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Original Article

Effects of curcumin on cell apoptosis in pulmonary ischemia/reperfusion injury in mice

Ziyin Luo[1*], Junhui Zhou[2*], Bingqian Xiang[1], Dong Song[1], Zhongqiu Lu[3], Longsheng Song[4], Wantie Wang[1]

[1]Ischemia/Reperfusion Injury Research Institute of Wenzhou Medical University, Wenzhou 325035, Zhejiang, China; [2]The Second Anesthesia Department of Henan Provincial Chest Hospital, Zhengzhou 450008, Henan, China; [3]Department of Emergency, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325035, China; [4]Division of Cardiovascular Medicine University of Iowa Carver College of Medicine, Iowa 52242, America. [*]Equal contributors.

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Abstract: Background: Endoplasmic reticulum stress (ERS) is known to play an important role in pulmonary ischemia/reperfusion injury (PIRI). The present study investigate the protection conferred by curcumin (CUR) from mice lung injury induced by PIRI and the underlying mechanisms. Methods: A model of PIRI *in situ* was established with unilateral lung *in vivo* C57BL/6J mice for 0.5 h ischemia by different hours (1 hour, 2 hours and 3 hours) of reperfusion. CUR was administered at different dosages (100 mg·kg^{-1}, 150 mg·kg^{-1} and 200 mg·kg^{-1}) 2 hours before ischemia. The lung tissue wet/dry weight (W/D), the total lung water content (TLW) of the left lung tissues were determined. The lung tissue morphology change and the ultrastructure change were observed by light microscopy and electron microscopy. The damage assessment (IQA) was taken. The structure changes and the apoptosis index (AI) of the lung tissues were evaluated by TUNEL method. The protein and mRNA expression of GRP78, CHOP, JNK and Caspase-12 in lung tissues were detected by Western blot and Reverse Translate-PCR. Results: Left lung W/D, TLW, lung damage, cell apoptosis and the expression levels of GRP78, CHOP, JNK and Caspase-12 were all gradually increased at 1 h, 2 h and 3 h after reperfusion. Pre-treatment of CUR (200 mg·kg^{-1} body wt) notably attenuated lung oedema, alveolar damage and pulmonary apoptosis after PIRI, and inhibited over-expression of CHOP, JNK and Caspase-12. Conclusion: Our data suggest that CUR reduced PIRI-induced apoptosis by relieving endoplasmic reticulum stress (ERS) through regulation of related signaling pathways to protect the lung.

Keywords: Curcumin, ischemia/reperfusion, GRP78, JNK, CHOP, Caspase-12, lung

Introduction

Pulmonary ischemia-reperfusion injury (PIRI) is one of the main reasons for lung dysfunction and structural injury in several clinical settings, such as lung trauma, segmentectomy of lung and lung transplantation. Now the mechanisms and pretreatment of PIRI has not been clearly elucidated. As various studies show, pulmonary apoptosis plays a key role in PIRI [1], lung injury can be alleviated by intervention in cell apoptosis [2]. In addition, endoplasmic reticulum stress (ERS) induces pulmonary apoptosis in PIRI has not been clearly elucidated.

The endoplasmic reticulum (ER) is the intracellular organelle controlling intracellular Ca^{2+} homeostasis, protein folding, lipid synthesis and protein maturation occur [3]. The ER is highly sensitive to various stimuli, such as oxidative stress, inflammatory response, ischemia, hypoxia and elevated protein synthesis [4, 5]. Such stresses ultimately cause accumulation of unfolded or misfolded proteins. This condition is generally known as ERS. Glucose-regulated protein-78 (GRP78) is an important ER chaperone. When ERS occurs, GRP78 dissociates from membrane receptors, which triggers the unfolded protein response (UPR), a pro-survival response to reduce the accumulation of unfolded proteins and restore normal ER function. However, If the ER stress is persistent and cannot be resolved, UPR does not directly cause cell death but rather initiate the activation of the c-Jun NH2 terminal kinase (JNK), C/EBP homologous protein (CHOP) and Caspases-12. Then it mediate apoptosis [6, 7]. However, the relationships between ERS and cell apoptosis

indued by ischemia-reperfusion in mouse lung tissue have not been reported.

Curcumin (CUR), a type of natural phenolic pigment, obtained from the plant Curcuma longa [8]. It has been well known to its various pleiotropic properties, including antioxidant action, anti-tumor, inhibited release of inflammatory cytokines and anti-apoptosis [9-11]. Previous studies have showed that curcumin has a protective effect against I/R injury in several organs [12, 13]. But the relevant mechanism remains unclear. Therefore, this study is aimed to explore whether regulation of ERS is one of the mechanisms by which CUR protects the lung tissue against I/R injury.

Materials and methods

Animals and experimental groups

Male C57BL/6J mice weighting 20-25 g were provided by the Experimental Animal Center of Shanghai, China. All animals experiments were performed in accordance with the guidelines for laboratory animal care of Wenzhou Medical University, Zhejiang, China. 108 mice were randomly divided into nine groups: control group (C group), Sham operation group (Sham group), ischemia for 30 minutes and reperfusion for 1 hour, 2 hours and 3 hours (I/R1h group, I/R2h group and I/R3h group); or animals with I/R3h and pretreated with curcumin at doses of 100 $mg{\cdot}kg^{-1}$, 150 $mg{\cdot}kg^{-1}$ and 200 $mg{\cdot}kg^{-1}$ and equivoluminal vehicle (I/R3h+CUR-100 group, I/R3h+CUR-150 group, I/R3h+CUR-200 group and I/R3h+DMSO group). In each group, 12 mice were euthanized after experimental time out. All the detections and measurements were finished by single or double blind method.

Surgery and experimental design

Curcumin, 250 mg per dose, was purchased from Sigma-Aldrich Co. LLC., US (Batch No. C7727). Curcuminoid content is ≥94%, and curcumin ≥80%.

The mouse model of PIRI was established as previous study described [14]: Mice were anesthetized with xylazine (0.01 $mg{\cdot}g^{-1}$) and ketamine (0.1 $mg{\cdot}g^{-1}$) intraperitoneally. The trachea was exposed by cutting on the center of the neck line above the chest. An endotracheal tube was fixed on the neck skin and retained 1 cm to link the animal to a breathing machine. Thorax was opened, and the hilum of left lung was dissociated. The left pulmonary hilum was occluded for 30 min with a microvascular clamp, then the clamp was opened and reperfusion was allowed for 1 h, 2 h and 3 h. The mice were euthanized after reperfusion finished. Then lung infarction, apoptosis, and the expression of ERS apoptotic factors were evaluated. In Sham group, thorax was just opened, left pulmonary hilum was not clamped.

Curcumin was dissolved in 10% dimethyl sulfoxide solvent (DMSO) of normal saline and diluted into 5 $mg{\cdot}mL^{-1}$ (I/R3h+CUR-100 group, 100 $mg{\cdot}kg^{-1}$), 7.5 $mg{\cdot}mL^{-1}$ (I/R3h+CUR-150 group, 150 $mg{\cdot}kg^{-1}$) and 10 $mg{\cdot}mL^{-1}$ (I/R3h+CUR-200 group, 200 $mg{\cdot}kg^{-1}$). The different doses of curcumin solution were injected intraperitoneally into mice 2 hours before ischemia and an equal volume of 10% DMSO instead in I/R3h+DMSO group. The procedures were the same in those I/R3h groups.

