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ORIGINAL ARTICLE

Preventive oral supplementation with glutamine and arginine has beneficial effects on the intestinal mucosa and inflammatory cytokines in endotoxemic rats

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Abstract The objective of this study was to evaluate the effect of oral supplementation with a combination of arginine and glutamine on the intestinal mucosa and inflammatory cytokines of lipopolysaccharide (LPS)-induced adult rats. Fifty Sprague–Dawley rats (average weight of 185 ± 15 g) were randomly divided into five groups: control group A (CA) and control group B (CB), both orally supplemented with 0.9% saline; group Arg, supplemented with 300 mg/kg day^{-1} arginine; group Gln, supplemented with 300 mg/kg day^{-1} glutamine; group AG, sup3plemented with 150 mg/kg day^{-1} arginine and 150 mg/kg day^{-1} glutamine. The experiment lasted for 2 weeks. Food intake and body weight were measured during the experiment. At 10.00 h of day 15, animals were injected with 4 mg/kg LPS (group CB, Arg, Gln, and AG) or sterile saline (group CA) after supplementation. Then at 14.00 h, all animals were killed and blood and tissue collected. The results showed that compared with group CB, arginine concentration tended to be increased ($P > 0.05$) in group Arg and AG, while there was no significant difference in glutamine concentration among the groups challenged with LPS. Oral supplementation with arginine or/and glutamine mitigated morphology impairment (lower villus height, $P < 0.05$) in the jejunum and ileum induced by LPS challenge. LPS administration resulted in a significant increase in TNF-α, IL-1β, IL-6 and IL-10 mRNA abundance. Arginine only significantly decreased TNF-α mRNA abundance in the ileum, while glutamine significantly decreased both TNF-α and IL-10 mRNA in the ileum. A combination of arginine and glutamine significantly decreased TNF-α and IL-1β mRNA abundance in both the jejunum and ileum, while they also significantly decreased anti-inflammatory IL-10 in the ileum. These results revealed that an oral supply of combined arginine and glutamine had more favorable effects on the intestinal mucosa and inflammatory cytokines than a supply of arginine or glutamine alone.

Keywords Arginine · Glutamine · Mucosal morphology · Inflammatory cytokines · Intestinal mucosa

Abbreviations

LPS	Lipopolysaccharide
DAO	Diamine oxidase
ET-1	Endothelin-1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
RT-PCR	Real-time polymerase chain reaction
ADG	Average daily weight gain
ADFI	Average daily food intake
CD	Crohn's disease
NT	Nucleotide

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Introduction

Arginine and glutamine are both considered as conditionally essential amino acids under inflammatory and many other conditions (Luiking et al. 2004; Newsholme et al. 2001). Arginine is a key amino acid in several metabolic pathways; it is used for the synthesis of polyamines and nitric oxide, and thus modulates the immuno-inflammatory response and wound healing (Coeffier and Dechelotte 2010; He et al. 2009; Li et al. 2009; Luiking and Deutz 2007). Arginine also enhances NT synthesis, stimulates proliferation and protects the intestinal cells and mucosa from LPS-induced damage (Baker 2009; Sukhotnik et al. 2004; Tan et al. 2009; Yamauchi et al. 2002). Since in adults nearly all of the dietary glutamine is catabolized in the intestine, glutamine plays an important physiological and pathological role in the gastrointestinal tract (Flynn et al. 2009; Stoll and Burrin 2006). It acts as an oxidative substrate in small intestine epithelial cells (Blachier et al. 2009; Windmueller and Spaeth 1975), a precursor for nucleotide synthesis, and a signal to proliferate and enhance cell survival in the intestine (Haynes et al. 2009; Rhoads and Wu 2009; Yamauchi et al. 2002). Moreover, several studies show that glutamine decreases LPS-induced bowel inflammation with the effects of reducing the production of the pro-inflammatory cytokines and improving the production of anti-inflammatory cytokines (Coeffier et al. 2003; Li et al. 2004; Garrett-Cox et al. 2009).

