Enteral glutamine pretreatment does not decrease plasma endotoxin level induced by ischemia-reperfusion injury in rats

Arda Demirkan, Erkin Orazakunov, Berna Savaş, M Ayhan Kuzu, Mehmet Melli

Abstract

AIM: To investigate whether oral glutamine pretreatment prevents impairment of intestinal mucosal integrity during ischemia-reperfusion (I/R) in rats.

METHODS: The study was performed as two series with 40 rats in each. Each series of animals was divided into four groups. The first group was used as a control. Animals in the second group were only pretreated with oral glutamine, 1 g/kg for 4 d. The third group received a normal diet, and underwent intestinal I/R, while the fourth group was pretreated with oral glutamine in the same way, and underwent intestinal I/R. Intestinal mucosal permeability to 51Cr-labeled EDTA was measured in urine in the first series of animals. In the second series, histopathological changes in intestinal tissue and plasma endotoxin levels were evaluated.

RESULTS: Intestinal I/R produced a significant increase in intestinal permeability, plasma endotoxin level and worsened histopathological alterations. After intestinal I/R, permeability was significantly lower in glutamine-treated rats compared to those which received a normal diet. However, no significant change was observed in plasma endotoxin levels or histopathological findings.

CONCLUSION: Although glutamine pretreatment seems to be protective of intestinal integrity, upon I/R injury, such an effect was not observable in the histopathological changes or plasma endotoxin level.
intracellular amino acid pool \[\text{[12]}\]. It is an important respiratory fuel, and nucleotide precursor for the gastrointestinal tract \[\text{[13]}\]. Gln-supplemented parenteral nutrition protects rats against morphological and functional mucosal injury \[\text{[14]}\], and improves survival in animals after intestinal I/R \[\text{[15]}\]. Some other experimental studies have also shown that intraluminal injection of Gln protects the mucosa, and diminishes the accumulation of neutrophils in the lamina propia of the small bowel during I/R \[\text{[16]}\]. Generation of reactive oxygen intermediates (ROI) during reperfusion is thought to be one of the major causes of intestinal mucosal injury \[\text{[17,18]}\]. Gln is also essential for the synthesis of the intrinsic ROI scavenger glutathione. Therefore, this protective action may be due to augmentation of ROI scavenging in intestinal mucosa \[\text{[19]}\].

In this study, we aimed to investigate the effects of orogastric Gln pretreatment on intestinal mucosal permeability, plasma endotoxin level and intestinal histopathology during intestinal I/R injury in rats.

**MATERIALS AND METHODS**

**Experimental design**
The experiment was performed in female Wistar albino rats weighing 200-230 g. It was approved by the Ankara University Ethical Committee, and was conducted according to the European Community guidelines for the use of experimental animals. Animals were fed with their ordinary diet and allowed to drink water ad libitum. Owing to the time difference between urine collection and blood sampling, the study had to be completed in two series of experiments (Table 1).

**Induction of intestinal I/R**
A rat model of transient mesenteric occlusion was used to obtain intestinal I/R. Rats were anesthetized with ketamine (80 mg/kg, im) and xylazine (10 mg/kg, im). Intestinal I/R was induced by 60 min occlusion, followed by 60 min reperfusion \[\text{[20]}\]. During the 2 h of the surgical procedure, animals were kept at room temperature, and given intraperitoneal fluid as 0.9% NaCl (10 mL/kg). The superior mesenteric artery (SMA) was exposed through a midline abdominal incision, and both this artery and the collateral branches coming from the celiac axis, and the inferior mesenteric artery were occluded withatraumatic vascular clamps (Vascu-Stat II original No. 1001-532-3; Scanlan International, St Paul, MN, USA) for 1 h, followed by 1 h reperfusion. Existence of pallor and absence of pulsation ensured mesenteric occlusion during the ischemic period. Recovery of pulsation and pink color were controlled in each animal when the clamps were removed. The existence of intestinal I/R in this model was also confirmed in our laboratory by the appearance of pulses at the marginal arteries (direct vision of mesenteric circulation by microscopy), as well as by fluorescein angiography in preliminary experiments \[\text{[21]}\].

