

EXPERIMENTAL STUDY

Effects of N-acetyl cysteine to improve acute lung injury in rats

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ABSTRACT

AIM: To study the mechanism of NAC to improve LPS induced acute lung injury.

METHODS: The 40 rats were divided into 4 groups included NC group, Model group, NAC group and DXM group. The rats of Model, NAC and DXM groups were injected LPS, NAC group and DXM group were respectively injected NAC (200 mg/kg) or DXM (70 mg/kg). Collecting the bronchoalveolar lavage fluid (BALF) from lung, and measuring the TGF- β 1 concentration of BALF and lung tissue in 4 groups; After executing the rats, taken the lung tissue to observe lung pathological morphology and evaluated TGF- β 1 expression of difference groups. Measuring the TLR-4 and NF- κ b in 4 groups by WB assay.

RESULTS: Compared with Model group, The NAC and DXM groups were improved in H&E staining, the TGF- β 1 concentration of NAC and DXM groups were significantly reduced in BALF and lung tissue ($p < 0.05$, respectively). TLR-4 and NF- κ b proteins of NAC and DXM groups were lower than that of Model group in IHC and WB assays ($p < 0.05$, respectively).

CONCLUSION: NAC had effects to protect LPS induced lung injury via TLR-4/NF- κ b signaling pathway (Fig. 5, Ref. 19). Text in PDF www.elis.sk.

KEY WORDS: NAC, TGF- β 1, TLR-4/NF- κ B signaling pathway, lung injury.

Introduction

Acute lung injury (ALI) is an inflammatory reaction characterized by massive neutrophil infiltration, extensive microvascular and alveolar epithelial damage, pulmonary edema, and pulmonary hemorrhage, and also is the early stage of acute respiratory distress syndrome (ARDS). Although the pathogenesis of ALI has been further understood in recent years, the mortality rate of ALI patients is still as high as 30–40% (1). Therefore, it is urgent to find effective methods to reduce the mortality of ALI patients.

N-Acetyl cysteine (NAC) is a structure containing a cysteine compound NAC with antioxidant, can inactivate the activity of oxidative metabolites, the structural stability of cell membrane and intracellular membrane, stable intracellular enzyme and protein function (2–6). This study investigated the effect of NAC on lipopolysaccharide induced lung injury and its mechanism.

Material and methods*Animals and reagents*

SD rats, SPF level, male, body weight (200 \pm 25) g (Shanghai silaike experimental animal limited liability company, China). LPS

(Sigma, USA, No. L2880-10MG, Batch number: BC86016X); NAC (Shanghai source leaf organism, China, No. S20137-25g, Batch number: H28J7S16826); DXM (Jiangxi bolaida Pharmaceutical Co., Ltd. China, No. 20160628), GL-21M High speed refrigerated centrifuge; BIO-RAD Electrophoresis tank and electrophoresis apparatus; Trizol Reagent (Invitrogen, USA). Antibody (Abcam, USA), Elisa kit (Sigma, USA).

Grouping and dose

The 40 rats were divided into 4 groups: Normal Control (NC) group (n = 10); Model group (Model) (n = 10); NAC treated group (NAC) (n = 10); Dexamethasone treated group (DXM) (n = 10). After the injection of LPS 12 h after exsanguination animal. With 20% urethane anesthetized rats, supine fixed on the operating table, model group sublingual intravenous injection of 5 mg/kg LPS in the rat model of acute lung injury, the control group normal saline to equal. NAC group were injected NAC (200 mg/kg) after injection LPS 1 h, DXM group were injected DXM (70mg/kg) after injection LPS 1 h, and Lung injury model group was given intravenous infusion of saline. After the animals were killed, they took a piece of lung tissue with sterile instruments and quickly frozen in liquid nitrogen, until using.

H&E staining

The fresh lung tissues were fixed by 10% (volume fraction) formaldehyde and embedded in paraffin sections. The morphological changes were observed by H&E staining with ordinary optical microscope.

