

# Biliverdin Protects the Isolated Rat Lungs from Ischemia-reperfusion Injury via Antioxidative, Anti-inflammatory and Anti-apoptotic Effects

Wen-Fang Tian<sup>1</sup>, Ping Weng<sup>1</sup>, Qiong Sheng<sup>1</sup>, Jun-Liang Chen<sup>1</sup>, Peng Zhang<sup>1</sup>, Ji-Ru Zhang<sup>2</sup>, Bin Du<sup>1</sup>, Min-Chen Wu<sup>1</sup>, Qing-Feng Pang<sup>1</sup>, Jian-Jun Chu<sup>2</sup>

<sup>1</sup>Department of Pathophysiology, Wuxi Medical School, Jiangnan University, Wuxi, Jiangsu 214122, China

<sup>2</sup>Department of Anesthesia, Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu 214122, China

## Abstract

**Background:** Biliverdin (BV) has a protective role against ischemia-reperfusion injury (IRI). However, the protective role and potential mechanisms of BV on lung IRI (LIRI) remain to be elucidated. Thus, we aimed to investigate the protective role and potential mechanisms of BV on LIRI.

**Methods:** Lungs were isolated from Sprague-Dawley rats to establish an *ex vivo* LIRI model. After an initial 15 min stabilization period, the isolated lungs were subjected to ischemia for 60 min, followed by 90 min of reperfusion with or without BV treatment.

**Results:** Lungs in the I/R group exhibited significant decrease in tidal volume ( $1.44 \pm 0.23$  ml/min in I/R group vs.  $2.41 \pm 0.31$  ml/min in sham group;  $P < 0.001$ ), lung compliance ( $0.27 \pm 0.06$  ml/cmH<sub>2</sub>O in I/R group vs.  $0.44 \pm 0.09$  ml/cmH<sub>2</sub>O in sham group;  $P < 0.001$ ; 1 cmH<sub>2</sub>O=0.098 kPa), and oxygen partial pressure (PaO<sub>2</sub>) levels ( $64.12 \pm 12$  mmHg in I/R group vs.  $114 \pm 8.0$  mmHg in sham group;  $P < 0.001$ ; 1 mmHg = 0.133 kPa). In contrast, these parameters in the BV group ( $2.27 \pm 0.37$  ml/min of tidal volume,  $0.41 \pm 0.10$  ml/cmH<sub>2</sub>O of compliance, and  $98.7 \pm 9.7$  mmHg of PaO<sub>2</sub>) were significantly higher compared with the I/R group ( $P = 0.004$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively). Compared to the I/R group, the contents of superoxide dismutase were significantly higher ( $47.07 \pm 7.91$  U/mg protein vs.  $33.84 \pm 10.15$  U/mg protein;  $P = 0.005$ ) while the wet/dry weight ratio ( $P < 0.01$ ), methane dicarboxylic aldehyde ( $1.92 \pm 0.25$  nmol/mg protein vs.  $2.67 \pm 0.46$  nmol/mg protein;  $P < 0.001$ ), and adenosine triphosphate contents ( $297.05 \pm 47.45$  nmol/mg protein vs.  $208.09 \pm 29.11$  nmol/mg protein;  $P = 0.005$ ) were markedly lower in BV-treated lungs. Histological analysis revealed that BV alleviated LIRI. Furthermore, the expression of inflammatory cytokines (interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor- $\alpha$ ) was downregulated and the expression of cyclooxygenase-2, inducible nitric oxide synthase, and Jun N-terminal kinase was significantly reduced in BV group (all  $P < 0.01$  compared to I/R group). Finally, the apoptosis index in the BV group was significantly decreased ( $P < 0.01$  compared to I/R group).

