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

ELSEVIER

World J Gastroenterol 2005;11(7):1070-1072
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

L-arginine administration ameliorates serum and pulmonary cytokine response after gut ischemia-reperfusion in immature rats

Ting-Liang Fu, Wen-Tong Zhang, Lan Zhang, Feng Wang, Yong Gao, Ming Xu

Ting-Liang Fu, Wen-Tong Zhang, Department of Pediatric Surgery, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China
Lan Zhang, Feng Wang, Clinical Nutrition Center and Department of Radiology, Affiliated Hospital of Binzhou Medical College, Binzhou 256603, Shandong Province, China
Yong Gao, Ming Xu, Binzhou Medical College, Binzhou 256603, Shandong Province, China
Supported by The Natural Scientific Foundation of Shandong Province, No. Q99C13
Correspondence to: Lan Zhang, Department of Radiology, Affiliated Hospital of Binzhou Medical College, 661 Huanghe Road, Binzhou 256603, Shandong Province, China. drfutl@sina.com
Telephone: +86-543-3256733
Received: 2004-06-08 Accepted: 2004-06-28

Abstract

AIM: Small intestinal ischemia-reperfusion (IR) has been demonstrated to result in both local mucosal injury and systemic injuries. The exact role of nitric oxide (NO) in intestinal IR is unclear. We propose that NO and some other cytokines change in the reperfusion period and these changes are associated with lung injury. The aim of this study was to determine the effect of supplementing NO substrate, L-arginine (L-arg), on serum and pulmonary cytokine production during small intestinal IR in immature rats.

METHODS: Immature rats underwent 60 min. of superior mesenteric artery occlusion followed by 90 min of reperfusion. L-arg (250 mg/kg) was given intravenously to the experimental group (IR+L-arg) which received L-arg after 45 min of intestinal ischemia. Serum and lung endothelin-1 (ET-1), NO, malondialdehyde (MDA), and tumor necrosis factor α (TNFα) were measured. Sham operation (SHAM) and intestinal IR (IR) groups were performed as control. The lavage fluid of the lung was collected by bronchoalveolar lavage (BAL) and white blood cells and polymorphonuclear cells (PMNs) were immediately counted to identify lung damage.

RESULTS: When L-arg was given during small intestinal IR, serum NO concentration increased significantly in IR+L-arg group (162.17±42.93 μmol/L) when compared with IR group (87.57±23.17 μmol/L, t = 3.190, P = 0.008 <0.01). Serum MDA reduced significantly in IR+L-arg group (8.93±1.50 nmol/L) when compared with SHAM (23.78±7.81 nmol/L, t = 3.243, P = 0.007<0.01) and IR (25.54±9.32 nmol/L, t = 3.421, P = 0.006<0.01). ET-1 level in lung tissues was significantly lower in IR+L-arg group (13.81±7.84 pg/mL) than that in SHAM (35.52±10.82 pg/mL, t = 2.571, P = 0.03<0.05) and IR (50.83±22.05 pg/mL, t = 3.025, P = 0.009<0.01) groups. MDA contents in lung tissues were significantly lower in IR+L-arg group (10.73±1.99 nmol/L) than in SHAM (16.62±2.28 nmol/L, t = 3.280, P = 0.007<0.01) and IR (21.90±4.82 nmol/L, t = 3.322, P = 0.007<0.01) groups. Serum and lung TNFα concentrations were not significantly different in three groups. NO contents in lung homogenates and white blood cell counts in BAL had no significant difference in three groups; but the percentage of PMNs in BAL was 13.50±8.92, 33.20±16.59, and 22.50±6.09 in SHAM, IR, and IR+L-arg groups, respectively.

CONCLUSION: Small intestinal IR induced increases of pulmonary neutrophil infiltration in immature rats. Neutrophil infiltration in lung tissues was reduced by L-arg administration but remained higher than in SHAM group. L-arg administration during intestinal IR enhances serum NO production, reduces serum MDA and lung ET-1 and MDA levels, resulting in the improvement of systemic endothelial function. L-arg supplementation before reperfusion may act as a useful clinical adjunct in the management of intestinal IR, thus preventing the development of adult respiratory distress syndrome, even multiple organ dysfunction syndrome (MODS).

