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Effects of curcumin or dexamethasone on lung ischemia-reperfusion injury in rats

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Abstract

The present study aims at investigating potential effects of curcumin (CUR) and dexamethasone (DXM) on ischemia-reperfusion (IR) induced lung injury in rats.

Experimental rats were pretreated with a single intraperitoneal dose of vehicle, CUR (50mg/kg or 200mg/kg) or DXM (5mg/kg) 2 h before anaesthesia and subjected to left lung hilus clamping with 90 min ischemia followed by 4 h of reperfusion.

Pretreatment with CUR (200 mg /kg) or DXM markedly attenuated IR-induced barrier disruption, lung edema, tissue inflammation, hypoxemia at 4 h after reperfusion, and oveactivation of NF-κB, inflammatory cytokines, MPO and malondialdehyde.

It seems that CUR attenuates acute lung injury probably through improving oxidative stress and inhibiting NF-κB-mediated expression of inflammatory cytokines. Thus, CUR may be an alternative therapy for improving the outcomes of IR induced lung injury.

Keywords: Curcumin; Lung; Ischemia-reperfusion (IR); Oxidative stress; Microvascular permeability; NF-κB

1. Introduction

Ischemia-reperfusion (I/R)-associated acute lung injury (I/R-ALI) occurs in many clinical situations such as lung transplantation and becomes life-threatening in about 20% of transplants, resulting in the primary dysfunction of implanted lungs and the mortality rate of 60% [1, 2].Such compromise usually appears at the early stage of lung transplantation, characterized by nonspecific alveolar damage, lung edema and hypoxemia, as reported in clinical studies [3]. It is important to elucidate the molecular mechanisms of I/R-ALI to develop an effective therapeutic strategy. For example, the redox-sensitive transcription factor nuclear factor-kappaB (NF-κB), which regulates genes encoding proinflammatory mediators, plays a pivotal role in the development of I/R injury. The inhibition of NF-κB activation could attenuate I/R-induced tissue injury [4, 5].

Curcumin (CUR) , an active component of the rhizome Curcuma longa, shows wide anti-inflammatory, antioxidant and bactericidal effects [6, 7]. CUR down-regulates the production of tumor necrosis factor (TNF) -α and interleukin (IL) -1 and inhibits the activation of NF-κB and activator protein-1 [7]. Dexamethasone (DXM) modulates enzyme systems and inhibits the formation of cytokines, arachidonic acid products, and NF-κB activation [8, 9]. Pretreatment with CUR or DXM was suggested to reduce I/R-induced injury in multiple organs/tissues [10-14]. It was proposed that CUR and others might have a similar mechanism to glucocorticoid in the treatment of IR injury [13]. The present study aims at investigating the effects of CUR or DXM on alveolar–capillary membrane disruption, lung edema, neutrophil lung tissue infiltration and hypoxemia in a rat model of I/R-ALI.

2. **Materials and methods**

2.1. Animals and surgical preparation

Male Sprague–Dawley rats (Animal Center of Fudan University, Shanghai, China) weighing between 250 and 300 g, were used. The animals had no access to solid food but free access to water 12 h before the experiments. The experimental protocol was approved by the Committee of Animal Care of Fudan University. All animals were handled in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health. Modified operations were performed as described previously[15]. Animals were anesthetized by an intraperitoneal injection of pentobarbital sodium at 50 mg/kg and intramuscular injection of atropine at 0.2 mg. After cannulation, rats were mechanically ventilated (Harvard Rodent Ventilator, Model 683, South Natick, MA, USA) with a standardized inspired oxygen content (FiO_2) of 60% at a rate of 75 breaths /min, the tidal volume at 10 ml/kg, positive end expiratory pressure (PEEP) at 2 cmH_2O and partial pressure of carbon dioxide in artery ($PaCO_2$) between 30 and 45 mmHg. A 22-gauge cannula inserted into the right carotid artery was used to monitor arterial blood gas (ABG) and draw blood samples. The animals were placed on their right sides, and a left anterolateral thoracotomy in the fifth intercostal space was performed. The left lung was mobilized atraumatically and the inferior pulmonary ligament was divided sharply. At this point, all animals received 50 U of heparin intravenously . After blood was harvested from the right carotid artery (CA) for ABG of preischemia, the pulmonary hilum was occluded with a non-crushing microvascular clamp, including the left main bronchus, artery and vein, for 90 min. After 90 min left lung ischemia, the clamp was removed and the lung was ventilated and reperfused for 4 h. At the end of the reperfusion period, ABG were measured from CA and left

pulmonary vein (LPV), and the arterial oxygen tension/inspired oxygen concentration (PO_2/FiO_2) value was compared to the preischemia value. The lung tissues were harvested for further analysis, after the experiment was terminated. Surgery and tissue analyses were blindly performed.

