

The Effects of Intravenous Glutamine on Bacterial Translocation and Intestinal Morphology in Experimental Pancreatitis

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Aim: The aim of this study was to investigate the effects of intravenous glutamine administration on bacterial translocation and intestinal morphology in rats with induced acute pancreatitis.

Materials and Methods: Forty-eight Wistar-Albino rats were divided into four groups (n = 12 for each group). Pancreatitis was induced with ligation of the main pancreatic duct except in the sham group, in which only periportal dissection was performed. Rats in the sham and control groups were exposed to standard rat pellet. Total parenteral nutrition (TPN) was administered solely to the pancreatitis + TPN group and together with glutamine (Gln) to the pancreatitis + TPN + Gln group. Rats were sacrificed at 48 h after experiment. Venous blood was obtained for blood culture and biochemistry. Tissue samples were obtained from liver, spleen, pancreas and lymph nodes from the mesentery for bacterial culture. Histopathologic examination was performed on tissue sections obtained from the pancreas.

Results: Formation of pancreatitis was demonstrated on microscopic examination. Amylase levels were significantly increased in pancreatitis-induced groups when compared to the sham group (P < 0.05). Bacterial translocation was observed in 1 rat (8%) in the sham group, in 7 rats (58%) in controls, in 8 rats (67%) in the pancreatitis + TPN group and in 3 rats (25%) in the pancreatitis + TPN + Gln group. Villus heights and numbers were significantly increased in TPN-administered groups compared to controls.

Conclusions: As Gln supplement in TPN treatment reduced the bacterial translocation and stimulated intestinal cell division and replication during the severe pancreatitis model, we suggest that application of Gln into TPN solutions can reduce possible septic complications associated with pancreatitis.

Key Words: Acute pancreatitis, bacterial translocation, glutamine, total parenteral nutrition, experimental surgery

DeneySEL Pankreatitlerde İntestinal Morfoloji ve Bakteriyel Translokasyonda İntervenöz Glutamin Etkileri

Amaç: Bu çalışmanın amacı bakteriyel translokasyonda i.v. glutamin uygulamasının etkilerini ve ratlardaki tetiklenmiş akut pankreatitlerde intestinal morfolojiyi araştırmaktır.

Yöntem ve Gereç: 48 adet Wistar-Albino rat (n = 12, her grup için) 4 ayrı gruba bölündü. Pankreatitler sham grubu dışında, ana pankreatobilier kanalın ligasyonu ile sağlandı. Sham gruba ise sadece periportal diseksiyon uygulandı. Sham grubundaki ve kontrol grubundakiler standart rat yemi verildi. Pankreatitli ve TPN grubuna yalnızca TPN uygulandı ve pankreatit + TPN + Gln grubuna Glutamin (Gln) ile birlikte uygulandı. Ratların 48s lik gözlemden sonra hayatlarına son verildi. Kan kültürleri ve biyokimyası için venöz kanları alındı. Karaciğerden, dalaktan, pankreastan ve mezenter lenf nodlarında bakteriyel kültür için doku örnekleri alındı. Pankreastan alınan doku kısımlarında ise histopatolojik inceleme yapıldı.

Bulgular: Pankreatitlerin şekilleri, mikroskopik incelemeler ile ortaya konuldu. Sham grupla karşılaştırıldığında (P < 0.05) pankreatit tetiklenmiş grupta amilaz düzeyleri belirgin artmıştı. Sham grupta bakteriyel translokasyon 1 ratta (% 8) görülürken, kontrol grubunda 7 (% 58), pankreatit + TPN + grubunda 8 ratta (% 67) ve pankreatitli + TPN + Gln grubunda ise 3 rat da (% 25) görüldü. Kontrol grubu ile kıyaslandığında villus yükseklikleri ve sayıları TPN alan grupta bariz artmıştı.