**Conclusion:** BV protects lung IRI through its antioxidative, anti-inflammatory, and anti-apoptotic effects.

**Key words:** Apoptosis; Biliverdin; Ischemia-reperfusion Injury; Lung; Pro-inflammatory Cytokines

## INTRODUCTION

Lung ischemia-reperfusion injury (LIRI) is a common pathological phenomenon in many clinical conditions such as lung transplantation, pulmonary embolism, cardiopulmonary resuscitation, and pulmonary thrombosis.<sup>[1]</sup> Pulmonary ischemia causes the imbalance between oxygen supply and metabolic demand, ultimately leading to hypoxic damages to tissues. Although the restoration of perfusion is essential for the preservation of lung function, the process can lead to a series of serious effects including pulmonary edema, hypoxemia, and respiratory failure.<sup>[2]</sup> Although many efforts have been made, there are currently no highly

effective therapies available for LIRI treatment in clinical practice.<sup>[3]</sup>

Reactive oxygen species (ROS) formation is considered to be involved in reperfusion-induced injury in ischemic

**Address for correspondence:** Dr. Qing-Feng Pang,  
Department of Pathophysiology, Wuxi Medical School, Jiangnan  
University, Wuxi, Jiangsu 214122, China  
E-Mail: qfpang@jiangnan.edu.cn

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lungs.<sup>[4]</sup> ROS induce lipid peroxidation and damage cellular membrane. Moreover, ROS are associated with inflammatory responses by inducing the expression of pro-inflammatory cytokines and leukocyte migration in the transplanted lung.<sup>[5]</sup> Based on the above-mentioned findings, antioxidants are a key treatment to attenuate LIRI.<sup>[6]</sup>

Heme oxygenase-1 (HO-1, encoded by the *HMOX1* gene) catalyzes the oxidative degradation of heme to biliverdin (BV), carbon monoxide, and ferrous iron. Both HO-1 and BV have been proven to have cytoprotective effect through the antioxidative and anti-inflammatory effects.<sup>[7,8]</sup> The expression and activation of HO-1 are induced by various stimuli, such as lipopolysaccharide, heme, and ROS.<sup>[9,10]</sup> It has been reported that HO-1 induction could prevent reperfusion-induced injury in ischemic tissues.<sup>[11,12]</sup> Noteworthy, microsatellite (GT)<sub>n</sub> dinucleotide length polymorphisms have been identified in the regulatory regions of the human *HMOX1* gene promoter, which may result in the impaired transcriptional regulation and decreased expression of HO-1 if individuals carry the long (L) allele [(GT)<sub>n</sub> ≥ 30] of this polymorphism. Patients with longer *HMOX1* promoters suffered from more severe injury cause by stress-associated diseases such as myocardial infarction than patients with shorter *HMOX1* promoters probably due to the lower HO-1 levels.<sup>[13]</sup> With respect to the patients with the longer *HMOX1* promoters, exogenous HO-1 byproduct supplement maybe is an important means to treat HO-1 associated diseases. Heme-derived byproducts, especially BV, exhibit cytoprotective effects on oxidative stress injury. Studies in animal models have demonstrated the cytoprotective effect of BV against I/R injury.<sup>[14-17]</sup> However, the detailed mechanism involved in BV-induced cytoprotection during LIRI still remains unclear. Therefore, we employed the isolated rat lungs to delineate the molecular mechanism responsible for the protective effect of BV against LIRI.

## METHODS

### Reagents and animals

Biliverdin hydrochloride (Frontier Scientific, Logan, UT, USA) was dissolved in 0.2 mol/L NaOH, and the solution was adjusted to a final pH of 7.4 with hydrogen chloride. L-glutathione reduced (GSH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Male Sprague-Dawley rats, weighing 200–230 g, were purchased from Jiangnan University Animal Center and housed in a room with a 12/12-h light/dark cycle. Rats had *ad libitum* access to food and water. Experiments were carried out according to protocols approved by the Animal Care and Use Committee of Jiangnan University.

### Ex vivo rat lung perfusion model

An isolated rat lung perfusion system (interleukin [IL]-2 type; Hugo Sachs Elektronik Harvard Apparatus, March Hugstetten, Germany) was used for *ex vivo* lung perfusion as previously reported.<sup>[18]</sup> Briefly, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital

(80 mg/kg) before surgery. After heparinization, heart–lung blocks were isolated and connected to the perfusion circuit and then perfused with a Krebs–Henseleit solution (2% bovine serum albumin, 0.1% glucose, pH 7.4). Tidal volume, airway compliance, and pulmonary vein oxygen partial pressure (PaO<sub>2</sub>) were measured during the perfusion.