2.2. Experimental groups

Animals were randomly divided into six experimental groups (n=12/group), including naive animals (Control), animals with sham operation and pretreated with vehicle (Sham), or animals with I/R-ALI and pretreated with vehicle (Vehicle), with CUR at 50 mg/kg (CUR-50), with CUR at 200 mg/kg (CUR-200) or with DXM at 5 mg/kg (DXM-5). Of them 36 was used for measurement of lung microvascular permeability and 36 for the lung function and biochemical assays, in order to avoid the contamination of blue. CUR (Sigma-Aldrich Co., St. Louis, MO) dissolved in dimethylsulfoxide (DMSO) and DXM (Tianjin Pharmaceutical Co., Tianjin, China) in physiologic saline was administered intraperitoneally 2 h prior to I/R induction. The dose of CUR and DXM used in this study was based on previous experiments and our preliminary studies [10, 14]. In pilot studies, dose ranges of CUR between 25 and 400 mg/kg were tested, and the dose of 200 mg/kg showed significantly inhibitory effects on I/R-induced changes in vascular permeability and CUR treatment in 200 mg/kg at 2h prior to anesthesia was more effective than that of after the induction of ischemia (data unshown). 50 and 200mg/kg were used as low and high doses of CUR in the current experiments, respectively.

2.3. Lung tissue oedema and morphology

The left lungs were harvested for measurements of lung tissue edema by lung wet-dry weight ratio and morphology by staining with hematoxylin and eosin. All microscopic sections were interpreted in a blind fashion by a pulmonary pathologist. Lung neutrophil sequestration was measured by counting lung tissue neutrophils and expressed as the number of neutrophis per 10 high-power fields of lung tissue, as described previously [16].

2.4. Lung microvascular permeability

The plasma leakage was measured by the Evan's blue dye (EBD), with minor modifications[17]. Animals received 30 mg/kg dye via the vein 30 min before the end of the reperfusion period. After then, a median sternotomy was performed and heparin (500 units) was infused into abdominal aorta. An 18-gauge cannula was inserted into the main pulmonary artery. The left atrial appendage and left ventricle were incised to allow free flow of effluent blood from the lung. The pulmonary vasculature was flushed with 50 ml of physiologic saline at 20 cm H_2O. After the dried tissues were immersed in 3 ml of formamide and homogenized, the homogenate was incubated at 37°C for 24 h and centrifuged at 5000 g for 30 min. The optical density of the supernatant was measured by spectrophotometry at 620 nm. For qualitative examination, lungs were sectioned into 10μm slices and examined by fluoromicroscopy.

2.5. Myeloperoxidase (MPO) activity and oxidative stress assay

The left lung was used to determine pulmonary tissue MPO activity. Tissue MPO activity was measured to quantify polymorphonuclear leukocyte accumulation in the lungs [18]. The

supernatant from lung homogenizing buffer was used for determinations of the enzymatic activities of xanthine oxidase (XO)[19], malondialdehyde (MDA)[20] and the total anti-oxidative capacity (TAOC)[21]. Activities of MPO, XO, MDA and TAOC in the lung tissue were determined using corresponding assay kit (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

2.6. NF- κB, TNF-α and IL-1β measurements

To observe changes in NF-κB activity, DNA binding activity was measured using an ELISA assay. Nuclear extracts of left lungs from various groups were prepared using the Nuclear Extract Kit (Active Motif, Carlsbad, CA, USA). P65 DNA binding activity was measured with 15 μg of nuclear extract with the Trans-AM kit according to the manufacturer's instructions (Active Motif)[11]. TNF-α and IL-1β levels in left lungs and serum were determined using a rat TNF-α ELISA kit and IL-1β ELISA kit (R&D Systems, Minneapolis, MN, USA), respectively, as previously described [22].

2.7 .Statistical analysis

The results are presented as mean ± S.D. and data was analyzed using SPSS version 11.5 statistical software (SPSS Inc., Chicago, IL, USA). Comparisons between multiple groups were performed by one-way ANOVA procedures, followed by the Bonferroni's post hoc test for intergroup comparisons, in the case of unequal variances, a non-parametric Kruskal–Wallis test was performed. The significance was considered as $P < 0.05$.

