International Journal of Surgery 12 (2014) 1181–1186

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

Enteral supplementation of alanyl–glutamine attenuates the up-regulation of beta-defensin-2 protein in lung injury induced by intestinal ischemia reperfusion in rats

Yun Li [a, b], Xiao-Bin Wu [b], Jia-Gen Li [a], Yi-Jia Lin [b], Hong-Lei Chen [b], Hu Song [b], Zhong-Hui Liu [b], Jun-Sheng Peng [b, *]

[a] Department of Oncological Surgery, Yinzhou People's Hospital, Ningbo, China
[b] Department of Gastrointestinal Surgery, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

HIGHLIGHTS

- IIR could upregulate the expression of BD-2.
- Glutamine was able to ameliorate IIR-induced injury of remote organs by limiting inflammatory reaction.
- The exact effect of immune-nutrients on BD-2 level in lung after IIR is unknown.
- Its relationship with inflammation and oxidative injury has not been elucidated.
- In IIR animal model, the alteration of BD-2 expression in the lung after oral supplementation of Ala–Gln was investigated.

ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form
27 July 2014
Accepted 3 August 2014
Available online 6 August 2014

Keywords:
Reperfusion injury
Intestines
Lung injury
Glutamine
Beta-defensin-2

ABSTRACT

Background: Beta-defensin-2 (BD-2), an endogenous antimicrobial peptide, plays a key role in immune response against microbial invasion. This study aimed to observe the effect of Alanyl–Glutamine (Ala–Gln) on BD-2 protein expression in pulmonary tissues after intestinal ischemia reperfusion (IIR) in rats and to investigate its correlations to pulmonary inflammatory and oxidative injury. **Methods**: Rats in IIR and the two treatment groups were subjected to intestine ischemia for 60 min and those in the treatment groups were administered orally with Ala–Gln or alanine (Ala) respectively. Lung tissues were harvested to detect the BD-2 protein expression. Concentrations of Tumor necrosis factor (TNF)-α and malondialdehyde (MDA) as well as superoxide dismutase (SOD) activity in lung tissues were determined simultaneously. **Results**: Ala–Gln attenuated the up-regulation of BD-2 expression ($p < 0.05$) and TNF-α ($p < 0.05$), MDA ($p < 0.05$) levels, as well as the reduction of SOD activity ($p < 0.05$) in lung tissues after IIR. But Ala did not exert significant effects. BD-2 protein in lung tissues was positively correlated to local TNF-α level ($p < 0.01$) and MDA concentration ($p < 0.01$) with statistical significance. **Conclusion**: Ala–Gln can relieve the IIR-induced up-regulation of BD-2 protein expression in the lung of rats, which involves anti-inflammation and anti-oxidation mechanisms.

1. Introduction

IIR is a potentially life-threatening condition which can be induced by various surgical procedures, such as liver and intestine transplantation, abdominal aortic surgery and postoperative ileus being the most common complication of abdominal surgery [1]. The systemic inflammatory response syndrome (SIRS) resulting from translocation of bacteria and endotoxin as a consequence of IIR can cause injuries to remote organs including lung, heart, kidney and liver, leading to multiple organ dysfunction syndrome (MODS) at the end stage [2]. Among these distant organ injuries, acute lung injury (ALI) or pneumonia develops frequently and is one of the most common postoperative complications of abdominal surgery [3]. IIR-induced ALI has been characterized by serious inflammation

* Corresponding author. Department of Gastrointestinal Surgery, The Sixth Affiliated Hospital of Sun Yat-sen University (Guangdong Gastrointestinal and Anal Hospital), 26 Yuancun Erheng Road, Guangzhou 510655, Guangdong Province, China.
E-mail address: pengjunsheng@tom.com (J.-S. Peng).

http://dx.doi.org/10.1016/j.ijsu.2014.08.003
1743-9191/

with significant recruitment of leucocytes and increased pulmonary permeability that can lead to severe pulmonary edema [4,5].

It is generally accepted that defensins play a critical and indispensable role in both innate and adaptive immune responses. Multiply injured Patients showed elevated concentrations of defensins in serum as compared to healthy donors [6]. Among these defense peptides, BD-2 serves as an endogenous antimicrobial peptide that was mainly produced by epithelial cells of skin and respiratory tract [7]. It has been demonstrated that BD-2 is expressed in rat lung [8] and plays an important role in maintaining host defense on the face of microbial invasion via its chemoattractant effect on dendritic cells and T cells. Furthermore, in some pulmonary inflammatory response BD-2 is up-regulated through some proinflammatory factors such as TNF-α and shows a protective effect against inflammatory injury. It was found that IIR could upregulate the expression of BD-2 and this phenomenon was positively correlated to TNF-α level in the lung [5].