The ratio of wet/dry weight (W/D) and total lung water content (TLW)

The apical portion of lung was excised and immediately weighed by an automatic electric balance (Sartorius, Goettingen, Germany), then desiccated at 70°C for 24 h and reweighed to calculate the ratio of wet/dry (W/D). The computational formula of TLW is as follows: TLW = (wet lung weight-dry lung weight)/dry lung weight.

Investigation of lung tissue morphology and alveolar damage quantitative evaluation index (IQA)

Lung tissues were stained with hematoxylin and eosin (H&E) and evaluated for morphological changes under an optical microscope. The analysis of IQA was according to previous literature [15]. Under 400 magnification of light microscope, 50 fields were randomly observed; the number of damaged alveoli and the total number of pulmonary alveoli were counted in every field, and the ratio between them was calculated as a quantitative evaluation of alveolar damage index, namely for IQA. The damaged alveoli was defined as those contained these phenomenons: erythrocytes, leukocytes or edema exudate. All the serious alveolar damage was evaluated by IQA and morphology changes were observed by optical microscope.

Table 1. Wet lung tissue to dry lung tissue (W/D), total lung water content (TLW), alveolar damage quantitative evaluation index (IQA) and apoptotic index (AI) of lung tissue in I/R groups

Group	W/D	TLW	IQA (%)	AI (%)
C	2.17±0.51	1.17±0.51	10.44±1.11	9.30±1.16
Sham	2.43±0.68	1.43±0.68	13.07±1.62	13.60±1.35
I/R1h	2.56±0.31	1.56±0.31	14.15±2.45	16.50±1.58
I/R2h	4.50±0.39$^{**,\#\#}$	3.50±0.39$^{**,\#\#}$	28.74±1.24$^{**,\#\#}$	25.50±3.06$^{**,\#\#}$
I/R3h	5.60±0.40$^{**,\#\#,+}$	4.60±0.40$^{**,\#\#,+}$	39.19±3.80$^{**,\#\#,++}$	35.10±1.79$^{**,\#\#,++}$

Data are presented as mean ± SD, n = 12. C: control; Sham: animals undergoing sham operation; I/R1h: animals undergoing 30 min ischemia and 1 h reperfusion; I/R2h: animals undergoing 30 min ischemia and 2 h reperfusion; I/R3h: animals undergoing 30 min ischemia and 3 h reperfusion. $^{*}P$<0.05, $^{**}P$<0.01 *vs* Sham group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* I/R1h group; ^{+}P<0.05, ^{++}P<0.01 *vs* I/R2h group.

Table 2. Wet lung tissue to dry lung tissue (W/D), total lung water content (TLW), alveolar damage quantitative evaluation index (IQA) and apoptotic index (AI) of lung tissue in CUR groups

Group	W/D	TLW	IQA (%)	AI (%)
Sham	2.43±0.68	1.43±0.68	13.07±1.62	13.60±1.35
I/R3h	5.60±0.40**	4.60±0.40**	39.19±3.80**	35.10±1.79
I/R3h+DMSO	5.32±0.41	4.32±0.41	37.92±2.22	35.10±2.23
I/R3h+CUR-100	4.22±0.51$^{\#}$	3.22±0.51$^{\#}$	31.59±1.80$^{\#}$	28.70±2.21$^{\#}$
I/R3h+CUR-150	3.65±0.26$^{\#\#,+}$	2.65±0.26$^{\#\#,+}$	22.89±1.71$^{\#\#,+}$	22.60±2.01$^{\#\#,+}$
I/R3h+CUR-200	2.64±0.26$^{\#\#,++,-}$	1.64±0.26$^{\#\#,++,-}$	18.80±2.11$^{\#\#,++,-}$	15.20±1.62$^{\#\#,++,-}$

Data are presented as mean ± SD, n = 12. Sham: animals undergoing sham operation; I/R3h: animals undergoing 30 min ischemia and 3 h reperfusion; I/R3h+DMSO: animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with DMSO vehicle; I/R3h+CUR-100: animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with 100 mg·kg^{-1} curcumin; I/R3h+CUR-150: animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with 150 mg·kg^{-1} curcumin; I/R3h+CUR-200: animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with 200 mg·kg^{-1} curcumin. $^{*}P$<0.05, $^{**}P$<0.01 *vs* Sham group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* I/R3h+DMSO group; ^{+}P<0.05, ^{++}P<0.01 *vs* I/R3h+CUR-100 group; ^{-}P<0.05, ^{--}P<0.01 *vs* I/R3h+CUR-150 group.

Examination for lung tissue ultrastructure by electron microscopy

Lung tissue that close to the left lung hilum were cut into pieces and saved in 2.5% glutaraldehyde. Then the samples were immersed 1% osmic acid, stained in 1% acetic acid. After dehydration in an ethanol solution, the samples were embedded in epon. Semithin sections and ultrathin sections of samples were dyed with lead citrate and uranyl acetate. Finally, the samples were observed under an electron microscope.

TUNEL

In Situ Cell Death Detection Kit (POD) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). The detection was performed and improved slightly according to the manufacturer's instructions. Positive cells were defined as those cells in whose nucleus existed deep brown particle. The number of apoptotic cells was counted at five high power field (×400) under a light microscope. At least 500 cells were observed in each section, and positive cells were counted in each 100 cells, namely for apoptotic index (AI).

RT-PCR

Total RNA was extracted from 100 mg lung tissue by using the Trizol kit (Invitrogen, USA) according to the manufacturer's instructions. The concentration and absorbance of RNA were determined. The cDNA was synthetized in accordance with the RT-PCR kit (Fermentas, USA). Perform PCR using the thermal cycling conditions outlined below: initial denaturation, 95°C, 1 min; denaturation, 95°C, 30 sec; annealing, 55°C (GAPDH and CHOP)/49°C (GRP78), 30 sec; extension, 72°C, 30 sec; final extension, 72°C, 10 min. The number of cycles was 33. The electrophoresis of PCR product was conducted in 1.5% agarose gel (Biowest, Spain), and photographed under ultraviolet light.