3. Results

3.1. Histopathology and lung injury score

Figure 1 demonstrated the different degrees of I/R-ALI. Lung tissue edema in I/R-ALI animals pretreated with vehicle or CUR at 50 mg/kg were more obvious than those in sham-operated animals (Fig. 1), while pre-treatment with CUR at 200 mg/kg or DXM prevented from I/R-induced lung tissue edema. Severity of ALI characterized by lung tissue edema, leukocyte infiltration, and hemorrhage in I/R-ALI animals were more obvious than sham-operated animals, while CUR at 200 mg/kg and DMX had preventive effects, as noticed morphologically in Fig. 1.

3.2. ABG and lung wet/dry weight ratio

Values of CA PO_2/FiO_2, LPV PO_2/FiO_2 (Fig. 2A), left lung water content (Fig. 2B) and right lung water content contra-lateral to I/R side (Fig. 2C) in all I/R-ALI animals were significantly lower or higher than those in sham-operated and Control ($p<0.05$ or 0.01, respectively). However, pre-treatment with CUR at 200 mg/kg and DXM significantly prevented from I/R-induced changes of LPV PO_2 ($p<0.05$ or 0.01, Fig. 2A) and left lung water content ($p<0.01$, Fig. 2C), as compared with vehicle pre-treatment.

3.3 Lung microvascular permeability

A significant increase in EBD extravasation was found in I/R left lungs of animals pretreated with vehicle, CUR or DXM, as compared with sham-operated and control animals ($p<0.01$, respectively, Fig. 3A). Pretreatment with CUR at 200 mg/kg or DXM significantly prevented

from I/R-induced increase in the capillary barrier permeability, as comparing with vehicle ($p<0.01$, respectively). Similar pattern of inhibitory effects of CUR or DXM was also noticed in the contra-lateral right lungs (Figure 3B).

3.4 Lung tissue neutrophilic infiltration and oxidative stress

Values of MPO activity (Fig. 4A) and number of neutrophils (Fig. 4B) in the left lung tissue increased significantly in all animals with I/R-ALI, as compared with sham-operated and Control animals ($p<0.01$, respectively), while pretreatment with CUR at both 50 and 200 mg/kg or DXM significantly prevented from I/R-increased MPO activity and neutrophil influx in lung tissue. Table 1 demonstrated that tissue levels of MDA and XO significantly increased and tissue levels of TAOC decreased in I/R-ALI animals pretreated with vehicle ($p<0.01$ vs Control or sham-operated animals, respectively). Both of CUR at 200 mg/kg or DXM showed significantly inhibitory effects on I/R-increased tissue levels of MDA. However, only pre-treatment with CUR at 200 mg/kg had significantly inhibitory effects on I/R-increased tissue levels of XO and I/R-decreased levels of TAOC, as compared with vehicle pre-treatment ($p<0.05$, Table 1).

3.5 NF-κB DNA Binding activity and cytokine alterations

Values of NF-κB activity in lung tissue was significantly higher in all operated animals than the Controls and all I/R-ALI animals than sham-operated those ($p<0.05$ or $p<0.01$, respectively, Table 2). Pretreatment with CUR 200 mg/kg or DXM significantly prevented from I/R-induced NF-κB over-activation by 64% and 76%, respectively. Table 2 describes that tissue and serum

levels of TNF-α and IL-1β significantly increased after induction of I/R, while CUR showed significantly inhibitory effects on I/R-induced serum TNF-α and IL-1β from 50 mg/kg and on tissue levels of TNF-α and IL-1β at 200 mg/kg. Pretreatment with DXM significantly prevented from both lung tissue and serum TNF-α and IL-1β.

4. Discussion

The present study demonstrates protective effects of CUR on I/R-induced lung edema, lung capillary endothelial barrier dysfunction, leukocyte infiltration, cytokine overproduction, and tissue injury, similar to DXM. The aim of using DXM in the present study was to evaluate protective effects of CUR on I/R-ALI where DXM is used as effective reference compound. However, we found that only CUR could prevent I/R-increased tissue levels of XO and decreased tissue levels of TAOC. This may indicate that CUR and DXM have anti-inflammatory effects through different pathways. For example, it is possible that CUR might have more preventive effects on oxidative stress. Another advantage is that CUR appeared non-toxic to humans up to 8,000 mg/day when taken by mouth for 3 months [23]. This provides a potential to use CUR for long-term therapy for I/R-ALI. We also noticed that left lung I/R caused an increase of capillary barrier permeability in the contra-lateral lung, which could be prevented by CUR or DXM pre-treatment.