### Experimental groups

The animals were randomly divided into four groups ( $n = 8$  in each group): sham control, I/R, BV, and GSH (positive control). In all groups, lungs were perfused for an initial 15 min of stabilization period after ventilation. In the sham group, lungs were continuously perfused for 90 min after the stabilization period. In other three groups, perfusion and ventilation were interrupted for 60 min after the stabilization period and then reperfused and ventilated for 90 min. In the I/R group, the Krebs–Henseleit solution was added to perfusate at the onset of reperfusion. In the BV or the GSH group, 10 μmol/L BV<sup>[19]</sup> or 4 mmol/L GSH was applied to perfusion at the onset of reperfusion.

### Histological studies

Histological analysis was performed as previously described.<sup>[20]</sup> Briefly, lung sections of paraffin-embedded lung tissues (4 μm in thickness) were stained with hematoxylin and eosin (H and E) and then examined under optical microscopy. All the sections were assessed by a single pathologist blinded to the group.

### Biochemical analysis

The activity of superoxide dismutase (SOD) and the levels of malondialdehyde (MDA) and adenosine triphosphate (ATP) were determined with commercially available kits (Nanjing Jiancheng Biotechnology, China) according to the manufacturer's instructions. Briefly, the lung samples were homogenized in RIPA lysis buffer. The supernatants were obtained by centrifugation at 12,000 ×g. The activity of SOD in the supernatants was measured at 550 nm of wavelength with a spectrophotometer. The contents of MDA and ATP were determined by reading the absorbance at 532 nm and 636 nm, respectively.

### RNA isolation and real-time polymerase chain reaction

Total RNAs were extracted with Trizol Regent (Invitrogen, CA, USA) according to the manufacturer's instructions. The extracted RNAs were reverse-transcribed into cDNA using a PrimeScript RT Reagent Kit (Takara, Dalian, China). Quantitative reverse-transcription polymerase chain reaction (RT-PCR) was performed using SYBR Fast qPCR Mix (Takara, Dalian, China) and signals were detected with Roche LightCycler 480 PCR system (Roche, USA), following the recommended protocols. Target gene expression was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase. The RT-PCR experiments were done in triplicate.

### Western blotting

Lung samples were homogenized in ice-cold RIPA buffer (50 mmol/L Tris, 150 mmol/L NaCl, 1% Triton

X-100, pH 7.4) containing protease inhibitor cocktail (Roche Life Science Co., USA). The whole cell lysates were obtained by centrifuging the suspensions for 10 min at 13,000  $\times g$  at 4°C. The lysates were loaded onto a 10% SDS-polyacrylamide gel for separation and then transferred to nitrocellulose membranes. The blots were probed with anti-p-Jun N-terminal kinase (JNK) (1:400; Santa Cruz Biotechnology, Inc., USA), anti-JNK (1:400; Santa Cruz Biotechnology, Inc., USA), and anti- $\beta$ -actin (1:400; Santa Cruz Biotechnology, Inc., USA) antibodies. After incubation with horseradish peroxidase-linked secondary antibodies, specific protein bands on the blots were visualized with the ECL chemiluminescence detection system (Millipore, USA) according to the manufacturer's manual.

### Terminal deoxynucleotidyl transferase mediated nick end labeling assay

Terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) staining was performed according to the manufacturer's protocol (KG Nanjing Ltd., Nanjing, China). Briefly, the pulmonary sections were treated with 20 g/ml proteinase K for 10 min, with 0.3% H<sub>2</sub>O<sub>2</sub> in methanol for 10 min and 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Then, the sections were incubated in the TUNEL reaction mixture. Finally, the sections were stained with diaminobenzidine solution for 10 min at room temperature. Five microscope fields of each section were picked to calculate apoptosis index (AI) using the formula: AI = positive cells/total cells  $\times$  100%.

### Statistical analysis

The data are expressed as means  $\pm$  standard deviation (SD). The nature of hypothesis testing was two tailed. For comparing the parameters (tidal volume, lung compliance, and PaO<sub>2</sub>) between groups during 90 min of observation, a two-way analysis of variance (ANOVA) for repeated measurements was used followed by the *post hoc* least significant difference test or Dunnett's *t*-test, treat I/R as a control and compared all other groups against it. There were no missing data points in this experiment. The sample size in the current study was justified based on our previous works. Eight animals in each group were appropriate to avoid intravariation in the experimental group. Significance was set at  $P < 0.01$ . SPSS version 13.0 software (SPSS Inc., Chicago, Illinois, USA) was used for all the statistical calculations.