Sonuç: TPN tedavisine Gln eklenmesi, bakteriyel translokasyonu azalttığı gibi, intestinal hücre bölünmesini ve replikasyonunu ağır pankreatitlerde stimüle etmektedir, TPN solusyonuna Gln ilavesinin pankreatitte muhtemel septik komplikasyonları önleyebileceğini düşünmekteyiz.

Anahtar Sözcükler: Akut pankreatit, bakteriel translokasyon, glutamin, TPN, deneysel cerrahi

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Introduction

Pancreatic sepsis is the most common cause of major morbidity and mortality associated with acute pancreatitis, with the pathogenesis of such infections remaining unknown (1). Bacterial translocation (BT) is known as the passage of viable bacteria or endotoxins from the gut to mesenteric lymph nodes (MLNs) and to other organs, which may commence or exacerbate septic states (2,3).

Translocation of organisms from the gastrointestinal tract to extraintestinal sites is known to be promoted by factors causing systemic insult or bowel injury (4). Several studies have demonstrated that intra-abdominal inflammation during acute pancreatitis promotes BT in the absence of obvious microscopic injury of the intestine (3). Glutamine (Gln) is known as the most significant energy source of enterocytes, and lowers the rate of endotoxemia and translocation by preserving mucosal integrity (5-9).

The aim of this study was to determine the effect of intravenously administered Gln on BT and intestinal mucosal integrity in an experimental model of acute pancreatitis in rats.

Materials and Methods

Experiments were conducted with adult, male Wistar rats weighing from 250 to 300 g. They were housed under constant temperature (22°C) and humidity, with 12 h dark/light cycles in Selçuk University, Experimental Research Laboratory. All studies were carried out under the guidelines of Selçuk University, Institutional Animal Ethics Committee.

Animals were randomized into four groups of 12 rats each (Table 1). All animals were fed with standard chow and tap water before the experiment. All animals in total parenteral nutrition (TPN) groups (Groups III and IV) were anesthetized with ketamine HCl (Ketalar™) (10 mg/kg), and a central venous catheter was placed via right internal jugular cut-down using aseptic technique. Catheters were protected from the movement of the animals. Animals underwent operation after anesthesia by ketamine HCl 10 mg/kg and xylazine (Rhompun, Bayer,

Leverkusen, Germany) 8 mg/kg intramuscularly. Following skin preparation with povidone iodine, laparotomy was performed via midline incision. Portal elements were dissected but pancreatic duct was not ligated in Group I (sham group). In Groups II, III and IV, retroduodenal surface was explored, the pancreaticobiliary duct (PBD) was ligated with 4-0 silk close to its entrance to the duodenum, and acute pancreatitis was induced (1,10). The abdomen was closed with 2-0 silk sutures in two layers.

After the operation, feeding regimens were continued according to groups for 48 h. Animals in Group I and II were fed with standard chow and tap water. Group III was treated with TPN. Those in Group IV were exposed to TPN with Gln (0.75 g/kg/day). TPN solution administered to Group III contained 25% dextrose and 4% amino acids, while the nutrition given to Group IV contained 25% dextrose, 2% amino acids and 2% Gln. Both TPN formulas were arranged as isonitrogenous and isoenergetic. Therapy was started 6 h after the induction of pancreatitis, and solutions were administered continuously via Harvard infusion pumps (A Harvard Bioscience Company, Massachusetts, USA).

All animals were sacrificed with an overdose of intravenous sodium thiopental at postoperative 48 h, and samples from peritoneum, liver, MLNs, spleen and blood were cultured under aerobic conditions. Biochemical and histological evaluations were performed. Small bowel sections from terminal ileum were prepared for villus measurement.

Testing for Translocation of Bacteria

Blood samples were inoculated in aerobic and anaerobic BacTec (Becton Dickinson Diagnostic Instrument Systems; Sparks, MD, USA) pediatric blood culture bottles. Bottles were incubated in automated blood culture system at 37°C, and growth was observed for 7 days. Samples with growth were stained with Gram stain and sub-cultured in blood agar and eosin-methylene blue (EMB) agar (Bio Mérieux Marcy l'Etoile, France) mediums for isolation of microorganisms.

