Archivum Immunologiae et Therapiae Experimentalis (2023) 71:23
https://doi.org/10.1007/s00005-023-00688-7

ORIGINAL ARTICLE

Expression of Angiopoietin-2 in Lung Tissue of Juvenile SD Rats with Lipopolysaccharide-Induced Acute Lung Injury and the Role of Ulinastatin

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Received: 8 February 2023 / Accepted: 12 October 2023 / Published online: 26 October 2023

Abstract

This study aimed to observe the expression of angiopoietin-2 (Ang-2) in the lung tissue of juvenile SD rats with lipopolysaccharide (LPS)-induced acute lung injury (ALI) and to clarify the role of ulinastatin (UTI). Ninety 18–21-day-old juvenile SD male rats were randomly divided into five groups ($n = 18$). ALI rat model was established by intraperitoneal injection of LPS (LPS 10 mg/kg), while the control group was given the same dose of normal saline. The UTI intervention group was given the injection of UTI (5000 U/mL) immediately after the injection of LPS, which was divided into UTI low-dose group (LPS + 5 ml/kg UTI), UTI medium-dose group (LPS + 10 ml/kg UTI), and UTI high-dose group (LPS + 20 ml/kg UTI).The respiratory status of each group of rats was observed, and six rats were randomly selected to be killed in each group at 6, 12, and 24 h, and the lung tissues were dissected and retained. The pathological changes of the lung tissues were observed by hematoxylin–eosin (HE) staining, the expression levels and locations of Ang-2 and vascular endothelial growth factor (VEGF) in lung tissue were observed by immunohistochemical staining, and the expressions of genes and proteins of Ang-2 and VEGF were detected by quantitative reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis. Three hours after intraperitoneal injection, rats in the model group developed shortness of breath and the developed respiratory distress progressed over time. The lung pathological changes in the model group were obvious compared with those in the control group, and gradually worsened with time, and the pathological changes of lung in the rats in the UTI intervention group were reduced compared with those in the model group. At different time points, the expressions of Ang-2 and VEGF in the lung tissue of rats in the model group were higher than those in the control group, and were lower in the UTI intervention group than those in the model group. The expressions of Ang-2 and VEGF protein were lower in the low-dose group of UTI group than those in the high-dose group of UTI group at different time points ($P < 0.05$), and the expressions of Ang-2 and VEGF protein in the low-dose group of UTI were significantly lower than those in the medium-dose group at 12 h and 24 h ($P < 0.05$). The expression of Ang-2 was increased in the lung tissue of juvenile SD rats with LPS-induced ALI, and was associated with the degree of lung injury. UTI might attenuate LPS-induced ALI by inhibiting the expression of Ang-2 in lung tissue, and the low dose was more obvious than the medium and high dose.

Keywords Acute lung injury · Angiopoietin-2 · Lipopolysaccharide · Rats · Ulinastatin · VEGF

Introduction

Acute lung injury (ALI) is a common problem of pediatric respiratory diseases, which can progress to acute respiratory distress syndrome (ARDS) (Li et al. 2022; Luo et al. 2022) with high mortality (Hsieh et al. 2022; Qiao et al. 2019) and lack of effective treatment (Kong et al. 2023; Song et al. 2022). Therefore, it is urgent to study effective therapeutic drugs for ALI. Studies have shown that the expression of angiopoietin-2 (Ang-2) in lung tissue of ALI is obviously increased, and inhibiting the expression of Ang-2 can

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alleviate ALI (Tang et al. 2017). Vascular endothelial growth factor (VEGF) can increase vascular permeability, leading to edema (Qiu et al. 2019), and the overexpression of VEGF in lung tissue can increase pulmonary microvascular permeability (Lange et al. 2012), and pulmonary edema caused by high pulmonary microvascular permeability is one of the main causes of ALI. The purpose of this study was to observe the expression of Ang-2 in lung tissue of juvenile SD rats with LPS-induced ALI, and to further clarify the effect and mechanism of ulinastatin (UTI) on ALI.