## RESULTS

### Biliverdin ameliorated lung ischemia-reperfusion injury-induced pulmonary dysfunctions

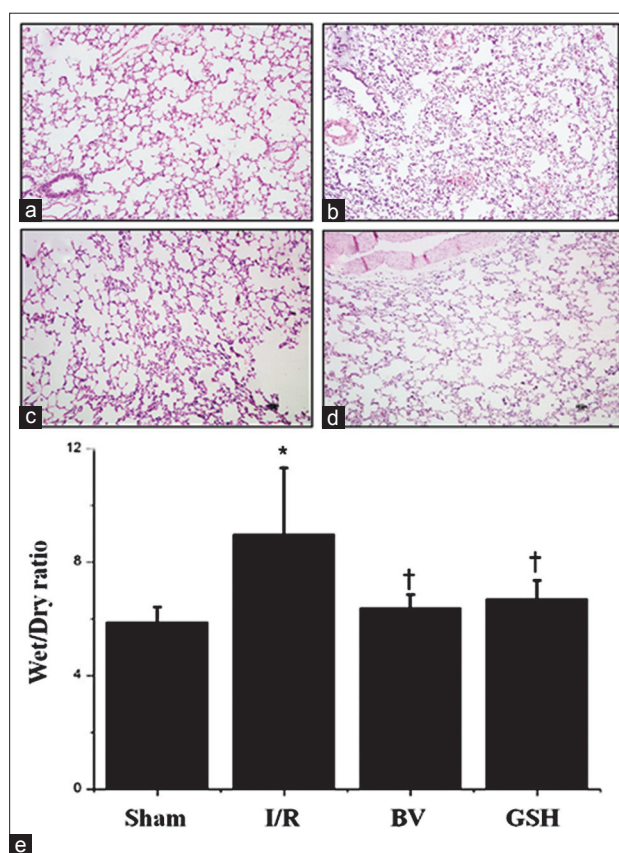
In the isolated ischemia-reperfusion rat model, lungs in I/R group exhibited a significant decrease in tidal volume, lung compliance, and PaO<sub>2</sub> levels after 90 min of reperfusion compared to the sham group ( $P < 0.001$ ) [Table 1]. In contrast, lungs in the BV group showed significantly increased tidal volume ( $P = 0.004$ ), lung compliance ( $P < 0.001$ ), and PaO<sub>2</sub> ( $P < 0.001$ ) as compared to the lungs in the I/R group treated with GSH or the sham group [Table 1].

### Biliverdin alleviated pulmonary oxidative stress

Predictably, as shown in Table 2, the I/R group had markedly decreased SOD activity ( $P < 0.001$ ) and ATP content ( $P < 0.05$ ) and increased MDA content ( $P < 0.001$ ) as compared to the sham group. BV or GSH treatment significantly restored SOD activity, increased ATP content, and reduced tissue MDA content, indicating their antioxidant activity ( $P < 0.05$ ).

### Biliverdin treatment attenuated lung edema and histological injury

The wet/dry ratios of lungs in BV group were significantly lower than those in the I/R group ( $P < 0.01$ ), indicating less severe edema in the tissue. Furthermore, in the H and E-stained lung sections, much lighter infiltration of immune cells was observed in BV- or GSH-treated group than that in the I/R group. Moreover, hemorrhage and severe edema were barely detectable in sections from groups treated either with BV or GSH. On the other hand, sections from the I/R group showed severe perivascular edema in almost all the vessels [Figure 1].



**Figure 1:** Histological changes of lung tissues. H and E staining was performed on the paraffin embedded sections (original magnification  $\times 200$ ). (a) Sham group; (b) I/R group; (c) biliverdin group; (d) GSH group. (e) Wet/dry ratio of the lungs after 90 min of reperfusion in each group ( $n = 8$ ). The results of wet/dry ratio were quantified and are shown as the mean  $\pm$  SD. \* $P < 0.01$  compared with the sham group. † $P < 0.01$  compared with the I/R group. I/R: Ischemia/reperfusion; SD: Standard deviation; BV: Biliverdin; GSH: Glutathione.