As depletion of both glutamine and arginine occur in stressed conditions and they are closely linked by metabolism (Blachier et al. 1993; Coeffier et al. 2010; Ekloulawson et al. 2009). Several previous experiments have assessed that supplementation with these two amino acids together resulted in certain additive effects (Chamorro et al. 2010; Gennari et al. 1995; Kul et al. 2009;) and studies in ex vivo experiments have suggested that giving arginine and glutamine decreased the production of pro-inflammatory cytokines (Lecleire et al. 2008). However, it remains to be investigated whether an enteral supply of combined arginine and glutamine has some synergistic and beneficial effects on the intestinal mucosa of endotoxemic rats. We conducted the present experiment to determine whether a combination of glutamine and arginine supplementation could favorably alleviate rats from endotoxemia.

Materials and methods

Animal and treatment

Fifty adult male Sprague–Dawley rats were chosen in this study. The animals were housed in individual cages and fed a ration prepared according to the recommendations of the American Institute of Nutrition-1993 for adult rats. The rats were maintained in a controlled environment at 22 ± 2°C and relative air humidity of 60 ± 10% under a 12-h light/dark cycle(lights off from 08:00 to 20:00 h), with water and food ration available ad libitum throughout the experiment (Kong et al. 2009; Ren et al. 2011; Yu et al. 2010). All animals were allowed to adapt to the experimental conditions for 1 week. After the adaption period, the animals ($n = 10$/group) were weighed and randomly allocated to five groups: control group A (CA) and control group B (CB), both orally supplemented with 0.9% saline; group Arg, supplemented with 300 mg/kg day^{-1} arginine; group Gln, supplemented with 300 mg/kg day^{-1} glutamine; group AG, supplemented with 150 mg/kg day^{-1} arginine and 150 mg/kg day^{-1} glutamine for 2 weeks, respectively. Each solution was given to the animals through gastric tube (gavage). Food intake and body weight were measured during the trial. At 22:00 h of day 14, all animals were deprived of food until being killed. At 10:00 h of day 15, after supplementation, animals were injected intraperitoneally with either 4 mg of LPS/kg body weight (group CB, Arg, Gln, and AG) or the same amount of 0.9% (w/v) saline (group CA). The LPS (*E. coli* serotype 055: B5; Sigma Chemical Inc., St Louis, MO, USA) was dissolved in sterile 0.9% NaCl solution (1 mg LPS/mL saline) and it was used as an inflammatory agent according to the model of Mercer et al. (1996). This dose was determined in our pilot studies to result in occasional shivering and diarrhea, but was not associated with death. At 14:00 h of day 15(4 h post-challenge), all animals were killed for evaluation of intestinal morphology and gene expression of inflammatory cytokines (Tan et al. 2009). This study was performed in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences (Yin et al. 1993). Arginine was obtained from Ajinomoto Inc. (Tokyo, Japan) and glutamine was supplied by Sangon Biotech (Shanghai) Co., Ltd.

Sample collection

After killing by decapitation, blood was collected and centrifuged for plasma separation, which was stored at −20°C for subsequent determination of arginine, glutamine, DAO and ET-1 concentrations (Deng et al. 2010; Kong et al. 2007; Yin et al. 2010b). The small intestine was removed and the jejunum and ileum were separated, thoroughly fluxed with sterile saline (Yang et al. 2011), immediately frozen in liquid nitrogen and then stored at −80°C for inflammatory cytokines mRNA analysis or fixed in 10% neutral buffered formalin for intestinal morphology.

Determination of arginine and glutamine in plasma

As much as 0.2 mL of plasma was mixed with 1.5 M $HClO_4$ in a 1.5-ml microcentrifuge tube. After 2 min, 0.5 mL H_2O and 0.1 mL of 2 M K_2CO_3 were added. The tubes were vortexed and centrifuged (10,000×*g* for 1 min) to obtain the supernatant fluid for analysis. The dilution factor for a sample is 5. The concentration of arginine was analyzed using high-performance liquid chromatography according to Wu and Meininger (2008). This method involved the precolumn derivatization of amino acids with *o*-phthaldialdehyde and fluorescence detection. Arginine in the samples was quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO, USA) (Yin et al. 2010a, 2011). Plasma glutamine was measured spectrophotometrically by the deamination/dehydrogenation method at 630 nm using the Glutamine Detection Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Distilled water, 2 μmol/L glutamine standard solution or plasma samples (scaled down by 10 times with sterilized saline) were mixed with the reagent of the kit in a 5-ml centrifuge tube, respectively, and the glutamine concentration in the plasma samples was calculated in terms of the OD value of pre-blended solutions according to the specification of the kit (Yin et al. 2010b).

