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Glucocorticoid combined with hyaluronic acid enhances glucocorticoid receptor activity through inhibiting p-38MAPK signal pathway activation in treating acute lung injury in rats

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Abstract. – OBJECTIVE: In order to seek an effective strategy for clinical treatment of acute lung injury (ALI), we are committed to explore the effect of combination therapy of glucocorticoid and hyaluronic acid on acute lung injury caused by an endotoxin (LPS) and its mechanism.

MATERIALS AND METHODS: Adult male Sprague-Dawley (SD) rats were divided randomly into 5 groups: normal group (n=8); LPS group (n=8); dexamethasone +LPS group (DXMS group, n=8); hyaluronic acid+ LPS group (HA group, n=8); dexamethasone +hyaluronic acid +LPS group (DXMS+HA group, n=8). Firstly, SD rat model with acute lung injury induced by LPS was established, and injected corresponding drugs according to the plan. Then, the expression of TNF-α, IL-8, IL-10, ICAM-1 and total protein were measured by ELISA, and the HE staining was used for detected the pathological change in lung tissue. Subsequently, the water content, dry and wet ratio and permeability in lung tissues of SD rats was assayed. Finally, the expression level of the glucocorticoid receptor (GR) was detected by RT-PCR, and activation of p-p38MAPK was determined by Western blotting.

RESULTS: The results showed that concentration of IL-8, IL-10 and ICAM-1 was significantly increased in BALF after LPS injection, and the results from HE staining showed it had widespread inflammation. However, lung structures in SD rats with inhalation lung injury were improved significantly after the injection of dexamethasone and hyaluronic acid, and the Pa02/Fi02, blood pressure and Cdyn were also increased. Moreover, lung water content, the ratio of wet and dry lung, and lung permeability index (LPI) was decreased after having treated the SD rats with a combination of dexamethasone and hyaluronic acid, and the apoptosis index was also decreased in the rats with LPS-induced ALI. Our data also suggested that TNF-α, IL-8, IL-10, intercellular cell adhesion molecule-1 (ICAM-1) and total protein was significantly declined in bronchoalveolar lavage fluid (BALF) of rats with LPS-induced acute lung injury after treated the SD rats with a combination of dexamethasone and hyaluronic acid. In addition, the data also implied that anti-inflammatory effect by inhibiting the activation of p38MAPK signal pathway induced by LPS through enhancement of the activity of GR, to further analyze the mechanism of the effect of combination therapy with dexamethasone and hyaluronic acid on acute lung injury in SD rats.

CONCLUSIONS: LPS-induced ALI in SD rats is relieved after treatment with a combination of dexamethasone and hyaluronic acid. In the process of its function, activated GR can represent anti-inflammatory effect and protect the lung tissue by inhibiting the activation/phosphorylation of p38MAPK, while hyaluronic acid can enhance micro-environment of alveolar tissue.

Key Words:
Glucocorticoid, Hyaluronic acid, Lipopolysaccharide, p38MAPK, Acute lung injury.

Introduction

Acute lung injury (ALI) is referred to structural damage and severe oxygenation dysfunction induced by a variety of pathogens, mainly presented as a clinical syndrome of respiratory distress and dispersive lung infiltration. ALI is developed rapidly in the clinic, and multiple organ dysfunction syndrome (MODS), as well as multiple organ failure (MOF), is induced by the end-stage of ALI, it can reach death rate of 40%-60%[1,2]. The pathogenic mechanism of ALI is the successively inflammatory reaction, and deposit of fibrous protein in lung tissue and vessel cavity caused by hypercoagulative state and low level of fibrinolytic function is one of its features[3]. Deposition of fibrous protein occurs not only in the restoring stage, but starts in the early stage, which decides the degree of fibrous proliferation. Deposition of the extravascular fibrous matrix is mainly located

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in alveolar cavities, namely hyaline membrane formation, which is a significant pathological feature of ALI. Now, many researchers[4] have considered that the abnormality of hypercoagulative state, fibrinolytic function and extravascular deposition of the fibrous matrix is closely relevant to the development of ALI.

Lipopolysaccharide (LPS), the main component of cell walls of G^- bacteria, is the major cause of the inflammatory reaction. After causing, LPS will cause septicemia, systemic inflammatory response syndrome and ALI. And the biological features of the lung make it the first and most attacked organ by LPS[5,6]. There is no effective treatment strategy has yet proved in declining its mortality rate despite the introduction of clinical treatments.

Besides the above treatments, glucocorticoids (GCs) are also widely used in clinical treatment of ALI[7,8]. Liu et al[9] have reported that CGs can affect multiple stages of ALI as an anti-inflammatory drug. It can sustain the stability of cell membrane, and decrease capillary permeability, eventually prevented cells from autolysis and necrosis. Müller et al[10] also reported that CGs might alleviate cell damage by inhibiting excessive inflammation and decreasing the release of endocrine hormones like catecholamine and inflammatory factors. However, the side effects of GCs, especially the effects on the immune system, has limited the feasibility of long-term usage of the medicine. Wigenstam et al[11] implied that the damage on the immune system could be prevented by controlling the dosage.