**Table 1: Results of lung function tested at different time points (mean ± SD, n = 8)**

Items	Groups	Ischemia 15 min	Reperfusion 30 min	Reperfusion 60 min	Reperfusion 90 min	Statistical value (LSD-t)	P
Tidal volume (ml/min)	Sham	2.73 ± 0.20	2.70 ± 0.13	2.61 ± 0.31	2.41 ± 0.31*	2.212	<0.001*
	I/R	2.69 ± 0.32	2.00 ± 0.41	1.96 ± 0.29	1.44 ± 0.23†	1.716	0.004†
	BV	2.71 ± 0.17	2.50 ± 0.29	2.47 ± 0.42	2.27 ± 0.37‡	0.462	0.209‡
	GSH	2.63 ± 0.79	2.54 ± 0.82	2.46 ± 0.83	2.11 ± 0.33§	0.122	0.737§
Compliance (ml/cmH <sub>2</sub> O)	Sham	0.56 ± 0.04	0.53 ± 0.02	0.50 ± 0.04	0.44 ± 0.09*	0.938	<0.001*
	I/R	0.57 ± 0.06	0.37 ± 0.09	0.37 ± 0.06	0.27 ± 0.06†	0.670	<0.001†
	BV	0.56 ± 0.07	0.46 ± 0.08	0.46 ± 0.09	0.41 ± 0.10‡	0.080	0.152‡
	GSH	0.52 ± 0.04	0.51 ± 0.04	0.49 ± 0.05	0.40 ± 0.08§	0.042	0.435§
PaO <sub>2</sub> (mmHg)	Sham	123.0 ± 11.0	121.0 ± 8.2	115.0 ± 7.7	114.0 ± 8.0*	21.872	<0.001*
	I/R	121.0 ± 9.4	86.5 ± 10.0	79.5 ± 11.0	64.1 ± 12.0†	16.812	<0.001†
	BV	128.0 ± 9.9	113.0 ± 9.7	110.0 ± 8.0	98.7 ± 9.7‡	5.060	0.011‡
	GSH	122.0 ± 8.9	111.0 ± 9.1	107.0 ± 7.1	98.0 ± 8.1§	1.858	0.737§

\*Sham group versus I/R group; †I/R group versus BV group; ‡Sham group versus BV group; §GSH group versus BV group. 1 cmH<sub>2</sub>O=0.098 kPa; 1 mmHg = 0.133 kPa. BV: Biliverdin; I/R: Ischemia/reperfusion; GSH: Glutathione; LSD: Least significant difference; SD: Standard deviation; PaO<sub>2</sub>: Oxygen partial pressure.

**Table 2: Contents of SOD, MDA, and ATP at 90 min after lung reperfusion (mean ± SD, n = 8)**

Groups	SOD (U/mg protein)	F	P	MDA (nmol/mg protein)	F	P	ATP (nmol/mg protein)	F	P
Sham	59.46 ± 7.56	10.977	<0.05	1.56 ± 0.12	19.596	<0.05	391.13 ± 65.13	16.917	<0.05
I/R	33.84 ± 10.15*	–	–	2.67 ± 0.46*	–	–	208.09 ± 29.11*	–	–
GSH	49.29 ± 7.76†	–	–	2.09 ± 0.36†	–	–	312.14 ± 44.59†	–	–
BV	47.07 ± 7.91‡	–	–	1.92 ± 0.25‡	–	–	297.05 ± 47.45‡	–	–

\*Sham group versus I/R group; †I/R group versus GSH group; ‡I/R group versus BV group. –: No data. BV: Biliverdin; I/R: Ischemia/reperfusion; GSH: Glutathione; SD: Standard deviation; SOD: Superoxide dismutase; MDA: Malondialdehyde; ATP: Adenosine triphosphate.

### Biliverdin treatment downregulated expression of pro-inflammatory proteins induced by lung ischemia-reperfusion injury

To test the anti-inflammatory effect of BV, we evaluated expression of the pro-inflammatory genes in each group. In the I/R group, the mRNA levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and IL-8 were significantly elevated compared to those in the sham group ( $P < 0.01$ ). In the BV group, treatment with BV downregulated the mRNA expression of IL-6 (about 2 folds), IL-1 $\beta$  (about 4 folds), TNF- $\alpha$  (about 3 folds), iNOS (about 3 folds), and COX-2 (about 4 folds) compared to those in the sham group [Figure 2].