Materials and Methods

Animals and Main Reagents

Ninety juvenile male SD rats (aged: 18–21 days, weight: 34–58 g, SPF) were purchased from Henan Experimental Animal Center (license No.: GBT 35892–2018), which were in accordance with the national experimental animal care regulations of China. LPS (Sigma, 055:B5) and UTI (Shanghai Aladdin Biochemical Technology Co., Ltd., AS: 80,449-31-6). Ang-2 antibody, TNF-α antibody, IL-6 antibody, and VEGF antibody were all purchased from Wuhan Doctor Biological Engineering Co., LTD. Immunohistochemical staining kit and DAB chromogenic kit were purchased from Beijing Zhongshan Jinqiao Biotechnology Company. Ultrapure RNA extraction kit and Trizol reagent were purchased from kangwei century (China). BCA protein quantitative kit was purchased from Solebao (China). GAPDH antibody was purchased from Cell Signaling Technology (USA).

Animal Grouping and Preparation of Animal Models

Ninety juvenile SD male rats were randomly divided into five groups ($n=18$). All rats were fed the sterile feed for 7 days, and were kept in a temperature-controlled (23.8 °C) and humidity-controlled (33.3%) room with 20 ventilation times per minute and 12 h light/dark cycle. During the whole experiment, these rats could get food and water freely. ALI rat model was established by intraperitoneal injection of LPS (LPS 10 mg/kg, 1 mg: 1 mL). The dose (Deng et al. 2020; Liu et al. 2021; Zhang et al. 2019) was determined by consulting the relevant literature. The control group was given the same dose of normal saline. In the UTI intervention group, rats were injected with UTI (5000 U/mL) immediately after intraperitoneal injection of LPS. The details are as follows: control group (NS group): rats were injected with normal saline (10 ml/kg); model group (LPS group): rats were injected with LPS (10 ml/kg); the UTI low-dose group (LPS + 5 ml/kg UTI): rats were injected with LPS (10 ml/kg) + UTI (5 ml/kg); the UTI medium-dose group (LPS + 10 ml/kg UTI): rats were injected with LPS (10 ml/kg) + UTI (10 ml/kg); the UTI high-dose group (LPS + 20 ml/kg UTI): rats were injected with LPS (10 ml/kg) + UTI (20 ml/kg). The respiratory status, mental state, diet, and stool of rats in each group were observed. The respiratory status was determined based on respiratory frequency, respiratory rhythm, and the presence of respiratory distress. The mental state was determined as follows: (1) normal—emotionally stable, flexible action, and eyes; (2) depression—depressed mood, slow action, and dull eyes.

Preparation of Lung Tissue Specimens

Six rats in each group were randomly selected at 6, 12, and 24 h after intraperitoneal injection, and killed by cervical vertebrae dislocation and dissected. Opened the chest wall quickly, took the middle lobe of the right lung, put half of it in a −80 °C refrigerator for later use, and fixed the other half with 4% paraformaldehyde for 48 h, then carried out HE staining and immunohistochemistry.

HE and Immunohistochemical Staining

After the right middle lobe of the lung was fixed with 4% paraformaldehyde, it was embedded in paraffin, and then cut into 5 μm thick sections. HE and immunohistochemical staining were performed, scanned with a digital sectioning scanner (Ningbo Jiangfeng Biological Information Technology Co., LTD.), the pathological changes of lung tissue and the expression of target protein and the distribution of positive cells were observed with K-Viewer software (KFBIO) at high magnification.

Quantitative Reverse Transcription Polymerase Chain Reaction

Thirty mg of lung tissue was taken from −80 °C refrigerator, and total RNA of lung tissue was extracted by Trizol reagent and RNA extraction kit. Reverse transcription products were synthesized according to the RT kit instructions. The following primers were designed: the forward primer of Ang-2 is 5′-GAGAACTACATCCAGGACAAC-3′, and the reverse primer is 5′-TGGGCTTCCACATCAGTCAGT-3′; the forward primer of VEGF is 5′-CTGACGGACAGACAGACA GACACC-3′, and the reverse primer is 5′-GAGCCCAGA AGTTGGACGAAAA-3′; the forward primer of GAPDH is 5′-ACGGCAAGTTCAACGGCACAG-3′, and the reverse primer is 5′-CGCCAGTAGACTCCACGACAT-3′. Reverse transcription polymerase chain reaction (RT-PCR) was performed, and a 20 μL reaction system was prepared in an eight even tubes, and then it was put on the machine. The first step was denaturation at 95 °C for 30 s, and then it went through 40 cycles (95 °C, 5 s, 60 °C, 30 s). The sample mRNA expression levels were calculated by $2^{-\Delta\Delta CT}$ method.