Anti-inflammation and/or anticoagulation is a common strategy in the treatment of ALI by further analysis of the pathogenesis of ALI. Saadat et al[12] showed that GCs had an anti-inflammatory and anti-coagulant effect. Yıldız et al[13] reported that hyaluronic acid (HA) could enhance the surface-activity in alveolus and antagonize the inhibitive effect of some inhibitors, respiratory mechanical parameters and gas exchange were significantly improved in the treatment of meconium-caused ALI when HA was used.

To explore an effective strategy for treatment of LPS-induced ALI, we combined dexamethasone with HA (both of lowered dosage); we expected that the strategy could enhance the efficiency of the drugs while lowering their side effects. In this study, we evaluated the efficacy of the two drugs by comparing the effect of the combination therapy. Then, we analyzed the mechanism of the combination therapy, and explore the effect of combination therapy on lung function and inflammatory reaction in LPS-induced ALS, thus evaluated its efficacy in the treatment of ALS, providing the basis for its clinical application.

Materials and Methods

Animals and Materials

Fifty healthy adult male Sprague-Dawley (SD) rats of specific pathogen free (SPF) grade with a body weight of 200-220 g were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. RNA extraction reagent (Trizol), LPS, hyaluronic acid for injection and dexamethasone were purchased from Sigma-Aldrich, St. Louis, MO, USA.

The reverse-transcription kit was purchased from Toyobo (Osaka, Japan) and the qRT-PCR kit was purchased from Takara (Otsu, Shiga, Japan). p38MAPK primary antibody, p-p38MAPK primary antibody, GC primary antibody was purchased from Santa Cruz Co (Santa Cruz, CA, USA). TUNEL kits were purchased from Takara (Otsu, Shiga, Japan). TNF-α, IL-8, IL-10, ICAM-1 ELISA assay kits were purchased from Millipore (Bedford, MA, USA).

Model Constructing and Grouping

Adult male SD rats were randomly divided into 5 groups, normal group (n=8), which were only injected with normal saline; LPS group (n=8), which were injected into 5 mg/kg LPS by tail vein injection; dexamethasone + LPS group (DXMS group, n=8), which were injected into 2 mg/kg dexamethasone before the LPS injection 30 min by intraperitoneal injection; hyaluronic acid+ LPS group (HA group, n=8), which were injected into 2 ml/kg hyaluronic acid before the LPS injection 30 min by intraperitoneal injection, dexamethasone +hyaluronic acid +LPS group (DXMS+HA group, n=8), which were injected into 2 mg/kg dexamethasone and 2 ml/kg hyaluronic acid before the LPS injection 30 min by intraperitoneal injection. Then, the SD rats were cultured into cages for 6h for a later experiment. Dosages and methods of administration in the experiment were in accordance with literature[14].

Collection of Bronchoalveolar Lavage Fluid (BALF)

SD rats were sacrificed at 12h, 24h and 36h after LPS injection by exsanguination, respectively. The lungs were exposed through a thoracotomy,

the right hilum of the lung was ligatured. Then 5ml pre-cooled PBS was used for lavaging the left lung for 3 times, recycling 90% of the lavage fluid. The bronco alveolar lavage fluid (BALF) was recycled and stored at -80°C.

Detection of GR Activity Using Electrophoretic Mobility Shift Assay (EMSA)

The probe sequence of the glucocorticoid receptor (GR) was showed as follows: P1: 5'-GTACAGGATGTTCT-3'; P2: 5'-AGAACATCCT-GTAC-3'. The probes were synthesized by Shanghai Shenggong Company. The marking of probes and extraction of nucleoprotein was conducted in accordance to the protocol of Gel Shift Assay Kit. 3 μg nucleoprotein, 10 μl DNA with buffer and 1 μl labeled probe were diluted with H_2O into a reaction volume of 20 μl. 4 μl loading buffer was added after incubation 30 min at 37°C. Reaction product has undergone 5% native polyacrylamide gel electrophoresis (PAGE). The gel was placed at -70°C for autoradiography. Retardant bands were density scanned. Then, the optical density of the junctional zone presented GR activity.

Analysis of cell Apoptotic Index

TUNEL method was used for detecting the epithelial cell in the BALF.

We detected apoptosis of the epithelial cell in the BALF using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). The procedure was followed as the kit instruction. Apoptosis index (AI) was calculated as follow, the percentage of positive cells in 500 cells by counted 5 high power fields (×200) of each slide.