### Biliverdin treatment decreased I/R-induced Jun N-terminal kinase phosphorylation

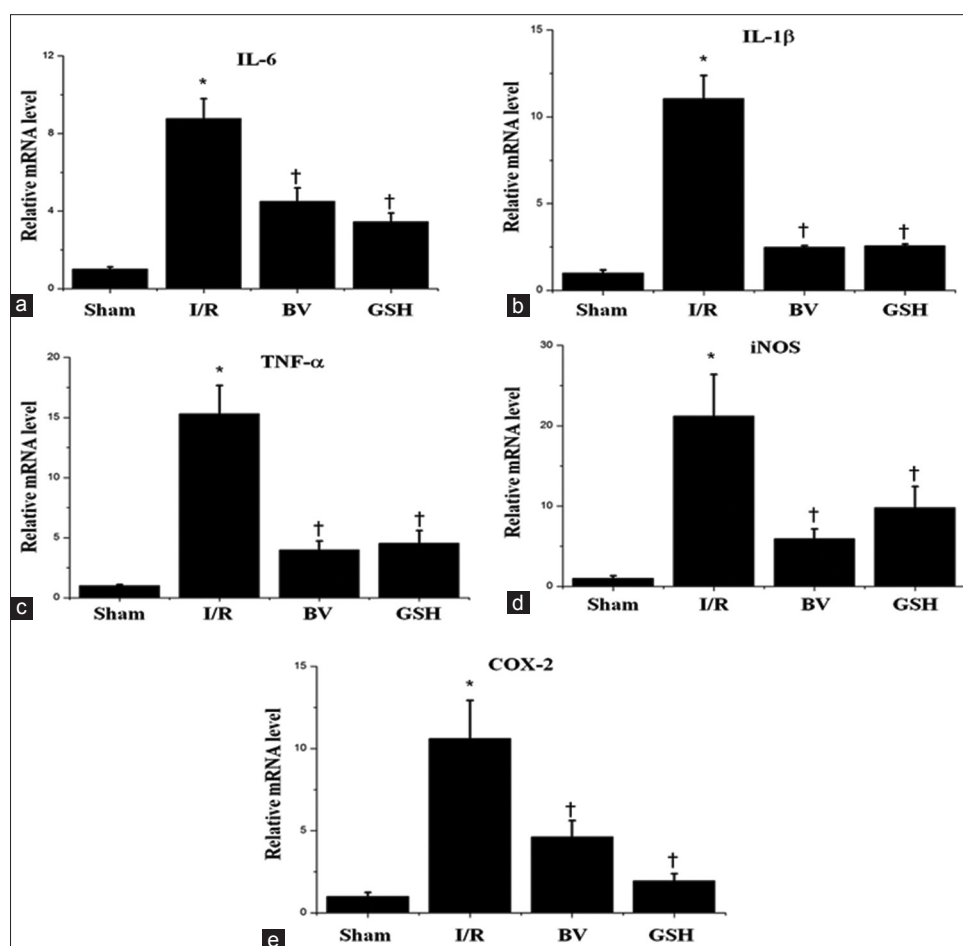
The c-Jun NH<sub>2</sub>-terminal kinase is activated by multiple forms of stress and has been proven to be a mediator of stress-induced apoptosis.<sup>[21]</sup> Manipulation of JNK/MAPK pathway could be a potential therapeutic target for acute respiratory distress syndrome (ARDS) in the context of suppressing inflammation.<sup>[22]</sup> Western blotting results showed that, like GSH, BV treatment decreased the phosphorylation of JNK which was greatly induced in the I/R group ( $P < 0.01$ ) [Figure 3].

### Biliverdin treatment protected lung cells against apoptosis

Compared to I/R lungs, the number of TUNEL-positive cells was evidently lowered in the BV group and GSH group ( $P < 0.01$ ) although the positive cell counting was still higher than that in the sham group [Figure 4].