Determination of DAO and ET-1 in plasma

Plasma DAO was measured spectrophotometrically by the deamination/dehydrogenation method using the DAO Detection Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma ET-1 concentration was assessed by TiterZyme Enzyme Immunometric Assay Kits (R&G Systems), Assays in samples and standards were conducted simultaneously according to the manufacturer's recommendations. The optical density was read at 450 nm for ET-1. Results of ET-1 concentration in plasma were expressed as pg/mL.

Intestinal morphology

Formalin-fixed jejunum and ileum samples were embedded in paraffin; Cross sections of the segments were cut approximately 5-μm thick with a microtome and stained with hematoxylin and eosin. In each section, the villus height and the associated crypt depth were measured using a light microscope with a computer-assisted morphometric system. Villus height was defined as the distance from the villus tip to the crypt mouth, and crypt depth from the crypt mouth to the base (Han et al. 2008; Hou et al. 2010, 2011).

Real-time PCR for IL-1β, IL-6, IL-10 and TNF-α in the jejunum and ileum

Total RNA in small intestine was isolated using the Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's recommendation. Total RNA was reversed into cDNA using a SuperScript First-Strand Synthesis System kit (Invitrogen Life Technologies). PCR amplification was performed in a total volume of 50 μl including Taq DNA polymerase and specific primers.

To amplify IL-1β, IL-6, IL-10, TNF-α (target gene) and GAPDH cDNA fragments, the following sequences of PCR primer pairs were used: forward 5′-GGCTTCCTTGTGCA AGTGT-3′, reverse 5′-CGAGATGCTGCTGTGAGATT-3′ for IL-1β (199 bp); forward 5′-AACTCCATCTGCCC TTCA-3′, reverse 5′-TTGCCATTGCACAACTCT-3′ for IL6 (245 bp); forward 5′-AGTCAGCCAGACCCACAT-3′, reverse 5′-GGCAACCCAAGTAACCCT-3′ for IL10 (140); forward 5′-CCACCACGCTCTTCTGTC-3′, reverse 5′- G CTACGGGCTTGTCACTC-3′ for TNFα (203 bp); forward 5′-GGCAAGTTCAACGGCACAG-3′, reverse 5′-CGCCA GTAGACTCCACGACAT-3′ for GAPDH (142 bp). The RT-PCR conditions were: 30 s denaturation at 94°C, 30 s annealing at 60°C, and 30 s extension at 72°C for 30 cycles. The relative quantification of gene amplification by RT-PCR was performed using cycle threshold (Ct) values. The comparative Ct value method was employed to quantitate expression levels for IL-1β, IL-6, IL-10 and TNF-α relative to those for GAPDH as described by Fu et al. (2006).

Statistical analysis

Data were presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was used to determine whether a significant difference was present among all treatment groups using SPSS 13.0. Statistical significance was considered at P less than 0.05 (Deng et al. 2009; Yin et al. 2004).

Results

Effects of arginine or/and glutamine on growth performance

There were no significant differences ($P < 0.05$) in the average daily weight gain (ADG) and average daily food intake (ADFI) between the control group and groups of oral supplementation with arginine or/and glutamine, though supplementation with arginine and glutamine tended to increase ADG and ADFI by 10.03 and 11.62%, respectively.

Plasma arginine and glutamine concentration

Figure 1 showed that LPS challenge caused a significant decrease in plasma arginine and glutamine concentration. Compared with CB, plasma arginine concentration was increased in group Arg and AG by 31% ($P > 0.05$) and 32.9% ($P > 0.05$), respectively. Plasma glutamine concentration did not differ among groups CB, Gln and AG.

Plasma DAO and ET-1 concentration

Figure 2 showed that LPS challenge caused a significant decease in plasma DAO concentration and a significant increase in plasma ET-1 concentration. Compared with CB, plasma DAO concentration in groups Arg, Gln and AG was increased by 38.6% ($P < 0.05$), 31.5% ($P < 0.05$) and 78.7% ($P < 0.01$), respectively, while ET-1 concentration was decreased by 41.4% ($P < 0.01$), 37.3% ($P < 0.01$) and 45.5% ($P < 0.01$), respectively.