Western Blot Detection

We took 50 mg of lung tissue from the frozen refrigerator at −80 °C, and extracted the histone according to the steps, and followed the instructions of BCA protein concentration determination kit to measure the protein concentration of the sample. After the development, the protein bands were analyzed by ImageJ software (National Institute of Mental Health, Bethesda, USA). The relative expression level of the target protein was calculated from the formula of the relative gray value (target protein/β-actin).

Statistical Analysis

Statistical software SPSS 25.0 was used to analyze the experimental data, and the measurement data were described as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for comparison among groups, and LSD or T3 test was used for comparison between groups. LSD test was used when the variance was even, and T3 test was used when the variance was uneven. $P < 0.05$ indicated statistically significant. Pearson linear correlation analysis was used to analyze the correlation of the measurement data, and the correlation was significant ($P < 0.05$).

Results

State of Rats

The rats in the control group were in good mental state, normal breathing state, normal diet, and stool; after 3 h of intraperitoneal injection, the rats of the model group experienced shortness of breath, less movement, less food, and loose stools, and the symptoms gradually worsened with time. The symptoms of the UTI intervention group were all between the control group and the model group.

Pathological Changes

In the control group, the lung tissue structure was intact, and did not have obvious inflammatory reaction. Model group: the widening of alveolar septum, destruction of alveolar structure, infiltration of inflammatory cells, and hemorrhage in the alveolar cavity, increasing lung pathological injury with time. The UTI intervention group: the widening of alveolar septum, destruction of alveolar structure, infiltration of inflammatory cells, and hemorrhage in the alveolar cavity in each group were significantly reduced compared with the model group in all time periods, and more severe than in the control group (Fig. 1).

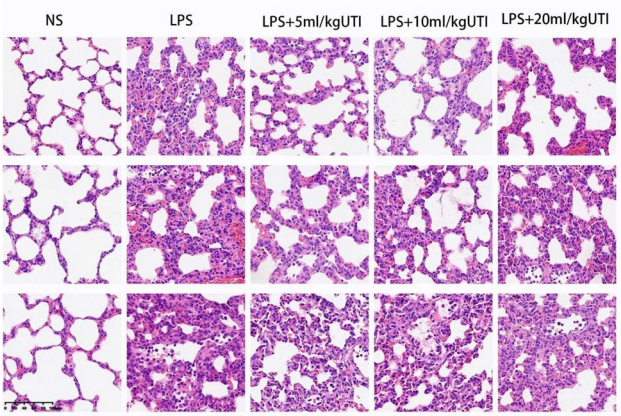

Fig. 1 Pathological changes in lung tissue of each group (hematoxylin–eosin staining, ×400). *Control group* NS group, *model group* LPS group, *the ulinastatin low-dose group* LPS + 5 ml/kgUTI, *the ulinastatin medium-dose group* LPS + 10 ml/kg UTI, *the ulinastatin high-dose group* LPS + 20 ml/kg UTI

Immunohistochemistry Results

Ang-2 protein was mainly expressed in bronchial epithelial cells and vascular endothelial cells, and VEGF was mainly expressed in alveolar septa cells and bronchial epithelial cells. The expressions of Ang-2 and VEGF protein were weak in the control group. At different time points, the expressions of Ang-2 and VEGF protein in the model group were higher than those in the control group, and the expressions of Ang-2 and VEGF protein in the low-, medium-, and high-dose groups of UTI were lower than those in the model group. (Fig. 2).

Expression of Ang-2 and VEGF mRNA

RT-PCR showed that the expressions of Ang-2 and VEGF mRNA in the control group had no significant change with time. The expressions of Ang-2 and VEGF mRNA in model group increased with time ($P < 0.05$) (Fig. 3).