**Measurement of intestinal mucosal permeability**
Intestinal mucosal permeability was measured on the basis of urinary radioactivity levels following oral administration of \[^{51}\text{Cr}-\text{EDTA}\]. \[^{51}\text{Cr}-\text{EDTA}\] was employed as a well accepted marker of mucosal integrity \[\text{[22,23]}\]. After 60 min reperfusion, rats were given 5 \(\mu\)Ci \[^{51}\text{Cr}-\text{EDTA}\] in 0.5 mL saline solution by the orogastric route. Urine samples were collected in metabolic cages for 6 h following the reperfusion period. During urine collection, animals did not receive any food; however, they were allowed to access tap water. The level of radioactivity in the urine samples of 500 \(\mu\)L was then determined by counting on a gamma counter (DPC Gambry CR, Los Angeles, USA). The amount of \[^{51}\text{Cr}-\text{EDTA}\] excreted in urine during 6 h was calculated as a percentage of the ingested dose.

**Measurement of plasma endotoxin level**
Plasma endotoxin level was measured by the colorimetric Limulus amebocyte lysate (LAL) test. The test was performed by using the Pyrochrome test kit (Pyroquant Diagnostik, Mörfelden, Germany). All glassware, solutions and surgical instruments used in the experiment were autoclaved at 121°C for 15 min. The non-pyrogenicity of solutions was tested using the LAL test (Charles River Endosafe, Charleston, SC, USA).

Venous blood samples (3-4 mL) were collected using heparin-coated pyrogen-free disposable syringes. Platelet-rich plasma (PRP) was prepared from the blood by centrifugation at 150 g for 10 min. Fifty microliters of PRP was transferred into a polystyrene plastic tube and kept frozen at -80°C until the assay. Frozen PRP samples were kept at room temperature for about 30 min before the assay. Fifty microliters of 0.18 mol/L NaOH was added to 50 \(\mu\)L PRP, and incubated at 37°C for 5 min. Next, 50 \(\mu\)L 0.32 mol/L perchloric acid was added, and incubated at 37°C for a further 10 min. To dissolve the formed precipitate, 100 \(\mu\)L 0.18 mol/L NaOH was added, and vortexed. Twenty-five microliters of the solution was transferred into sterile non-pyrogenic microplates (Pyroquant Diagnostik), and 25 \(\mu\)L 0.2 mol/L Tris/HCl buffer (pH 8.0) was added to the wells \[\text{[24]}\]. Finally, 50 \(\mu\)L pyrochrome test solution was added to all wells, and mixed for 30 s. Plates were incubated at 37°C. Optical density was read at 405 nm. Standard curves from 0.04 to 1.28 EU/mL were used to evaluate the concentration of endotoxin.

| Table 1 Characteristics of study groups |
|----------------------------------------|
| Groups | Measurement of intestinal permeability by \[^{51}\text{Cr}-\text{EDTA}\] | Measurement of plasma endotoxin levels and histopathological changes |
|--------|-----------------------------|-----------------------------|
| I (Control) | – | – |
| II (Gln) | – | + |
| III (I/R) | – | + |
| IV (I/R and Gln) | + | + |
| Time-matched, sham-operated animals undergoing laparotomy and dissection of the SMA without occlusion served as controls (group I). The Gln group (group II) was pretreated with Gln (1 g/kg per day) by the orogastric route for 4 d \[\text{[20]}\]. Gln was prepared in 0.9% NaCl for daily use. Intestinal I/R group (group III) underwent 1 h intestinal ischemia, and 1 h reperfusion. In the I/R and Gln group (group IV), I/R periods and Gln administration were the same as in Gln (II) and I/R (III) groups.

World J Gastroenterol 2008; 14(3): 464-470

ISSN 1007-9327  CN 14-1219/R  World J Gastroenterol  January 21, 2008  Volume 14  Number 3
Results were calculated by using non-linear regression of a four-parameter logistic model.

Histopathological assessment of ileal tissues
After the collection of blood samples, ileal tissue samples, 10 cm proximal to the cecum, were harvested and evaluated for histopathological changes. Sections were stained with hematoxylin and eosin, and were examined by light microscopy by two pathologists in a blinded manner. Mucosal injury was scored on a scale from 0 to 5, as described by Chiu et al.

Statistical analysis
The SPSS program was used for statistical analysis. Comparison of the various protocols on the changes in intestinal permeability was made by one-way analysis of variance (ANOVA) following a Bonferroni post-hoc test. Changes in plasma endotoxin levels and intestinal histopathology were determined using a Kruskal-Wallis test following a multiple comparison post-hoc test. Data on changes in intestinal permeability were presented as means ± SEM. Data of plasma endotoxin levels and intestinal histopathology were presented as medians. P < 0.05 was considered statistically significant.

