

Original Article

Effects of glutamine on alveolar epithelial barrier function after cardiopulmonary bypass

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Abstract: Objective: This study is to investigate the effects of glutamine (Gln) on alveolar epithelial barrier function after cardiopulmonary bypass (CPB). Methods: A total of 40 rats were randomized into 5 equal groups: sham operation (SH), CPB, Gln 1 (G1), Gln 2 (G2), and Gln 3 (G3). Three days before operation, rats in SH and CPB groups were injected with 0.5 ml of saline, while rats in G1, G2, and G3 groups were respectively injected with 2 ml 0.15 g/kg, 0.45 g/kg and 0.75 g/kg of Gln. CPB was constructed in groups CPB, G1, G2, and G3. Alveolar-arterial oxygen difference (A-aDO₂) and the levels of respiratory index (RI), interleukin-6 (IL-6), interleukin-10 (IL-10), tumornecrosisfactor- α (TNF- α), lipopolysaccharide (LPS), matrix metalloprotein-9 (MMP-9), extravascular lung water (EVLW), malonic dialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), aquaporin-1 (AQP-1), and aquaporin-4 (AQP-4) were detected. Results: A-aDO in CPB, G1 and G2 groups was lower than that in SH group, and was higher in groups G1, G2 and G3 than in CPB, all with significant difference (P<0.05). RI in CPB and G1 was significantly lower than that in SH, whereas was higher in G1, G2 and G3 than in CPB group (P<0.05). EVLW, LPS, MMP-9, MDA, MPO, IL-6, IL-10, and AQP-4 in CPB, G1, G2, and G3 groups were higher than that in SH group, however, were lower in G1, G2, and G3 groups comparing with that of CPB group (P<0.05). SOD and AQP-1 in CPB, G1, G2, and G3 groups were lower than that in SH group, and in G1, G2 and G3 groups were higher than that in CPB group (P<0.05). TNF- α in G1 group was higher than in SH group, while in G1, G2, and G3 groups was lower than that in CPB group (P<0.05). Conclusion: Gln pretreatment was helpful for releasing lung injury, systemic inflammatory response syndrome (SIRS), and ischemia-reperfusion injury which are secondary to CPB.

Keywords: Glutamine, cardiopulmonary bypass, lung injure, barrier function

Introduction

Cardiopulmonary bypass (CPB) is important in open heart surgery. With the development of CPB technology, myocardium preservation, and cardiac surgery, the incidence and death rate of complications of open heart surgery greatly decreased, however, lung injury was still one of the main complications. During CPB, the lung was the only organ without protection of effective cooling and would collapse and be injured by the high temperature. Chemoweth [1] and Kirklin [2] consider that systemic inflammatory response is induced by CPB. Kirklin and colleagues [2] argue that the systemic inflammatory response induced by CPB is similar to that of local inflammation on the wound part. In

1990s, Steinbery [3] and cremer [4] illustrated the association between CPB and SIRS together with the underlying mechanism. CPB could active leukocyte and platelet, thereby leading to a series of pathophysiological changes including immune factors release and endothelial cell proliferation. These changes would enhance the risk of perfusion syndrome and aggravate the disease, and even lead to acute respiratory distress syndrome (ARDS) or multiple organ dysfunction syndrome (MODS).

Glutamine (Gln), as a neutral amino acid (AA), is with the richest content in free AA pool of body, and has special metabolic and regulating function on tissue and cells. Furthermore, Gln is a critical regulatory factor in protein metabolism

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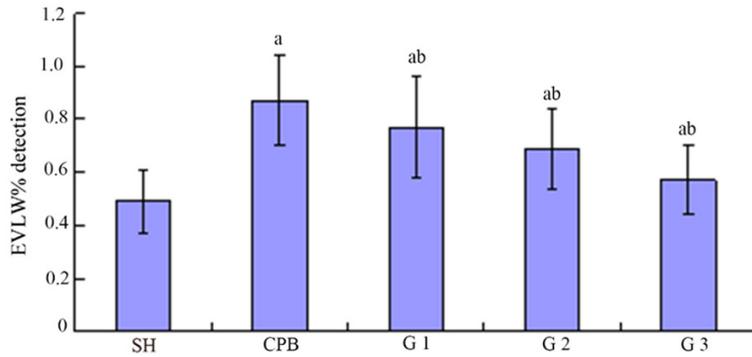


Figure 1. EVLW% detection. a, comparing with SH group, $P < 0.05$; b, comparing with CPB group, $P < 0.05$.

and immune function. Under the stress states including operation, severe trauma, sepsis, acute hemorrhagic necrotizing pancreatitis and so on, there are obvious changes on Gln consumption and emission nets in different organs. This could result in Gln disorder of the flow and metabolism, followed by protein, lipid, and glucose and energy metabolism abnormality and organ dysfunction, even failure. Under physiological or severe stress status, Gln is the important fuel and regulatory factor sustaining the structure and function in different kinds of cells in lung tissue, including endothelial cell, alveolar epithelial cell, macrophage, and lymphocyte. This function is helpful for protecting lung from injuries like biological endotoxin, oxygen free radical, and cytotoxin. In the present study, by constructing the CPB model with rat and giving exogenous Gln, the effects of different dosages of Gln on alveolar permeability and AQP protein expression during CPB were observed and compared. We intend to investigate the protective mechanism of Gln on lung and provide evidences for clinical application of Gln.