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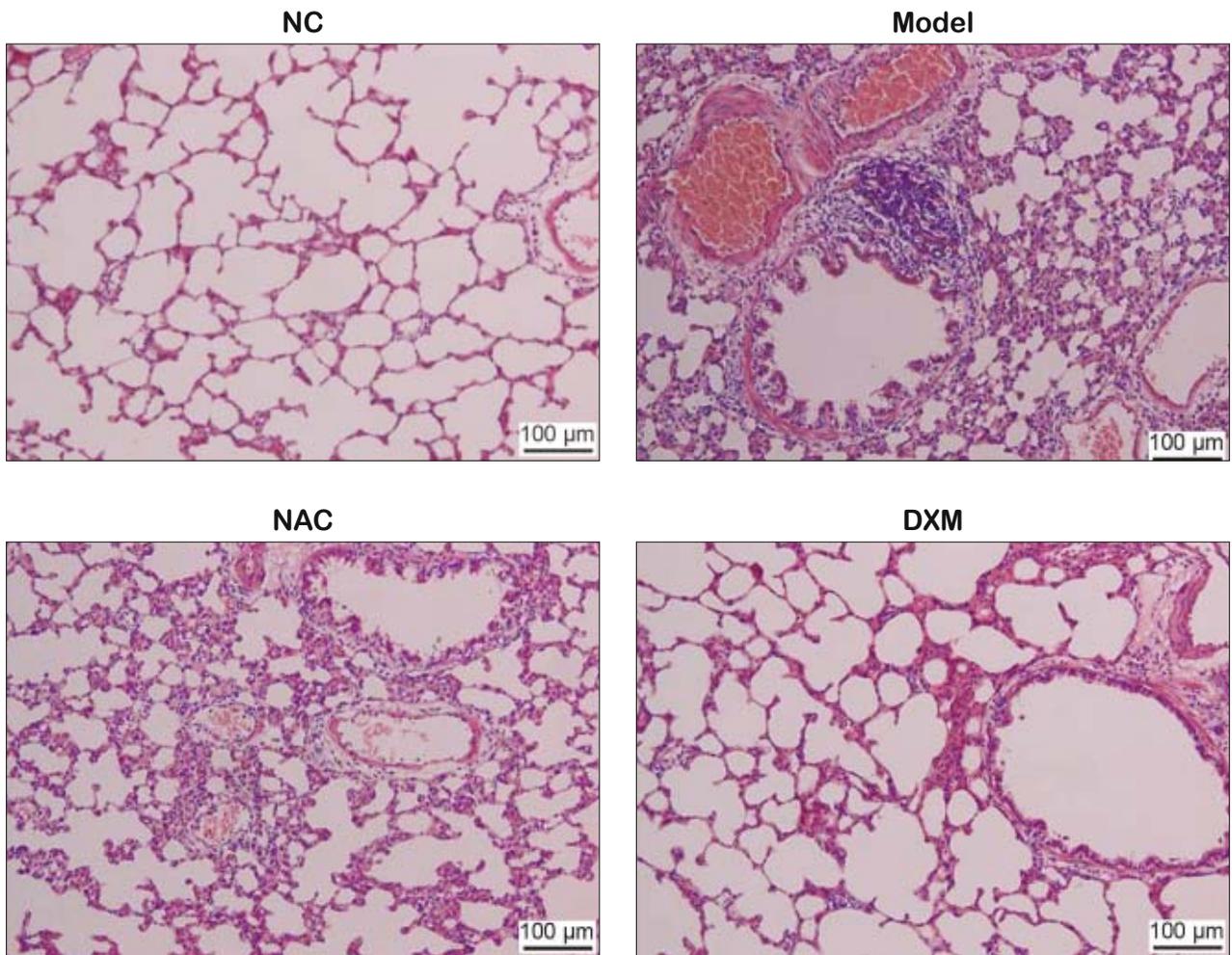


Fig. 1. The morphology of lung tissue in optical microscope ($\times 100$).

Elisa testing

The expression of TGF- $\beta 1$ in bronchoalveolar lavage fluid and lung tissue of rats in each group were detected according to the kit specification.

Western blotting (7)

According to the related literature introduction method, Bio-rad image analysis software was used to detect the optical density of strip, and GAPDH was used as the internal reference.

Immuno histochemistry

Each group of mice were left lung, 10 % Formaldehyde Solution fixed, routine paraffin sections after immunohistochemical analysis of lung tissue TLR-4 and NF- κ b expression levels. B I2000 image analysis system was used to record the number of positive cells.

Statistical processing and analysis

Data are presented as the mean \pm standard deviation (SD) values. Statistical analyses between the two groups were evalu-

ated using one way ANOVA with SPSS 19.0 (SPSS Inc., Chicago, USA). $p < 0.05$ was considered statistical significant.