Detection of Lung Tissue Sections Using HE Staining

The lung tissues were fixed by 10% formaldehyde solution and douched using a saline buffer. Then it was dehydrated with 95%-100% alcohol in automatic hydro-extractor at room temperature for overnight. The pathological tissues were sliced slices into 4 μm. The slices were dewaxed using xylene for 10 min. Afterwards, it was douched by the saline buffer. The slices were stained with hematoxylin for 5 min and then 0.5% eosin for 30s. Then, the slices were dehydrated using alcohol again and dealcoholized with xylene. Neutral gum was dropped on it and the cover ship was replaced. The slices were observed under light microscope and photographed.

Detection of Wet/Dry Ratio (W/D) in Lung Tissue

The anterior right lobe of the lung was taken out from rat after it was sacrificed. The blood on the surface was rapidly wiped with filter paper. Then the lung tissues were weighed, which was named wet weight of lung tissue (*W*). When the lung tissues were baked until constant weight at 80°C and weighed on an electronic balance, which was named dry weight (*D*). The wet/dry ratio of lung tissue = W/D.

Detection of TNF-a, IL-8, IL-10 and ICAM-1 content by ELISA

Total serum TNF-α, IL-8, IL-10 and ICAM-1 content were determined using highly specific enzyme-linked immunosorbent assays (ELISA) (eBioscience Inc., San Diego, CA, USA). All samples were performed thrice. Standard curves were produced by the OD values of the standard samples, the TNF-α, IL-8, IL-10 and ICAM-1 content were calculated.

Detection of GC mRNA Expression Using RT-PCR

Total RNA was extracted from lung tissue of SD rats. The quality of RNA was determined by Nano Drop-2000 (Thermo Scientific, Waltham, MA, USA). For GR mRNA detection, we designed a specific RT primer specifically hybridizing with GR according to the GR sequence from NCBI database. GADPH was the normalized internal reference gene.

Detection of GC, p38MAPK and p-p38MAPK Protein Expression Using Western blotting

The samples were collected and extracted with radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific, Waltham, MA, USA) according to the manual procedures. Total protein samples were separated through 8-12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). The primary antibodies GC (Abcam, Cambridge, UK; rabbit, 1:500), p38MAPK (Cell Signaling Technology, rabbit, 1:1000), p-p38MAPK (Cell Signaling Technology, rabbit, 1:1000), GAPDH (Cell Signaling Technology, rabbit, 1:1000). The membranes were incubated with primary antibody at 4°C for overnight. The next day they have been incubated with horseradish peroxidase-conjugated secondary antibody

Table I. The gas exchange indices assay of SD rats with ALI (x±s)

Index	Normal	ALI	DXMS	HA	DXMS+ HA
PaO_2 (mmHg)	103.43±6.82	81.89±2.46#	95.35±3.25*	93.94±1.93*	99.38±2.14*
$PaCO_2$ (mmHg)	45.87±1.39	59.91±2.86#	55.36±2.76*	54.89±1.98*	58.83±2.74*
PH	7.40±0.05	7.25±0.09#	7.35±0.06*	7.34±0.04*	7.40±0.05*
PaO_2/FiO_2	512.39±23.83	335.93±18.94#	450.24±21.38*	472.84±26.61*	500.12±19.36*
Blood pressure(mmHg)	18.35±0.58	13.59±0.74#	15.89±0.37*	16.13±0.18*	17.78±0.41*
Cdyn[L/(kg.pa)]	0.0081±0.0007	0.0038#±0.0007	0.0070±0.0008*	0.0071±0.0006*	0.0079±0.0005*

Note: #means $p<0.01$ compared to Normal group; *means $p<0.01$ compared with ALI group.

for 2 hours. The bound antibody was detected with a chemo-fluorescence detection kit (Amersham, Piscataway, NJ, USA). The representative images were shown.

Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data was represented as mean ± standard deviation ($\bar{x} \pm s$). The data were analyzed by normal distribution and homogeneity of variance test. Pairwise comparisons were proceeded using LSD-t test. $p < 0.05$ was considered as significant difference.

Results

Combination Therapy of Dexamethasone and Hyaluronic Acid Improved Pathological Structures of Lung Tissue in SD Rats with Ali Induced by LPS

To study the pathological effect of combination therapy of dexamethasone (DXMS) and hyaluronic acid (HA) in the lung tissue of SD rats, we observed the pathological structure of lung tissue of SD rats in different groups under a microscope. There was no inflammatory cells infiltration in the alveolar and interstitial spaces of the SD rats in the normal group. However, the alveolar structure was damaged severely in ALI group, alveolar collapsed, pulmonary interstitial edema broadened, and a large number of red blood cells and inflammatory cells infiltrated in the alveolar cavity. We also found that the alveolar structure is partly destroyed, pulmonary in interstitial edema, and there are a number of red blood cells and inflammatory cells scattered in the alveolar cavity in the DXMS group. Then, the alveolar structure was damaged in HA group, there was pulmonary edema, and pulmonary interstitial was broadened, and a large number of red cells were distributed in the alveolar and interstitial spaces of the lung. Moreover, the alveolar structure in DXMS+HA group was relatively complete, only had slight edema in the interstitium, and there was no evident infiltration of red cells and inflammatory cells in the alveolar cavity (Table I).