Glutamine (Gln) has been proved to be able to ameliorate IIR-induced injury of remote organs including the mechanism of limiting inflammatory reaction [9–11]. Alanyl–Glutamine (Ala–Gln), an artificially synthetic dipeptide containing Gln, is commonly used in clinical practice. However, the exact effect of immune-nutrients administration on BD-2 level in lung after IIR and its relationship with local inflammation and oxidative injury has not been elucidated clearly. In the present study, the rat model of superior mesenteric artery occlusion and reperfusion was employed to investigate the alteration of BD-2 protein expression in the treatment of IIR-induced lung injury via oral supplementation of Ala–Gln.

2. Materials and methods

2.1. Animals

This study was approved by Animal Care Committee of Ooo University. All of the animal care and experimental protocols were performed in accordance with National Institutes of Health guidelines for the use of experimental animals. Forty healthy male Sprague–Dawley (SD) rats weighing between 200 and 250 g were used in the current study. They were kept in an air-conditioned room with a 12-h light–dark cycle. They were allowed standard rat chow (free of Gln and Ala) and tap water ad libitum.

2.2. Experimental design and establishment of IIRI model

Rats were randomly divided into sham operation (S), IIR, Ala–Gln and Ala groups, each of which contained 10 rats. Those in Ala–Gln group and Ala group were administered orally with Ala–Gln (Sino-Swed Pharmaceutical Co., Ltd, Wuxi, China) or Ala (Sigma–Aldrich, St Louis, MO, USA) at the dose of 0.6 g/kg d 3 days before the ischemia procedure followed by the reperfusion period of 72 h, except the day on which ischemia was conducted. Meanwhile rats in S group and IIR group have been administered with physiological saline orally at the same points.

After fasted for 12 h before the operation, animals were anesthetized with pentobarbital (30 mg/kg, intraperitoneal injection). The model of IIR was treated by occlusion of the superior mesenteric artery. Through a midline laparotomy, the superior mesenteric artery was located and isolated from the surrounding tissues, and a vascular clip was placed around the vessel near the aortic origin. The abdominal incision was then temporarily closed, and the rat was placed on a heating pad during the ischemic period. After 60 min, the abdominal incision was opened, and the vascular clip was removed. Reperfusion was noted by visual inspection of the intestine in each animal, after which the abdominal incision was permanently closed. A sham operation on control rats was carried out as above but without placing the vascular clip on the superior mesenteric artery.

2.3. Samples of lung tissues

After the reperfusion procedure of 72 h (3 day), the rats were reanesthetized with intraperitoneal pentobarbital (75 mg/kg) and sacrificed by blood depletion. Then thoracic cavity of each animal was opened, the right lower lung was harvested for immunohistochemistry examination. The right lower lungs were utilized for the detections of BD-2 protein expression and distribution by immunohistochemistry, immunofluorescence and western blotting techniques, while the left upper and lower lungs were for the determinations of TNF-a concentration, SOD activity and MDA level. All the lung tissues were snap frozen with liquid nitrogen and stored at −80 °C until analysis.

2.4. Immunohistochemistry staining of BD-2

Pulmonary tissues stored in ultra low temperature freezer were transferred to −20 °C refrigerator for about 20 min and then mounted on opti-mum cutting temperature compound (SAKURA, USA) until slicing at 8 μm. The cryosections were fixed in ice-cold acetone for 15 min and incubated with 3% hydrogen peroxide at room temperature for 10 min to block endogenous peroxidase activity. After blocking with phosphate buffered sodium containing 5% fetal bovine serum, the slides were incubated overnight with a 1:1000 dilution of polyclonal primary antibody against BD-2 (Abbiotec, USA). The labeling was visualized by using horseradish peroxidase (HRP)-conjugated secondary antibody (Invitrogen, USA). All incubation steps were performed in a humidified chamber. Then the sections were stained with diaminobenzidine (DAB) + Substrate Buffer (Zhongshan Goldenbridge Biotechnology Co., Ltd, Beijing, China) according to the manufacturer's instruction and counterstained with hematoxylin for 3 min and mounted with glass coverslips. Examination was performed under light microscopy.

2.5. Immunofluorescence detection of BD-2

The preparation and fixation of cryosections was conducted as described previously. The sections were incubated with a 1:800 dilution of polyclonal primary antibody against BD-2 overnight at 4 °C and then with fluorescein isothiocyannate (FITC)- secondary antibody against rabbit (Invitrogen, USA) at the same dilution for 1 h at 37 °C. Mounting medium for fluorescence with DAPI (Vector laboratories Inc. USA) was added for staining of nucleuses just before the examination the fluorescent signals of BD-2 protein under fluorescence microscope.