Materials and methods

CPB model construction

A total of 40 male adult SD rats weighted 400 to 500 g were obtained from the animal center of the General Hospital of Shenyang Military. The rats were randomized into 5 equal groups: sham operation (SH) group, CPB group, Gln 1 group, Gln 2 group, and Gln 3 group. Three days before operation, the rats in SH and CPB groups were injected with 0.5 ml of saline via tail vein. The rats in G1, G2, and G3 groups were injected with 2 ml of Gln according to 0.15 g/kg, 0.45 g/kg, 0.75 g/kg, separately [5]. The rats

in SH group received open chest and intubation without CPB. A total of 15 ml of non-blood priming containing 12 ml of 6% glyoxyethyl starch, 2 ml of 5% NaHCO_3 , 1 ml of 20% mannitol, and 150 IU/kg heparin sodium was applied. As for G1, G2, and G3 groups, corresponding concentration of Gln was added in the non-blood priming.

After 6 h of fasting and water deprivation, the rats were anesthetized with 10% chloral hydrate (0.3 ml/100 g) via intraperitoneal injection. The tracheal tube was inserted and the rodent ventilator was connected. The limbs of rat were connected to electrocardiogram lead for electrocardiographic (ECG) monitoring. The transducer was inserted with 2 cm of depth for monitoring central temperature. Pure oxygen was inhaled with respiratory rate of 60 breaths/min, I:E = 1:2, PEEP = 8~10 ml/kg, and $P_{\text{ET}}\text{CO}_2 = 35\sim 45$ mmHg. The venous channel was constructed via left femoral vein puncture with 22G trocar. And 6% glyoxyethyl starch (0.5 ml/h) was injected with electric infusion pump. The real-time arterial pressure was monitored by tail artery puncture with 24G trocar with pressure sensor connected with monitor. Right femoral artery puncture was carried out with 22G trocar and CPB perfusion. Heparin sodium (300 IU/kg) was given via vein after right carotid artery puncture. A 18G trocar with porous was placed in cavity by right cervical vein puncture and detected ACT 400~500 s to construct the CPB (**Figure 1**). The rats in SH group received the operation mentioned above but without CPB. A 35 ml/(kg·min) of flow was adopted at the beginning, and increased to 100~120 ml/(kg·min) gradually. A 1~2 ml of blood volume in the blood-collecting utensil was sustained. The ventilator was not used after CPB beginning, and oxygenator (0.05 m^2) was used for oxygen supply ($\text{FiO}_2 = 1.0$). Blood gas management was used to adjust the medicine according to the blood gas analysis to sustain MAP > 60 mmHg, pH 7.35~7.45, PaCO_2 35~45 mmHg, and base excess (BE) -3~3 mmol/l. One hour after CPB, the mechanical ventilation was recovered. When the hematocrit (Hct) was above 0.25, the flow was gradually decreased until stop. The pipelines inserted in the heart was extracted successively,

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while mechanical ventilation was sustained and the rectal temperature was kept at 36.5~37.5°C. The blood in blood-collecting utensil was infused and a proper amount of dopamine or blood was supported according to the actual situation. Anesthesia deepness was monitored according to plantar reflex and intraperitoneally injection of 1% pentobarbital sodium (50 mg/kg) was performed while necessary. Blood gas analysis was performed with 0.1 ml of arterial blood at different points: the beginning of CPB (T_0), blocking aorta (T_1), opening aorta (T_2), after CPB (T_3), and 2 h after CPB (T_4). All the rats were sacrificed 2 h after CPB, and the lung tissue was collected for further study. This study was consented by the Animal Ethics Committee of First Affiliated Hospital of China Medical University.

Lung tissue sample

After sacrifice, the trachea was exposed and the chest was open. Inferior lobe of right lung was taken and the extravascular lung water (EVLW) was detected by gravity method [6]. The lung tissue was then weighted, added distilled water with the same mass of the lung tissue, and made into homogenate with machine and ultrasound. The homogenate was weighted and centrifuged at 5,000 g for 30 min, then placed at 4°C for 1 h. The supernatant was collected and hemoglobin (Hb) was detected. The arterial blood, tissue homogenate, and the supernatant were dried at 80°C for 72 h and the water percentage (%) was calculated. EVLW was calculated according to the formulas:

Homogenate Hb = Supernatant Hb × (Homogenate water content%/Supernatant water content%); Blood weight = Homogenate weight × Homogenate Hb/Blood Hb; Blood water weight = Blood weight × Blood water content%; Total pulmonary water (TPW) = Homogenate water content% × Homogenate weight-supplementary water; and EVLW = TPW - Blood water weight.

Left lung tissue was taken and fixed in 4% paraformaldehyde for paraffin sections. The tissue that was 1 cm away from the upper lobe of right lung was cut into cubes (1 mm³), fixed by 2.5% glutaraldehyde, embedded and cut into sections (4 μm). After staining with sodium acetate and lead citrate, the slices were observed the ultrastructure changes of the lung tissue under H-7200 transmission electron microscope (TEM).

Detection of respiratory function

Blood gas analysis was used for estimating the respiratory function. Alveolar-arterial oxygen difference (A-aDO₂) and respiratory index (RI) were calculated using the formulas of $A-aDO_2 = (FiO_2 \times 713 - 5/4 \times PaCO_2) - PaO_2$ and $RI = PA-aDO_2/PaO_2$.

Spectrophotometry detection

Malondialdehyde (MDA), myeloperoxidase (MPO) and superoxide dismutase (SOD) were detected by spectrophotometry. Briefly, 100 mg lung tissue was taken and made into 5% homogenate with machine and ultrasound. Spectrophotometry was then carried out strictly according to the specification of the kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Radio-immunity detection

Serum IL-6, IL-10 and TNF-α were detected according to the radioimmune outfit (Tianjin Jiuding Medical Bioengineering Co., Ltd. Tianjin, China). The data and standard curve were analyzed and made by GC-2016 software V3.3 with GC-2016v radioimmunity analyzer.