Lymph nodes and tissue samples taken from peritoneum, liver, spleen and pancreas were placed in pre-weighted tubes containing 5 ml thioglycolate medium

(Merck Diagnostica, Darmstadt, Germany) for quantitative culture. Tissue samples were homogenized and inoculated in blood agar and EMB agar medium (Bio-Merieux, Marcy l'Etoile, France) for aerobic culture. Plaques were incubated at 37°C for 24-48 h. The organisms grown under these conditions were identified by using the standard microbiologic methods and API identification (API 20 E; Bio Mérieux 69280, Marcy l'Etoile, France) tests.

Histologic Examination

The terminal ileum was removed after sacrifice. Specimens were fixed in 10% formalin in 0.15 M phosphate buffer (pH = 7.2) and were embedded in paraffin. They were stained with hematoxylin and eosin, and examined under light microscope. Morphometric analysis was performed using an eyepiece micrometer. Mean villus heights and numbers were measured in each specimen.

In histopathologic examination of the pancreas, interstitial edema, infiltration of inflammatory cells, hemorrhagic areas, and necrosis were investigated. One point was given for each finding, and the severity of pancreatitis was determined by adding the obtained points. The overall scores were between 0-4, with 0 representing no pancreatitis, 1: mild pancreatitis, 2-3: moderate pancreatitis, and 4: severe pancreatitis (11).

Plasma Assays

Venous blood samples were obtained before sacrifice, and they were processed using an auto-analyzer (Type 717, Hitachi, Tokyo, Japan) for the measurement of

serum amylase, aspartate transaminase (AST), and alanine transaminase (ALT) levels. Assays were performed using reagent kits and standard applications.

Statistical Analysis

Statistical analysis was performed using SPSS version 10.0 for Windows 98. Changes in the groups were compared with the one-way ANOVA and Fisher's exact test. Differences between groups were analyzed with the Student's *t*-test. Differences were considered to be significant if *p* values were less than 0.05.

Results

All of the rats in Groups II, III and IV were found to have pancreatitis at the second laparotomy, 48 h after PBD ligation. Moderate (*n* = 8/12 in Group II, *n* = 7/12 in Group III and *n* = 8/12 in Group IV) to severe pancreatitis (*n* = 4/12 in Group II, *n* = 5/12 in Group III and *n* = 4/12 in Group IV) was observed. In these groups, parenchymal inflammation-necrosis, peripancreatic fat necrosis, various degrees of bile reflux, and peritoneal fluid accumulation were observed microscopically and macroscopically. In addition to these findings, serum pancreatic amylase and liver transaminase levels were increased in pancreatitis-induced groups (Table 1).

All peritoneal swabs were negative. BT rates in all groups are shown in Table 2. Rats in Group II (acute pancreatitis) and Group III (acute pancreatitis + TPN) had significantly higher rates of BT than Group IV (acute pancreatitis + TPN + Gln). Incidences of BT to the MLNs, liver, spleen, and blood in all groups are shown in Table

Table 1. Biochemical findings of experiments.

Groups	Amylase (IU/L)	AST	ALT
I (Sham)	62 ± 18*	80 ± 12*	87 ± 23*
II (Pancreatitis)	3180 ± 965	428 ± 24	388 ± 41
III (Pancreatitis + TPN)	3300 ± 1062	400 ± 32	396 ± 15
IV (Pancreatitis + TPN + Gln)	3207 ± 983	396 ± 30	403 ± 13

**P* < 0.05 vs group II, III and IV (one-way ANOVA).

AST: Aspartate transaminase. ALT: Alanine transaminase.

Table 2. Bacterial translocation ratios of the groups.

Groups	Ratios	Median (cfu/ml)
I (Sham)	1/12	10 ²
II (Pancreatitis)	7/12	10 ^{5*}
III (Pancreatitis + TPN)	8/12	10 ^{5*}
IV (Pancreatitis + TPN + Gln)	3/12	10 ²

*P < 0.001 vs controls (Fisher's exact test)
cfu: Colony forming units.