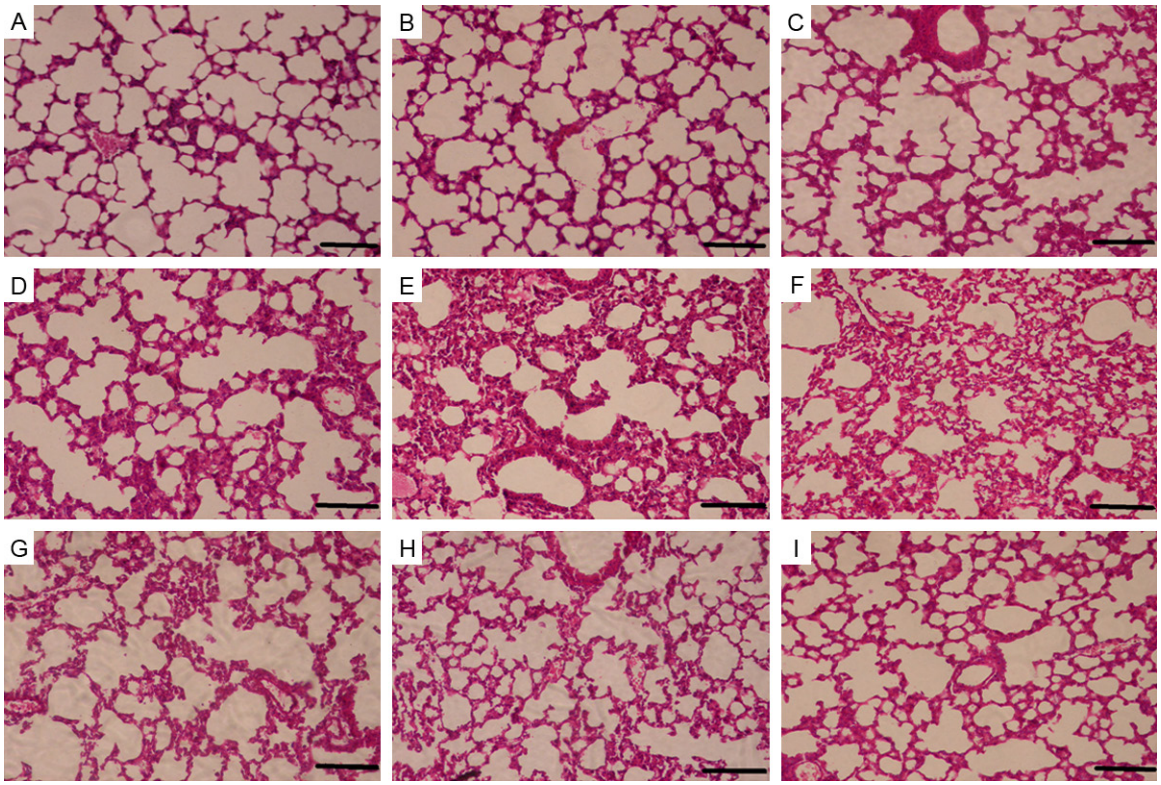

Figure 1. Light morphological changes in lung tissue of mice in various groups (Conventional haematoxylin and eosin stained, H&E). Lung tissues from (A) mice in control group; or (B) mice undergoing a sham operation; or animals undergoing 30 min ischemia and (C) 1 h, (D) 2 h or (E) 3 h reperfusion; or animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with (F) DMSO vehicle, (G) 100 mg·kg^{-1} curcumin, (H) 150 mg·kg^{-1} curcumin, or (I) 200 mg·kg^{-1} curcumin. Scale bar, 50 μm.

The expression levels of targeted mRNA were normalized to GAPDH mRNA level. Primers were synthesized and purified by Shanghai Sangong Bioengineering Company (CN). The PCR primer sequences were: GRP78 forward: 5'-GAT AAT CAA CCA ACT GTT AC-3', reverse: 5'-GTA TCC TCT TCA CCA GTT GG-3', and the length of amplification was 575 bp. CHOP forward: 5'-CAC CTA TAT CTC ATC CCC AGG A-3', reverse: 5'-ACC ACT CTG TTT CCG TTT CCT A-3', and the length of amplification was 217 bp. caspase-12 forward: 5'-CTG ACA GCT CCT CAT GGA CTC-3', reverse: 5'-GCC AGC AAA CTG CAT TAA CTC, and the length of amplification was 309 bp. JNK forward: 5'-TGA CGC CTT ATG TGG TGA CT-3', reverse: TGA TGT ATG GGT GCT GGA GA-3', and the length of amplification was 420 bp. GAPDH forward: 5'-ACT TGA AGG GTG GAG CCA AA-3', reverse: 5'-CCA GGA AAT GAG CTT GAC A-3', and the length of amplification was 530 bp. The RT-PCR data were analyzed using the relative gene expression method. In brief, the data are presented as the fold change in gene expression normalized to the endogenous reference gene (GAPDH) and relative to a calibrator.

Western blot analysis

About 100 mg of frozen lung tissue was suspended in 400 μl ice-cold RIPA lysis buffer (including 1 mM PMSF), homogenized, and centrifuged, removing insoluble material. Supernatant protein concentrations were determined by the BCA Protein Assay Reagent (Beyotime Institute of Biotechnology, Jiangsu, CN). After samples were denatured, equal amounts of protein (20 μl) were separated using SDS-PAGE (10% Tris-glycine or 5% Bis-Tris gels, Invitrogen, US). Then the membranes were blocked with 5% skim milk solution, followed by overnight incubation at 4°C with the appropriate primary antibody. The membranes were probed the following day with secondary antibodies (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd, CN) for 1 h, followed by chemiluminescent detection of peroxidase, chemical development and

