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.