Table 3. Bacterial translocation incidences in all groups.

Groups	MLN	Liver	Spleen	Pancreas	Blood
I	1	1	1	1	0
II	7	6	4	4	1
III	8	7	6	6	3
IV	3	2	1	1	0

P < 0.001 vs. controls (Fisher's exact test).
MLN: Mesenteric lymph nodes.

3. Most of the organisms were translocated to the MLNs in pancreatitis-induced groups. *E. coli* constituted the leading translocated organism. *Proteus mirabilis* was found to be another commonly translocated organism in the experimental groups (Table 4).

Mean villus heights and numbers decreased significantly in Group II compared to others (Table 5). Those in the TPN-administered groups were not significantly different; however, mean villus heights and numbers increased in Gln-enriched TPN-administered animals compared to TPN-administered rats.

Discussion

The clinical course of pancreatic sepsis, a rare complication seen after acute pancreatitis, is generally devastating (up to 80% mortality) (12). Despite the improvements in surgical and radiological approaches for local control of pancreatic infections or medical treatment of those patients (TPN, intensive antibiotic treatment, etc.), the presence of higher morbidity and mortality rates remains unresolved (12-14).

The spectrum of organisms in pancreatic infection consists of *Escherichia*, *Enterobacter*, *Enterococcus*,

Table 4. Translocated microorganisms.

	MLN	Liver	Spleen	Pancreas	Blood	Total
<i>E. coli</i>	11	8	6	6	4	35
<i>Proteus mirabilis</i>	8	7	6	6	1	28
<i>K. pneumoniae</i>	2	2	1	1		6
<i>Salmonella</i> spp	1					1
Total	22	17	13	13	5	70

MLN: Mesenteric lymph nodes.

Table 5. Villus characteristics of groups.

Groups	Villus height (mm)	Villus number/cm
I (Sham)	0.131 ± 0.02	94 ± 4
II (Pancreatitis)	0.043 ± 0.03*	35 ± 2*
III (Pancreatitis + TPN)	0.096 ± 0.008	70 ± 3
IV (Pancreatitis + TPN + Gln)	0.106 ± 0.015	79 ± 6

P < 0.05 vs. other groups (one-way ANOVA).

Pseudomonas, *Proteus*, *Bacteroides* or *Klebsiella* species (15,16). As in cases with hemorrhagic shock (4) or major burns (17), the gut is a potential source of pancreatic sepsis in that enteric microorganisms translocate to extraintestinal sites without direct manipulation of the gastrointestinal tract (1-3). Disruption of indigenous intestinal microflora, intestinal mucosal damage and alterations in immunity of the host are all considered as the principal factors that promote the passage of bacteria across the mucosal barrier (17-19). Similar changes in bowel mucosa and reticuloendothelial system during acute pancreatitis may potentially promote translocation (1,20). Another hypothesis for BT in acute pancreatitis is intraperitoneal inflammation (2). The mechanisms by which intraperitoneal inflammation promotes BT have not yet been defined but may involve the release of inflammatory mediators or cytokines (2,21). In addition, direct injury to the intestine during intraperitoneal inflammation may disrupt intestinal barrier function. Examination of the possible mechanisms in BT during acute pancreatitis is not the main scope of this study. However, our data clearly demonstrated translocation of certain Gram-negative species during acute pancreatitis. Forty-eight hours after the onset of acute pancreatitis, *E. coli* was found to be the leading translocated organism, and bacteria of the indigenous flora were present in the MLN of every culture-positive animal, as reported previously by Runkel et al. (1).