MPO is an enzyme whose level of activity is known to be directly proportional to the number of neutrophils present [18]. The present study demonstrated that I/R increased neutrophilic infiltration in lung tissue evidenced by both increased MPO activity and number of neutrophils in the tissue. CUR at high dose or DXM had anti-neutrophil influx. NF-κB is involved in the control of many genes in the early processes of immune and inflammatory responses [9], including the activation of a number of immune response proteins (e.g. TNF-α, IL-1β, IL-6, and IL-8). NF-κB activation is tightly regulated by its endogenous inhibitor, IκB, which complexes with NF-κB in the cytoplasm. CUR has been shown to inhibit phosphorylation and proteolytic

degradation of IκB and prevent the release and nuclear transmigration of NF-κB [24], similar to DXM treatment in cold-preserved alveolar epithelial cells [8]. The present study demonstrates that I/R-induced over-activation of NF-κB and hyper-production of inflammatory cytokines in both system and local tissue.

Although both warm lung I/R and prolongation of the duration of cold transplantation present partially common pathological characteristics of I/R injury, lung transplantation-associated lung injury also includes graft rejection, injuries induced by lung flushing with organ preservation solution and complicated surgery manipulation. Cold preservation consumes less metabolic substrate than warm ischemia, which could prolong the preservation time of the lungs. In addition, the flushing with preservation solutions before cold storage could alleviate the radicals and mediators of inflammation release after reperfusion than warm ischemia. In addition, lung I/R can also exist in multiple pathophysiological conditions, e.g. shock, hemorrhage, and trauma. Clinical and experimental studies have shown that I/R induces a rapid release of proinflammatory cytokines in the lung [3]. It is possible that ischemia triggers the activation of donor macrophages, resulting in the release of proinflammatory cytokines and the development of I/R-ALI during the early phase of reperfusion [25]. That CUR or DXM could reduce levels of TNF-α and IL-1β after reperfusion indicates that they may inhibit activation of macrophages at the early phase of reperfusion. It seems that systemic levels of cytokines wer more sensitive to CUR pretreatment.

Oxidative stress plays an important part in experimentally induced reperfusion injury models [26] and IR-induced lung injury [27]. One important mechanism leading to the production of radicals is the accumulation of hypoxanthine and the conversion of the enzyme xanthine dehydrogenase into XO during anoxia, with the degradation of hypoxanthine into superoxide after reoxygenation [28]. XO derived superoxide formation accounts for major contribution of total superoxide produced during IR injury[26]. The recent study also showed XO played a prominent role in acute lung injury because of its ability to generate radicals, such as activation of lung XO contributed to the development of capillary permeability related to ventilator-induced lung injury [29]. Our results and others previously [30] demonstrated that only CUR could prevent from I/R-induced XO over-activity, which indicates that CUR may directly prevent the production of superoxide during lung I/R. Thus, CUR has the potential to improve oxidative stress induced lipid peroxidation reaction .

The balance between radical production and endogenous antioxidants is disturbed by increasing generation of radicals and reducing antioxidant defenses during I/R lung, resulting in severe oxidative stress, confirmed by a reduction in TAOC after IR injury. Antioxidant treatment should reverse oxidative stress and the subsequent inflammatory effects through NF-κB activation. The present study showed that CUR or DXM reduced the concentration of MDA during I/R, while only CUR showed preventive effects on I/R-induced reduction of TAOC activity. The inhibitory effect of DXM on I/R-induced lipid peroxidation in the lung may result from anti-inflammatory activity rather than from direct anti-oxidant activity.

CUR is a kind of monomer extracted from natural plants, while DXM is a drug by chemosynthesis. Their usage and dosage were both different in present experiment. CUR is liposoluble and need to be dissolved by DMSO. Although the present study showed preventive effects of both CUR and DXM, it should pay more attention to understand potential mechanisms by which CUR may be involved in the metabolism and balance of oxidative and anti-oxidants and to explore clinical application of CUR for preventing oxidative stress. It should be investigated whether CUR has preventive and therapeutic effects on long-term lung I/R injury, organ dysfunction and mortality rate of I/R-ALI.