Key words: Intestine; Ischemia-reperfusion; Nitric oxide; L-arginine; Rat

Fu TL, Zhang WT, Zhang L, Wang F, Gao Y, Xu M. L-arginine administration ameliorates serum and pulmonary cytokine response after gut ischemia-reperfusion in immature rats. *World J Gastroenterol* 2005; 11(7): 1070-1072
http://www.wjgnet.com/1007-9327/11/1070.asp

INTRODUCTION

Intestinal IR has been demonstrated to result in both intestinal mucosal injury and systemic injuries[1,2]. The exact role of NO in intestinal IR is unclear[1]. In the literature, several studies indicated NO as being either beneficial or detrimental to IR-induced injury[1,2]. An inflammatory process mediated by activated neutrophils resulting in capillary dysfunction and macromolecular leakage is proposed in recent years[3]. One proposed mechanism involves the combination of endothelial-derived NO with superoxide, producing the powerful oxidant peroxynitrite (ONOO-). This molecule may act locally to cause intestinal mucosal injury following

IR[1]. Tavaf-Motamen *et al*[4] demonstrated that inhibition of constitutive form of NO synthase (cNOS) with N^G-nitro-L-arginine (L-NNA) accelerated the post-ischemia neutrophil activation and induced lung injury.

The focus of the present study was to investigate if supplement of L-arg would ameliorate systemic and lung cytokine production after small intestinal IR in immature rats. Thus, the effects of L-arg administration on serum and lung endothelin-1 (ET-1), NO, MDA, TNFα concentrations were quantitatively measured.

MATERIALS AND METHODS

Animals

Experiments were performed on male Sprague-Dawley rats (25-day-old), weighing 84-110 g. Twelve hours prior to experimentation, animals were fasted and free access to water was allowed. Anesthesia was induced with intraperitoneal ketamine (15 mg/kg) injection, and maintained with intermittent boluses of intraperitoneal ketamine (10 mg/kg). Animals were placed on a heating pad set at 37 ℃.

Induction of intestinal IR models

Through a midline abdominal incision, the superior mesentery artery (SMA) was dissected in all the three groups. Animals in SHAM group were treated identically, omitting the SMA occlusion. At 45 min after laparotomy, 0.5 mL of normal saline was injected slowly into the femoral vein. For animals in IR group, SMA was occluded with an atraumatic microvascular clamp for 60 min. Then the clamp was removed. Reperfusion of the intestine was confirmed by the return of pulsatile mesenteric blood flow. At 45 min after ischemia, 0.5 mL of normal saline was injected slowly into the femoral vein. In IR+L-arg group, 45 min after ischemia, 0.5 mL of L-arg (250 mg/kg) was slowly administered intravenously.

Sampling procedures

After 90 min of reperfusion, 1.5 mL of blood was obtained from jugular artery and animals were euthanized with 0.5 mL of 10% potassium chloride intravenously. Then a thoracotomy was immediately performed. The left lobe base of lung was harvested and immediately homogenized on ice with nine volumes of normal saline. The homogenates of the lung tissue and blood were spun by centrifugation at 1 000 r/min at 4 ℃ for 10 min. The lungs were then excised en bloc, and BAL was performed through the trachea. The lavage fluid was collected immediately to count white blood cells and PMNs.

Specimen measurements

Serum and supernatants of the lung homogenates were stored at -20 ℃ for analysis. The concentration of ET-1, and TNFα in serum and supernatants was analyzed using radioimmunoassay kits (supplied by Beijing East Asia Immunology Institute, China). The concentration of NO and MDA was monitored by NO and MDA assay kits (supplied by Nanjing Jiancheng Bioengineering Institute, China) respectively. Pulmonary neutrophil infiltration was assessed by counting the number of white blood cells and PMNs in BAL collection.

Statistical analysis

All data are presented as mean±SD. Significance was determined using *t* test. *P* less than 0.05 was considered significant.

RESULTS

Serum ET-1, MDA, NO, and TNFα levels

Serum MDA levels decreased significantly in IR+L-arg group, while serum NO levels increased significantly after the administration of L-arg. Serum ET-1 levels in IR+L-arg group were not different from those in the other two groups. Serum TNFα concentration had no significant difference in the three groups (Table 1).