Restoration of impaired mucosal integrity may be hampered by the lack of enteral alimentation during the severe acute pancreatitis episode, which has been shown to play a decisive role in preserving the intestinal barrier function (21-23). The presence of delayed enteral nutrition secondary to gut paralysis and recurrent episodes of abdominal pain during severe acute

pancreatitis necessitates a prolonged period of TPN treatment. Absence of intestinal stimulation during TPN treatment promotes BT from the gut (24). To strengthen the improving effects of TPN on gut mucosal barrier, several agents have been examined (growth factors, trophic gut hormones, and specific nutrients). Gln, an essential substrate for the gut in intestinal stress situations, has been well studied for this goal (5,6,8,25). The net effect of Gln on reduction of BT was clearly observed in our study, as in previous experiments. Postoperative survey of animals in this study was restricted to 48 hours, which was shorter than the period in the previous studies. Despite this limited follow-up, the improving effects of Gln supplement were seen on villus morphology and translocation rates. The mechanism of Gln on BT remains unclear. Increased apoptosis during the deprivation of Gln and increased villus height and number during the treatment with Gln support the theory that Gln supplies metabolic energy and nucleotide bases required for cell division and replication of intestinal mucosa (7,26). Alteration in gut immune function during the Gln treatment -a point not assessed in our study- may be an important pathway for Gln (5,24,27). In addition, studies on gut perfusion have also demonstrated that Gln increases splanchnic blood supply and mucosal capillary blood flow in the ascending colon (8,28).

In conclusion, when compared to other medical treatment options in acute pancreatitis (selective gut decontamination, intravenous imipenem treatment, etc.), Gln-enriched TPN treatment reduces the prevalence of pancreatic infections as appropriately as other medical modalities in experimental models (6,29). Our study supports the latter and the inspiring theory proposed by Foitzik et al. (6), that is, the stimulation of intestinal cell division and replication with Gln supplement.

References

1. Runkel NS, Moody FG, Smith GS, Rodriguez LF, LaRocco MT, Miller TA. The role of the gut in the development of sepsis in acute pancreatitis. *J Surg Res* 1991; 51: 18-23.
2. Medich DS, Lee TK, Melhem MF, Rowe MI, Schraut WH, Lee KK. Pathogenesis of pancreatic sepsis. *Am J Surg* 1993; 165: 46-50.
3. Tarpila E, Nyström PO, Franzen L, Ihse I. Bacterial translocation during acute pancreatitis in rats. *Eur J Surg* 1993; 159: 109-13.
4. Kale IT, Kuzu MA, Berkem H, Berkem R, Acar N. The presence of hemorrhagic shock increases the rate of bacterial translocation in blunt abdominal trauma. *J Trauma* 1998; 44: 171-4.
5. Burke DJ, Alverdy JC, Aoye E, Moss GS. Glutamine-supplemented total parenteral nutrition improves gut immune function. *Arch Surg* 1989; 124: 1396-9.
6. Foitzik T, Stuffer M, Hotz HG, Klünnert J, Wagner J, Warshaw AL et al. Glutamine stabilizes intestinal permeability and reduces pancreatic infection in acute experimental pancreatitis. *J Gastrointest Surg* 1997; 1: 40-7.
7. Erbil Y, Berber E, Ozarmagan S, Seven R, Eminoglu L, Calis A et al. The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. *Hepatogastroenterol* 1999; 46: 2791-5.