Enzyme linked immune response (ELISA)

Serum MMP-9, total and free, including TIMP binding, was detected with ELISA kit (R & D Systems, Inc). Simply stated, the specimens were divided into two copies in parallel and one copy was diluted with a 1:10 ratio pre testing. The OD was detected at the wavelength of 450 nm with microplate reader (Thermo Fisher Scientific, MA, USA) and the content of MMP-9 was calculated according to the standard curve.

Immunohistochemistry (IHC)

All the samples were fixed by 40 g/l formaldehyde, embedded by paraffin and cut into series of sections (4 μm). Hematoxylin and eosin (HE) staining and immunohistochemical staining were carried out, separately. AQP-1 was labeled by streptavidin-peroxidase (SP) method according to the specification of the IHC kit (Thermo Fisher Scientific, Inc. Shanghai, China). DAB staining and hematoxylin counterstaining nucleus were carried out. PBS instead of primary antibody was taken as negative control. The sections were observed under microscopy.

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Table 1. EVLW% detection ($X \pm S$, $n = 8$)

Groups	SH	CPB	G1	G2	G3
EVLW%	0.49±0.12	0.87±0.17 ^a	0.77±0.19 ^{a,b}	0.69±0.15 ^{a,b}	0.57±0.13 ^{a,b}

Note: a, comparing to SH group, $P = 0.026$; b, comparing to CPB group, $P = 0.0037$.

Table 2. Serum LPS and MMP-9 expressions ($X \pm S$, $n = 8$)

Groups	LPS (EU/ml)	MMP-9 ($\mu\text{g/L}$)
SH	0.363±0.114	65.48±14.59
CPB	0.923±0.194 ^a	277.14±34.27 ^a
G1	0.726±0.158 ^{a,b}	223.36±28.42 ^{a,b}
G2	0.543±0.174 ^{a,b}	180.72±19.25 ^{a,b}
G3	0.459±0.233 ^{a,b}	123.55±26.37 ^{a,b}

Note: a, comparing to SH group, $P = 0.015, 0.024$; b, comparing to CPB group, $P = 0.001, 0.007$.

Western blotting (WB) detection

A total of 10 μg protein of each sample was analyzed by 12.5% separating gel with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to polyvinylidene fluoride (PVDF) membrane by Trans-Blot instrument. Primary antibody (1:4000, Cell Signaling Technology, Inc) was added and followed by secondary antibody (1:5000, Sigma) incubation. Electrochemiluminescence (ECL) immunoassay was used for detection. β -actin was used as an internal control. The protein bands were analyzed by Quantity One software.

Real-time quantitative PCR (RT-qPCR)

The right middle lobe of lung tissue was extracted and RNA was extracted using TRIzol reagent (Takara, Dalian, China). Reverse transcription was performed using 2 μg RNA as template. *NADPH* was used as the internal reference. The primer sequences used were as follows: *NADPH*: TTC CAG CCT TCC TTC CTG G (5'-3'), TTG CGC TCA GGA GGA GCA AT (3'-5'), *AQP-1*: CTT ACC TCC AGG ACC CTT CC (5'-3'), TAG CTC ATC CAC ACG TGC TC (3'-5'), *AQP-4*: CGG TTC ATG GAA ACC TCA CT (5'-3'), and CAT GCT GGC TCC GGT ATA AT (3'-5').

Statistical analysis

All the measuring data were presented as mean \pm standard deviations ($X \pm S$) and analyzed by SPSS 11.0 software (SPSS Inc, Chicago, IL, USA). One-way ANOVA and chi-square test

were used for analyzing comparisons among groups. $P < 0.05$ was considered as significant difference.

Results

EVLW detection

To identify the severity of lung injury, EVLW in each group was detected. The EVLW in CPB, G1, G2 and G3 groups were significantly higher than that in SH group ($P < 0.05$). The EVLW in G1, G2 and G3 groups was significantly lower than that in CPB group and decreased gradually ($P < 0.05$) (**Table 1**; **Figure 1**). The result demonstrated that CPB could increase the permeability of lung tissue and Gln could improve the lung epithelial barrier function and relieve pulmonary edema.

Serum lipopolysaccharide (LPS) and MMP-9 expressions

In order to reveal the possible mechanism of Gln on CPB injury rats, serum LPS and MMP-9 were measured. Serum LPS in G1, G2 and G3 groups were higher than in SH group while LPS in G1, G2 and G3 were significantly lower than in CPB group ($P < 0.05$) (**Table 2**; **Figure 2A**).

Serum MMP-9 in G1, G2, G3 and CPB groups were higher than in SH group and serum MMP-9 in G1, G2 and G3 groups were lower than that in CPB group ($P < 0.05$) (**Table 2**; **Figure 2B**). Together, the results showed that pretreatment of Gln might reduce acute lung injury after CPB in rats by decreasing MMP-9.

A-aDO₂ and RI detection

To learn about the situation of respiratory function, A-aDO₂ and RI detection was performed. As illustrated in **Tables 2, 3**, A-aDO₂ in G1, G2, G3, and CPB groups were lower than that in SH group and A-aDO₂ in G1, G2, and G3 groups were significantly higher than that in CPB group ($P < 0.05$). RI in G1 and CPB groups were lower than SH group ($P < 0.05$). RI in G1, G2 and G3 were significantly higher than that in CPB group ($P < 0.05$) (**Table 3**). To sum up, the results argued that PB could decrease the diffusion function of lung tissue while Gln could improve the lung tissue dispersion.