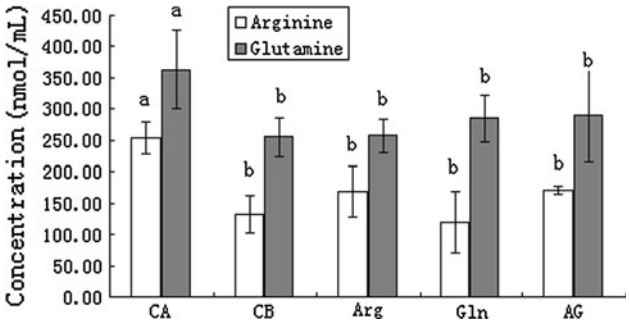

Fig. 1 Effects of oral supplementation with arginine or/and glutamine on plasma arginine and glutamine concentrations after 4 h of LPS challenge Values are expressed as mean ± SEM, $n = 7$. Mean values with different letters differ ($P < 0.05$). *CA* control rats treated with saline, *CB* control rats treated with LPS, *Arg* rats supplied with arginine and treated with LPS, *Gln* rats supplied with glutamine and treated with LPS, *AG* rats supplied with arginine and glutamine and treated with LPS

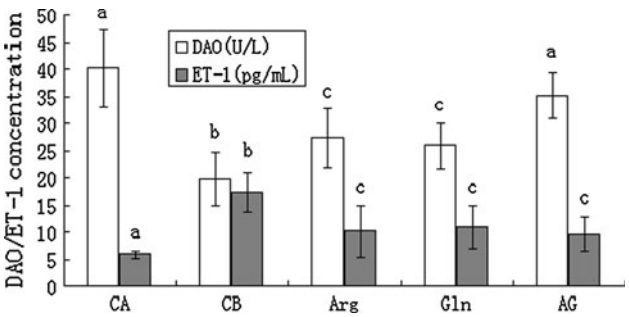

Fig. 2 Effects of oral supplementation with arginine or/and glutamine on plasma DAO and ET-1 concentrations in LPS-induced rats. Values are expressed as mean ± SEM, $n = 7$. Mean values with different letters differ ($P < 0.05$). *CA* control rats treated with saline, *CB* control rats treated with LPS, *Arg* rats supplied with arginine and treated with LPS, *Gln* rats supplied with glutamine and treated with LPS, *AG* rats supplied with arginine and glutamine and treated with LPS

Intestinal morphology

Figure 3 showed that LPS challenge caused a significant decrease in villus height and crypt depth in the jejunum and ileum, while oral supplementation with arginine or/and glutamine attenuated these effects. Compared to group CB, villus height was significantly enhanced in group Arg, Gln and AG in the jejunum (199 ± 15 vs. 259 ± 16 μm in Arg, 251 ± 18 μm in Gln, 287 ± 14 μm in AG; $P < 0.05$) and ileum (126 ± 14 vs. 178 ± 18 μm in Arg, 166 ± 10 μm in Gln, 208 ± 19 μm in AG; $P < 0.05$). Of the four groups administrated LPS, group AG had the highest villus. Compared to group CB, there was a trend toward an increase in jejunal and ileal crypt depth in groups Arg, Gln and AG; Although this trend did not achieve statistical significance in the jejunum and ileum in groups Arg and Gln, the difference in the jejunum crypt depth between groups CB and AG was significant (155 ± 21 vs. 177 ± 13 μm; $P < 0.05$).

Inflammatory cytokine production

The data for mRNA expression of TNF-α, IL-1β, IL-6 and IL-10 are shown in Tables 1 and 2.

Compared with CA, TNF-α mRNA abundance of group CB was significantly increased in the jejunum (5.6-fold higher; $P < 0.05$) and ileum (2.35-fold higher; $P < 0.05$). Compared with CB, LPS-induced TNF-α mRNA abundance of groups Arg and Gln tended to be decreased by 26.8% ($P > 0.05$) and 29.8% ($P > 0.05$), respectively, in the jejunum, whereas TNF-α mRNA abundance of group AG was significantly decreased by 42.9% ($P < 0.05$). LPS-induced TNF-α mRNA abundance of the three groups was significantly decreased ($P < 0.05$) in the ileum.