2.6. Assessment of TNF-α level in lungs

TNF-α concentration in the lung were assessed by using commercially available ELISA kits according to the manual book provided by the manufacturer (Dakewe biotech Co., Ltd, China). Pulmonary tissues were homogenised on ice with physiological saline (1:9 w/v) and then centrifuged for 10 min at 3000 rpm/min. Supernatants were collected for the further assessment. Briefly, 0.1 ml supernatants were incubated with the 50 μl biotinylated antibody for 90 min at 37 °C and then washed four times. 0.1 ml streptavidin- HRP was added for per sample and incubated for 30 min at 37 °C. After washed for anther four times the substrate solution was added. The reaction system was kept from light for 20 min at 37 °C. The optical density at 450 nm was detected with a microplate reader.

2.7. Assessment of SOD activity and MDA concentration in lungs

Homogenization of lung tissues was prepared as above. Commercially available kits for detections of SOD activity and MDA concentration were used based on the protocol provided by the manufacturer (Nanjing Jiancheng Corp., China).

2.8. Western blotting analysis for detection of BD-2 protein

Lung tissues were homogenised with ice-cold RIPA Buffer (Thermo, USA) containing 1% Protease Inhibitor (cocktail kit, Thermo, USA). The homogenates were vortexed for 5 s and incubated on ice for 1 min. After vortexed for another 5 s, the homogenates were centrifuged at 14,000 rpm, 4 °C for 5 min. The supernatants were collected as tissue extracts and kept at −80 °C. Protein concentration was determined by BCA protein assay kit (Thermo Fisher Scientific Inc., USA). Aliquots of 30 mg protein of each rat were electrophoresed on a 12% SDS-polyacrylamide gel for 1.5 h at 120 V and transferred onto a nitrocellulose membrane. The membrane was incubated with blocking solution (Tris buffered saline with Tween 20 containing 5% nonfat milk) for 1 h at room temperature and then probed with primary antibody against BD-2 (Abbiotec, USA) overnight at 4 °C. After washed with TBST (Tris buffered saline with Tween 20, 1000:1 of v/v) for 3 times, the membrane was incubated with a horseradish peroxidase-labeled secondary antibody for 1 h at room temperature. Protein bands were visualized with chemiluminescence detection. Optical density of protein bands corresponding to BD-2 were quantified by Image-Pro Plus software.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (IBM, USA). All data were expressed as Means ± SD. One-way analysis of variance (ANOVA) was used for multiple comparison. LSD-t test was used for intra-group comparison or Tamhane's T2 test was used if equal variances were not assumed. The relationships of BD-2 protein expression level with TNF-α and MDA concentration in pulmonary tissues were evaluated by Pearson correlation test. Differences at $p < 0.05$ were considered significant.

3. Results

3.1. Immunohistochemistry staining of BD-2

In S group, BD-2 had a constitutive expression in which both intra-cellular and ecto-cellular immunoreactivity were observed somewhat. In IIR group, the expression of BD-2 in lung was increased significantly and the distribution was mainly in bronchiole epitheliums and pulmonary mesenchymal compartments. Expression level of BD-2 was decreased significantly in Ala–Gln group compared to IIR group. However, there is no marked difference between Ala and IIR group (Fig 1).

