. 356:545-549, 2000.
26. Weiner HL. Oral tolerance for the treatment of autoimmune diseases. *Annu Rev Med*. 48:341-351, 1997.
27. Daniel D, Wegmann DR. Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide. *Proc Natl Acad Sci USA*. 93:956-960, 1996.
28. Zhang ZJ, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA*. 98:10252-10256, 1991.
29. Polanski M, Melican NS, Zhang J, Weiner HL. Oral administration of the immunodominant B-chain of insulin reduces diabetes in a co-transfer model of diabetes in the NOD mouse and is associated with a switch from Th1 to Th2 cytokines. *J Autoimmune*. 10:339-346, 1997.
30. Bergerot I, Fabien N, Maguer V, Thivolet C. Oral administration of human insulin to NOD mice generates CD4(+) T cells that suppress adoptive transfer of diabetes. *J Autoimmune*. 7:655-663, 1994.
31. Bergerot I, Arreaza GA, Cameron MJ, et al. Insulin B-chain reactive CD4+ regulatory T cells induced by oral insulin treatment protect from type 1 diabetes by blocking the cytokine secretion and pancreatic infiltration of diabetogenic effector T-cells. *Diabetes*. 48:1720-1729, 1999.