PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(17):2592-2594
World Journal of Gastroenterology
 ISSN 1007-9327

• BRIEF REPORTS •

Glutamine supplemented parenteral nutrition prevents intestinal ischemia- reperfusion injury in rats

Guo-Hao Wu, Hao Wang, Yan-Wei Zhang, Zhao-Han Wu, Zhao-Guang Wu

Guo-Hao Wu, Hao Wang, Yan-Wei Zhang, Zhao-Han Wu, Zhao-Guang Wu, Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai 200032, China
Correspondence to: Guo-Hao Wu, Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai 200032, China. wugh@zshospital.net
Telephone:+86-21-64041990 Ext. 2365 **Fax:** +86-21-64038472
Received: 2004-02-02 **Accepted:** 2004-02-24

Abstract

AIM: To examine whether glutamine prevents the injury to the intestinal mucosa after intestinal ischemia-reperfusion (I/R) in rats.

METHODS: Thirty male Sprague-Dawley rats were randomly divided into 3 groups: a standard parenteral nutrition (PN) group (n = 10); an I/R-PN group (n = 10); an I/R-glutamine enriched PN (I/R-Gln) group (n = 10). The superior mesenteric artery (SMA) was clamped. After 60 min of ischemia, reperfusion was initiated and infusion was started. All rats received isocaloric and isonitrogenous nutritional support for 48 h. Spleen, liver, mesenteric lymph nodes (MLN), and intestinal segments were removed for morphological and biochemical analyses, and blood samples were collected for bacterial culture and measurement of endotoxin levels. The permeability of intestinnal mucosa was assayed by measurement of D-(-)-lactate levels in plasma.

RESULTS: In I/R-PN group, extensive epithelial atrophy was observed, mucosal thickness, villous height, crypt depth and villous surface area were decreased significantly compared with PN group, whereas these findings did not occur in the I/R-Gln group. The incidence of intestinal bacterial translocation to spleen, liver, MLN, and blood was significantly higher in I/R-PN group than that in other groups. Plasma endotoxin levels significantly increased in the I/R-PN group compared with the I/R-Gln group. Remarkably higher values of D-(-)-lactate were also detected in PN group compared with that in I/R-Gln group.

CONCLUSION: Glutamine protects the morphology and function of intestinal mucosa from injury after I/R in rats.

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 (17): 2592-2594

http://www.wjgnet.com/1007-9327/10/2592.asp

INTRODUCTION

Ischemia-reperfusion (I/R) of the gut is a common event in a variety of clinical conditions, such as trauma, burn, septic shock, heart or aortic surgery, and liver or small bowel transplantation[1,2]. Intestinal I/R results in many adverse events to the small intestine such as edema and disruption of the structural and functional mucosa. This injury includes mucosal and vascular permeability, change bacterial translocation, and a high death rate[3,4]. It has been proposed that most of the mucosal injury resulted from I/R are mediated by reactive oxygen derived free radicals produced and released when hypoxic tissues are reoxygenated during reperfusion. Clinical and experimental studies suggest that I/R-induced intestinal injury play a role in the pathogenesis of systemic inflammation, respiratory failure, and multiple-organ failure (MOF)[5].

Glutamine (GLN) is the primary metabolic fuel of small intestinal enterocytes, and has been shown to be an essential metabolic component of the proliferative response of enterocytes. Studies have shown that glutamine reduced atrophy of intestinal mucosa in rats on total parenteral nutrition (TPN), prevented intestinal mucosal injury accompanying small bowel transplantation, chemotherapy, and radiation. It was also reported that glutamine supplemented TPN prevented intestinal I/R injury, and improved survival after intestinal I/R in animal models[6,7]. Thus, GLN treatment seems to be a preventive and therapeutic method for gut I/R-induced organ injury. However, despite the positive result from previous studies, glutamine has not been used extensively to treat preexisting intestinal I/R injury. The purpose of this study was to examine whether glutamine prevented damage to the intestinal mucosa after intestinal I/R in rats.

MATERIALS AND METHODS

Experimental protocol/procedures

Thirty male Sprague-Dawley rats weighing 350 to 450 g were employed after a period of acclimatization. After an overnight fast, the rats were anesthetized with sodium pentobarbital 25 mg/kg intraperitoneally. Through a midline laparotomy, the superior mesenteric artery (SMA) was carefully isolated and clamped at its origin from the abdominal aorta. After 60 min of ischemia, the clamp was removed from the SMA and reperfusion was initiated. The abdominal incision was closed and infusion was started immediately. A silastic catheter was inserted through the right jugular vein, tunneled subcutaneously, and brought out through the skin of midscapular region. The rats were randomly divided into 3 groups: a standard parenteral nutrition (PN) group (n = 10); an I/R-PN group (n = 10); an I/R-glutamine enriched PN (I/R-Gln) (n = 10). The SMA of sham rats in PN group was isolated but not clamped. All rats were maintained in individual metabolic cages, and received parenteral nutritional support for 48 h. The composition of the TPN solution is shown in Table 1. Both solutions were isocaloric (174.3 kcal/kg·d) and isonitrogenous (1.0 g/kg·d). After 48 h' nutritional support, a 20-cm long intestinal segment was obtained from a point 10 cm distal to the ligament of Treiz for morphological and biochemical analysis. Spleen, liver, MLN, and blood samples were collected for bacterial culture and measurement of endotoxin levels.