## DISCUSSION

This study was aimed to investigate the protective role of BV in LIRI using an *ex vivo* rat lung I/R model. Our results showed BV protected lung tissues from I/R-induced injury in the isolated lung perfusion model. Treatment of BV during reperfusion attenuated I/R lung injury by increasing tidal volume and PaO<sub>2</sub>, decreasing lung wet/dry ratio, and improving tissue histological appearances. Consistent with the results from functional and histological analysis, restoring the activity of SOD, increasing the content of ATP, and decreasing the level of MDA implied that BV exhibited beneficial effect on I/R injured lungs, partially through the antioxidative effects. To further explore the underlying mechanism of BV protective effects, we assessed the expression of several pro-inflammatory genes and JNK phosphorylation in lungs tissue. The expression of all target genes and JNK phosphorylation was increased in the I/R group,



**Figure 2:** Relative mRNA expression of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , iNOS, and COX-2 in lung tissues. (a) Relative mRNA level of IL-6. (b) Relative mRNA level of IL-1 $\beta$ . (c) Relative mRNA level of TNF- $\alpha$ . (d) Relative mRNA level of iNOS. (e) Relative mRNA level of COX-2. After 90 min of reperfusion, lung tissues were collected for determination of pro-inflammatory gene expression by real-time polymerase chain reaction ( $n = 8$ ). The results were quantified and are shown as the mean  $\pm$  SD. \* $P < 0.01$  compared with the sham group. † $P < 0.01$  compared with the I/R group. I/R: Ischemia/reperfusion; BV: Biliverdin; GSH: Glutathione; IL-6: Interleukin-6; IL-1 $\beta$ : Interleukin-1 $\beta$ ; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; iNOS: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2; SD: Standard deviation.

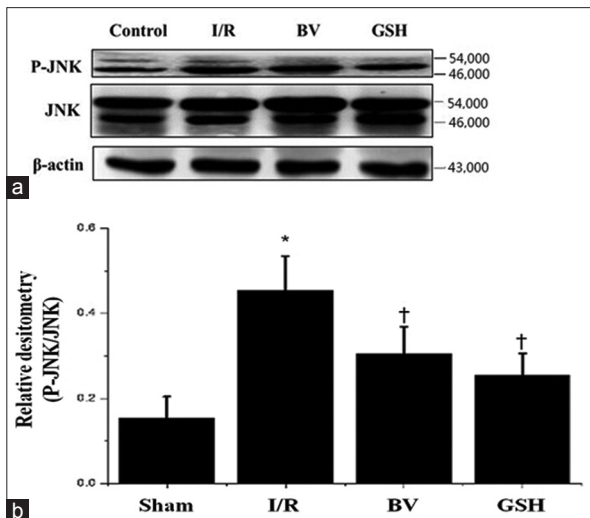
while BV treatment abrogated that inflammation response and decreased I/R-induced JNK phosphorylation. Furthermore, we evaluated the anti-apoptotic effect of BV in LIRI. As we expected, reperfusion caused severe lung cell apoptosis in the I/R group while BV markedly downregulated the AI.

BV is a product of the breakdown of heme moiety of hemoglobin by the heme oxygenase system. Thereafter, BV is rapidly reduced to bilirubin by biliverdin reductase (BVR), which is excreted in bile and urine. It is well accepted that both BV and bilirubin are potential antioxidants related to cytoprotection against oxidative damages. More experiments need to be designed to demonstrate the involvement of conversion of BV to bilirubin in the protective effect against LIRI. Furthermore, whether bilirubin exerts the cytoprotective effect against LIRI remains to be elucidated.

GSH, a well-accepted endogenous oxygen-free radical scavenger, was chosen to serve as a positive control in

our study.<sup>[23]</sup> The protective effect of BV on I/R-injured lungs was comparable to that of GSH. As previously reported BV has a cytoprotective role in experimental animal models for several types of disorders.<sup>[14-16]</sup> It is well known that ROS is an inflammatory stimulus and activates nuclear factor- $\kappa$ B (NF- $\kappa$ B), a pathway implied in IRLI pathogenesis.<sup>[24]</sup> Moreover, in cultured HEK293A cells, BV at 5  $\mu$ mol/L inhibited TNF- $\alpha$ -induced NF- $\kappa$ B activation.<sup>[25]</sup> Our study demonstrated that the cytoprotection of BV on I/R-induced lung injury in the isolated lung perfusion model relied on its antioxidative and anti-inflammatory effects.