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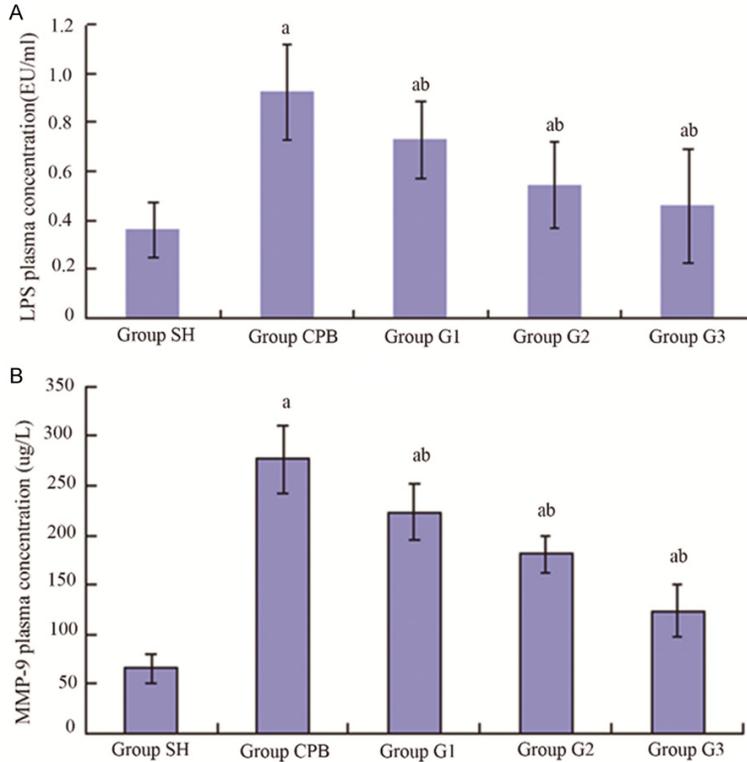


Figure 2. Expression level of serum LPS (A) and MMP-9 (B) in each group. a, comparing with SH group, $P < 0.05$; b, comparing with CPB group, $P < 0.05$.

Table 3. A-aDO₂ and RI detection ($\bar{X} \pm S$, n = 8)

Groups	A-aDO ₂ (mmHg)	RI
SH	158.74±19.68	0.78±0.27
CPB	115.35±14.57 ^a	0.69±0.24 ^a
G1	132.42±17.39 ^{a,b}	0.73±0.21 ^{a,b}
G2	138.24±16.27 ^{a,b}	0.75±0.34 ^b
G3	142.53±15.53 ^b	0.76±0.42 ^b

Note: a, comparing to SH group, $P = 0.032, 0.016$; b, comparing to CPB group, $P = 0.019, 0.006$.

Table 4. MDA, MPO and SOD expressions in lung tissue ($\bar{X} \pm S$, n = 8)

Groups	MDA mmol/g	MPO U/g	SOD U/g
SH	10.87±1.42	2.98±0.74	98.14±3.32
CPB	40.23±1.31 ^a	17.42±0.73 ^a	47.24±2.71 ^a
G1	29.42±2.71 ^{a,b}	13.45±0.38 ^{a,b}	68.52±3.48 ^{a,b}
G2	18.54±1.87 ^{a,b}	11.27±0.57 ^{a,b}	72.32±2.57 ^{a,b}
G3	14.32±3.42 ^{a,b}	9.24±0.46 ^{a,b}	79.47±4.31 ^{a,b}

Note: a, comparing to SH group, $P = 0.011, 0.027, 0.015$; b, comparing to CPB group, $P = 0.0009, 0.014, 0.008$.

MDA, MPO and SOD expressions in lung tissue

In order to study the expression of oxidation-reduction related enzymes, spectrophotometry

was carried out. As shown in **Table 4** and **Figure 3**, MDA of lung tissue in G1, G2, G3, and CPB groups were higher than that in SH group, and MDA in G1, G2, and G3 groups were lower than that in that CPB group ($P < 0.05$). MPO and SOD showed a similar tendency. In conclusion, the results illustrated that Gln could alleviate the oxidative stress caused by CPB to some extent.

Serum TNF- α , IL-6 and IL-10 expressions

To determine the expression of immune-associated factors, radio-immunity detection was undertaken. Serum TNF- α and IL-6 in G1, G2, G3, and CPB groups were higher than that in SH group while in G1, G2, and G3 groups were significantly lower than that in CPB group ($P < 0.05$) (**Table 5**; **Figure 4**). As for serum IL-10, G1, G2, G3, and CPB groups

were lower than in SH group and G1, G2 and G3 groups were higher than that of CPB group ($P < 0.05$). In total, the results suggested that Gln could reduce systemic inflammatory response induced by CPB.

Lung tissue morphological changes

In order to observe the morphological changes of lung tissue, HE staining and transmission electronic microscopy observation were performed. The alveolar wall in CPB group 2 h after CPB thickened obviously, with congestion and hemorrhage in alveoli and large amount of inflammatory cell infiltration (**Figure 5A-D**). In G1 and G2 groups, the exudation increased, the alveolar wall thickened slightly, with a few of inflammatory cell infiltration and telangiectasis. The hemorrhage in G1 and G2 were less than in CPB group. The alveolar cavity was clear in G3 with alveolar wall thickening.