Compared with CA, IL-1β mRNA abundance of group CB was significantly increased in the jejunum (4.83-fold higher; $P < 0.05$) and ileum (3.24-fold higher; $P < 0.05$). Compared with CB, oral supplementation with arginine or/and glutamine decreased IL-1 mRNA abundance by 24, 9.9 and 57.8% ($P < 0.05$) in the jejunum and by 18, 11.9 and 56.6% ($P < 0.05$), respectively, in the ileum, and there was a significant difference between groups Arg/Gln and AG.

LPS administration resulted in a significant increase in IL-6 mRNA abundance of group CB in the jejunum (2.86-fold higher, $P < 0.05$) and ileum (4.31-fold higher; $P < 0.05$) compared with group CA. Although oral supplementation with arginine or/and glutamine tended to decrease IL-6 mRNA abundance by 16.8, 3.8 and 33.2% in the jejunum and by 17.3, 12.6 and 24.6%, respectively, in

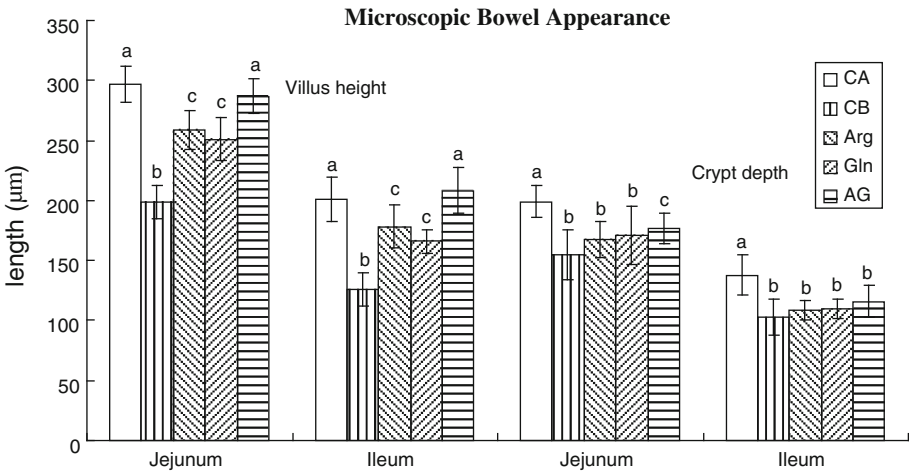

Fig. 3 Effects of oral supplementation with arginine or/and glutamine on intestinal morphology after 4 h of LPS challenge in rats ($n = 7$). Mean values with different letters differ ($P < 0.05$). *CA* control rats treated with saline, *CB* control rats treated with LPS, *Arg* rats supplied with arginine and treated with LPS, *Gln* rats supplied with glutamine and treated with LPS, *AG* rats supplied with arginine and glutamine and treated with LPS

Table 1 Effects of oral supplementation with arginine or/and glutamine on the growth performance of normal rats

	Initial body weight (g)	Final body weight (g)	Average daily gain (g)	Average daily feed intake (g)
CA	180.17 ± 10.98	221.83 ± 19.73	2.97 ± 1.55	19.45 ± 1.75
CB	183.67 ± 4.13	225.83 ± 27.12	3.01 ± 1.94	19.64 ± 3.83
Arg	181.17 ± 5.23	236.67 ± 19.63	3.25 ± 0.83	20.54 ± 2.51
Gln	185 ± 9.44	231.17 ± 21.15	3.3 ± 1.49	20.2 ± 3.35
AG	183.5 ± 10.39	238.17 ± 26.29	3.29 ± 1.45	21.8 ± 5.41

Values are expressed as mean ± SEM, $n = 9$

CA and *CB* control rats, *Arg* rats supplied with arginine, *Gln* rats supplied with glutamine, *AG* rats supplied with arginine and glutamine

Table 2 Effects of oral supplementation with arginine or/and glutamine on intestinal TNF-α, IL-1β, IL-6 and IL-10 mRNA abundance of rats after 4 h of *Escherichia coli* lipopolysaccharide (LPS) challenge