At different time points, the expressions of Ang-2 and VEGF mRNA in the model group were higher than those in the control group, and were lower in the UTI low-dose group than those in the model group, all of the differences were statistically significant ($P < 0.05$). At different time points, the expression of Ang-2 mRNA in the middle- and high-dose groups of UTI was lower than that in the model group ($P < 0.05$). At 6 h and 12 h, the expression of VEGF mRNA in the UTI medium-dose group was lower than that in the model group ($P < 0.05$). At 6 h, the expression

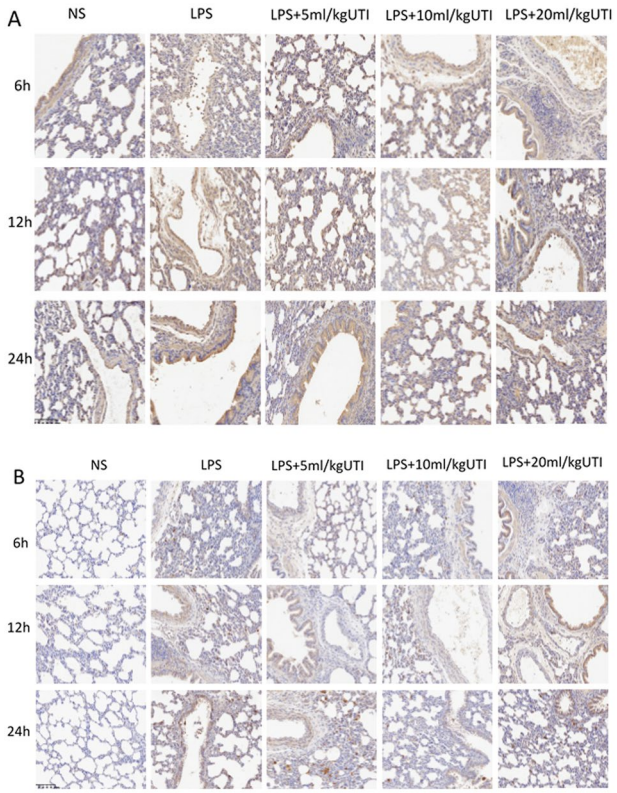

Fig. 2 The expressions of Ang-2 and VEGF in lung tissue of each group (×400). Dark brown was strongly positive, brown was moderately positive, and light yellow was weakly positive. **A** Ang-2, **B** VEGF

of VEGF mRNA in the UTI high-dose group was lower than that in the model group ($P<0.05$) (Fig. 4, Tables 1 and 2).

Bivariate Pearson test showed that the expressions of Ang-2 and VEGF mRNA in lung tissue were positively correlated, and the correlation was significant ($P<0.05$). (Fig. 5).

Expression of Ang-2 and VEGF Protein

Western blot analysis showed that the expressions of Ang-2 and VEGF protein in the control group had no obvious change with time. The expressions of Ang-2 and VEGF protein in model group increased with time ($P<0.05$) (Fig. 6).

At different time points, the expressions of Ang-2 and VEGF protein in the model group were higher than those in the control group ($P<0.05$), and the expressions of Ang-2 and VEGF protein in the UTI low-dose group were lower than those in the model group ($P<0.05$). At 6 h, the expression of Ang-2 protein in the UTI medium-dose group was significantly lower than that in the model group ($P<0.05$). At different time points, the expression of VEGF protein in the UTI medium-dose group was lower than that in the model group ($P<0.05$). At 24 h, the expression of VEGF protein in the UTI high-dose group was significantly lower than that in the model group ($P<0.05$).

At different time points, the expressions of Ang-2 and VEGF protein in UTI low-dose group were lower than those in UTI high-dose group ($P<0.05$). At 12 h and 24 h, the expressions of Ang-2 and VEGF protein in low-dose UTI group were significantly lower than those in middle-dose UTI group ($P<0.05$) (Fig. 7, Tables 3 and 4).

Bivariate Pearson test showed that the expressions of Ang-2 and VEGF protein in lung tissue were positively correlated, and the correlation was significant ($P<0.05$) (Fig. 8).