PaO_2/FiO_2, Blood Pressure and Cdyn were up-Regulated significantly After Receiving the Combination Therapy of Dexamethasone and Hyaluronic Acid in SD Rats with ALI Induced by LPS

Our data showed that PaO_2/FiO_2 was 512.39 ± 23.83 in the normal group by blood gas analysis, much higher than the ALI group, which was 335.93 ± 18.9 ($p < 0.05$). After injection of DXMS or HA, the data showed that PaO_2/FiO_2 were up-regulated, which were 450.24 ± 21.38 and 472.84 ± 26.61, respectively, which were still lower than that the normal group ($p < 0.05$, $p < 0.05$). However, PaO_2/FiO_2 in DXMS+HA group was up-regulated significantly compared to the ALI group, which was slightly lower than that the normal group ($p < 0.01$). Also, PH values were similar among all groups. Our findings also showed that blood pressure in the ALI group was significantly lower than the normal group ($p < 0.05$), and blood pressure in the DXMS group and the HA group was up-regulated slightly, while the difference between that of the DXMS+HA group and the normal group was insignificant ($p > 0.05$) (Table I).

Combination Therapy of Dexamethasone and Hyaluronic Acid can Reduce Significantly lung water content, Lung W/D and LPI in rats with ALI Induced by LPS

Our results revealed that lung water content in the ALI group (82.72 ± 3.03%) was significantly

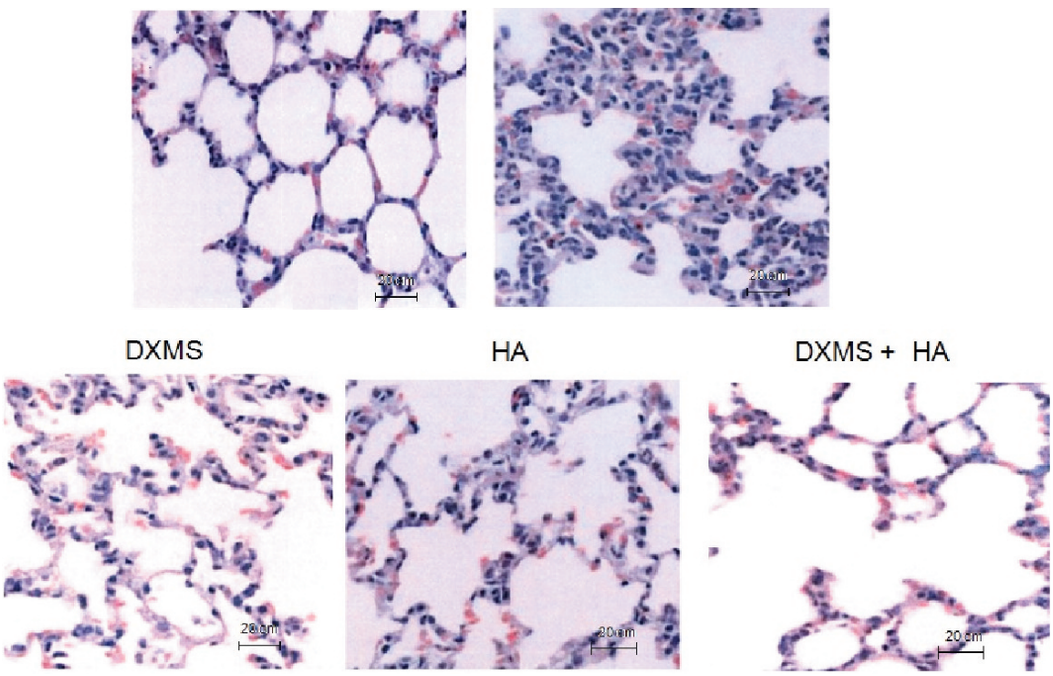

Figure 1. The pathological change of lung tissue after combination treatment of DXMS and HA under 400× light microscope.

higher than the normal group ($p < 0.01$). Lung water content in the DXMS group (75.93 ± 2.73%), the HA group (77.31 ± 2.58%) and the DXMS+HA group (75.22 ± 1.95%) were all significantly lower than the ALI group ($p < 0.05$) (Table II). In addition, the lung W/D in the ALI group (4.47 ± 0.32) was significantly higher than the normal group (3.74 ± 0.21) ($p < 0.01$). Lung W/D in DXMS group (3.93 ± 0.28), HA group (3.89 ± 0.19) and DXMS+HA group (3.79 ± 0.13) were all significantly lower than that the ALI group ($p < 0.01$). Moreover, LPI in the ALI group (3.59 ± 0.31) was significantly higher than that the normal group (1.87 ± 0.13) ($p < 0.01$). And LPI in the DXMS group (2.34 ± 0.28), HA group (2.59 ± 0.26) and DXMS+HA group (2.01 ± 0.23) were all significantly lower than that the ALI group ($p < 0.01$). Our study indicated that combination therapy of DXMS and HA could relieve LPS-induced ALI by stabilizing the blood pressure and quickly alleviating lung damage and edema.