	CA	CB	Arg	Gln	AG
Jejunum					
TNF-α	1 ± 0.4^{a}	5.6 ± 1.4^{b}	4.1 ± 2.73^{b}	3.93 ± 3.04^{ab}	3.2 ± 1.7^{c}
IL-1β	1 ± 0.54^{a}	4.83 ± 2.14^{b}	3.67 ± 1.53^{b}	4.35 ± 1.24^{b}	2.04 ± 0.38^{c}
IL-6	0.83 ± 0.22^{a}	2.86 ± 1.88^{b}	2.38 ± 0.77^{b}	2.75 ± 1.47^{b}	1.91 ± 1.08^{ab}
IL-10	1 ± 0.53^{a}	4.54 ± 2.08^{b}	4.42 ± 0.88^{b}	3.27 ± 2.04^{b}	3.2 ± 1.92^{b}
Ileum					
TNF-α	0.81 ± 0.2^{a}	1.9 ± 0.72^{b}	1.15 ± 0.47^{a}	1.33 ± 0.43^{a}	0.83 ± 0.31^{a}
IL-1β	0.96 ± 0.54^{a}	3.11 ± 1.62^{b}	2.55 ± 1.6^{b}	2.74 ± 1.17^{b}	1.35 ± 0.66^{a}
IL-6	0.83 ± 0.34^{a}	3.58 ± 1.56^{b}	2.96 ± 1.31^{b}	3.13 ± 1.25^{b}	2.7 ± 1.18^{b}
IL-10	1 ± 0.48^{a}	4.31 ± 2.56^{b}	3.94 ± 2.33^{bc}	2.02 ± 1.07^{ac}	1.88 ± 0.98^{a}

Values are expressed as mean ± SEM, $n = 6$. Mean values with different letters differ ($P < 0.05$)

CA control rats treated with saline, *CB* control rats treated with LPS, *Arg* rats supplied with arginine and treated with LPS, *Gln* rats supplied with glutamine and treated with LPS, *AG* rats supplied with arginine and glutamine and treated with LPS

the ileum, the difference between the groups administered LPS did not reach significance.

LPS administration resulted in a significant increase in IL-10 mRNA abundance of group CB in the jejunum (4.54-fold higher; $P < 0.05$) and ileum (4.31-fold higher; $P < 0.05$) compared with group CA. Compared with group CB, IL-10 mRNA abundance of groups Arg, Gln and AG were decreased by 8.6% ($P > 0.05$), 53.1% ($P < 0.05$) and 56.4% ($P < 0.05$), respectively, in the ileum; however, there was no significant difference in the jejunal IL-10 mRNA abundance among groups Arg, Gln and AG.

Discussion

During sepsis, the endogenous synthesis of arginine and glutamine is severely reduced (Luiking et al. 2003; Souba and Austgen 2001). Previous studies have shown that extracellular arginine content rapidly decreased in animal model of sepsis induced by LPS, and arginine concentration in plasma was lower in septic patients (Lortie et al. 2004; Luiking et al. 2003). Moreover, recent evidences demonstrate that plasma glutamine concentration is decreased in the early phase of endotoxemia in rats (Garrett-Cox et al. 2009). In the present study, we found that plasma arginine and glutamine concentrations were both significantly decreased after 4 h of LPS challenge. In our previous study, oral supplementation with glutamine did not increase either plasma glutamine or arginine concentration, while oral supplementation with arginine increased arginine concentration (Liu et al. 2011; Wu et al. 2010), though only by 31%. This might be because nearly all of the enteral glutamine and most of the arginine did not enter the portal circulation, as they were metabolized in the intestine and their consumption was increased under sepsis induced by LPS (Lortie et al. 2004; Garrett-Cox et al. 2009; Stoll and Burrin 2006). Our data also suggested that the combination of arginine and glutamine was not able to increase the plasma arginine concentration more markedly than arginine alone, which suggested that all of the enteral glutamine and most of arginine were used by the intestine of LPS-induced rats.