Discussion

ALI is a lung lesion caused by various direct and indirect injuries inside and outside the lung, such as severe pneumonia and sepsis (Cao et al. 2022). It's main clinical manifestations are progressive hypoxemia and respiratory distress, and it's pathological features are inflammatory reaction (Niemiec et al. 2022) and increased pulmonary capillary permeability (Blázquez-Prieto et al. 2021).

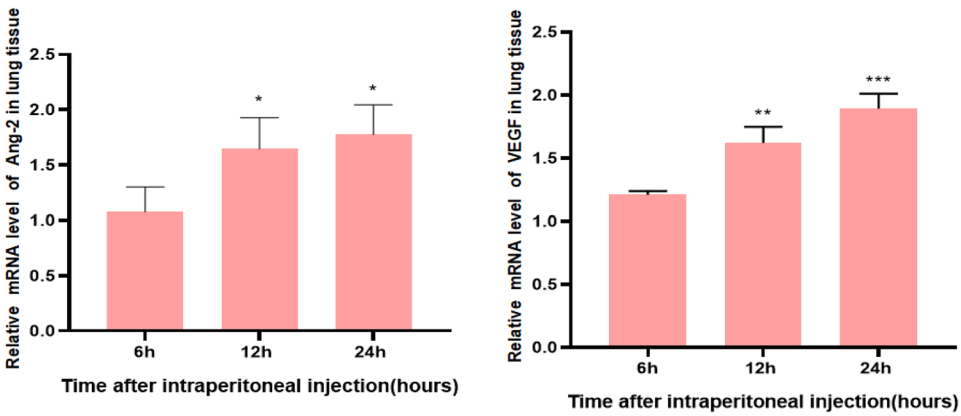

Fig. 3 The data of model group. *Compared with the 6 h time point: *$P<0.05$; **$P<0.01$; ***$P<0.001$

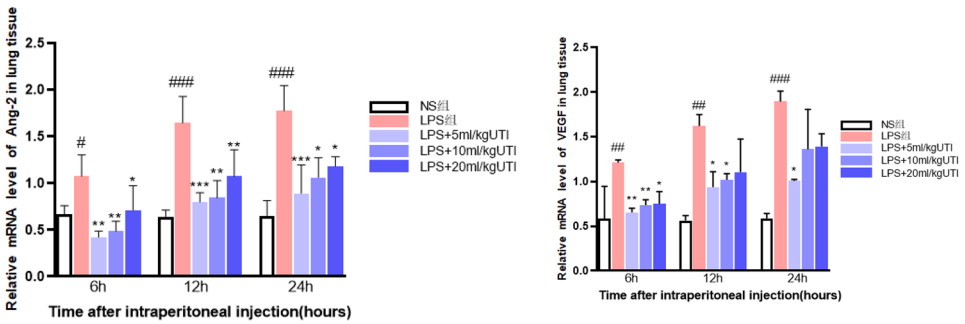

Fig. 4 The expression of VEGF mRNA in different groups. #Compared with the control group: #$P<0.05$; ##$P<0.01$; ###$P<0.001$. *Compared with the LPS group: *$P<0.05$; **$P<0.01$; ***$P<0.001$

Table 1 Comparison of Ang-2 mRNA expression in lung tissue of each group ($\bar{x} \pm s$)

	6 h	12 h	24 h	*F* value	*P* value
NS group	0.67 ± 0.09	0.64 ± 0.07	0.65 ± 0.16	0.032	0.968
LPS group	1.07 ± 0.23[a]	1.64 ± 0.28[a]	1.77 ± 0.27[a]	6.053	0.036
LPS + 5 ml/kg UTI group	0.42 ± 0.07[b]	0.80 ± 0.10[b]	0.89 ± 0.30[b]	5.350	0.046
LPS + 10 ml/kg UTI group	0.49 ± 0.10[b]	0.84 ± 0.18[b]	1.06 ± 0.21[a,b]	8.513	0.018
LPS + 20 ml/kg UTI group	0.71 ± 0.26[b]	1.07 ± 0.28[a,b]	1.18 ± 0.10[a,b]	3.383	0.104
F	6.803	10.997	10.793		
P	0.007	0.001	0.001		

The "*F*" and "*P*" in the last row refer to the results of the comparison between the five groups

The "*F*" and "*P*" in the last column refer to the results of the data comparison between the three time points