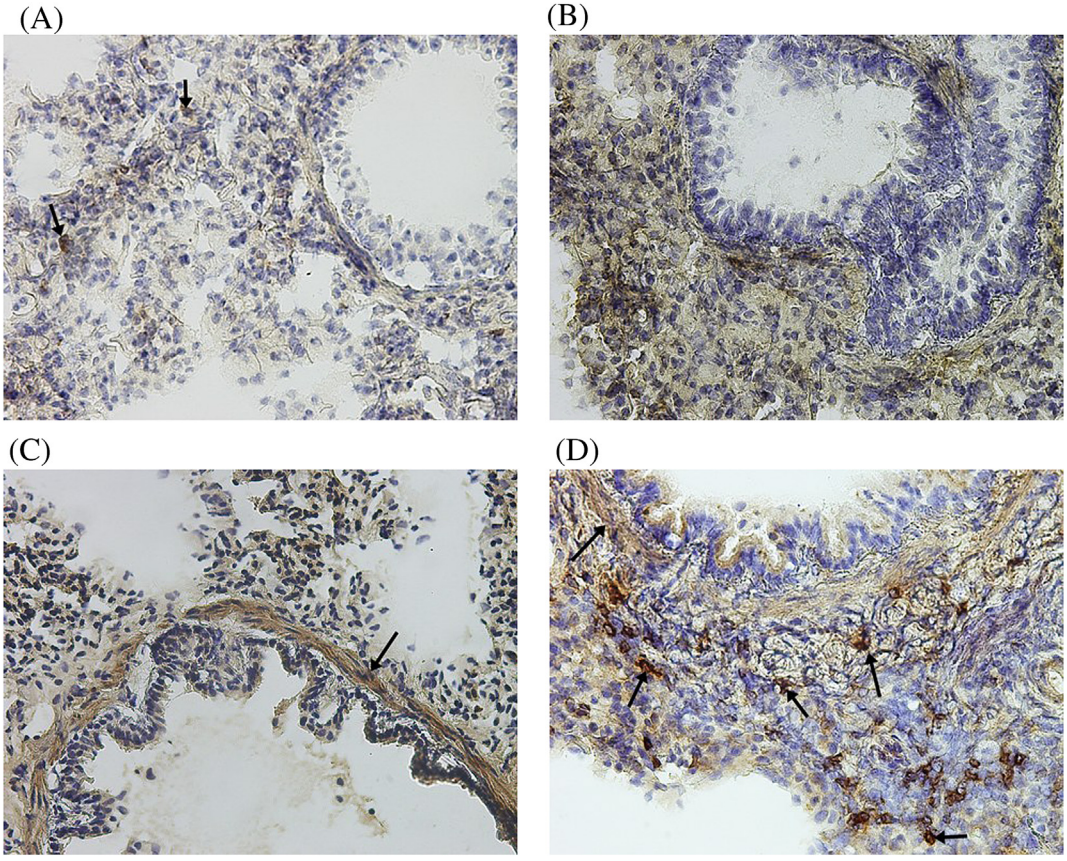

Fig. 1. Expression of BD-2 protein in lungs assessed by immunohistochemistry staining under the light microscopy (400×). The brown color indicated BD-2 positive staining. S group showed a very low level of BD-2 expression (as shown by arrows). (A); Apparent positive staining was observed mainly in bronchiole epithelium and pulmonary interstitium in Ala and IIR groups (as shown by arrows). (C and D); In Ala–Gln group, the positive staining of BD-2 was much weaker than that in IIR and Ala groups (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Immunofluorescence detection of BD-2

Immunofluorescence revealed the laminated expression of BD-2 protein along bronchiole wall more distinctly than the immunohistochemistry technique. S group showed a monoptychial expression of BD-2 in bronchiole wall; Significantly enhanced signals were observed in IIR and Ala groups, which were visualized as multilaminar in bronchiole wall; In Ala–Gln group, the distribution of BD-2 protein was similar to S group and the fluorescent intensity was much weaker than that in IIR and Ala groups (Fig 2).

3.3. BD-2 protein expression detected by western blotting analysis

Western blotting demonstrated that weak positive signal was found in the S group and a significant up-regulation in band intensity corresponding to BD-2 protein was observed in IIR and Ala group. The gray scale of band corresponding BD-2 protein was decreased significantly in Ala–Gln group as compared with IIR and Ala groups. The alterations of BD-2 protein expression in lung detected by western blotting were in line with the results of immunohistochemistry and immunofluorescence studies (Fig 3).

3.4. Changes of TNF-α level in lungs

IIR led to significant increases in concentrations of TNF-α in lung tissues as compared with S group ($p < 0.01$). TNF-α concentration in Ala–Gln group was lower significantly than that in IIR group ($p < 0.05$). However, there was no significant difference between Ala and IIR group ($p > 0.05$) (Fig 4).

3.5. Changes of SOD activity and MDA concentration in lungs

In IIR groups, the SOD activity in pulmonary tissues was decreased ($p < 0.01$) and MDA concentration was increased ($p < 0.05$) significantly compared to S group. In Ala–Gln group, changes of SOD activity and MDA concentration were attenuated markedly ($p < 0.05$). However, there was no significant difference in these two parameters between Ala and IIR group ($p > 0.05$) (Fig 5).

3.6. Correlation analysis

BD-2 protein expression level detected by western blotting in lung tissues was significantly positively correlated to local TNF-α level ($r = 0.934$, $p < 0.01$) and MDA concentration ($r = 0.891$, $p < 0.01$).

4. Discussions

Ala–Gln is used as the substitute of Gln in clinical practice because of its advantages over the latter, which include more stableness in aqueous solution and the property of withstanding high temperature sterilization. Thus oral supplementation of Ala–Gln was used to assess the effects of immune-nutrient on IIR-related lung injury and Ala was used in current research to exclude the effects of common amino acid.