In conclusion, the present study confirmed the protective effects of curcumin on I/R-induced lung inflammation, capillary barrier dysfunction, tissue edema and injury□similar to steroid. CUR reduces I/R-induced acute lung injury probably through improvement of oxidative stress and NF-κB-mediated expression of inflammatory cytokines. Thus, CUR may be an alternative of therapeutic strategies for improving the outcome of IR induced lung injury.

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Figure legends

FIGURE 1. Morphological evaluation of left lungs after 90 min ischemia and 4 h reperfusion. Lung histopathological slices were stained with conventional H&E. Magnification was 20 ×.

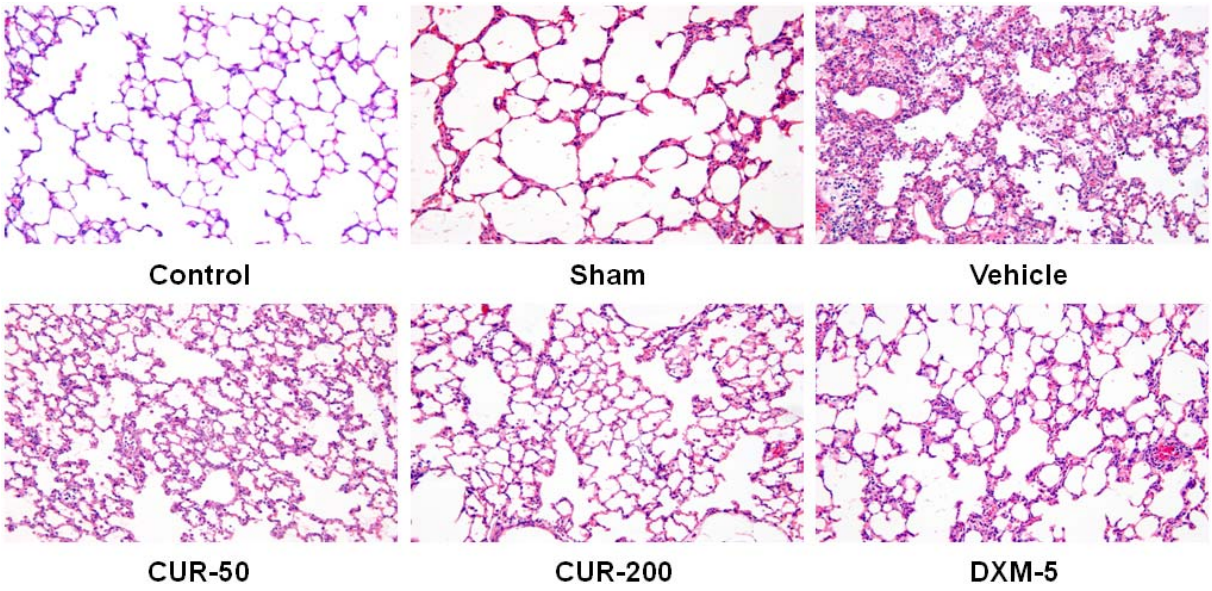


FIGURE 2. The arterial oxygen tension/inspired oxygen concentration (PO_2/FiO_2) ratio in left pulmonary vein (LPV) (A)and lung wet/dry weight ratio of the left lungs (B) and right lungs (C). ## stands for p□0.01 vs Control group, * and ** for p□0.05 and 0.01 vs. sham-operated group, and † and †† for p□ 0.05 and 0.01 vs. I/R-ALI group with vehicle, respectively.