Results

H&E staining

Observed lung tissue, NC group, NAC and DXM group showed no abnormal lung appearance changes; The lung volume of model group increased, dark red, scattered in the size of the red spots, cut loose; There was red liquid overflow; NAC and DXM intervention group than the model group lesions alleviated. The morphological changes of lung tissue were observed by light microscope. The control group, NAC and DXM intervention group had intact lung structure, no alveolar edema, clear alveolar space. In the model group, the lung tissues were alveolar space widened, a little bleeding, exudation and inflammatory cell infiltration, interstitial congestion and edema, infiltration of inflammatory cells. NAC and DXM intervention group lung tissue inflammatory cells infiltration, exudation

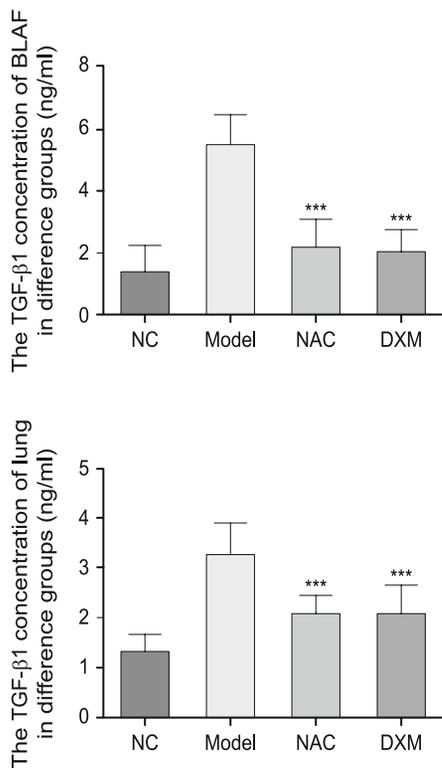


Fig. 2. The TGF-β1 concentration of difference groups in BLAF and lung tissue, ***: $p < 0.05$, compared with Model group.

and bleeding and other morphological changes than the model group (Fig. 1).

TGF-β1 concentration of lung tissue and BALF

Compared with Model group, the TGF-β1 concentration of NAC and DXM groups were significantly reduced in lung tissue and BALF ($p < 0.05$, respectively). The data was shown in Figure 2.

IHC assay

That phosphorylation of TLR-4 and NF-κb in the cytoplasm showed light brown light microscope, in the expression of many parts, such as the nuclear envelope, dark brown (Figs 3 and 4). Compared with NC group, TLR-4 and NF-κb positive degrees of Model group were significantly increased ($p < 0.05$, respectively); Compared with Model group, The TLR-4 and NF-κb positive degrees of NAC and DXM groups were significantly down-regulation ($p < 0.05$, respectively). There were no significantly differences between NAC and DXM groups.

WB assay

The NF-κB and TGF-β1 protein expression of NAC and DXM groups were significantly lower compared with those of Model group ($p < 0.05$, respectively). The data was shown in Figure 5.

Discussion

ALI is caused by a variety of predisposing factors except cardiogenic the acute hypoxemic respiratory disorder, with similar