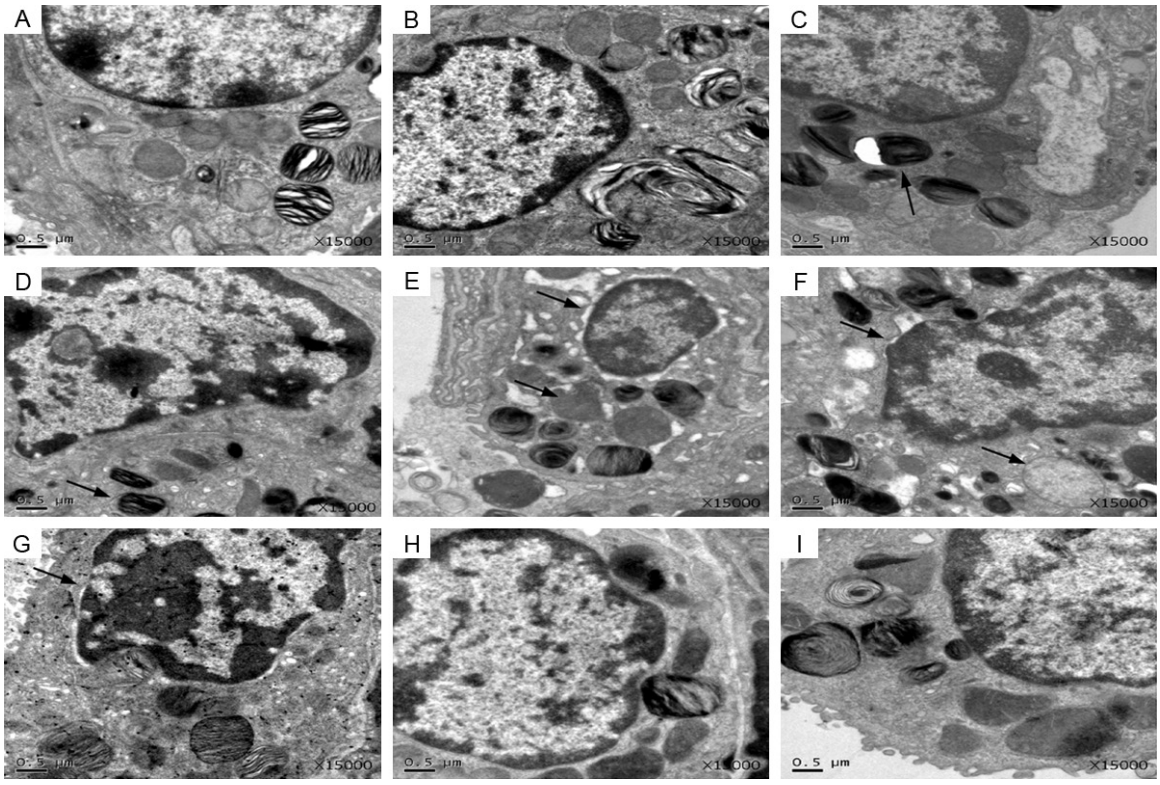

Figure 2. Ultrastructural changes in lung tissue of mice in various groups (transmission electron microscope,TEM). Lung tissues from (A) mice in control group; or (B) mice undergoing a sham operation; or animals undergoing 30 min ischemia and (C) 1 h, (D) 2 h or (E) 3 h reperfusion; or animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with (F) DMSO vehicle, (G) curcumin at 100 mg·kg^{-1}, (H) curcumin at 150 mg·kg^{-1}, or (I) curcumin at 200 mg·kg^{-1}. Scale bar, 0.5 μm.

photographic fixing. Films were scanned. Densitometry was performed using Quantity One software (Bio-Rad, US).

Statistical analysis

Data were statistically analyzed using SPSS 17.0 (SPSS Inc., US). All measurements were repeated three times and presented as means ± standard deviation (SD). Data were all tested using the t test. P<0.05 was considered statistically significant.

Results

Curcumin induces changes in lung W/D and TLW

Table 1 shows that, compared with control group, W/D and TLW in Sham group had no significant differences (P>0.05). Compared with Sham group, W/D and TLW were increasingly higher in I/R2h group and I/R3h group (P<0.05 or P<0.01), but not increased in I/R1h group (P>0.05). Compared to I/R3h+DMSO group, W/D and TLW were decreasingly lower in I/R3h+CUR-100 group, I/R3h+CUR-150 group and I/R3h+CUR-200 group (P<0.05 or P<0.01, respectively, **Table 2**).

Curcumin reduces PIRI pathological changes

We investigated whether curcumin provides protection against PIRI in mice when the pulmonary hilum of left lung is clamped for 30 minutes. Lungs were harvested for histopathological assessment of lung damage. The different types of lung injuries, including interstitial edema, inflammatory cell infiltration, rupture of pulmonary alveolar interval and erythrocyte exudation, existed in I/R groups and I/R3h+CUR groups (**Figure 1**). The degree of lung injury was increasingly more serious in I/R2h group and I/R3h group (P<0.01, respectively, **Table 1**). Treatment with curcumin, the damage was significantly decreased in I/R3h+CUR-100 group, I/R3h+CUR-150 group and I/R3h+CUR-200

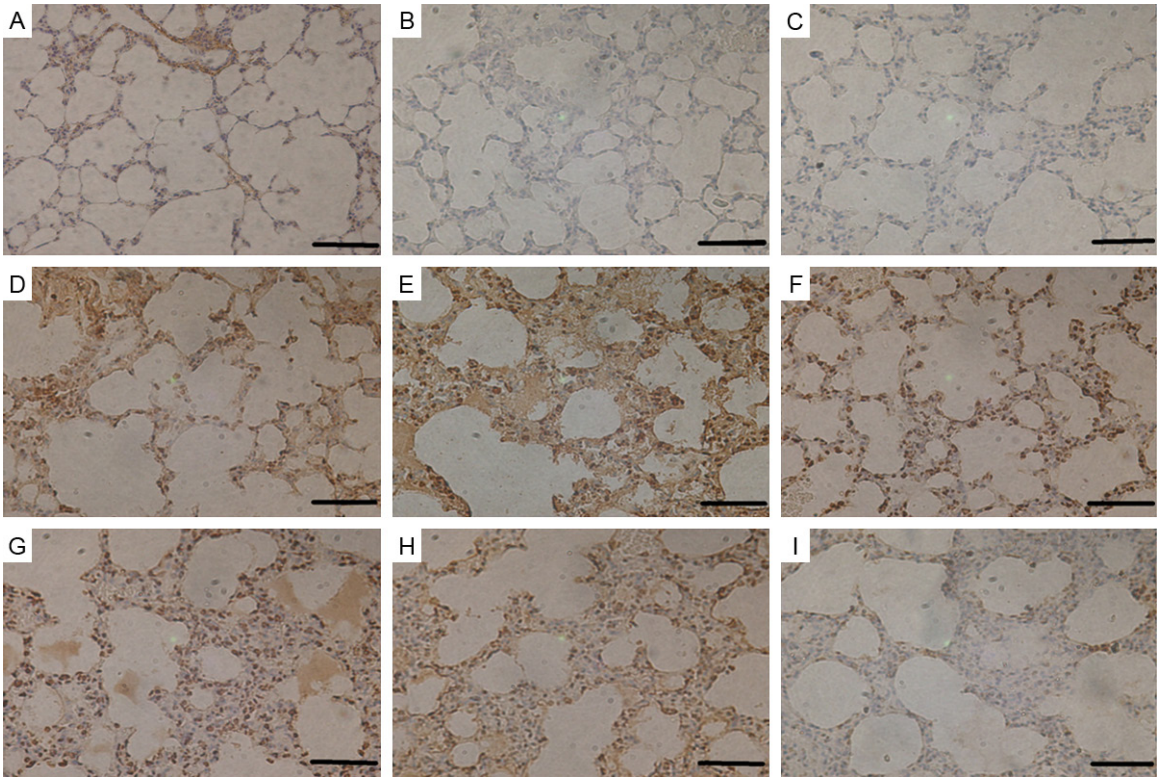

Figure 3. Apoptosis in lun tissue of mice in various groups detected by TUNEL method (POD). Lung tissues from (A) mice in control group; or (B) mice undergoing a sham operation; or animals undergoing 30 min ischemia and (C) 1 h, (D) 2 h or (E) 3 h reperfusion; or animals undergoing 30 min ischemia and 3 h reperfusion and pre-treated with (F) DMSO vehicle, (G) 100 mg·kg^{-1} curcumin, (H) 150 mg·kg^{-1} curcumin, or (I) 200 mg·kg^{-1} curcumin. Scale bar, 25 μm.

group (P<0.05 or P<0.01, respectively, **Table 2**).