Combination Therapy of Dexamethasone and Hyaluronic acid Can Decrease the Apoptotic Index of SD rat ALI Induced by LPS

In order to further study the effect of the combination therapy of dexamethasone and hyaluronic acid on the apoptotic index of rats with ALI, the apoptotic index of SD rats in different groups was assayed. Apoptotic index in ALI group (16.99 ± 2.34%) was significantly higher than that the normal group (3.01 ± 0.14%) ($p < 0.01$). The apoptotic index in DXMS group (11.67 ± 1.23%) and HA group was significantly lower

Table II. The ventilation indices assay of SD rats with ALI in different groups (x±s).

Index	Normal	ALI	DXMS	HA	DXMS+ HA
Lung water content (%)	74.28±2.97	82.72±3.03#	75.93±2.73*	77.31±2.58*	75.22±1.95*
W/D	3.74±0.21	4.47±0.32#	3.93±0.28*	3.89±0.19*	3.79±0.13*
lung permeability index(×103)	1.87±0.13	3.59±0.31#	2.34±0.28*	2.59±0.26*	2.01±0.23*

Note: #means $p<0.01$ compared to normal group; *means $p<0.01$ compared to the ALI group.

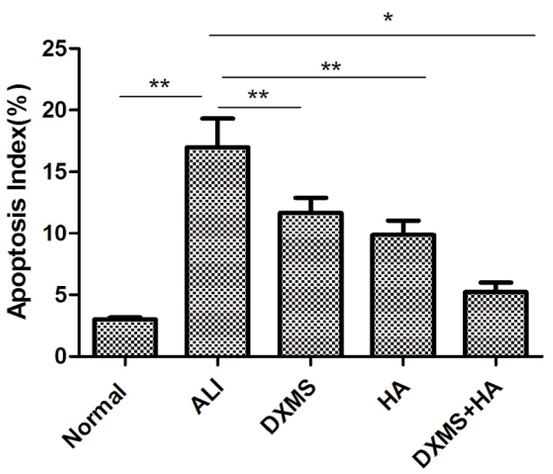

Figure 2. Effect of combination therapy of glucocorticoid and hyaluronic acid on apoptosis index of ALI rats. $^{*}p<0.05$, $^{**}p<0.01$.

than that the ALI group (9.88 ± 1.18%) ($p < 0.01$). However, there was rarely a difference between DXMS group and the HA group for the apoptotic index ($p > 0.05$). In addition, the apoptotic index in DXMS+HA group was 5.22 ± 0.77%, which was significantly lower than the ALI group, the DXMS group and HA group ($p < 0.01$, $p < 0.05$, $p < 0.05$), which was similar to the normal group ($p > 0.05$) (Figure 2). Our research revealed that the combination therapy of DXMS and HA could remarkably decrease lung damage by lowering the apoptotic index of SD rats with ALI.

Combination therapy of dexamethasone and Hyaluronic Acid Decreases the total Protein and concentration of TNF-α, IL-8, IL-10 and ICAM-1 in BALF in SD rats with LPS-Induced ALI

In order to measure the inflammation in the lung of SD rats with ALI after the combination therapy of dexamethasone and hyaluronic acid. Our results displayed that the total protein and concentration of TNF-α, IL-8, IL-10 and ICAM-1 in BALF of the ALI group were all significantly increase in SD rat with ALI induced by LPS, compared to the normal group ($p < 0.01$), which proved SD rat ALI models were successfully prepared. Moreover, the concentration of TNF-α IL-8 and ICAM-1 in DXMS group, HA group and DXMS+HA group were all significantly lower than that the ALI group after surgery 12h, 24h and 36h ($p < 0.05$, $p < 0.05$, $p < 0.05$), and only slightly higher than that the normal group. Total proteins in the DXMS+HA group were significantly lower than that the ALI group after surgery 12h and 36h ($p < 0.05$) (Figure 3). Our research revealed that the combination therapy of DXMS and HA could markedly relieve ALI of SD rats by inhibiting the release of pro-inflammatory cytokines.