RESULTS

Effect of Gln pretreatment on intestinal permeability during I/R injury
To investigate the effect of intestinal I/R and Gln pretreatment on intestinal permeability, renal clearance of 51Cr-EDTA was assessed. Statistically significant differences were detected in intestinal permeability in the I/R group compared to the control group (8.6% ± 1.7% vs 2.4% ± 1.1%, P < 0.001). Gln pretreatment significantly lowered the increased intestinal permeability due to intestinal I/R (8.6% ± 1.7% vs 5.3% ± 1.3%, P < 0.001). There was no statistically significant difference in intestinal permeability between the control and Gln groups (Figure 1).

DISCUSSION
Ischemia and subsequent reperfusion is one of the major causes of cell injury. The mechanisms of I/R injury are complex, and are likely to differ with respect to the duration of ischemia, the specific tissue involved, and the species studied. The small intestine may experience ischemia and reperfusion during septic shock, hemorrhagic shock, or cardio-vascular surgery. These clinical situations can result in some serious postoperative complications such as delay in anastomotic healing. Intestinal mucosa is known to be sensitive to I/R injury. Its basal high rate of oxygen use renders the intestine relatively incapable of increasing oxygen transport in cases of hypoxic stress, and thus is
In conclusion, although Gln pretreatment reversed increased intestinal permeability, it did not prevent an increase in plasma endotoxin levels or histopathological alterations in intestinal I/R. Further studies are necessary to clarify the effects of different doses and administration periods of Gln on plasma endotoxin levels and histopathological changes in intestinal I/R.

ACKNOWLEDGMENTS

The authors would like to thank Professor Ongun Onaran for helpful criticism of the manuscript.

COMMENTS

Background

Impairment of microcirculation during I/R in the gastrointestinal tract may diminish intestinal mucosal integrity and cause an increase in intestinal permeability. Increased plasma endotoxin levels after I/R are a major threat to many surgical patients. Different Gln treatments are known to have protective effects on mucosal integrity in intestinal I/R.

Research frontiers

Injury to the intestinal barrier results in an increase in permeability to intraluminal substances. Correlation between morphological alterations and the degree of increased intestinal permeability is uncertain. Recent studies have demonstrated that morphological alterations after intestinal mucosal injury cannot reflect the function of the intestinal barrier.

Innovations and breakthroughs

Recent studies suggest that gastrointestinal epithelial cells contain specific transport systems for lipopolysaccharides.
Applications
Intestinal permeability can be measured by many different in vitro and in vivo methods. Endotoxin passing through the intestinal mucosa into the circulation indicates loss of the intestinal barrier function. Measurement of plasma endotoxin level ensures the assessment of real pathogenic factors that arise in the systemic circulation as a result of intestinal mucosal injury.

Terminology
Bacterial translocation: indigenous bacteria that colonize the gastrointestinal tract can cross the epithelial mucosa to infect distant organs. Under normal conditions, the epithelial lining prevents the escape of these bacteria from the gut lumen. Intestinal permeability: relates to the properties and function of the epithelial barrier that enables unmediated passage of substances through the intestinal mucosa. The use of intestinal permeability tests for screening of intestinal disease and assessment of treatment efficacy, to understand the normal intestinal physiology and pathogenesis of disease, is described and reviewed. There is now a need for research into the basic mechanisms of regulatory control of the intestinal barrier function.

Peer review
Peer reviewers considered this to be a very interesting paper with a great deal of potential clinical benefit. These basic results may be of value in clinical applications in organ or cellular transplantation.