A similar pattern of changes were noted in Ultra-structure of Alveolar II type cells function. After PIRI, Swollen mitochondria, disappeared or emptied lamellar bodies, condensed cytoplasm and nuclear chromatin in type II alveoli appeared in I/R2h group and I/R3h group, especially in I/R3h group. With the treatment of curcumin, The damage of lung tissue in I/R3h+CUR-200 group was notably ameliorated. The ultrastructure of type II alveoli and capillary endotheliocyte verged to normality (**Figure 2**).

Curcumin restrains cell apoptosis in PIRI

To evaluate lung damage at the cellular level, we used terminal deoxynucleotidyl transferase-mediated digoxigenindeoxyuridine nick-end labeling (TUNEL) staining to detect dead alveolar epithelial cells and vascular endothelial cells. PIRI significantly increased TUNEL-positive cells, compared with Sham group (P<0.01), especially in I/R3h group (P<0.01, **Table 1**). Apoptosis cells were mainly alveolar epithelial cells and vascular endothelial cells (**Figure 3**). Treatment with curcumin, apoptotic indexes in I/R3h+CUR-100 group, I/R3h+CUR-150 group and I/R3h+CUR-200 group were decreased progressively (P<0.05 or P<0.01), especially in I/R3h+CUR-200 group (P<0.01, **Table 2**).

Curcumin relieves endoplasmic reticulum stress induced by PIRI

GRP78, CHOP, JNK and Caspase-12 are markedly high expression when severe ERS occurs. To investigate the molecular mechanisms of curcumin-induced lung protection, we assessed GRP78, CHOP, JNK and Caspase-12 expression. There were no significant differences in the expression levels of GRP78, CHOP, JNK and Caspase-12 in control group and Sham group. Along with extension of reperfusion time, the levels of targeted mRNA and protein were gradually higher (P<0.05), especially in I/R3h group (P<0.05). After curcumin treatment, The levels of GRP78 were all notably increased (P<0.05). The expression levels of CHOP, JNK and Cas-

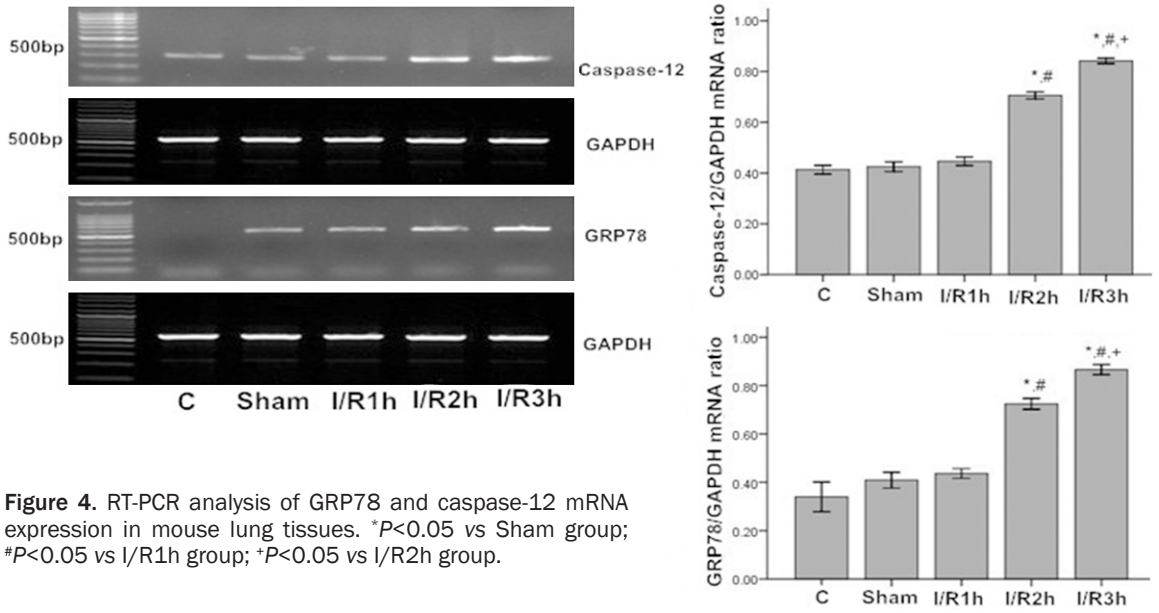

Figure 4. RT-PCR analysis of GRP78 and caspase-12 mRNA expression in mouse lung tissues. *P<0.05 *vs* Sham group; #P<0.05 *vs* I/R1h group; +P<0.05 *vs* I/R2h group.

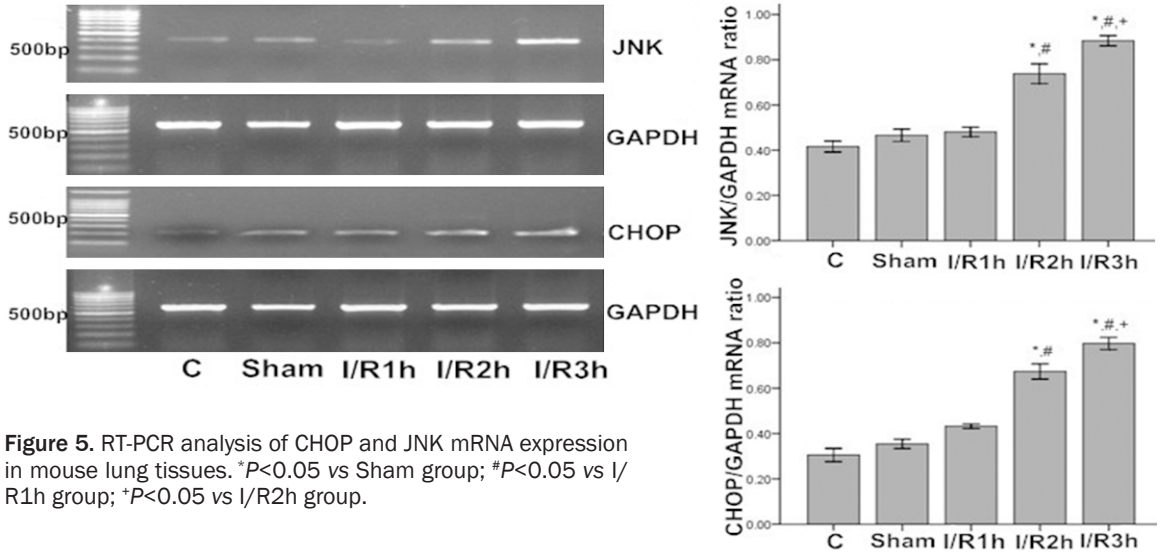

Figure 5. RT-PCR analysis of CHOP and JNK mRNA expression in mouse lung tissues. *P<0.05 *vs* Sham group; #P<0.05 *vs* I/R1h group; +P<0.05 *vs* I/R2h group.

pase-12 in I/R3h+CUR-100 group, I/R3h+CUR-150 group and I/R3h+CUR-200 group were reduced progressively (P<0.05), especially in I/R3h+CUR-200 group (P<0.05). The above is as seen in **Figures 4-11**.