At the same point in time: [a]compared with the control group, *P* value < 0.05. [b]compared with the LPS group, *P* value < 0.05

Table 2 Comparison of VEGF mRNA expression in lung tissue of each group ($\bar{x} \pm s$)

	6 h	12 h	24 h	*F* value	*P* value
NS group	0.59 ± 0.36	0.56 ± 0.06	0.59 ± 0.05	0.013	0.987
LPS group	1.21 ± 0.03[a]	1.62 ± 0.13[a]	1.89 ± 0.12[a]	34.070	0.001
LPS + 5 ml/kg UTI group	0.65 ± 0.05[b]	0.94 ± 0.17[b]	1.01 ± 0.02[a,b]	10.135	0.012
LPS + 10 ml/kg UTI group	0.74 ± 0.06[b]	1.02 ± 0.07[a,b]	1.36 ± 0.45	4.153	0.074
LPS + 20 ml/kg UTI group	0.75 ± 0.14[b]	1.11 ± 0.37	1.39 ± 0.14[a]	5.235	0.048
F	5.745	11.057	14.753		
P	0.011	0.001	0.000		

Note: At the same point in time: [a]compared with the control group, *P* < 0.05. [b]compared with the LPS group, *P* < 0.05

We hypothesized that the improvement of ALI by UTI might be related to the expressions of Ang-2 and VEGF in lung tissue. In this study, the ALI model of juvenile SD rats was induced by LPS, and UTI was used to treat it. The expressions of Ang-2 and VEGF in lung tissue of juvenile SD rats with LPS-induced ALI were observed, and the role and mechanism of UTI in ALI were further discussed.

We found that the rats in the model group developed shortness of breath after 3 h of intraperitoneal injection, and developed respiratory distress over time, which was consistent with the clinical manifestations of ALI. In the model group, HE staining showed that alveolar septa were widened, alveolar structure was destroyed, inflammatory cells infiltrated, and alveolar cavity was bleeding. With the increase of time, the pathological damage of lung became worse, which was in line with the pathological changes of ALI (Cui et al. 2021; Sun et al. 2022). The clinical manifestations and lung pathological changes in the rats showed that the animal model of ALI was successfully established.

Angiopoietin (Ang) is a group of endothelial growth factors, which can regulate inflammation and vascular permeability when combined with its receptor Tie, and it is essential for normal vascular development and angiogenesis. Ang-2 is a marker of endothelial injury, which plays an important role in many inflammatory diseases, promotes the inflammatory reaction and leads to the loss of vascular integrity

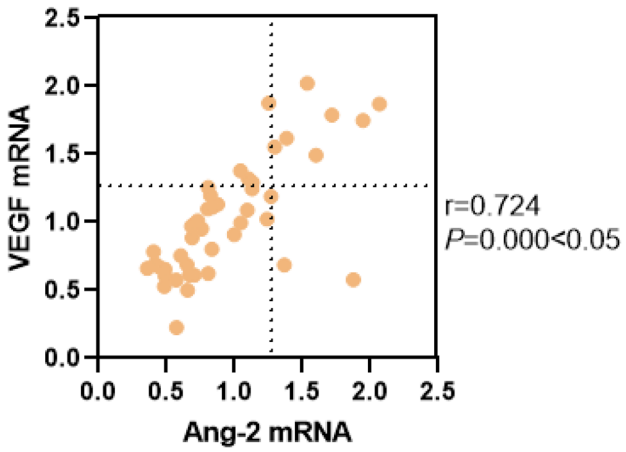

Fig. 5 The correlation between VEGF mRNA and Ang-2 mRNA expression. $P<0.05$: $0.3<|r|<0.5$, weak correlation; $0.5<|r|<0.7$, moderate correlation; $0.7<|r|<0.9$, strong correlation