REFERENCES
1 Stechmiller JK, Treloar D, Allen N. Gut dysfunction in critically ill patients: a review of the literature. Am J Crit Care 1997; 6: 204-209
2 Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970; 101: 478-483
3 Parks DA, Granger DN. Contributions of ischemia and reperfusion to mucosal lesion formation. Am J Physiol 1986; 250: G749-G753
4 Vatistas NJ, Nieto JE, Van Hoogmoed L, Gardner I, Snyder JR. Use of an isolated intestinal circuit to evaluate the effect of ischemia and reperfusion on mucosal permeability of the equine jejunum. Vet Surg 2003; 32: 52-61
5 Langer JC, Sohal SS, Brennerhassett P. Mucosal permeability after subclinical intestinal ischemia-reperfusion injury: an exploration of possible mechanisms. J Pediatr Surg 1995; 30: 568-572
6 Wu GH, Wang H, Zhang YW, Wu ZH, Wu ZG. Glutamine supplemented parenteral nutrition prevents intestinal ischemia-reperfusion injury in rats. World J Gastroenterol 2004; 10: 2592-2594
7 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: 403-404
8 Doig CJ, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. Am J Respir Crit Care Med 1998; 158: 444-451
9 Deitch EA. Bacterial translocation: is it of clinical significance? Gastroenterology 1990; 98: 243-244
10 Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology 1995; 108: 1566-1581
11 Sorkine P, Szold O, Halpern P, Gutman M, Greenland M, Rudick V, Goldman G. Gut decontamination reduces bowel ischemia-induced lung injury in rats. Chest 1997; 112: 491-495
12 Ziegler TR, Smith RJ, Byrne TA. Potential role of glutamine supplementation in nutrition support. Clin Nutr 1993; 12 Suppl 1: S82-S90
13 Haque SM, Chen K, Usui N, Liboisy O, Okuyama H, Masunari A, Cui L, Nezu R, Takagi Y, Okada A. Alanyl-glutamine-dipeptide-supplemented parenteral nutrition improves intestinal metabolism and prevents increased permeability in rats. Ann Surg 1996; 223: 334-341
14 Tazuke Y, Wasa M, Shimizu Y, Wang HS, Okada A. Alanyl-glutamine-supplemented parenteral nutrition prevents intestinal ischemia-reperfusion injury in rats. JPEN J Parenter Enter Nutr 2003; 27: 110-115
15 Ikeda S, Zarzar BL, Johnson CD, Fukatsu K, Kodak SA. Total parenteral nutrition supplementation with glutamine improves survival after gut ischemia/reperfusion. JPEN J Parenter Enter Nutr 2002; 26: 169-173
16 de Aguilar-Nascimento JE, Gurgel Marques C, Carvalho Mariano A, Bicudo Salomao A, de Souza Neves J. Benefits of intraluminal injection of glutamine for intestinal mucosa during ischemia-reperfusion. Eur Surg Res 2003; 35: 352-356
17 Nilsson UA, Lundgren O, Haglund E, Bylund-Fellenius AC. Radical production during in vivo intestinal ischemia and reperfusion in the cat. Am J Physiol 1989; 257: G409-G414
18 Schoenberg MH, Beger HG. Oxygen radicals in intestinal ischemia and reperfusion. Chem Biol Interact 1990; 76: 141-161
19 Harward TR, Coe D, Souba WW, Klingman N, Seeger JM. Glutamine preserves gut glutathione levels during intestinal ischemia/reperfusion. J Surg Res 1994; 56: 351-355
20 Jensen JC, Schaefer R, Nwokedi E, Bevans DW 3rd, Baker ML, Pappas AA, Westbrook KC, Klimberg VS. Prevention of chronic radiation enteropathy by dietary glutamine. Ann Surg Oncol 1994; 1: 157-163
21 Demling RH. Enteral glutamine administration prevents the decrease in cell energy charge potential produced in ileum after a skin burn in the rat. J Burns Care Rehabil 2000; 21: 275-279; discussion 274
22 Kojima M, Iwakiri R, Wu B, Fujise T, Watanabe K, Lin T, Amemori S, Sakata H, Shimoda R, Oguzu T, Ootani A, Tsunada S, Fujimoto K. Effects of antioxidant agents on apoptosis induced by ischemia-reperfusion in rat intestinal mucosa. Aliment Pharmacol Ther 2003; 18 Suppl 1: 139-145
23 Koksoy C, Kuzu MA, Ergun H, Demirpence E, Zulfikaroglu B. Intestinal ischemia and reperfusion impairs vasomotor functions of pulmonary vascular bed. Ann Surg 2000; 231: 105-111
24 Bjarnason I, Smethurst P, Levi AJ, Peters TJ. Intestinal permeability to 51Cr-EDTA in rats with experimentally induced enteropathy. Gut 1985; 26: 579-585
25 Reuter BK, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohelial circulation. Gastroenterology 1997; 112: 109-117
26 Inada K, Endo S, Takahashi K, Suzuki M, Narita T, Yoshida T, Suda H, Komuro T, Yoshida M. Establishment of a new perchloric acid treatment method to allow determination of the total endotoxin content in human plasma by the limulus test and clinical application. Microbiol Immunol 1991; 35: 303-314
27 Conover WJ. Multiple comparison test. In: Practical nonparametric statistics. 2nd ed. New York: John Wiley & Sons, 1980: 229-239
28 Bulkley GB. Free radicals and other reactive oxygen metabolites: clinical relevance and the therapeutic efficacy of antioxidant therapy. Surgery 1993; 113: 479-483
29 Tedros T, Traber DL, Heggars JP, Herndon DN. Effects of interleukin-1alpha administration on intestinal ischemia and reperfusion injury, mucosal permeability, and bacterial translocation in burn and sepsis. Ann Surg 2003; 237: 101-109
30 Chang JX, Chen S, Ma LP, Jiang LY, Chen JW, Chang RM, Wen LQ, Wu W, Jiang ZP, Huang ZT. Functional and morphological changes of the gut barrier during the restitution process after hemorrhagic shock. World J Gastroenterol 2005; 11: 5485-5491
31 Juel IS, Solligard E, Lyng O, Stromholm T, Tvedt KE, Johnsen H, Nygge P, Saether OD, Aadal P, Gronbech JE. Intestinal injury after thoracic aortic cross-clamping in the pig. J Surg Res 2004; 117: 283-295
32 Bjorck M, Troeng T, Bergqvist D. Risk factors for intestinal ischaemia after aortoiliac surgery: a combined cohort and case-control study of 2624 operations. Eur J Vasc Endovasc Surg 1997; 13: 531-539
33 Kologlu M, Yorganci K, Renda N, Sayek I. Effect of local and www.wignet.com
remote ischemia-reperfusion injury on healing of colonic anastomoses. *Surgery* 2000; 128: 99-104