Discussion

As studies have indicated, pneumonocyte apoptosis played a crucial role in PIRI, and there were prevention and cure function for PIRI by intervening apoptosis. This study showed that few apoptotic cells were seen in C group and Sham group, but apoptotic cells appeared in I/R group, and with the extension of reperfusion time, apoptotic cells were increasing, especially in I/R3h group. Moreover, given curcumin, apoptotic cells were significantly reduced. In addition, lung vascular endothelial cells and alveolar epithelial cells were the major apoptotic cells, indicating that one or two types of these cells may play an important role in PIRI [16].

Endoplasmic reticulum (ER) is quite sensitive to the changes of homeostasis in organism. Oxidation, ischemia and anoxia etc. can disturb ER steady-state, and lead to folding of protein

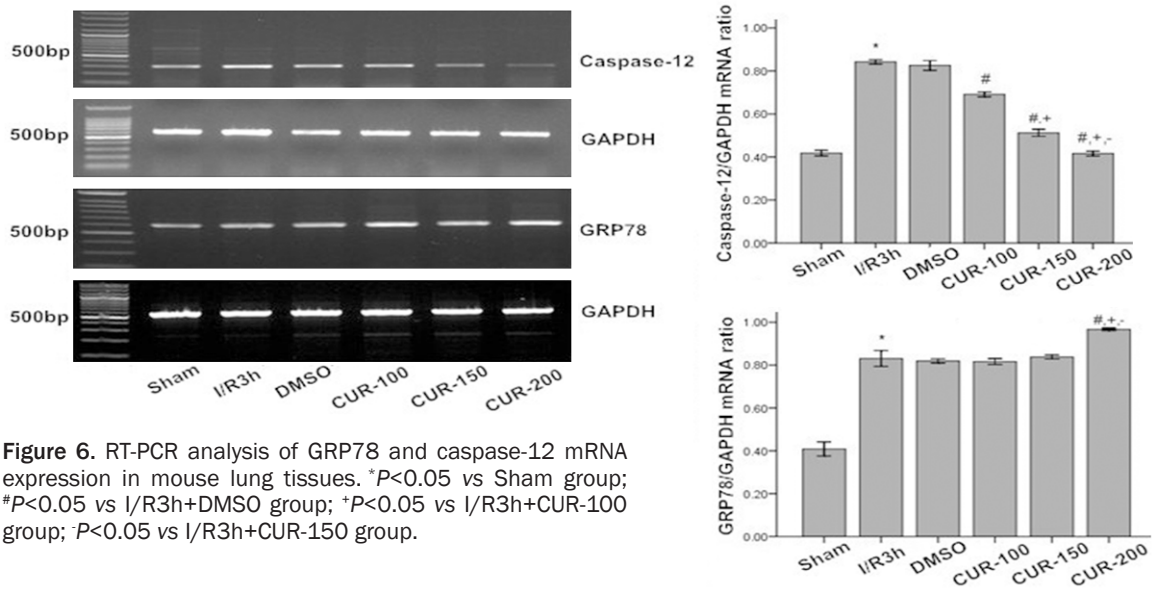

Figure 6. RT-PCR analysis of GRP78 and caspase-12 mRNA expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R3h+DMSO group; $^{+}P<0.05$ *vs* I/R3h+CUR-100 group; $^{-}P<0.05$ *vs* I/R3h+CUR-150 group.

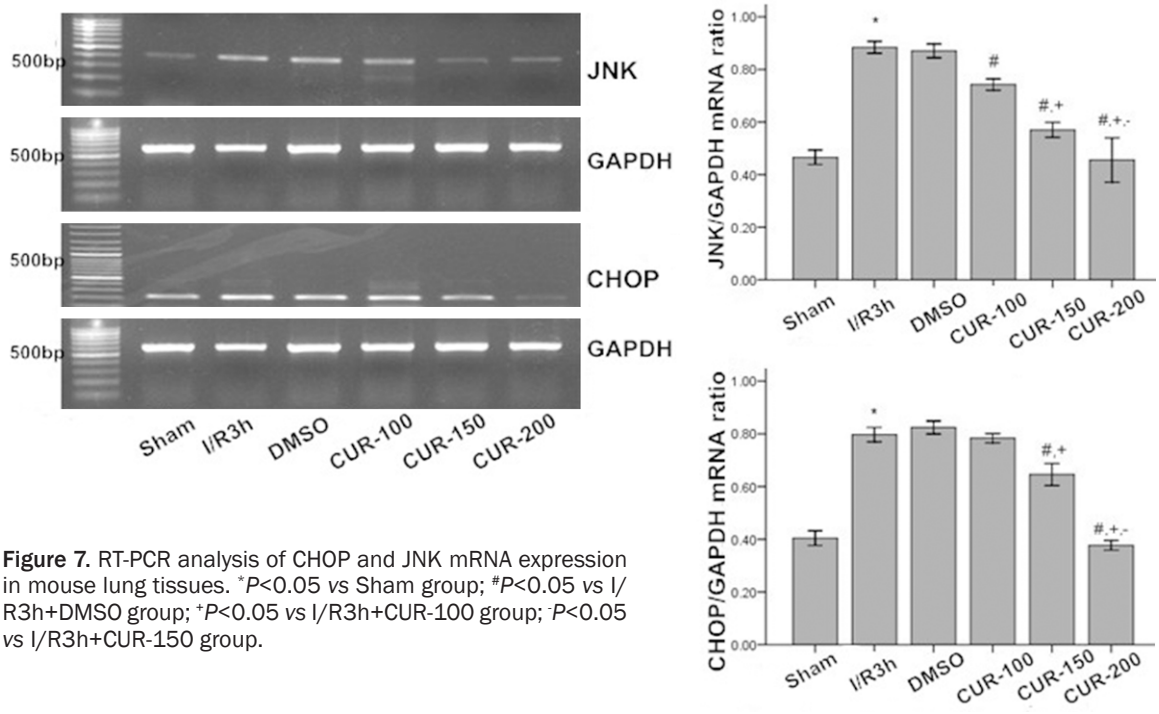

Figure 7. RT-PCR analysis of CHOP and JNK mRNA expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R3h+DMSO group; $^{+}P<0.05$ *vs* I/R3h+CUR-100 group; $^{-}P<0.05$ *vs* I/R3h+CUR-150 group.

by mistake or accumulation of unfolded protein, initiating UPR. Some degree of UPR has protection for organisms, but continued and excessive UPR induces ER-associated apoptosis mediated by CHOP, Caspase-12 and JNK. GRP78 and CHOP are traditional markers. GRP78, called Bip (immunoglobulin binding protein), is a chaperonin in ER. UPR can induce notable up-regulation of GRP78, which is combined with mistaken folded and unfolded proteins, regain correct conformation of proteins, maintain homeostasis. Induced transcription of GRP78 has a key role in protection against apoptosis induced by ERS. CHOP, namely for GADD153 (growth arrest-and DNA damage-inducible gene 153), is an important signal molecular of pro-apoptosis. Its expression level is very low in normal cells, but it will increase highly induced by ERS [17]. In brief, on the one hand, up-regulation of GRP78 can relieve the