Combination Therapy of Dexamethasone and Hyaluronic acid up-regulates GR Activity in SD Rats with LPS-Induced ALI

In order to explore the mechanism of combined treatment, our study displayed that GR activity of the ALI group was significantly decreased, compared to the normal group ($p < 0.05$), and the GR activity in SD rats with ALI was significantly promoted in the combination therapy of DXMS and HA ($p < 0.01$). However, when only treatment using DXMS or HA, our data showed that the GR activity up-regulated in the DXMS group ($p < 0.05$), while the effect of HA was insignificant ($p > 0.05$) (Figure 5A). Then the expression of GR mRNA and protein in lung tissue of SD rats with ALI were detected. Our results displayed that neither the combination therapy of DXMS and HA nor the treatment of dexamethasone alone showed significant effect could up-regulate the GR mRNA and protein expression in SD rats with ALI (Figure 5B and 5C). In addition, compared to the DXMS group, the GR activity was remarkable decreased in the DXMS+HA group.

Dexamethasone combined with hyaluronic Acid Inhibits the Activation of p38MAPK Pathway induced by LPS by Enhancing the Activity of GR

Previous studies have shown that p38MAPK pathway and NF-kB pathway are important in anti-inflammation reactions, but the mechanism of the anti-inflammation effect of DXMS combined with HA is still unknown. We detected the phosphorylation of p38MAPK and NF-kB in different by western blotting. Our findings showed that the p-p38MAPK expression in ALI group was significantly up-regulated ($p < 0.05$), compared to the normal group, and the expression of p-p38MAPK in DXMS group and DXMS+HA group was all significantly down-regulated, compared to the ALI group ($p < 0.05$, $p < 0.05$) (Figure. 5). However, the expression of NF-kB among groups was rarely statistically significant ($p >$