Diamine oxidase (histaminase) is an enzyme found in high concentrations in the intestinal mucosa of humans and other mammalian species. Plasma DAO, known also as histaminase, appears to come primarily from the small intestine in many mammalian species and may serve as a useful plasma marker of mucosal maturation and also of mucosal integrity in the rat (Buffoni 1966; Luk et al. 1980). Studies on acute gastroenteritis in children show that serum DAO activity is significantly lower when compared with control patients (Forget et al. 1985). ET-1, a vasoconstrictor peptide produced by vascular endothelial cells, exerted widely biological effects on the neuroendocrine systems such as the gastrointestinal tract and cardiovascular system (Yanagisawa et al. 1988). ET-1 is presently thought to be an endogenous pathogenicity factor released when the small intestine is in such conditions as ischemia and anoxia, and it may cause intestinal mucosal injury. Our results showed that LPS challenge caused a significant increase in DAO concentration and a significant decrease in ET-1 concentration, while an oral supply of arginine and glutamine significantly blunted these changes, which demonstrated that arginine and glutamine had an effect on protecting the intestinal mucosal barrier.

The small intestine is a target during sepsis and LPS results in morphologic damage to the epithelium in both the jejunum and ileum (Mercer et al. 1996). In support of these views, we found that the villus height and crypt depth of the small intestine were significantly decreased 4 h after treatment with intraperitoneal LPS, and the ileal villi often appeared to have bizarre shape changes in the form of partial loss, sloughing and vacuolization, as shown in Fig. 4.

Arginine degradation by arginases results in the release of polyamine products in cells, including enterocytes of the intestinal villus and crypt (Blachier et al. 1991, 1995; Wu et al. 2000a, Wu et al. 2000b). These polyamines are essential for cell growth, proliferation and the synthesis of proteins and nucleic acids (Blachier et al. 2007; Wu et al. 2009). They are also involved in the repair of the extracellular matrix, cell adhesion and different signaling processes, contributing to wound healing and tissue repair (Moinard et al. 2005). Previous studies have shown that a dietary supplementation of arginine attenuates the decrease of crypt cell proliferation and the increase of villus cell apoptosis caused by the LPS challenge in the model of adult rats and weaned pigs (Liu et al. 2008; Sukhotnik et al. 2004), and cell culture results also demonstrate that arginine may stimulate cell proliferation and prevent LPS-induced enterocyte damage (Tan et al. 2010).

Glutamine serves as an essential precursor for the synthesis of proteins, purine and pyrimidine nucleotides, NAD^+ and aminosugars (Krebs et al. 1980). Glutamine is also a fuel for rapidly dividing cells, including activated lymphocytes (Ardawi et al. 1983; Krebs 1980) and intestinal epithelial cells (Windmueller and Spaeth 1975, 1980; Wu et al. 1995). Moreover, glutamine is required for the maintenance of gut-associated lymphatic tissues, intestinal integrity and the prevention of bacterial translocation from the gut and is an important energy source for proliferation of intestinal intraepithelial lymphocytes (Alverdy 1990; Dugan et al. 1994; Wu 1996). In addition, as a key factor in maintaining mucosal structure, glutamine may have special effects on the maintenance of tight junctions and permeability of intestinal mucosa (Panigrahi et al. 1997). Several studies have elucidated that dietary supplementation with glutamine prevented jejunal atrophy (an indicator by villus height) and mitigated the overall disruption (Wu et al. 1996; Yi et al. 2005).

In the current study, we discovered that an oral supply of arginine or glutamine alone significantly enhanced the villus height of the jejunum and ileum in LPS-challenged rats. What is more, a combination of glutamine and arginine supplementation had additional beneficial effects in improving villus height in the jejunum and ileum. There might be several contributing factors. Firstly, given that arginine and glutamine not only played an important role in