8. Foitzik T, Kruschewski M, Kroesen AJ, Hotz HG, Eibl G, Buhr HJ. Does glutamine reduce bacterial translocation? : a study in two animal models with impaired gut barrier. *Int J Colorectal Dis* 1999; 14: 143-9.
9. Zulfikaroglu B, Zulfikaroglu EE, Ozmen MM, Ozalp N, Berkem R, Erdogan S et al. The effect of immunonutrition on bacterial translocation and intestinal villous atrophy in experimental obstructive jaundice. *Clin Nutr* 2003; 22(3): 277-81.
10. Sahin M, Yol S, Ciftci E, Ozer S, Aköz M, Yilmaz O et al. Does large-bowel enema reduce septic complications in acute pancreatitis? *Am J Surg* 1998; 176: 331-4.
11. Sahin M, Ozer S, Vatansev C, Vatansev H, Aksoy F, Dilsiz A et al. The impact of oral feeding on the severity of acute pancreatitis. *Am J Surg* 1999; 178: 394-8.
12. Lumson A, Bradley EL. Secondary pancreatic infections. *Surg Gynecol Obstet* 1990; 170: 459-67.
13. Maravi-Poma E, Gener J, Alvarez-Lerma F, Olaechea P, Blanco A, Domínguez-Muñoz JE. Early antibiotic treatment (prophylaxis) of septic complications in severe acute necrotizing pancreatitis: a prospective, randomized, multicenter study comparing two regimens with imipenem-cilastatin. *Intensive Care Med* 2003; 29: 1974-80.
14. Werner J, Uhl W, Buchler MW. Surgical treatment of acute pancreatitis. *Curr Treat Options Gastroenterol* 2003; 6: 359-67.
15. Beger HG, Bittner R, Block S, Buchler M. Bacterial contamination of pancreatic necrosis. *Gastroenterology* 1986; 91: 433-8.
16. Holden JL, Berne TV, Rosoff L. Pancreatic abscess following acute pancreatitis. *Arch Surg* 1976; 111: 858-61.
17. Deitch EA, Maejima K, Berg R. Effect of oral antibiotics and bacterial overgrowth on the translocation of the GI tract microflora in burned rats. *J Trauma* 1985; 25: 385-92.
18. Redan JA, Rush BF, Lysz TW, Smith S, Machiedo GW. Organ distribution of gut-derived bacteria caused by bowel manipulation ischemia. *Am J Surg* 1990; 159: 85-9.
19. Maddaus MA, Wells CL, Platt JL, Condie RM, Simmons RL. Effect of T cell modulation on the translocation of the bacteria from the gut and mesenteric lymph node. *Ann Surg* 1988; 207: 387-98.
20. Widdison AL, Karanjia ND, Reber HA. Routes of spread of pathogens into the pancreas in a feline model of acute pancreatitis. *Gut* 1994; 35: 1306-10.
21. Han X, Fink MP, Yang R, Delude RL. Increased iNOS activity is essential for intestinal epithelial tight junction dysfunction in endotoxemic mice. *Shock* 2004; 21: 261-70.
22. Edmiston CE, Condon RE. Bacterial translocation. *Surg Gynecol Obstet* 1991; 173: 73-83.
23. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. *Arch Surg* 1990; 125: 403-4.
24. Alverdy JC, Aoye E, Moss GS. Total parenteral nutrition promotes bacterial translocation from the gut. *Surgery* 1988; 104: 185-90.
25. Alverdy JA, Aoye E, Weiss-Carrington P, Burke DA. The effect of glutamine-enriched TPN on gut immune cellularity. *J Surg Res* 1992; 52: 34-8.
26. Papaconstantinou HT, Hwang KO, Rajaraman S, Hellmich MR, Townsend CM Jr, Ko TC. Glutamine deprivation induces apoptosis in intestinal epithelial cells. *Surgery* 1998; 124: 152-60.
27. Arndt H, Kullmann F, Reuss F, Schölmerich J, Palitzsch KD. Glutamine attenuates leukocyte-endothelial cell adhesion in indomethacin-induced intestinal inflammation in the rat. *J Parenter Enteral Nutr* 1999; 23: 12-8.
28. Houdijk AP, Van Leeuwen PA, Boermeester MA, Van Lambalgen T, Teerlink T, Flinkerbusch EL et al. Gln-enriched enteral diet increases splanchnic blood flow in the rat. *Am J Physiol* 1994; 267: 1035-40.
29. Foitzik T, Fernandez-del Castillo C, Ferraro MJ, Mithöfer K, Rattner DW, Warshaw AL. Pathogenesis and prevention of early pancreatic infection in experimental acute necrotizing pancreatitis. *Ann Surg* 1995; 222: 179-85.