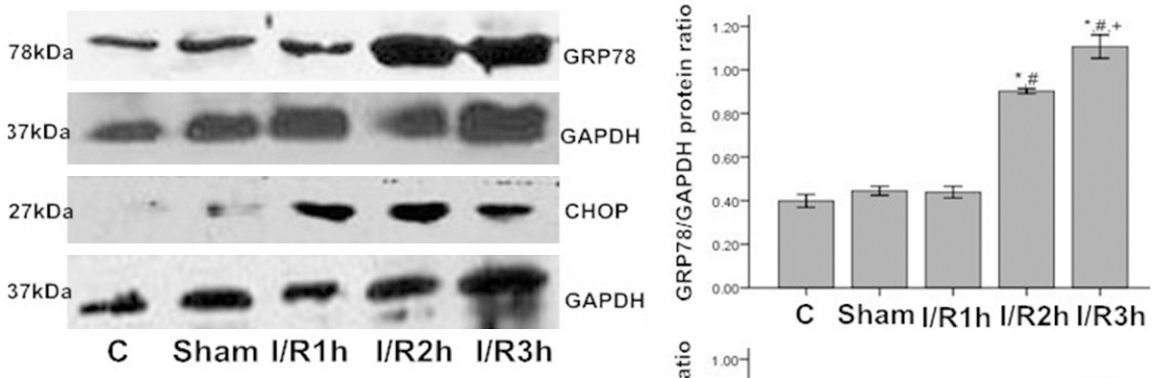

Figure 8. Western bloting analysis of GRP78 and CHOP protein expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R1h group; $^{+}P<0.05$ *vs* I/R2h group.

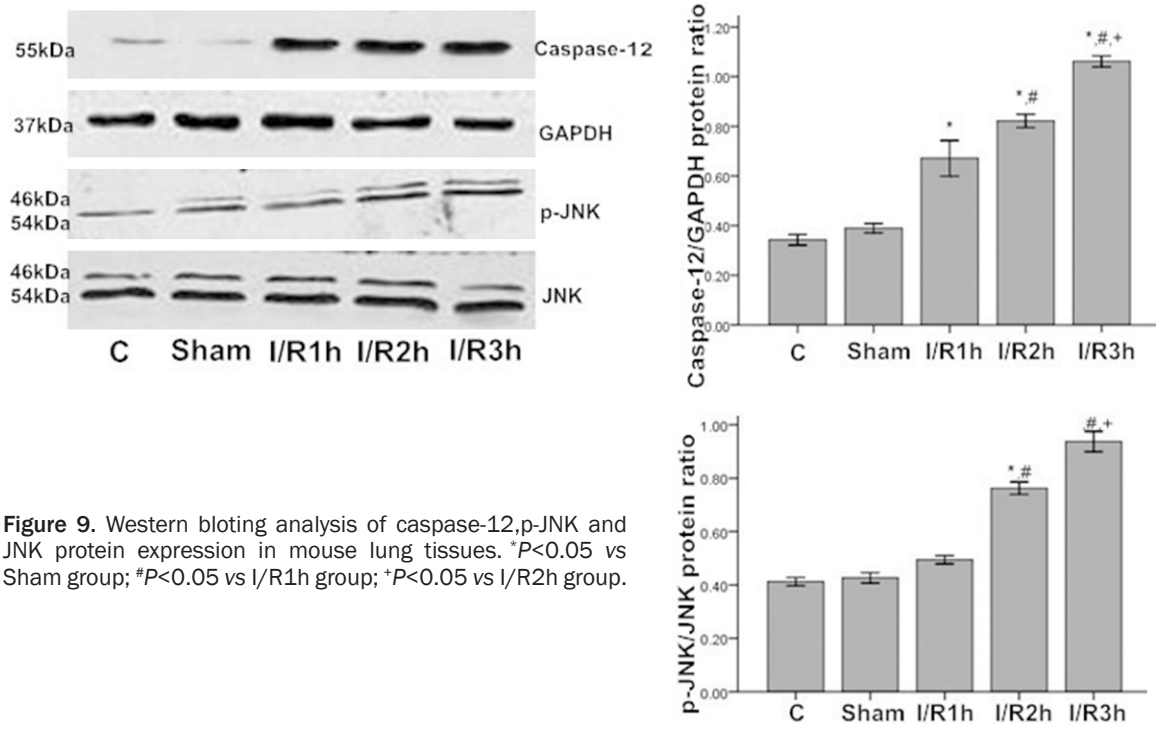

Figure 9. Western bloting analysis of caspase-12,p-JNK and JNK protein expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R1h group; $^{+}P<0.05$ *vs* I/R2h group.

load of unfolded protein in ER, and have a protective effect; on the other hand, up-regulation of CHOP will activate pathways of cell apoptosis, and bring a damage effect. When serious ERS occured, inositol-requiring protein-1 (Ire1) activated by c-jun NH_2-terminal inhibitory kinase (JIK) binds to TNF receptor-associated factor-2 (TRAF-2), and complex of the two proteins recruits apoptosis signal regulating kinase-1 (ASK1) to activate JNK pathways, leading to cell apoptosis [18]. Furthermore, the inhibition of apoptosis attributes to depressing the JNK pathway [19]. Caspase-12 exists in ER membrane, and its precursor in the form of proen-