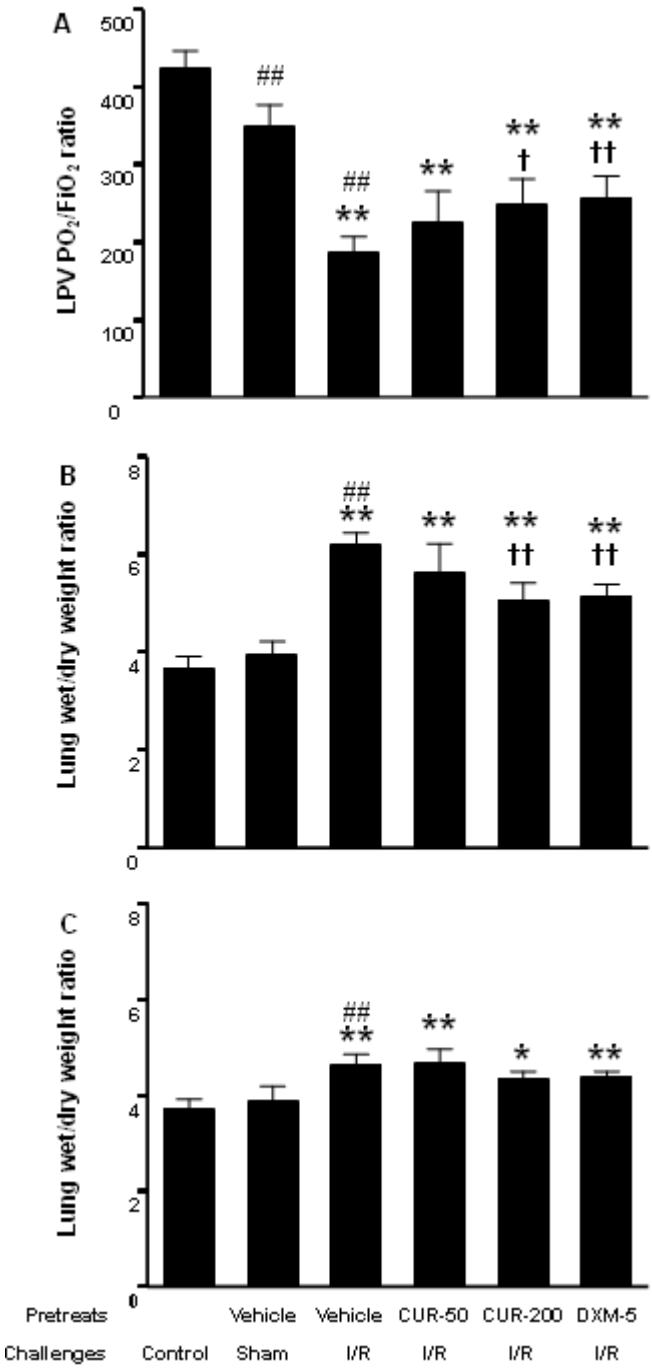


FIGURE 3. Assessment of the lung microvascular permeability by quantification of Evan's blue into the parenchymal tissue of left lungs (A) And right lungs (B). ## stands for p□0.01 vs Control group, ** for p□0.01 vs. sham-operated group, and †† for p□ 0.01 vs. I/R-ALI group with vehicle, respectively. Spontaneous red fluorescence of EBD noticed in lung tissue section (C). No extravasation of Evan's blue dye with a competent alveolar-capillary membrane was

observed in animals with sham operation and vehicle (C1), obvious extravasation of the dye into the parenchyma in I/R-ALI animals with vehicle(C2), and slight extravasation of the dye into the parenchyma in I/R-ALI animals with CUR at 200 mg/kg(C3).