As shown in **Figure 5E-H**, transmission electronic microscopy observation showed that the ultrastructure was damaged obviously in lung tissue of CPB group, with unclear, edematous and thickening blood-air barrier. There were defects in a few of alveolar epithelial cells with

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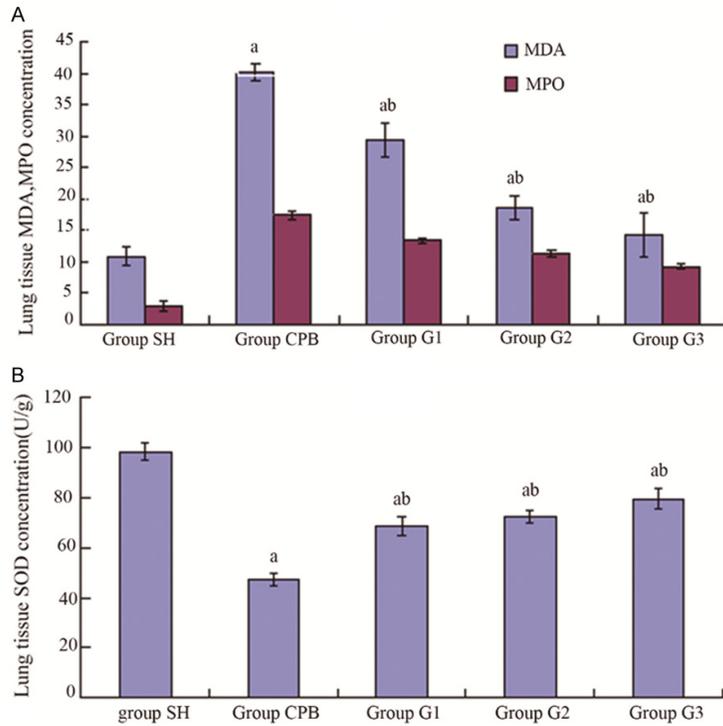


Figure 3. MDA, MPO (A) and SOD (B) expressions in lung tissue. a, comparing with SH group, $P < 0.05$; b, comparing with CPB group, $P < 0.05$.

Table 5. Serum TNF- α , IL-6 and IL-10 expressions ($\bar{X} \pm S$, $n = 8$)

Groups	TNF- α (ng/ml)	IL-6 (pg/ml)	IL-10 (ng/ml)
SH	1.93 \pm 0.84	20.21 \pm 7.49	792.34 \pm 72.37
CPB	4.12 \pm 0.37	85.32 \pm 9.47 ^a	270.68 \pm 58.24 ^a
G1	2.51 \pm 0.25 ^{a,b}	50.14 \pm 7.23 ^{a,b}	547.6 \pm 48.27 ^{a,b}
G2	2.17 \pm 0.32 ^b	43.57 \pm 6.52 ^{a,b}	598.34 \pm 63.42 ^{a,b}
G3	1.98 \pm 0.23 ^b	35.23 \pm 5.78 ^{a,b}	678.42 \pm 86.93 ^{a,b}

Note: a, comparing to SH group, $P = 0.026, 0.031, 0.029$; b, comparing to CPB group, $P = 0.008, 0.022, 0.013$.

fracture in the basement membrane of capillary endothelium. The mitochondrial structure was damaged with bubbles and decreasing lamellar bodies. The mitochondrial structure in G1, G2, and G3 were basically complete and the damage was slighter than that in CPB group. In line with this, it is possible to assume that Gln could reduce lung injury induced by CPB.

AQP expression in lung tissue by IHC

To unravel the expression of AQP in lung tissue, IHC was carried out. AQP-1 expressed highly in lung tissue of SH group with the brown granules in endothelial cells of capillary around airway and alveolus. The AQP-1 expression in G1, G2,

and G3 groups decreased gradually, while the AQP-1 expression in CPB group decreased the most, with weakened staining comparing with SH group (**Table 6; Figure 6**).

AQP-4 in lung tissue of SH group appeared weakly positive staining with a few of light yellow staining cells, which distributed mainly in ATII cell. The AQP-4 expression in G1, G2, and G3 increased gradually, while in CPB group increased the most with brownish yellow particles and strong staining (**Table 6; Figure 6**). Collectively, IHC result indicated that AQP-1 protein was highly expressed after CPB, whereas AQP-4 protein expression was low.

AQP-1 and AQP-4 expressing by WB

To further understand the amount of AQP at the protein and mRNA level, WB together with RT-PCR were performed. AQP-1 in SH group was with high density, while in G1, G2 and G3 significantly decreased gradually with low activity and integral optical density ($P < 0.05$). At 2 h after CPB, the AQP-1 expression decreased further ($P < 0.05$) (**Figure 7**). AQP-4 in SH group was with low density. At 60 min after CPB, AQP-4 expression significantly decreased with high activity and increasing integral optical density ($P < 0.05$). At 2 h after CPB, comparing with 60 min after CPB, AQP-4 expression increased further ($P < 0.05$) (**Figure 7**).

At 2 h after CPB, AQP-1 mRNA expression decreased significantly ($P < 0.05$). Comparing with open aorta, AQP-1 mRNA decreased significantly 2 h after CPB ($P < 0.05$) (**Figure 7**). With open aorta and at 60 min after CPB, AQP-4 mRNA expression increased significantly ($P < 0.05$). Comparing with open aorta, AQP-4 mRNA increased 2 h after CPB ($P < 0.05$) (**Figure 7**). In conclusion, the results indicated that Gln could decrease the expression of AQP-1 protein and

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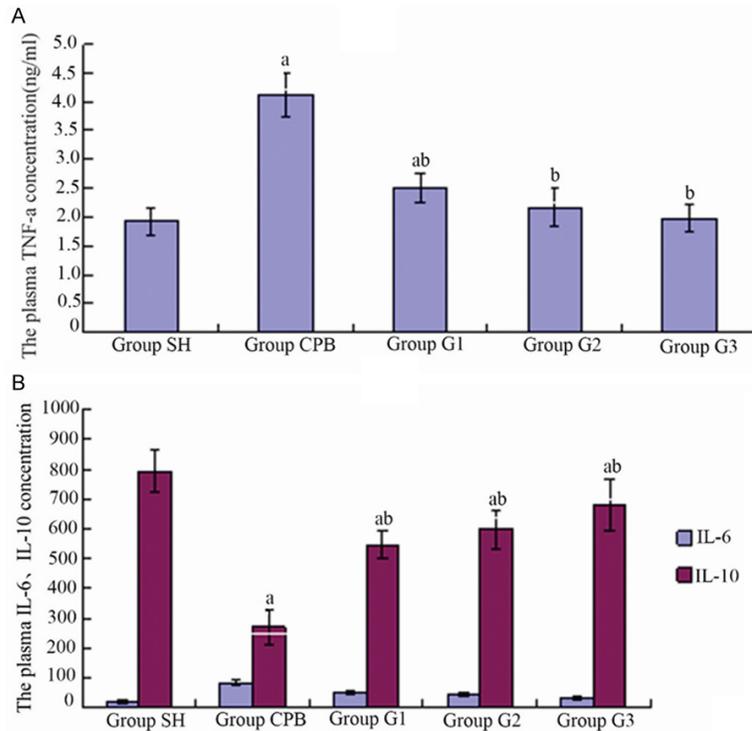