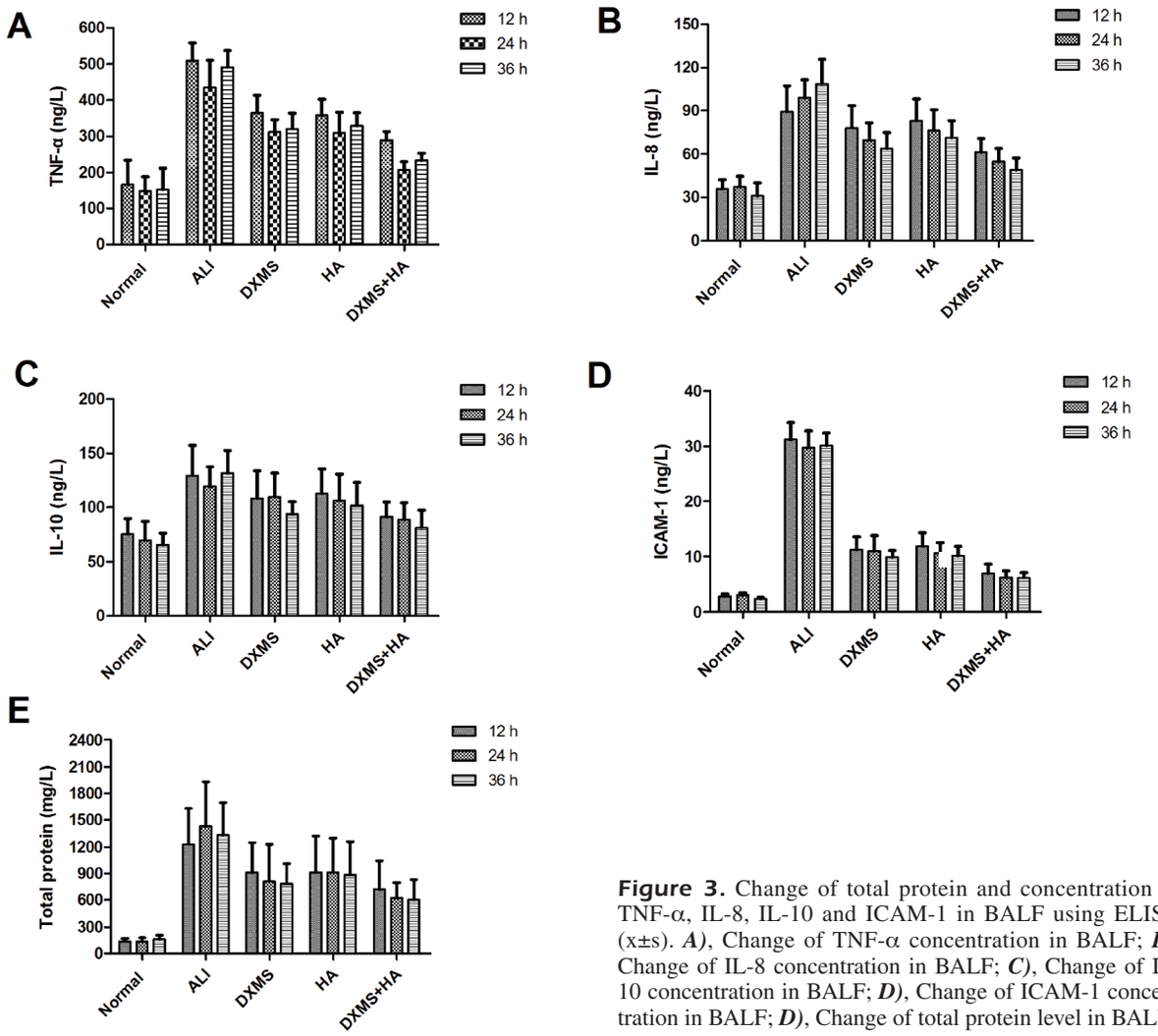

Figure 3. Change of total protein and concentration of TNF-α, IL-8, IL-10 and ICAM-1 in BALF using ELISA (x±s). ***A)***, Change of TNF-α concentration in BALF; ***B)***, Change of IL-8 concentration in BALF; ***C)***, Change of IL-10 concentration in BALF; ***D)***, Change of ICAM-1 concentration in BALF; ***D)***, Change of total protein level in BALF.

0.05). Our work implied that DXMS combined with HA could inhibit the activation of p38MAPK pathway induced by LPS by enhancing the activity of GR.

Discussion

Acute lung injury (ALI) is a common illness in the clinic, whose basic characteristics are the extensive inflammatory reaction in lung marked by the excessive release of inflammatory factors[15]. Now the mortal rate of ALI is still high due to the complexity of pathogenesis. Lipopolysaccharide (LPS) is the major component of endotoxin can induce ALI by injuring pulmonary vascular endothelial cells, activating leukocytes or binding with its receptors on macrophages[16,17]. The deterioration of ALI often leads to multiple organ dysfunction syndrome (MODS) or multiple organ failure (MOF).

Glucocorticoid (GC) has been widely applied to the treatment of inflammatory diseases due to its anti-inflammatory effect which can bind to the glucocorticoid receptor (GR). Moreover, GC can be used to treat a variety of diseases due to glucocorticoid interferes with variously inflammatory pathways induced by cytokines[18]. GR belongs to the nuclear receptors superfamily. It stays in the cytoplasm when inactive, and become active once it binds with the corresponding hormone, then moves into the nucleus and exerts its anti-inflammatory effect. However, the mechanism of the anti-inflammatory ef-

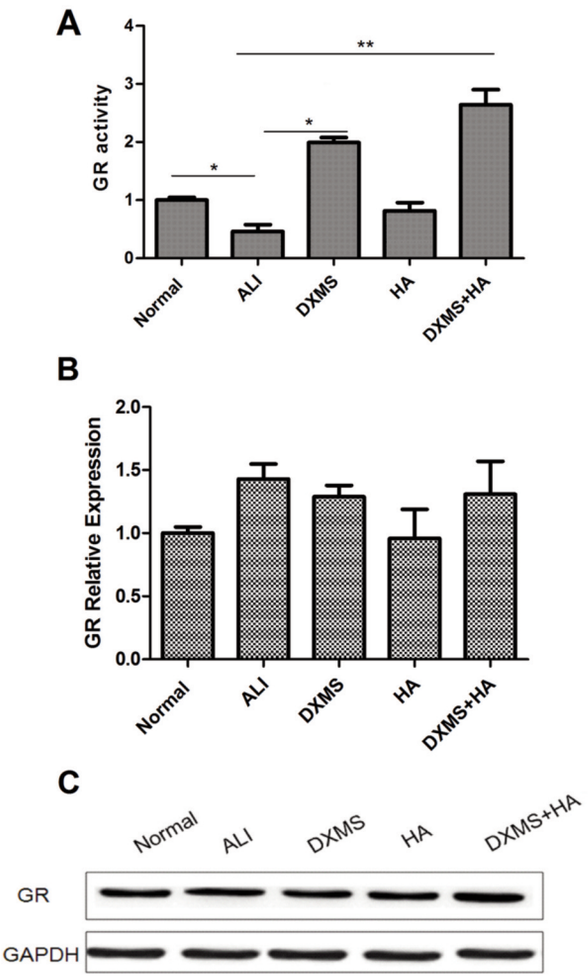

Figure 4. The effect of combination therapy of glucocorticoid and hyaluronic acid on activity and expression level of GR in SD rats with ALI. ***A)***, GR activity assay in lung tissue of SD rats with ALI; ***B),*** GR mRNA expression in lung tissue of SD rats with ALI; ***C)***, GR protein expression in lung tissue of rats with ALI.

fect of GR is rather complex, either intervenes the synthesis of inflammatory mediators by binding with DNA, or inhibits the inflammatory reaction by interacting with other transcription factors[19,20]. In our article, we found that GR played an important role in the process of declined apoptotic rate and released cytokines in SD rats with ALI induced by LPS.