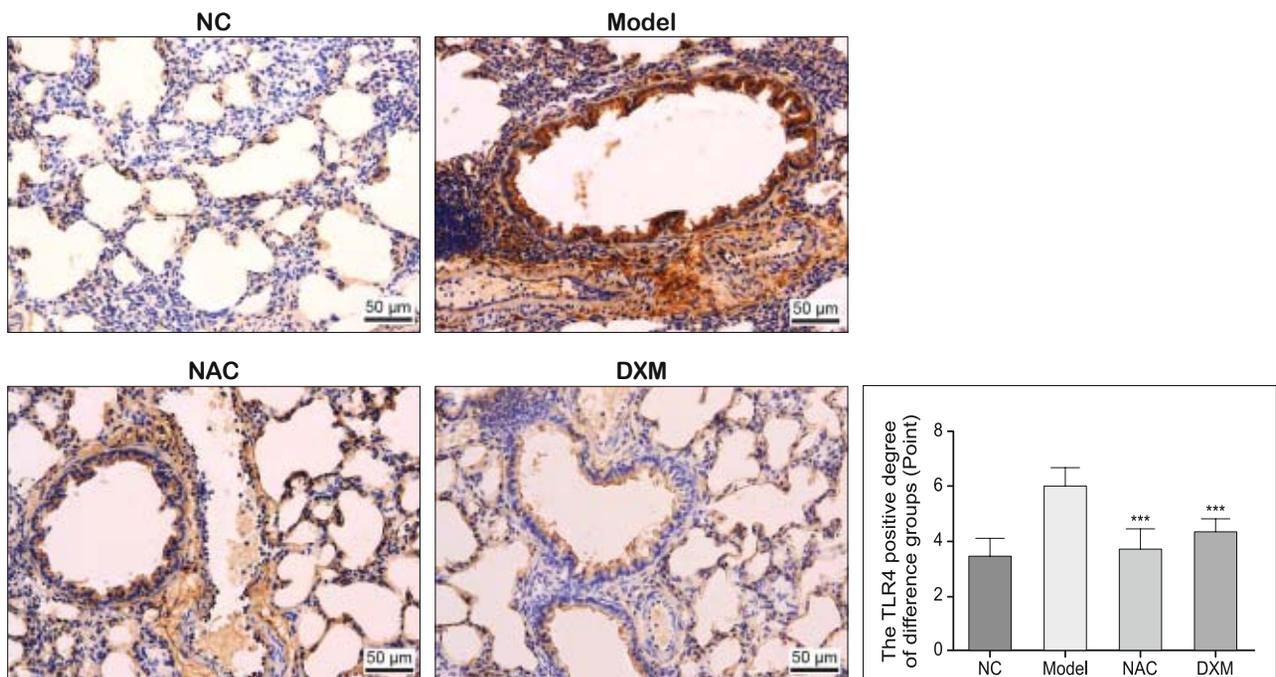


Fig. 3. The immunohistochemical staining by using TLR-4 antibody in the lung tissue of mice 12 h after resectively treated ($\times 200$). ***: $p < 0.05$, compared with Model group.

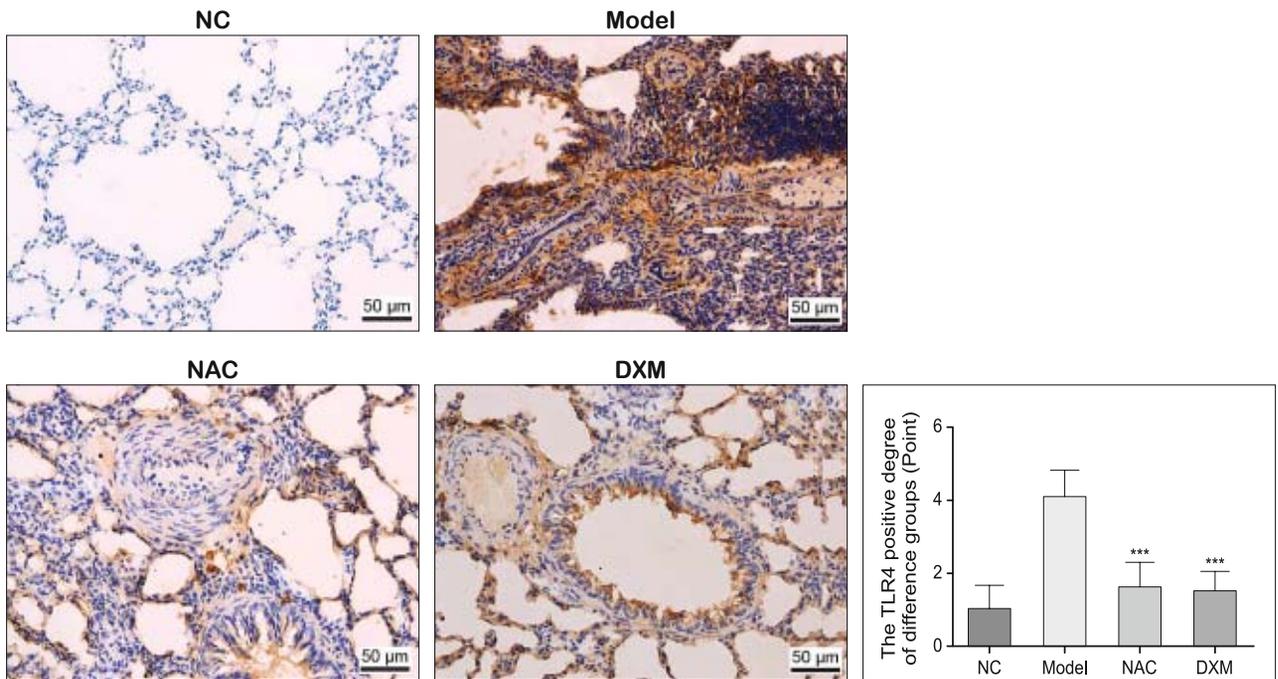


Fig. 4. The immunohistochemical staining by using NF- κ B antibody in the lung tissue of mice 12 h after resectively treated ($\times 200$). ***: $p < 0.05$, compared with Model group.