Figure 4. Serum TNF- α (A), IL-6 and IL-10 (B) expression. a, comparing with SH group, $P < 0.05$; b, comparing with CPB group, $P < 0.05$.

increase the expression of AQP-4 protein in the lung tissue of CPB rats.

Discussion

Martin et al. [7] found that EVLW could reflect the change of water permeability in lung tissue at the early stage of injury. In our study, EVLW increased in lung tissue of rat after CPB, indicating that CPB increases the permeability by damaging the barrier function of alveolar epithelium. The increase of A-aDO₂ and RI suggested that CPB could induce the decrease of diffusing capacity by edema in alveolar epithelium, which would lead to lung damage. The results showed that CPB would hurt the barrier function in alveolar epithelium by some pathways.

Usually, the lung is influenced firstly during the generating process of multiple organ dysfunctions, as well as the organ with the easiest failure. Acute respiratory distress syndrome/acute lung injury (ARDS/ALI) is one of the important pathological features that the heightened permeability of alveolar-capillary barrier could lead to alveolar and pulmonary interstitial edema. By detecting the oxidative stress factors includ-

ing MDA, MPO, and SOD, inflammatory factors including IL-6, IL-10, and TNF- α , and proteins including MMP-9, AQP-1, and AQP-4, we presumed that AQP, MMP-9, inflammatory factors, and peroxidation injury would participate in the barrier function damage of alveolar epithelium after CPB.

Under physiological or serious stress state, Gln was the key substrate and regulatory factor, which sustained the structure and function of the cells such as epithelial cell, alveolar epithelial cell, macrophage, and lymphocyte in lung tissue. Gln was helpful for protecting the lung from the damage of biological endotoxin, oxygen free radical, and cytotoxin. Austgen et al. [8] observed that the metabolic rule of lung damage was induced by endotoxin and found that after 30

min of intravenously endotoxin injection, Gln in lung tissue increased 1 fold, however, there was no change on the activity of glutamine synthetase (GS) in lung tissue. At 2 h after injection of endotoxin, no significant Gln releasing was found comparing with the control. At 12 h after injection of endotoxin, although the activity of GS in lung tissue increased about 2 folds, Gln decreased significantly comparing with the control and 30 min after injection. In the same time, there was pulmonary interstitial and alveolar lung edematous with 21% of increased weight. Singleton et al. [9] considered that the activation mentioned above was by enhancing the activity and expression of the heat shock factor-1 (HSF1) in lung tissue.

The studies mentioned above focused on the supplying of Gln for protecting lung by different ways after traumatic stress. As we all know, the demand of Gln would increase when the body was under stress state, which would lead to the lack of Gln and induce many complications to influence the prognosis. Could the supplement of enough Gln before stress reaction block or release the inflammatory reaction and lung damage earlier? We carried on the Gln intervention 3 days before CPB, and added equal

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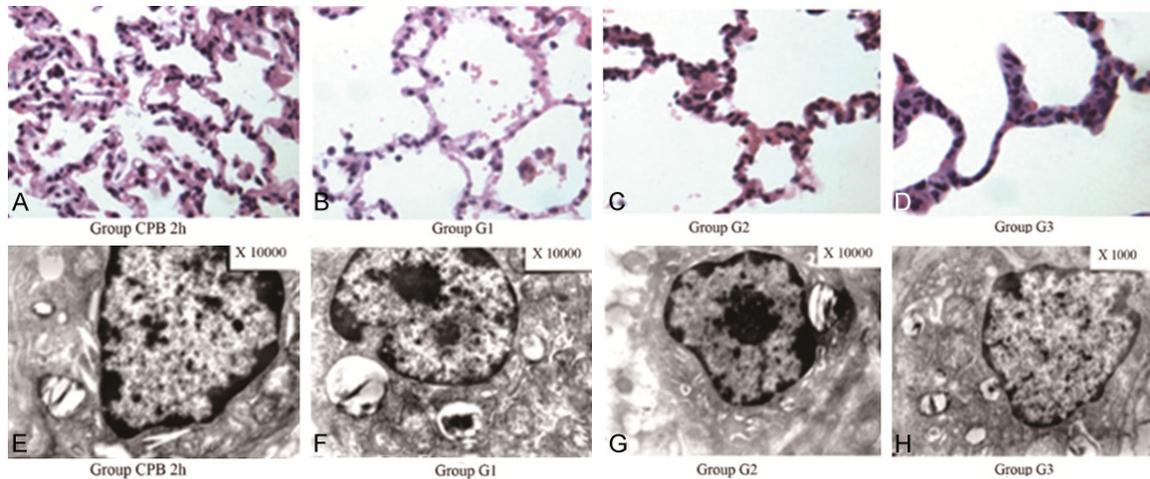


Figure 5. HE staining (A-D) and transmission electronic microscopy observation (E-H) of mouse lung tissue.