34 Schoenberg MH, Beger HG. Reperfusion injury after intestinal ischemia. *Crit Care Med* 1993; 21: 1376-1386

35 Hammerman C, Goldschmidt D, Caplan MS, Kaplan M, Schimmel MS, Eidelman AI, Branski D, Hochman A. Amelioration of ischemia-reperfusion injury in rat intestine by pentoxifylline-mediated inhibition of xanthine oxidase. *J Pediatr Gastroenterol Nutr* 1999; 29: 69-74

36 Ameno H, Tani T, Hanasawa K, Kodama M. New method for the detection of bacterial translocation using intestinal permeability with polyethylene glycol 4000. *Eur Surg Res* 2000; 32: 25-29

37 Drewe J, Beglinger C, Fricker G. Effect of ischemia on intestinal permeability of lipopolysaccharides. *Eur J Clin Invest* 2001; 31: 138-144

38 Caty MG, Guice KS, Oldham KT, Remick DG, Kunkel SI. Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. *Ann Surg* 1990; 212: 694-700

39 Mangino MJ, Anderson CB, Murphy MK, Brunet E, Turk J. Mucosal arachidonate metabolism and intestinal ischemia-reperfusion injury. *Am J Physiol* 1989; 257: G299-G307

40 Hagiwara M, Kataoka K, Arimochi H, Kuwahara T, Ohnishi Y. Role of unbalanced growth of gram-negative bacteria in ileal ulcer formation in rats treated with a nonsteroidal anti-inflammatory drug. *J Med Invest* 2004; 51: 43-51

41 Banerjee AK, Peters TJ. Experimental non-steroidal anti-inflammatory drug-induced enteropathy in the rat: similarities to inflammatory bowel disease and effect of thromboxane synthetase inhibitors. *Gut* 1990; 31: 1358-1364

42 Kinouchi T, Kataoka K, Bing SR, Nakayama H, Uejima M, Shimono K, Kuwahara T, Akimoto S, Hiraoka I, Ohnishi Y. Culture supernatants of Lactobacillus acidophilus and Bifidobacterium adolescentis repress ileal ulcer formation in rats treated with a nonsteroidal antiinflammatory drug by suppressing unbalanced growth of aerobic bacteria and lipid peroxidation. *Microbiol Immunol* 1998; 42: 347-355

43 Medeiros AC, Chacon DA, Sales VS, Egito ES, Brandao-Neto J, Pinheiro LA, Carvalho MR. Glucan and glutamine reduce bacterial translocation in rats subjected to intestinal ischemia-reperfusion. *J Invest Surg* 2006; 19: 39-46

44 Klimberg VS, Salloum RM, Kasper M, Plumley DA, Dolson DJ, Hautamaki RD, Mendenhall WR, Bova FC, Bland KI, Copeland EM 3rd. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. *Arch Surg* 1990; 125: 1040-1045

45 Fox AD, Kripke SA, De Paula J, Berman JM, Settle RG, Rombeau JL. Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis. *JPEN J Parenter Enteral Nutr* 1988; 12: 325-331

46 Souba WW, Herskowitz K, Klimberg VS, Salloum RM, Plumley DA, Flynn TC, Copeland EM 3rd. The effects of sepsis and endotoxemia on gut glutamine metabolism. *Ann Surg* 1990; 211: 543-549; discussion 549-551

47 Tomita M, Ohkubo R, Hayashi M. Lipopolysaccharide transport system across colonic epithelial cells in normal and infective rat. *Drug Metab Pharmacokinet* 2004; 19: 33-40