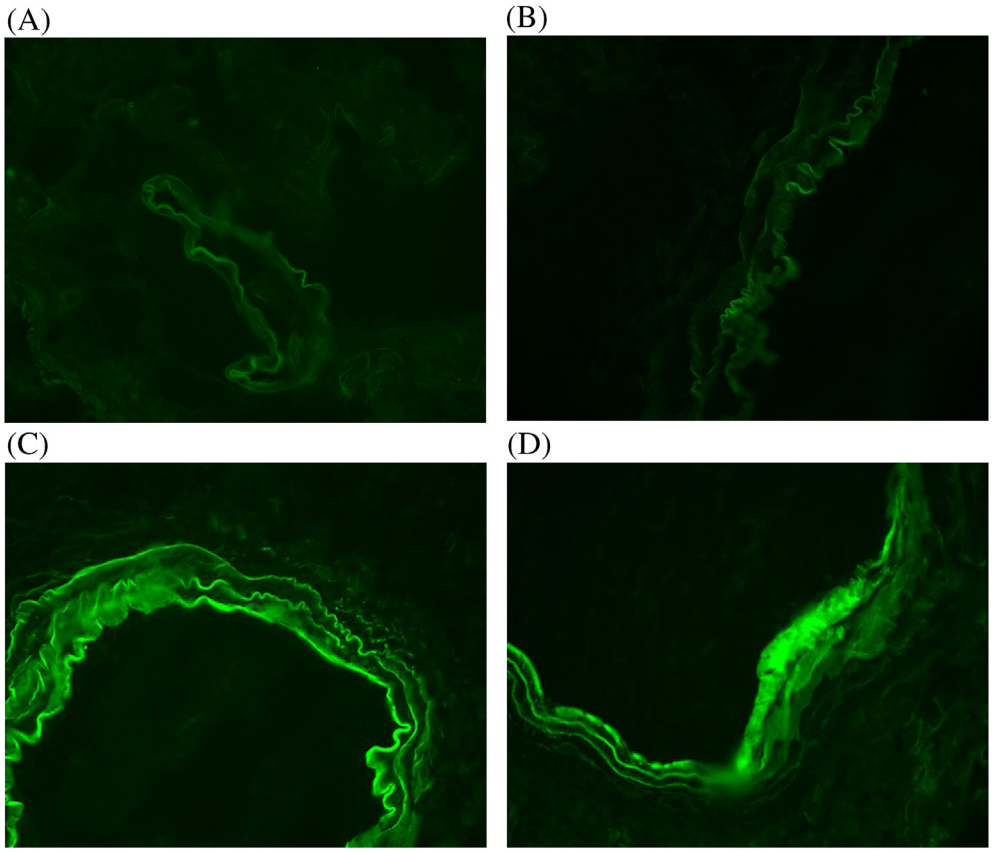

Fig. 2. Expression signals of BD-2 protein in lungs assessed by immunofluorescence detection (400×). The laminated expressions of BD-2 along bronchiole wall were displayed distinctly. S group showed a monoptychial expression of BD-2 in bronchiole wall (A); Stronger signals were observed in Ala and IIR groups, which were visualized as multilaminar in bronchiole wall (C and D); In Ala–Gln group, the distribution of BD-2 protein were similar to S group and the fluorescent intensity was much weaker than that in IIR and Ala groups (B).

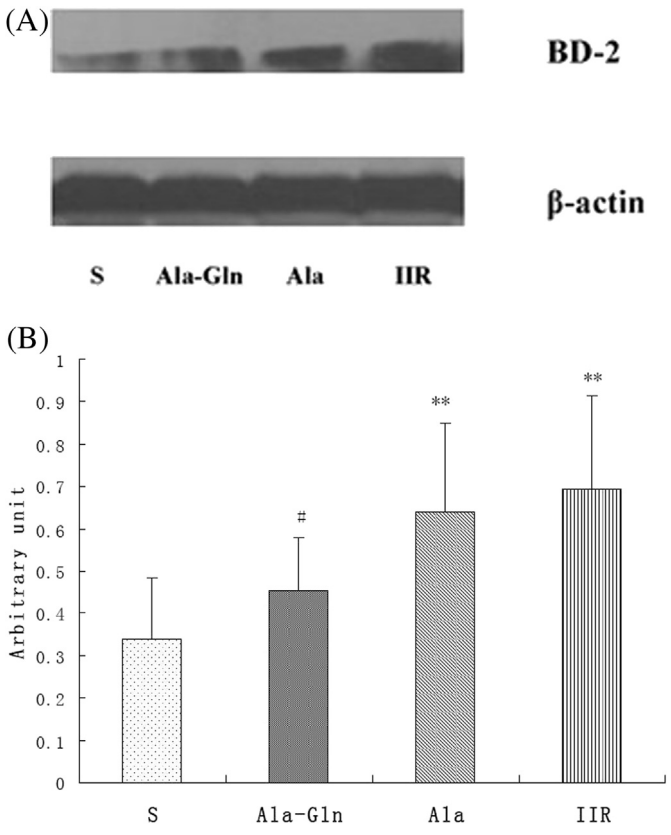

Fig. 3. Western blotting analysis of BD-2 expression in the lung tissues. A: The classical bands of BD-2 and β-actin in the four groups. The prominent bands of BD-2 protein were indicated at approximately 7 kDa. The intensity of BD-2 protein in Ala and IIR groups was prominent over that in S and Ala–Gln groups. B: The gray scales of bands corresponding to BD-2 protein were normalized as the ratios to the same parameters of β-actin. The expression of BD-2 protein in Ala and IIR groups was increased significantly compared to S group ($p < 0.01$), while the down-regulation of the micromolecular protein was marked in Ala–Gln group as compared with IIR group ($p < 0.05$). $^{**}p < 0.01$ *vs* S group; $^{\#}p < 0.05$ *vs* IIR group. $n = 10$ rats per group.