Table 6. The optical density (OD) value of AQP-1 and AQP-4 in lung tissue ($X \pm S$, $n = 8$)

	SH	CPB	G1	G2	G3
AQP-1	78.42±23.45	23.19±5.81 ^a	39.24±4.78 ^{a,b}	45.37±6.51 ^{a,b}	53.47±7.65 ^{a,b}
AQP-4	13.52±3.15	69.48±13.69 ^a	47.21±9.65 ^{a,b}	39.52±6.51 ^{a,b}	30.57±7.56 ^{a,b}

Note: a, comparing to SH group, $P = 0.043$; b, comparing to CPB group, $P = 0.012$.

Gln in the priming solution, for considering that the patients would receive CPB 3 days after hospitalizing. The equal Gln adding in the priming solution was as the supplement of the losing Gln during CPB with stress reaction.

Our results showed that the lung damage was lighter in G1, G2, and G3 groups than the CPB group, with decreasing alveolar exudation and inflammatory cell infiltrate. The lung damage in G3 group was the lightest. Under the electron microscope, there was decreasing vacuole and lightening mitochondria swelling. Comparing with SH group, the lung damage was still obvious in G1, G2, and G3 groups, however, the damage degree was decreasing. Moreover, the function of epithelial barrier was improved, with obviously decreased EVLW and releasing pulmonary edema. Additionally, the MDA and MPO in lung tissue and serum TNF- α and IL-6 were both significantly lower than that of CPB group. These results indicated that although the Gln pretreatment could not protect lung from stress reaction after CPB in some extent, it could lighten and release the lung ischemia-reperfusion injury and systemic inflammatory response syndrome. The supplement of Gln after CPB could reduce the incidence of complications and improve the organ function, which was helpful

for the prognosis of the patients.

The MDA and MPO in G1, G2, and G3 groups were lower than that in CPB group, but still higher than

that in SH group, while the SOD was significantly higher than that in CPB group and lower than in SH group. These results indicated that Gln could release the stress reaction induced by ischemia-reperfusion injury after CPB in some extent. Glutathione (GSH) system was one of the main mechanisms of oxidative stress reaction release. As the important reducing agent that protecting lung damage induced by peroxidation, 90% of the non-protein thiol in cell was GSH. When with sepsis, there was serious oxidative stress reaction in the body [10]. The supplement of exogenous Gln could increase the synthesis of glutathione and the body antioxidant capacity [11]. Giving rats endotoxin and Gln at the same time could induce HSP and release acute lung injury (ALI) [12, 13].

Serum TNF- α , IL-6, and LPS in G1, G2, and G3 groups were lower than that in CPB group, but were still higher than that in SH group, while IL-10 was significantly higher than that in CPB but lower than that in SH group. These results indicated that Gln pretreatment could release systemic inflammatory reaction after CPB.

Many studies showed that Gln could enhance the immunocyte function and reduce inflammatory factor releasing. Gln could down-regulate

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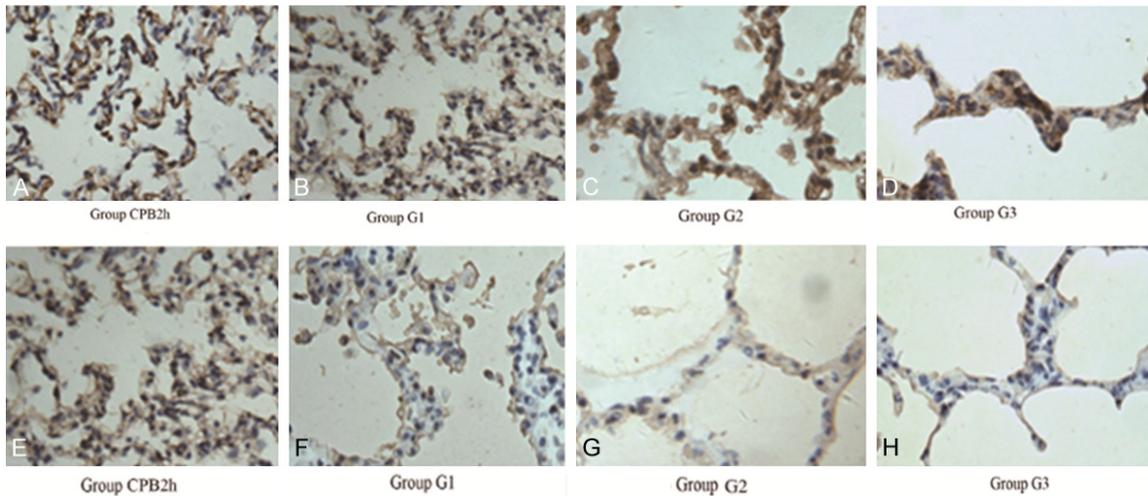


Figure 6. AQP-1 (A-D) and AQP-4 (E-H) expression in lung tissue.