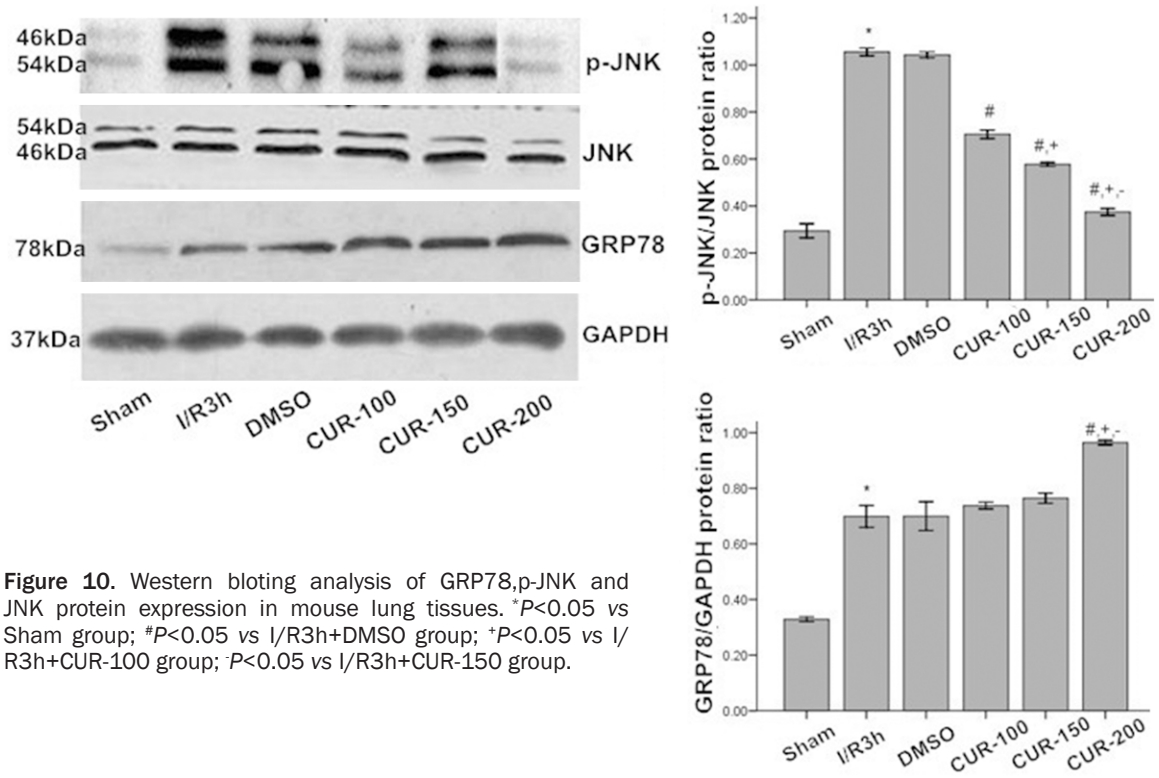

Figure 10. Western bloting analysis of GRP78,p-JNK and JNK protein expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R3h+DMSO group; $^{+}P<0.05$ *vs* I/R3h+CUR-100 group; $^{-}P<0.05$ *vs* I/R3h+CUR-150 group.

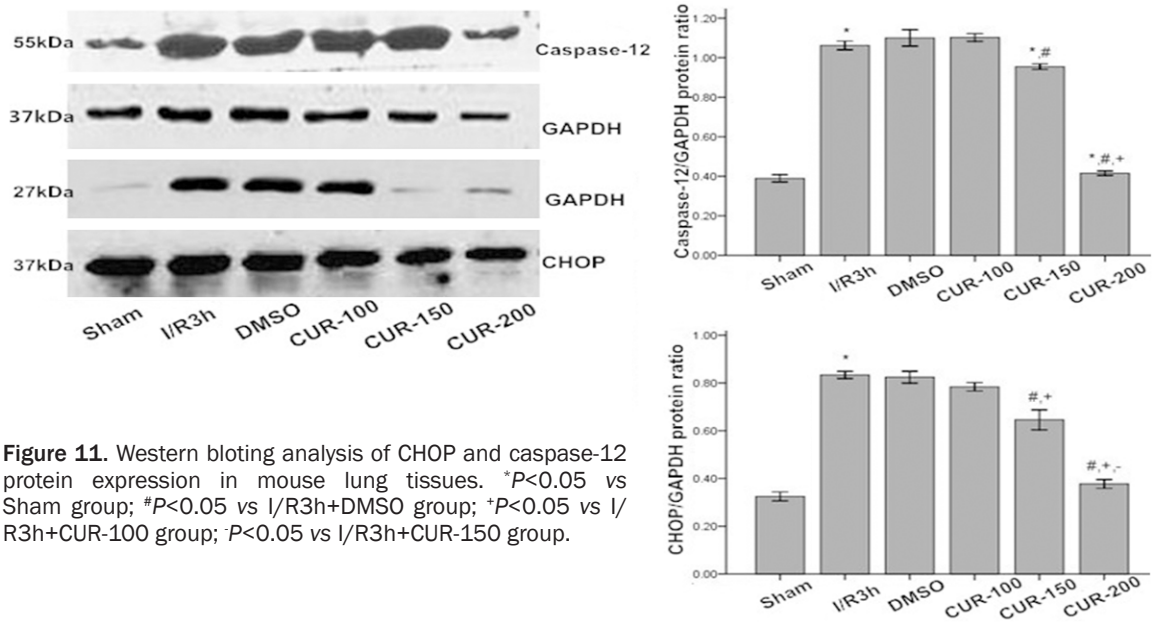

Figure 11. Western bloting analysis of CHOP and caspase-12 protein expression in mouse lung tissues. $^{*}P<0.05$ *vs* Sham group; $^{\#}P<0.05$ *vs* I/R3h+DMSO group; $^{+}P<0.05$ *vs* I/R3h+CUR-100 group; $^{-}P<0.05$ *vs* I/R3h+CUR-150 group.

zyme binds to TRAF α. The combination of Ire1 and TRAF α causes complex of TRAF α and Caspase-12 precursor disintegrate, Caspase-12 is activated by Ca^{2+}-dependent protease. The activated Caspase-12 further activates Caspase-9 and Caspase-3, causing cell apoptosis [20].

The study showed that GRP78 expressed very little in control group and Sham group, but increased gradually at I/R1h, I/R2h, I/R3h. After pretreated with curcumin, the level of GRP78 was in state of high expression, which confirmed that up-regulation of GRP78 had vigorous protection effect. Meanwhile, the expres-

sion levels of CHOP, Caspase-12 and JNK were increasing along with extension of reperfusion time, but down-regulated by curcumin pretreatment. So it was concluded that CHOP, Caspase-12 and JNK in excessive ERS participated in pathways of cell apoptosis and caused lung injury. The conclusions of this research were in agreement with those previously reported [21, 22].

Curcumin is a traditional Chinese medicine monomer extracted from Curcuma L. including carcuma longa, curcuma aromatica and zedoaria etc. which can promote blood circulation and remove blood stasis. It has been reported that curcumin has great protection against I/R injury involved in various organs, which was related to antioxidation, inhibit the release of inflammatory cytokine and anti-apoptosis. This experiment is focus on the effects of curcumin on the role of CHOP, caspase-12 and JNK of ERS in PIRI. The concentration of curcumin in this study was determined by previous reaserch [10]. As the results of this study show, given curcumin, W/D, TLW, IQA and AI in I/R3h+CUR-100 group, I/R3h+CUR-150 group and I/R3h+CUR-200 group were all decreasingly reduced. The results of light microscope and electron microscope indicated that morphological structure of lung tissue was less damaged. TUNEL showed that apoptotic cells were significantly decreased. The expression levels of CHOP, Caspase-12 and JNK were notably down-upregulated, while the level of GRP78 was still increasing. What mentioned above confirmed that curcumin can against with pneumonocyte apoptosis, lighten PIRI, which may be related to down-regulation of CHOP, Caspase-12 and JNK in excessive ERS induced by I/R. However, I/R injury is the consequence of combined action by various factors, the optimum efficiency of treatment cannot be acquired in the case of simple intervention to a certain part of I/R injury. Therefore the machanisms of curcumin against PIRI should be comprehensive, need to explore with great efforts.

This study has investigated possible mechanisms and pretreatments of PIRI. I/R induces excessive ERS in lung tissue, in which CHOP, Caspase-12 and JNK may mediate cell apoptosis, thus leading to lung tissue injury; Curcumin has notable effects on lung protection against I/R injury, which may be related to down-regulation of CHOP, Caspase-12 and JNK in excessive ERS and inhibition of cell apoptosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Wantie Wang, Ischemia/Reperfusion Injury Research Institute of Wenzhou Medical University, Wenzhou 325035, Zhejiang, China. E-mail: wwt@wmu.edu.cn

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