As reported previously, Gln treatment could decrease IIR-induced acute lung injury and various mechanisms probably employed have been explored [9,10]. However, to our knowledge, the present study is the first to observe the changes of lung BD-2 protein expression in the treatment of IIR related lung injury in rats by using immunohistochemistry, immunofluorescence and western blotting techniques.

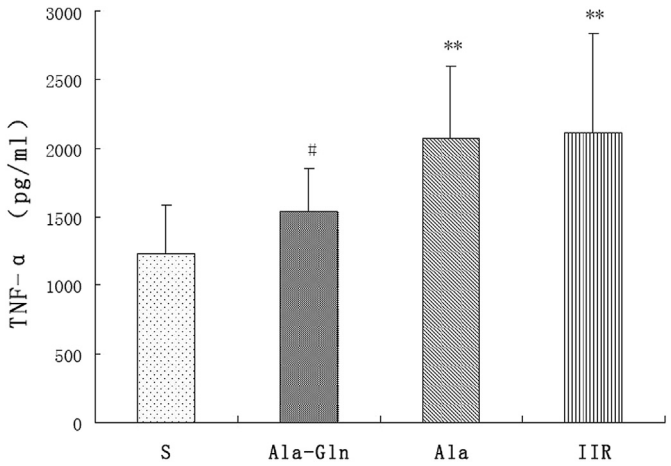

Fig. 4. Changes of TNF-α concentration in rat lung tissues. Data are mean ± S.D. $^{**}p < 0.01$ *vs* S group; $^{\#}p < 0.05$ *vs* IIR group. $n = 10$ rats per group.

BD-2 exerts a broad spectrum antimicrobial activity by destroying the cytoplasmic membrane of microorganisms and can be induced by various kinds of inflammatory stimuli. Abundant researches have demonstrated that BD-2 is a favorable factor involved in adaptive defense against the lung injury resulting from some diseases [12,13]. It was well established that BD-2 has chemoattractant effect on immature dendritic cell and $CD4^+$ T cell via chemokine receptor CCR6 [7]. So promoting recruitment of dendritic cell and T cell to the site of inflammatory response is one of the most important mechanisms by which BD-2 inhibits inflammatory injury of local organs.

The present study revealed that the up-regulated level of BD-2 protein in the lung after IIR was decreased by oral administration of Ala–Gln in rats. This is a novel finding. There are two explanations mainly for this phenomenon. Firstly, BD-2 in pulmonary tissue is correlated tightly to local microbial invasion and proinflammatory mediators, it can be induced by a variety of stimuli including TNF, IL-1 and lipopolysaccharide (LPS) [14]. In vitro, bacterial infections in the lung can enhance BD-2 expression [15]. Nuclear factor-κB (NF-κB), an transcription facilitative factor of proinflammatory cytokines, can up-regulate the expression of BD-2 in pulmonary epithelial cells through the actions of IL-1 and TNF-α [16]. Secondly, the disruption of intestinal barrier after