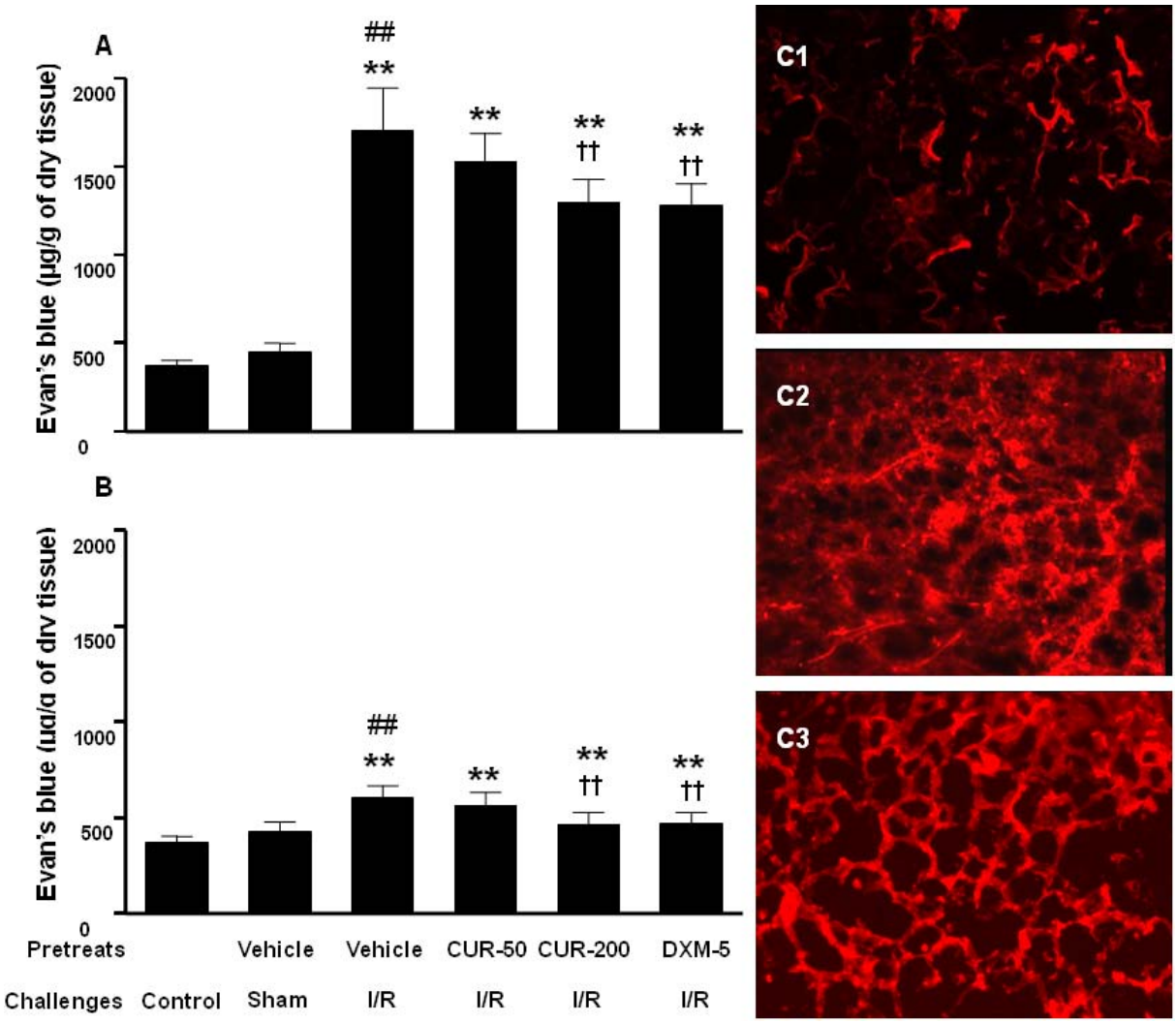
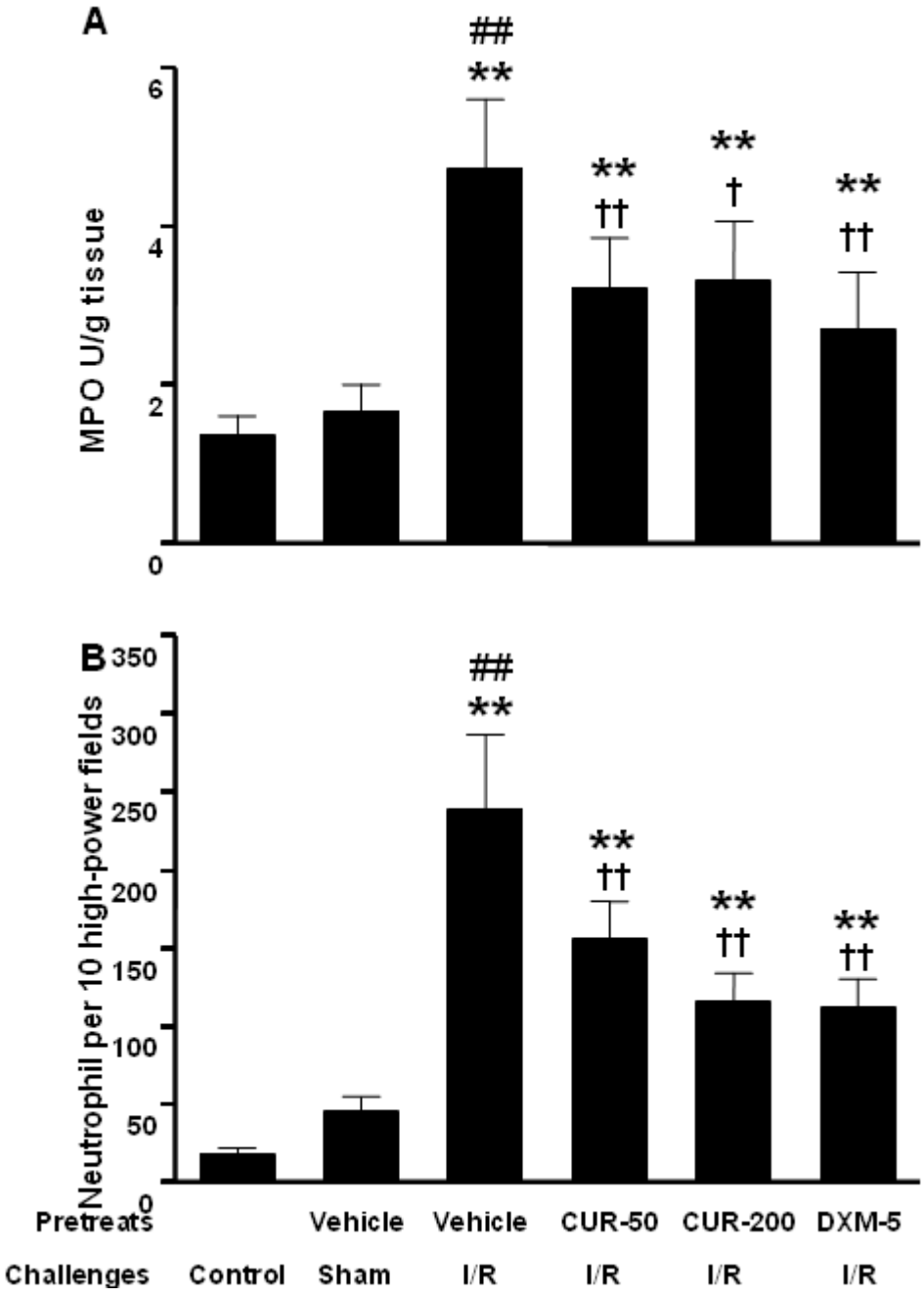