Previous studies showed that BV treatment protected lung injury induced by various stimuli. Kosaka *et al.*<sup>[26]</sup> demonstrated that administration of BV before hemorrhagic shock and resuscitation suppressed lung injury in rats. In a rat model of lung transplantation, BV treatment significantly ameliorated lung graft injury.<sup>[27]</sup> In addition, preservation of the lung grafts in low-potassium dextran



**Figure 3:** Expression and activation of JNKs in I/R injured lung tissue. (a) The expression of phospho-JNK was examined by Western blotting. (b) The protein levels of phosphor-JNK were normalized by the level of total JNK and the level of  $\beta$ -actin. The results were quantified and are shown as the mean  $\pm$  SD. \* $P < 0.01$  compared with the sham group. † $P < 0.01$  compared with the I/R group. I/R: Ischemia/reperfusion; BV: Biliverdin; GSH: Glutathione; JNK: Jun N-terminal kinase; SD: Standard deviation; I/R: Ischemia/reperfusion.

supplemented with BV at 4°C improved the lung graft function.<sup>[19]</sup> All of those cytoprotective effects of BV were exerted through its anti-inflammatory and antioxidant mechanisms. Our results and those from the previous reports suggested that BV was a potential molecule in protecting lungs from I/R injury.

There are some limitations in our study. First, we employed an isolated perfused lung model to mimic any hemodynamic effects on lung injury, but the model could not provide information regarding the interactions with other organs. Second, we only investigate the effect of BV at 10  $\mu$ mol/L and dose–response experiments were absent in our study. It would be important in the future to screen for the most effective dose of BV and the beneficial effect of continuous infusion of BV. Third, the specific inhibitors of JNK or COX-2 should be applied for clarifying the direct effect of BV on the relative signaling pathways.

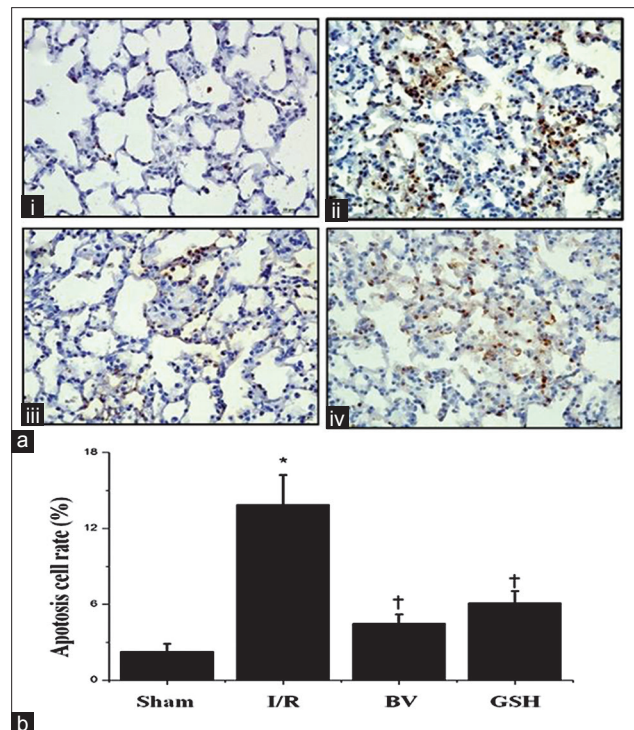
In summary, our results demonstrated that BV, at 10  $\mu$ mol/L concentration, rescued rat lungs from I/R injury *ex vivo* through its antioxidative, anti-inflammatory, and anti-apoptotic effects. The results suggested that BV might be a potential compound to treat LRI.

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Nil.

### Conflicts of interest

There are no conflicts of interest.



**Figure 4:** The dUTP nick end labeling stain of pulmonary tissue in each group. (a) Histological images of TUNEL stained cells in rat lungs tissue (original magnification  $\times 400$ ); (i) sham group; (ii) I/R group; (iii) biliverdin group; (iv) GSH group. (b) Apoptosis index in each group. \* $P < 0.01$  compared with the sham group. † $P < 0.01$  compared with the I/R group. I/R: Ischemia/reperfusion; BV: Biliverdin; GSH: Glutathione; TUNEL: Terminal deoxynucleotidyl transferase mediated nick end labeling.

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