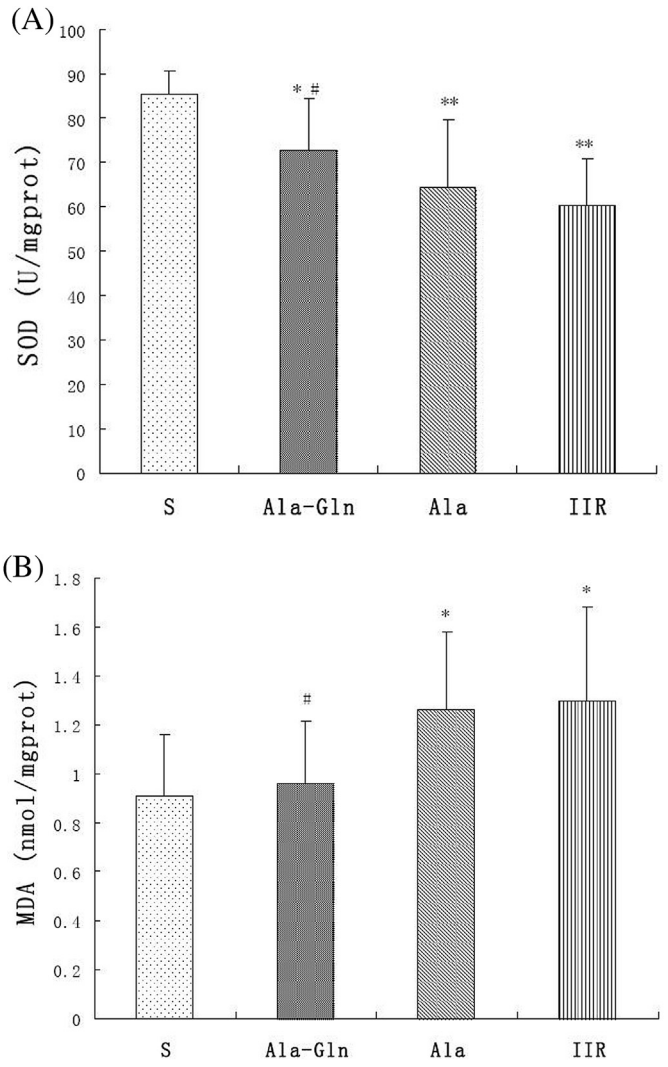

Fig. 5. Changes of SOD activity (A) and MDA concentration (B) in rat lung tissues. Data are mean ± S.D. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* S group; $^{\#}p < 0.05$ *vs* IIR group. $n = 10$ rats per group.

IIR and consequent translocations of bacteria and endotoxin play a central role in the development of systemic inflammation that can result in damage to remote organs. Under traumatic and stress conditions, Gln is the preferred fuel for the enterocyte and is beneficial for maintaining intestinal mucosal barrier function. After Gln administration, prevention against increased intestinal permeability [17] and inhibition of bacteria and endotoxin translocations to lung from gut after IIR were observed [18]. In the current study, by utilizing rat model we revealed that Ala–Gln was capable of attenuating the up-regulation of TNF-α concentration in lung tissues caused by IIR, which was responsible for the reduction of BD-2 protein expression by Ala–Gln administration.

In addition, the great generation of free radicals released from polymorphonuclear leukocytes (PMNL) mobilized at the initial stage of reperfusion process [19] could lead to oxidative damage directly in the lung. Gln has protective effects against redox disturbance and oxidative stress resulting from free radicals by generating glutathione (GSH), a major antioxidant and a vital component of a host's defense. Gln can exert a potent inhibitive effect in nitric oxide (NO) biosynthesis, which is thought to be involved in ischemia reperfusion injury [20]. In this study, administration of Ala–Gln attenuated the IIR-induced down-regulation of SOD activity in rats that is a highly specific scavenger of superoxide. Meanwhile, we observed that the lung MDA level, which was considered as an important marker of lipoperoxidation associated to oxidative stress [21], was increased after IIR and could be down-regulated by Ala–Gln oral administration. These effects were due to the function of Ala–Gln in maintaining redox homeostasis under the condition of serious trauma. Moreover, it was demonstrated in present study that the changed trend of BD-2 protein expression in the lung after application of Ala–Gln was paralleled with the alterations of local concentration of TNF-α and MDA. The observation suggested that Ala–Gln could modify the level of BD-2 in IIR-induced lung injury in rats via limiting pulmonary inflammatory and oxidative injury. Similarly to the previous research focusing on the relationship between BD-2 protein and airway inflammation [13], it also can be concluded from the results of the current study that the expression level of BD-2 protein is able to serve as a useful marker of pulmonary injury induced by IIR. However, in order to reveal the exact functions of BD-2 protein in the development and treatment of IIR-induced lung injury further studies employing specific BD-2 inhibitor or gene knock-out models are required.

In conclusion, oral administration of Ala–Gln can exert a protection against IIR-induced lung injury in rats and relieve the IIR-induced up-regulation of BD-2 protein expression in the lung through anti-inflammation and anti-oxidation mechanisms.

Ethical Approval

This study was approved by Animal Care Committee of Sun Yat-sen University. All of the animal care and experimental protocols were performed in accordance with National Institutes of Health guidelines for the use of experimental animals.

Funding

This study was supported by the Science & Technology Planning Foundation of Guangdong Province, China PR, No. 2008B030301105 and the PhD Start-up Fund of Natural Science Foundation of Guangdong Province, China PR, No. 10451008901005513.

Author contribution

Yun Li: Study design and writing.
Xiao-Bin Wu: Data collections.
Jia-Gen Li: Data collections.
Yi-Jia Lin: Data analysis.
Hong-Lei Chen: Data analysis.
Hu Song: Data analysis.
Zhong-Hui Liu: Quality control.
Jun-Sheng Peng: Study design.

Conflicts of interest

No conflicts of interest.

Acknowledgments

None.