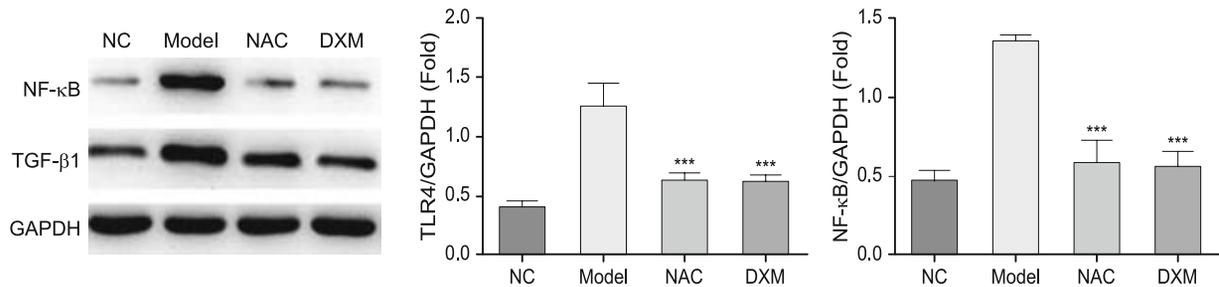


Fig. 5. The relative protein expression of difference groups. ***: $p < 0.05$, compared with Model group.

pathophysiological changes and ARDS, but its pathogenesis has not been fully elucidated. In recent years, it is believed that the inflammatory mediators induced by pulmonary inflammatory cell aggregation and pulmonary capillary membrane permeability increase are the main causes of ALI.

The present study showed that the expression of TGF-1 was significantly up-regulated in lung tissue of rats with acute lung injury, which may be activated by acute lung injury, mainly monocytes, releasing a large number of TGF- β 1. However, TGF- β 1 is monocyte chemotactic factor, could make more mononuclear cells, fibroblasts and mast cell accumulation at the site of injury, resulting in more TGF- β 1 in local, and by increasing the number of inflammatory cells and retention time, promote inflammatory reaction (8). In addition, TGF- β 1 can induce fibroblast proliferation, differentiation and promote collagen synthesis, which may be an important reason for the formation of pulmonary fibrosis after acute lung injury. Related studies had shown that hypoxia

could induce increased expression of TGF- β 1 in pulmonary vessels, and TGF- β 1 could induce the differentiation of fibroblasts into myoblasts leading to the occurrence of hypoxic pulmonary vascular remodeling (9, 10). Therefore, the increasing TGF- β 1 expression played an important role in the occurrence and development of ALI, and targeting it for treatment is expected to delay the occurrence of ALI and reduce the severity of ALI.

TGF-1 was a multifunctional cytokine which played an important role in pulmonary fibrosis, pulmonary hypertension, chronic obstructive pulmonary disease and acute lung injury (11–13). Research shows: In alveolar epithelial and pulmonary artery endothelial cells, TGF- β 1 was able to consume reduced glutathione (GSH), thereby reducing its concentration. In cultured fibroblasts, glomerular epithelial cells TGF- β 1 also reduced GSH concentration (14–16). NAC is a kind of strong antioxidant, the molecule contains SH functional group, it can oxidize and reduce the two sulfur bond in biological macromolecules, thus directly protect-

ing the activity of biological macromolecules. In addition, NAC enters the cells after deacetylation, formation of cysteine, glutathione synthetase in under the action of the formation of glutathione (GSH), increase the content of GSH in tissues, indirect protective effect. This study found that NAC could effectively inhibit the expression of TGF- β 1 in lung tissue.

TLR-4/NF- κ B signaling pathway plays an important role in regulating the gene expression of inflammatory mediators involved in ALI, TLR-4/NF- κ B signaling pathway stimulation is closely related to the occurrence of ALI, NF- κ B plays a key role in intracellular signal transduction, The injury of related tissues induced by inflammatory could be reduced by inhibiting TLR-4/NF- κ B expression (17–19). In our study, we found that NAC had effects to reduce TGF- β 1 concentration in BALF and lung tissue by suppressing TLR-4/NF- κ B expression.

In conclusion, NAC had effects to improve lung injury (reduce TGF- β 1 concentration) by suppressing TLR-4/NF- κ B signaling pathway.

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