FIGURE 4. Neutrophilic infiltration assessed by lung myeloperoxidase (MPO) activity and number of neutrophils per 10 high-power fields of lung tissue. ## stands for p□0.01 vs Control group, ** for p□0.01 vs. sham-operated group, and †and †† for p□ 0.05 and 0.01 vs. I/R-ALI group with vehicle.



Tables

TABLE 1. Levels of xanthine oxidase (XO), malondialdehyde (MDA), and total anti-oxidative capability (TAOC) in lungs after 90 min ischemia and 4 h reperfusion.

Groups	XO	MDA	TAOC
	□U/g protein□	□nmol/mg protein□	□U/mg protein□
Control	9.31±1.32	0.72± 0.09	3. 05± 0.37
Sham	11.25±1.48	0.79± 0.12	2.60± 0.23
Vehicle	15.99±1.51$^{\#\#}$**	1.28± 0.15$^{\#\#}$**	1.82± 0.22$^{\#\#}$**
CUR-50	14.99±1.90**	1.07± 0.15**	1.91± 0.25**
CUR-200	12.48±1.32†	0.90± 0.06††	2.24± 0.19†
DXM-5	13.36±1.95	0.73± 0.12††	2.08± 0.28**

stands for p□0.01 vs Control group, ** for p□0.01 vs. sham-operated group, and † and p †† for p□ 0.05 and 0.01 vs. I/R-ALI group with vehicle.

TABLE 2. Levels of NF-κB activities, tumor necrosis factor (TNF) -α and interleukin (IL) 1 –β in lung tissue (t) and serum (s) after 90 min ischemia and 4 h reperfusion.

Groups	NF-κB	tTNF-α	sTNF-α	tIL1-β	sIL1-β
	□OD□	□pg/mg protein□	(pg/ml)	(pg/mg protein□	(pg/ml)
Control	0.30± 0.07	4.71±0.79	10.59±1.84	15.89±3.25	16.60±3.08
Sham	0.52± 0.13$^{\#}$	6.41± 0.36	13.14±1.76	21.18± 5.87	20.87±3.64
Vehicle	1.68± 0.15$^{\#\#}$**	16.48± 4.63$^{\#\#}$**	41.28±8.32$^{\#\#}$**	48.74± 8.97$^{\#\#}$**	59.08±10.59$^{\#\#}$**
CUR-50	1.40± 0.25**	12.18± 3.02*	28.27±4.66**††	40.34± 8.93**	37.28±7.31**††
CUR-200	1.11± 0.17**††	10.70± 2.58†	18.15±4.16††	35.76± 6.38*†	24.37±3.39††
DXM-5	0.98± 0.16**††	7.70 ± 2.00††	16.17±3.95††	29.67± 4.17††	25.21±4.42††

and ## stand for p□005 and 0.01 vs Control group, * and ** for p□0.05 and 0.01 vs. sham-operated group, and † and p †† for p□ 0.05 and 0.01 vs. I/R-ALI group with vehicle.