References

[1] A.A. Ay, S. Kutun, H. Ulucanlar, O. Tarcan, A. Demir, A. Cetin, Risk factors for postoperative ileus, J. Korean Surg. Soc. 81 (2011) 242–249.
[2] D.F. Ben, X.Y. Yu, G.Y. Ji, D.Y. Zheng, K.Y. Lv, B. Ma, et al., TLR4 mediates lung injury and inflammation in intestinal ischemia-reperfusion, J. Surg. Res. 174 (2012) 326–333.
[3] S.J. Kwag, J.G. Kim, W.K. Kang, J.K. Lee, S.T. Oh, The nutritional risk is a independent factor for postoperative morbidity in surgery for colorectal cancer, Ann. Surg. Treat. Res. 86 (2014) 206–211.
[4] R.H. Turnage, K.S. Guice, K.T. Oldham, Pulmonary microvascular injury following intestinal reperfusion, New Horiz. 2 (1994) 463–475.
[5] K.X. Liu, S.Q. Chen, H. Zhang, J.Y. Guo, Y.S. Li, W.Q. Huang, Intestinal ischaemia/reperfusion upregulates beta-defensin-2 expression and causes acute lung injury in the rat, Injury 40 (2009) 950–955.
[6] S. Lippross, T. Klueter, N. Steubesand, S. Oestern, R. Mentlein, F. Hildebrandt, et al., Multiple trauma induces serum production of host defence peptides, Injury 43 (2012) 137–142.
[7] S. Jäger, E.F. Stange, J. Wehkamp, Antimicrobial peptides in gastrointestinal inflammation, Int. J. Inflam. 2010 (2010) 910283.
[8] O. Froy, A. Hananel, N. Chapnik, Z. Madar, Differential expression of rat beta-defensins, IUBMB Life 57 (2005) 41–43.
[9] G.Z. He, L.G. Dong, X.F. Chen, K.G. Zhou, H. Shu, Lymph duct ligation during ischemia/reperfusion prevents pulmonary dysfunction in a rat model with ω-3 polyunsaturated fatty acid and glutamine, Nutrition 27 (2011) 604–614.
[10] G. Guiqi, Pre-treatment with glutamine attenuates lung injury in rats subjected to intestinal ischaemia–reperfusion, Injury 42 (2011) 72–77.
[11] D. Kazantzidou, K. Tsalis, K. Vasiliadis, H. Kaldrymidou, G. Papageorgiou, K. Koliakou, et al., Alanine-glutamine dipeptide pretreatment protects rat renal function from small intestine ischemia-reperfusion injury, Minerva Chir. 65 (2010) 515–525.
[12] Q. Shu, Z. Shi, Z. Zhao, Z. Chen, H. Yao, Q. Chen, et al., Protection against Pseudomonas aeruginosa pneumonia and sepsis-induced lung injury by overexpression of beta-defensin-2 in rats, Shock 26 (2006) 365–371.
[13] T. Hiratsuka, H. Mukae, H. Iiboshi, J. Ashitani, K. Nabeshima, T. Minematsu, et al., Increased concentrations of human beta defensins in plasma and bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis, Thorax 58 (2003) 425–430.
[14] T. Suzuki, C.W. Chow, G.P. Downey, Role of innate immune cells and their products in lung immunopathology, Int. J. Biochem. Cell. Biol. 40 (2008) 1348–1361.
[15] S. Scharf, S. Hippenstiel, A. Flieger, N. Suttorp, P.D. N'Guessan, Induction of human β-defensin-2 in pulmonary epithelial cells by Legionella pneumophila: involvement of TLR2 and TLR5, p38 MAPK, JNK, NF-κB,and AP-1, Am. J. Physiol. Lung Cell. Mol. Physiol. 298 (2010) L687–L695.
[16] Y. Tsutsumi-Ishii, I. Nagaoka, Modulation of human β-defensin-2 transcription in pulmonary epithelial cells by lipopolysaccharide-stimulated mononuclear phagocytes via proinflammatory cytokine production, J. Immunol. 170 (2003) 4226–4236.
[17] S.M. Haque, K. Chen, N. Usui, Y. Iiboshi, H. Okuyama, A. Masunari, et al., Alanyl-Glutamine dipeptide-supplemented parenteral nutrition improves intestinal metabolism and prevents increased permeability in rats, Ann. Surg. 223 (1996) 334–341.
[18] G.H. Wu, H. Wang, Y.W. Zhang, Z.H. Wu, Z.G. Wu, Glutamine supplemented parenteral nutrition prevents intestinal ischemia-reperfusion injury in rats, World J. Gastroenterol. 10 (2004) 2592–2594.
[19] J. Hamar, I. Rácz, M. Cíz, A. Lojek, E. Pállinger, J. Furész, Time course of leukocyte response and free radical release in an early reperfusion injury of the Superior mesenteric artery, Physiol. Res. 52 (2003) 417–423.
[20] J.M. Matés, C. Pérez-Gómez, I. Núñez de Castro, M. Asenjo, J. Márquez, Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death, Int. J. Biochem. Cell. Biol. 34 (2002) 439–458.
[21] E. Barocelli, V. Ballabeni, P. Ghizzardi, F. Cattaruzza, S. Bertoni, C.A. Lagrasta, et al., The selective inhibition of inducible nitric oxide synthase prevents intestinal ischemia-reperfusion injury in mice, Nitric Oxide 14 (2006) 212–218.