Table 1 Effects of L-arg on ET-1, MDA, NO, and TNFα levels in serum

Group	*n*	ET-1 (pg/mL)	MDA (nmol/L)	NO (μmol/L)	TNFα (ng/mL)
SHAM	6	226.01±37.91	23.78±7.81	108.17±56.28	3.20±0.39
IR	7	229.68±68.20	25.54±9.32	87.57±23.17	2.66±0.54
IR+L-arg	6	246.50±31.44	8.93±1.50[b]	162.17±42.93[d]	3.76±0.95

[b]*P*<0.01 *vs* either SHAM or IR group; [d]*P*<0.01 *vs* IR group.

Lung tissue ET-1, MDA, NO, and TNFα levels

ET-1 and MDA levels in lung tissue were significantly lower in IR+L-arg group thanthose in SHAM and IR groups. Lung NO concentrations had no significant difference in three groups (Table 2).

Table 2 Effects of L-arg on ET-1, MDA, NO, and TNFα levels in lung homogenates

Groups	*n*	ET-1 (pg/mL)	MDA (nmol/L)	NO (μmol/L)	TNFα (ng/mL)
SHAM	6	35.52±10.82[a]	16.62±2.28[b]	8.12±3.20	1.24±0.19
IR	7	50.83±22.05[b]	21.90±4.82[b]	4.97±0.05	1.07±0.17
IR+L-arg	6	13.81±7.84	10.73±1.99	4.98±0.82	0.91±0.33

[a]*P*<0.05 *vs* IR+L-arg group; [b]*P*<0.01 *vs* IR+L-arg group.

PMN counting of lung lavage fluid

White blood cell counts had no significant differences in three groups, but the percentage of PMNs was 13.50±8.92, 33.20±16.59, and 22.50±6.09 in SHAM, IR, and IR+L-arg groups, respectively. IR significantly increased pulmonary neutrophil infiltration in immature rats. Neutrophil infiltration was reduced significantly by L-arg infusion before reperfusion. Pulmonary neutrophil infiltration in IR+L-arg group remained above that in SHAM group, but not significantly.

DISCUSSION

The lung is one of the very important target organs in MODS caused by severe injury[1,2,5-7]. It has been found that, in addition to the direct trauma, the lung could also be damaged by indirect injury such as gut, liver IR[8]. Activated neutrophils are one of the primary mediators of local and

remote tissue damage after intestinal IR[1,3]. Neutrophil infiltration has been shown to be important in the development of pulmonary edema and microvascular leakage in animal models[5]. The present results showed that 1 h of intestinal ischemia followed by 90 min of reperfusion in immature rats induced lung neutrophil infiltration. When L-arg was given before reperfusion, lung neutrophil infiltration reduced but remained higher than that in the control group.

The mechanism of lung injury induced by intestinal IR, is poorly understood. The pathophysiology of lung injury associated with intestinal IR involves a variety of inflammatory and vasoactive mediators[5]. The production of large amounts of NO, a free radical produced by the inducible isoform of NO synthase, has been implicated as a cytotoxic factor in pathophysiological processes[9,10]. In animal models, the gut has been proposed as a cytokine-producing organ after IR-type injuries. Under the condition of an inadequate mucosal blood flow, the gut barrier function can be progressively impaired and invaded by bacteria or endogenous endotoxin[5]. This process is associated with the activation of systemic inflammatory mediators including bacteriotoxin, inflammatory mediators, such as TNFα and other cytokines. Studies of intestinal IR linking the local process to the resulting systemic changes have identified NO as a potential mediator[1,2]. In this regard, inhibitors of endogenous NO production greatly exacerbate the increase in epithelial permeability and cardiovascular dysfunction in the reperfused post-ischemia intestine, while administration of NO donors prevents the early rise in epithelial permeability and tissue dysfunction. Luo *et al* suggested that endogenous NO may play a role in protecting intestinal integrity after intestinal IR. Li *et al* recently reported that exotic melatonin can improve the hepatic function after reperfusion and plays a definitely protective role in liver IR by increasing the activities of superoxide dismutase, and decreasing the cumulation of MDA in liver reperfusion tissue. Yang *et al* studied the role of bFGF and TGFβ mRNA expression and basic fibroblast growth factor in the intestinal IR process. The data manifested that the endogenous bFGF and TGFβ expression appeared to be up-regulated in the lung following intestinal IR. Both growth factors might be involved in the process of lung injury and repair. In the current experiment, we investigated the contribution of L-arg to the lung damage induced by intestinal IR. The results demonstrated that in 1 h of intestinal ischemia followed by 90 min of reperfusion in immature rat models, L-arg administration significantly reduced serum and lung MDA levels when compared with SHAM group and IR group. L-arg administration during intestinal IR also increased serum NO concentration significantly. But there was no significant difference of lung TNFα concentrations in the three groups. It is suggested that intestinal IR-mediated lung injury is oxygen-dependent and the protective effect of L-arg supplementation in intestinal IR is probably related to increasing intravascular NO formation[6,10].