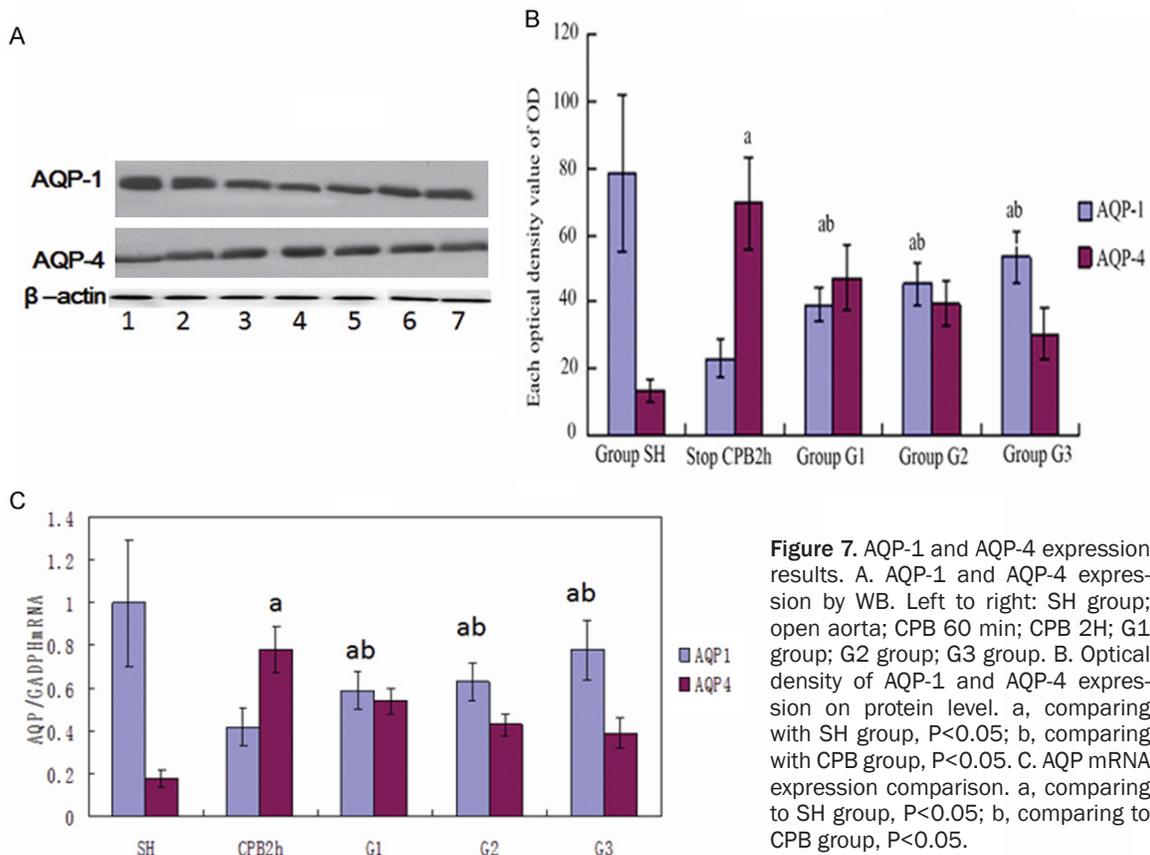


Figure 7. AQP-1 and AQP-4 expression results. A. AQP-1 and AQP-4 expression by WB. Left to right: SH group; open aorta; CPB 60 min; CPB 2H; G1 group; G2 group; G3 group. B. Optical density of AQP-1 and AQP-4 expression on protein level. a, comparing with SH group, $P < 0.05$; b, comparing with CPB group, $P < 0.05$. C. AQP mRNA expression comparison. a, comparing to SH group, $P < 0.05$; b, comparing to CPB group, $P < 0.05$.

TNF- α , IL-6, and IL-8, and up-regulate IL-10 [14]. Paul et al. [15] found that Gln could directly reduce TNF- α which was related to the HSP72 overexpression in human peripheral blood monocytes. The decrease of inflammatory factor releasing and improvement of the cell survival by Gln in the rats with sepsis were also

confirmed by other researchers. For example, Yoshitaka et al. [16] pretreated rats with Gln before CPB and found that serum IL-6 and IL-8 decreased significantly.

Recently, the functions of MMPs in the lung tissue induced by CPB have drawn more and more

attentions. The neutrophil played an important role in the pathophysiological process of ALI induced by CPB [17]. The proteolytic enzyme released by neutrophil was the main factor leading to alveolar capillary basement membrane injury, as well as the final effector of ALI. Among the damage factors released by the active neutrophil, the plasma proteinase was considered to be linked to ALI and acute respiratory distress syndrome (ARDS) such as the elastase and matrix metalloproteinase [18]. In 2001, Steinberg et al. [19] showed that MMP-9 concentration and activation both increased at the end of CPB, and the inhibitor of MMP-9 could protect and release the lung damage induced by CPB with the animal experiments. For further identifying the actions of MMP-9 in ALI, Warner et al. [20] constructed the mouse model of ALI induced by endotoxin with MMP-9 knockdown and the wild type, separately. The results demonstrated that comparing with the mice of wild type, the severity of lung damage of those with MMP-9 knockdown was lighter, and the pulmonary edema and hemorrhage were less, which indicated that MMP-9 could increase the permeability and aggravate ALI by acting on the alveolar capillary basement membrane. In our study, serum MMP-9 was lower in G1, G2 and G3 groups than that in CPB group, but still higher than that in SH group, which indicated that Gln pretreatment could release ALI after CPB by down-regulating MMP-9.

Aquaporin (AQP) is also important in ALI. Many correlated injury factors could lead to lung damage, disturbance of lung fluid balance, damage of lung-water barrier, increase of permeability, and even pulmonary edema with severity. Pulmonary edema has the characteristics of alveolar interstitial fluid gathering and when pulmonary edema occurs, there is disorder in water-transportation and AQPs function. Therefore, to understand the AQPs function on the treatment of pulmonary edema clinically is important. Tabbutt et al. [21] found that 6 h after CPB, AQP-1 mRNA expression increased, as well as IL-8 and ICAM-1. In our study, we identified that Gln was with protective function on lung injury after CPB. In G1, G2, and G3 groups, AQP-1 mRNA expression decreased while AQP-4 increased, which indicated that Gln pretreatment could influence the expression of AQPs and release ALI after CPB. However, further investigation on the mechanism of

AQPs acting in ALI after CPB, and the association of AQPs with systemic inflammatory response syndrome (SIRS) and ischemia-reperfusion injury is needed.

Disclosure of conflict of interest

None.

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