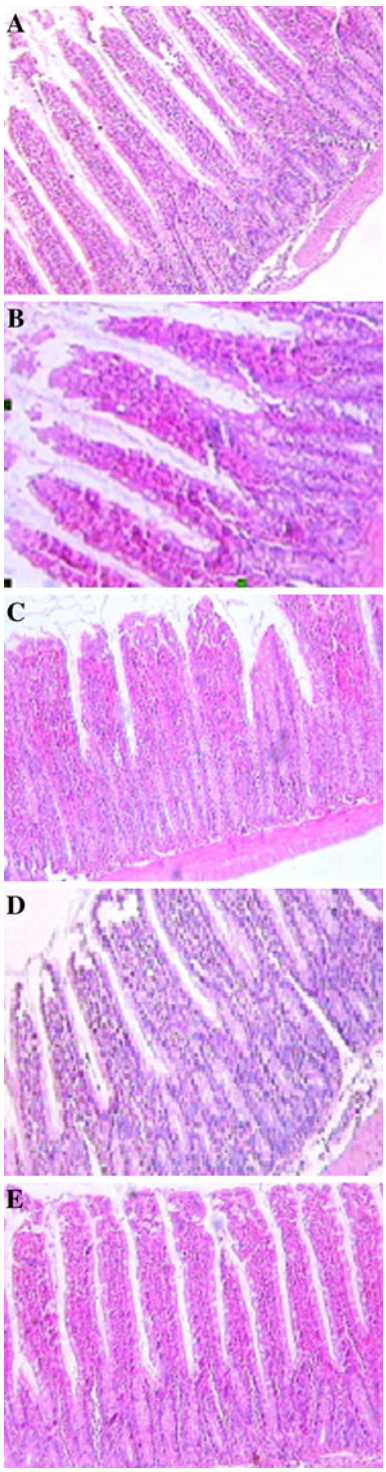

Fig. 4 Representative staining of jejunal mucosal morphology after 4 h of LPS challenge in rats. **a** Control rats treated with saline, **b** control rats treated with LPS, **c** rats supplied with arginine and treated with LPS, **d** rats supplied with glutamine and treated with LPS, **e** rats supplied with arginine and glutamine and treated with LPS

the synthesis of proteins and DNA, cell growth and proliferation, but also had effects on improving gut barrier function, combined administration of arginine and glutamine could have synergistic effects on the maintenance of intestinal integrity (Coeffier et al. 2010). Secondly, arginine significantly stimulated cell growth and proliferation only in the presence of glutamine, and NT synthesis from glutamine increased with arginine supplementation (Yamauchi et al. 2002).

Arginine and glutamine exert beneficial effects on the modulation of inflammatory cytokines, which can have a negative influence on gut integrity and epithelial function when excessively produced (Coeffier et al. 2010; Mckay 1999). Several studies have demonstrated these effects of arginine and glutamine on the model of sepsis. Liu et al. (2008) reported that dietary arginine alleviated the elevation of jejunal IL-6 mRNA abundance, and jejunal and ileal TNF-α mRNA abundance induced by LPS challenge in weaned pigs. Wischmeyer et al. (2001) reported that intravenous glutamine significantly decreased plasma TNF-α and IL-1β, and decreased plasma IL-10, although this difference was not significant in an adult rat endotoxemia model. Subsequently, Garrett-Cox et al. 2009 found that glutamine blunted plasma TNF-α and IL-10 concentrations in infant rat in response to LPS, whereas Li et al. (2004) demonstrated that glutamine blunted LPS-induced TNF-α increase in the intestine but not in the plasma.

Moreover, combined pharmacological doses of arginine and glutamine decrease TNF-α and the main proinflammatory cytokines released in active colonic CD biopsies, showing the possible effects of enteral supply of combined arginine and glutamine during active CD (Lecleire et al. 2008). However, there are very few in vivo experiments that have studied the additional effects of arginine and glutamine in the small intestine of the sepsis model, and the need for such studies has previously been stated. Thus, we performed the present study in LPS-induced rats supplied with both arginine and glutamine. The results of the study suggested that an enteral supply of arginine or glutamine alone tended to decrease jejunal TNF-α, IL10 and IL-1β, IL-6 in both the the jejunum and ileum, and they significantly decreased ileal TNF-α. Surprisingly, enteral supply of both arginine and glutamine significantly decreased proinflammatory TNF-α and IL-1β mRNA abundance in both jejunum and ileum, and they also significantly decreased the anti-inflammatory IL-10 in the ileum, showing the advantage of using both together.

In conclusion, this study showed that a combined supply of arginine and glutamine exerted more favorable effects on LPS-induced damage in the rat small intestine than arginine or glutamine alone. Our findings could be a support for oral supplementation with combined arginine and glutamine for early endotoxemia and sepsis patients.

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