The GC protective effect for lung depends on its binding with GR, however, the pathological mechanism of activated GR exert its anti-inflammatory effect is rarely known. Previously studies[21,22] show that p38MAPK pathway is important in the signal transduction of LPS induction. The activation of p38MAPK promotes the production of inflammatory cytokines in ALI induced by LPS. Li et al[23] have reported that GR in macrophage regulates the inflammatory reaction of TLR4 by selective inhibition of p38MAPK. The expression of phosphated p38MAPK in lung tissue is significantly up-regulated after the injection of LPS, along with the release of cytokines and aggregation of inflammatory cells. Many scholars[24,25] consider that p38MAPK pathway plays an important role in the release of cytokines induced by LPS. GC has been widely used in the treatment of ALI for a long time. However, the efficacy of GC in the treatment of ALI has been questioned, and its side effects limit the application in the clinic[26,27]. In this study, we proved that dexamethasone (DXMS) could decrease the mortal rate of ALI induced by LPS. Meanwhile, the decreased concentration of cytokines in BALF and the pathological improvement of lung tissue further confirmed that DXMS has strongly protective effect for the lung.

Hyaluronic acid (HA) is a straight-chain glycosaminoglycan with a high molecular weight which is synthesized and secreted into alveolar space by type II alveolar epithelial cells[28,29]. Chang et al[30] indicated that HA might enhance the activity of pulmonary surfactants and nullify certain inhibitors, which can significantly promote respiratory mechanical parameters and gas exchange in the treatment of toxin-caused lung injury when HA was used. In the process, HA may increase the adsorption rate of pulmonary by aggregating the PS microstructure. HA dissolves in water slowly yet completely, forming a viscous solution which is colorless or with a slight tint of ivory. And it is the major component in alveolar extracellular matrix[31,32].

In our study, we limited the dosage of glucocorticoid strictly to evade its side effects, simultaneously injected into HA with low molecular

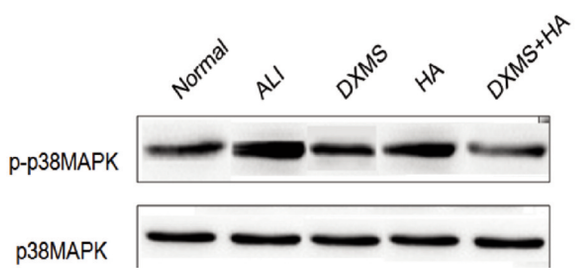

Figure 5. The expression and phosphorylation of p38MAPK by Western blotting.

weight that had undergone sulfonation and degradation in the treatment of SD rats with ALI. Our findings showed that lung structure of SD rats with LPS-induced ALI was improved significantly after using the combination therapy of DXMS and HA. The results also showed that combination therapy of DXMS and HA significantly improved pathological and biological indices in SD rats with ALI. The analysis of recycled BALF showed that combination therapy of DXMS and HA decreased the total protein and concentration of inflammatory cytokines, such as TNF-α, IL-8, IL-10 and ICAM-1, indicating that inflammatory reaction in SD rats was inhibited significantly.

Furthermore, red the mechanism of the effect dexamethasone combined with hyaluronic acid holds on the alleviation of symptoms in SD rats with LPS-induced ALI was explored in our study. Our work displayed that GR activity was up-regulated significantly after treatment of DXMS solely, while treatment of HA increased GR activity only slightly, implied that HA can significantly improve GR activity in SD rats with ALI after the treatment of DXMS combined with HA, while has little influence on GR activity by itself. Other studies have shown that p38MAPK pathway and NF-kB pathway are important in anti-inflammation reactions of GC, but whether it correlates with the mechanism of the anti-inflammation effect of DXMS combined with HA is still unknown[33]. Then, the phosphorylation level of p38MAPK and NF-kB in different groups was measured by Western blotting. After the treatment of DXMS, expression of p38MAPK in lung tissue of SD rats was decreased significantly, while the treatment of HA only slightly decreased. Our research revealed that DXMS inhibited the activation of p38MAPK signal pathway induced by LPS by enhancing the activity of GR, while HA had rarely significant function in the process, implied our conjecture that hyaluronic acid might provide an ideal environment for the functioning of glucocorticoid on the surface of alveoli.

Conclusions

In this work, our data proved that the efficacy of dexamethasone combined with hyaluronic acid could significantly alleviate symptoms in rats with LSD-induced ALI. The effect of dexamethasone is GR-dependent, activated GR further inhibits the activation/phosphorylation of p38MAPK, thus exerts its anti-inflammatory effect and protects lung tissue, while hyaluronic acid provided the desired function environment in the process.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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