ET-1 is a peptide which is produced in the intestinal vascular endothelium, and it has been identified as an important participant in IR-induced vasoconstriction in circulation[9]. Durakbasa *et al* reported that NO and ET had a feedback effect on each other both under physiological conditions and in IR injury. In this study, lung ET-1 level was also significantly lower in IR+L-arg group than that in SHAM and IR groups. It is suggested that L-arg alleviates lung damage by reducing lung ET-1 level and improving pulmonary endothelial function.

In summary, the present study shows that L-arg administration before intestinal IR increases serum NO content, reduces serum and lung MDA and ET-1 levels, and attenuates systemic and pulmonary endothelial dysfunction. L-arg supplementation before reperfusion may act as a useful clinical adjunct in the management of intestinal IR injury, thus preventing the development of adult respiratory distress syndrome, even MODS[1,3].

ACKNOWLEDGEMENTS

The skillful statistical assistance of Mr. He-Ming Zhang, Director of Computer Office of Affiliated Hospital of Binzhou Medical College, is gratefully acknowledged.

REFERENCES

1 **Ward DT**, Lawson SA, Gallagher CM, Conner WC, Shea-Donohue T. Sustained nitric oxide production via l-arginine administration ameliorates effects of intestinal ischemia-reperfusion. *J Surg Res* 2000; **89**: 13-19
2 **Turnage RH**, Kadesky KM, Bartula L, Myers SI. Intestinal reperfusion up-regulates inducible nitric oxide synthase activity within the lung. *Surgery* 1995; **118**: 288-293
3 **Kalia N**, Brown NJ, Wood RF, Hopkinson K, Fairburn B, Pockley AG. Effects of intestinal ischemia-reperfusion injury on rat peripheral blood neutrophil activation. *Dig Dis Sci* 2003; **48**: 1677-1684
4 **Tavaf-Motamen H**, Miner TJ, Starnes BW, Shea-Donohue T. Nitric oxide mediates acute lung injury by modulation of inflammation. *J Surg Res* 1998; **78**: 137-142
5 **Grotz MR**, Deitch EA, Ding J, Xu D, Huang Q, Regel G. Intestinal cytokine response after gut ischemia: role of gut barrier failure. *Ann Surg* 1999; **229**: 478-486
6 **Kozlov AV**, Sobhian B, Duvigneau JC, Costantino G, Gemeiner M, Nohl H, Redl H, Bahrami S. Nitric oxide synthase inhibitors affect nitric oxide synthesis in normoxic but not in ischemic organs during intestinal ischemia and early reperfusion. *J Lab Clin Med* 2002; **140**: 303-311
7 **Vlasov TD**, Smirnov DA, Nutfullina GM. Preconditioning of the small intestine to ischemia in rats. *Neurosci Behav Physiol* 2002; **32**: 449-453
8 **Gong JP**, Tu B, Wang W, Peng Y, Li SB, Yan LN. Protective effect of nitric oxide induced by ischemic preconditioning on reperfusion injury of rat liver graft. *World J Gastroenterol* 2004; **10**: 73-76
9 **Nankervis CA**, Schauer GM, Miller CE. Endothelin-mediated vasoconstriction in postischemic newborn intestine. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G683-G691
10 **Chen JC**, Chen HM, Shyr MH, Fan LL, Chi TY, Chi CP, Chen MF. Selective inhibition of inducible nitric oxide in ischemia-reperfusion of rat small intestine. *J Formos Med Assoc* 2000; **99**: 213-218