Histologic evaluation and biochemical analysis

Intestinal samples corresponding to the first 5 cm of the resected intestinal segment were fixed in 40 g/L formaldehyde. All samples were embedded in paraffin and stained with hematoxylin and eosin. Three paraffin sections were prepared from each fixed tissue sample, and each slide was analyzed. A blinded

observer performed the histologic analyses using the histologic scoring system. Mucosal wall thickness, villous height, crypt depth and villous surface area were measured under light microscope. All measurements were made in triplicate, and mean values were obtained. The 15 cm of the intestinal segment left was sampled, and the mucosal sample was immediately scraped from the underlying muscular layer with a glass slide. The mucosa was weighed and stored at -70 °C until analysis. DNA and RNA content was measured by the fluorometric method. Protein content was determined by the bicichoninic acid method.

Intestinal permeability

The permeability of intestinal mucosa was assayed by measurement of D-lactate levels in plasma. The procedure used a glycine-hydrazine buffer at pH 9.5 and 25 °C. The assay was based on the enzymatic oxidation of D-lactate with a specific D-lactic dehydrogense coupled to reduction of NAD^+ with the spectrophotometric measurement of NADH at 340 nm. Plasma was separated from the blood sample by centrifugation and stored at -70 °C until analysis. Quantification of D-lactate was performed by fluorescence spectrometry as described by Shimojo *et al*.[8]. The measurements were made with a Beckman DU fluorescence spectrophotometer at 340 nm. D-lactate standard stock solution was 0.47 mg/mL Li lactate (0.44 mg/mL D-lactic acid) and was diluted for standard curves. D-lactic dehydrogenase was from Sigma Biochemicals and was diluted with water to about 600 U/mL. Sigma assay kit 826-UV was used for D-lactate analysis.

Bacterial translocation measurements

Rats were killed, and their mesenteric lymph nodes, spleens and livers were removed. The tissues were transferred to grinding tubes containing sterile BHI to detect aerobic bacteria or sterile 10 A broth to detect lactobacilli. The tissues were homogenized, and BHI tubes were incubated aerobically, whereas the 10 A broth tubes were incubated in 100 mL/L carbon dioxide at 37 °C. After incubation, 0.2 mL of the tissues homogenates was spread on blood agar plates to detect aerobic bacteria, *E. coli* or lactobacilli. The tissues homogenates were Gram- stained to confirm that bacteria present in the homogenates grew on the agar plates.

Statistical analysis

Data were analyzed using standard statistical software (SPSS 10.0). For normally distributed data, a paired Student's *t* test was used for statistical analysis. A probability value less than or equal to 0.05 was considered statistically significant. All data were expressed as mean±SE.

RESULTS

The mortality rate was 0% (0/10), 28.6% (4/14), and 16.7% (2/12) in the PN, I/R-PN, and I/R-Gln groups, respectively. It was significantly higher in I/R-PN group compared with that in the PN group and I/R-Gln group (P<0.05).

In the I/R-PN group, extensive mucosal damage characterized by extensive edema, leukocytic infiltration, epithelial sloughing, mucosal ulceration of villous tips, and mucosal atrophy was observed. This did not occur in the PN group and I/R- Gln group, and no histologic difference was noted between the PN group and I/R- Gln group. Mucosal wall thickness, villous height, crypt depth and villous surface area decreased significantly in the I/R-PN group compared with those in the PN group and I/R-Gln group (P<0.05; Table 2). Mucosal wet weight, protein, DNA content and RNA content decreased significantly in the I/R-PN group compared with those in the PN group and I/R- Gln group (P <0.05; Table 3).

Plasma D-lactate levels significantly increased in the I/R-PN group (0.15±0.04 mmol/L) compared with those in the PN group (0.08±0.02 mmol/L; P<0.05) and I/R- Gln group (0.10±0.02 mmol/L; P<0.05). No significant difference was observed between PN group and I/R- Gln group.

The incidence of intestinal bacterial translocation to MLN, spleen, liver, and blood was significantly higher in I/R-PN group compared with that in PN group and I/R- Gln group. There was no significant difference between PN group and I/R- Gln group (P<0.05; Table 4). The plasma endotoxin levels increased in I/R-PN group (14.5±0.12 pg/mL) compared with PN group (9.2±0.8 pg/mL; P<0.05) and I/R- Gln group (10.5±0.9 pg/mL; P<0.05).

Table 1 Composition of parenteral nutrition solutions (per 100 mL)

	PN	I/R- Gln
Glucose (g)	17.9	17.9
Fat emulsion (g)	5.6	5.6
Amino acid (g)	5.8	2.2
Ala-Glu (g)	0.0	3.6
Nitrogen (g)	0.93	0.93
NPC (kcal)	122.0	122.0
NPC/N	131.2	131.2

Table 2 Morphologic parameters

	PN (n = 10)	I/R-PN (n = 10)	I/R- Gln (n = 10)
Mucosal wall thickness (μm)	566.5±37.4[a]	418.2±39.6	564.4±59.3[a]
Villous height (μm)	404.2±39.1[a]	290.8±48.8	396.4±60.5[a]
Crypt depth (μm)	214.5±29.4[a]	136.6±20.2	204.9±38.6[a]
Villous surface area (mm^2)	0.118±0.010[a]	0.065±0.006	0.112±0.012[a]

[a]P<0.05 *vs* I/R-PN; PN: parenteral nutrition; I/R: ischemia-reperfusion Gln: glutamine.

Table 3 Mucosal wet weight and biochemistry

	PN (n = 10)	I/R-PN (n = 10)	I/R- Gln (n = 10)
Mucosal wet weight (mg/cm)	74.6±16.3[a]	55.2±12.0	82.8±18.2[a]
Protein content (mg/cm)	3.25±0.92[a]	1.73±0.60	3.16±0.47[a]
DNA content (mg/cm)	1.42±0.20[a]	0.64±0.12	1.33±0.17[a]
RNA content (mg/cm)	4.53±0.58[a]	3.26±0.78	4.45±0.84[a]

[a]P<0.05 *vs* I/R-PN; PN: parenteral nutrition; I/R: ischemia-reperfusion Gln: glutamine.

Table 4 Bacterial translocation measurements

	PN (n = 10)	I/R-PN (n = 10)	I/R- Gln (n = 10)
Rate of BT	10% (1/10)	100% (10/10)	20% (2/10)
MLN	1	9	2
Blood	1	7	1
Liver	0	5	0
Spleen	0	4	1

BT: bacterial translocation, MLN: mesenteric lymph mode.

DISCUSSION

Ischemia of the gut is a common event after trauma and a significant predisposing factor of MOF. Intestinal I/R injury results in

many adverse events to the small intestine such as interstitial edema and disruption of the structural and functional mucosa. These increase the permeability of the intestinal mucosa and promote bacterial translocation. Clinical and experimental studies suggest that I/R-induced intestinal injury plays a role in the pathogenesis of systemic inflammation, respiratory failure, and MOF[9,10]. Glutamine is the primary metabolic fuel of small amino acid pool in the body, and has been shown to be an essential metabolic component of the proliferative response of enterocytes. Studies showed that glutamine supplementation of TPN improved survival after gut I/R injury[6,11], reduced atrophy of intestinal mucosa in rats on total parenteral nutrition[12], prevented intestinal mucosal injury accompanying short bowel, small bowel transplantation, chemotherapy, and radiation[13-17]. In the present study, the mortality rate was 28.6% in the I/R-PN group and apparent mucosal damage, such as epithelial sloughing and mucosal ulceration was observed histologically. A 100% survival was achieved in the PN group, 16.7% in I/R-Gln group, and only minor histologic changes of the small intestine were observed in PN and I/R-Gln groups. The I/R-Glin rats had a greater villous height, more mucosal protein, DNA and RNA content, and a lower degree of intestinal permeability compared with the I/R-TPN rats. This suggests that Gln supplemented TPN plays an important role in the maintenance of intestinal structure and barrier function in I/R-injured rats.

The intestinal mucosa, especially in the region of villi, is especially susceptible to hypoxia because of the microvascular architecture and its high energy demand. Intestinal ischemia perpetuates the bacteremic condition by promoting translocation of bacteria into the circulation. Several experimental and clinical studies have shown that intestinal ischemia decreases barrier function of the gut and enhances translocation of bacteria and toxin[18,19]. In our I/R model, the SMA of rats was clamped for 60 min, and the rats were killed 48 h after intestinal I/R. Mesenteric lymph nodes, spleens, livers and blood were then cultured quantitatively. Almost all MLN had positive cultures and grew significantly great numbers of enteric bacteria, spread to the blood, liver and spleen in I/R-PN group. The most common bacterium discovered from solid viscera was *E. coli*, other species included *enterococcus, pseudomonas, proteus, and staphylococcus*. While, only 20% MLN cultures were positive, less than 10% spread to blood and spleen in I/R-Gln group. Additionally, plasma D-lactate and endotoxin levels increased significantly in I/R-PN group compared with that in I/R-Gln group. These findings indicated that the increase of intestinal permeability and the incidence of bacterial translocation in the intestinal I/R rats were prevented by glutamine supplementation.

In conclusion, the present study demonstrates that glutamine supplemented TPN protects rat intestine from morphologic and functional mucosal injury after intestinal I/R. These results suggest that glutamine would be clinically useful in the treatment of intestinal I/R injury.

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Edited by Zhang JZ **Proofread by** Zhu LH and Xu FM