(Zhong et al. 2020). The role of Ang-2 in ALI is mainly manifested in inflammatory response and vascular leakage (Lymperopoulou et al. 2015). VEGF is a growth factor that promotes angiogenesis activity, can increase vascular permeability, and plays an important role in angiogenesis and homeostasis. Immunohistochemistry showed that the expressions of Ang-2 and VEGF protein were weak in the control group and stronger in the model group. RT-PCR and Western blot showed that the expressions of Ang-2 and VEGF in lung tissue of the model group were higher than those in the control group at different time points (P value <0.05). Bivariate Pearson test showed that both the expressions of Ang-2 and VEGF mRNA and the expressions of Ang-2 and VEGF protein in lung tissue were positively correlated, with significant correlation (P value <0.05). These data indicated that the expressions of Ang-2 and VEGF in lung tissue were involved in the occurrence and development of ALI. At the same time, we found that the expressions of Ang-2 and VEGF mRNA, and the expressions of Ang-2 and VEGF protein in lung tissue of the model group increased with time (P value <0.05), indicating that the expression of Ang-2 and VEGF in lung tissue was related to the degree of lung pathological injury, and increased with the aggravation of lung pathological injury.

UTI is a broad-spectrum serine protease inhibitor, which can reduce vascular permeability (Ao et al. 2022; Li et al. 2019), block the release of inflammatory factors (Wei et al. 2017), effectively reduce the mortality of sepsis patients (Yang et al. 2020), and be used in the treatment of ALI (Jin et al. 2022; Wu et al. 2019). HE staining showed that the alveolar septum widening, congestion, inflammatory cells infiltration, and erythrocyte exudation in the UTI intervention groups were significantly less than those in the model group, which indicated that UTI could improve the lung pathological injury of ALI. Recent studies have found that drugs targeting Ang-2 and VEGF are shown to be complementary in the treatment of tumors, and superior to drugs that target one of them alone, with antiangiogenic effects (Biel and Siemann 2016). In ALI, Ang-2 and VEGF are both related to vascular permeability, so we have conducted relevant studies on whether the improvement of ALI by UTI has the targeted relationship with the expression of Ang-2 and VEGF in lung tissue.

Extensive injury of pulmonary vascular endothelial cells and alveolar epithelial cells leads to ALI, showing inflammation of lung tissue, increase of vascular permeability, and leakage of plasma. Ang-2 acted as a biomarker of lung injury and an amplifier of local inflammation and vascular injury (Gottlieb et al. 2022). Honokiol could improve LPS-induced ARDS by inhibiting Ang-2 expression (Chen et al. 2018). Hydrogen sulfide ameliorated ALI induced by infrarenal aortic cross-clamping by inhibiting inflammation and Ang-2 release (Tang et al. 2017). Therefore, targeting the Ang-2 pathway may be a potential therapeutic approach for ALI. Immunohistochemistry showed that Ang-2 protein was

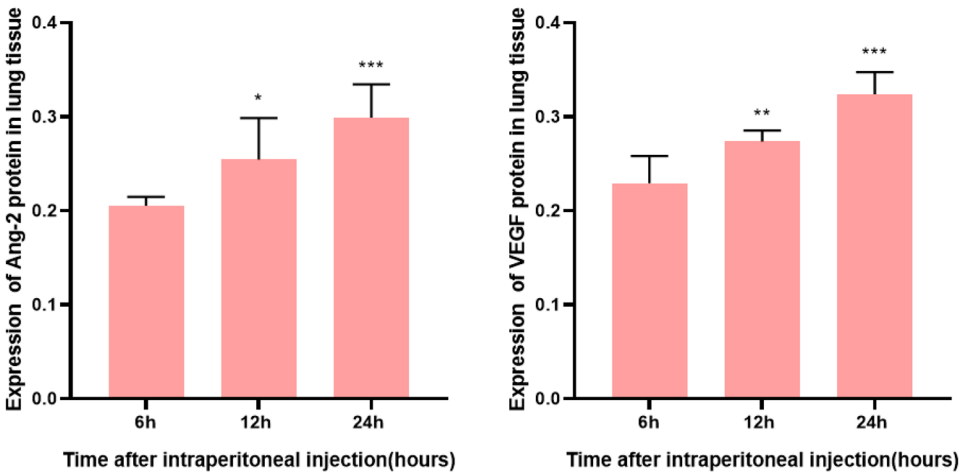

Fig. 6 The expression of Ang-2 and VEGF in model group. *Compared with the 6 h time point: $*P<0.05$; $**P<0.01$;$***P<0.001$

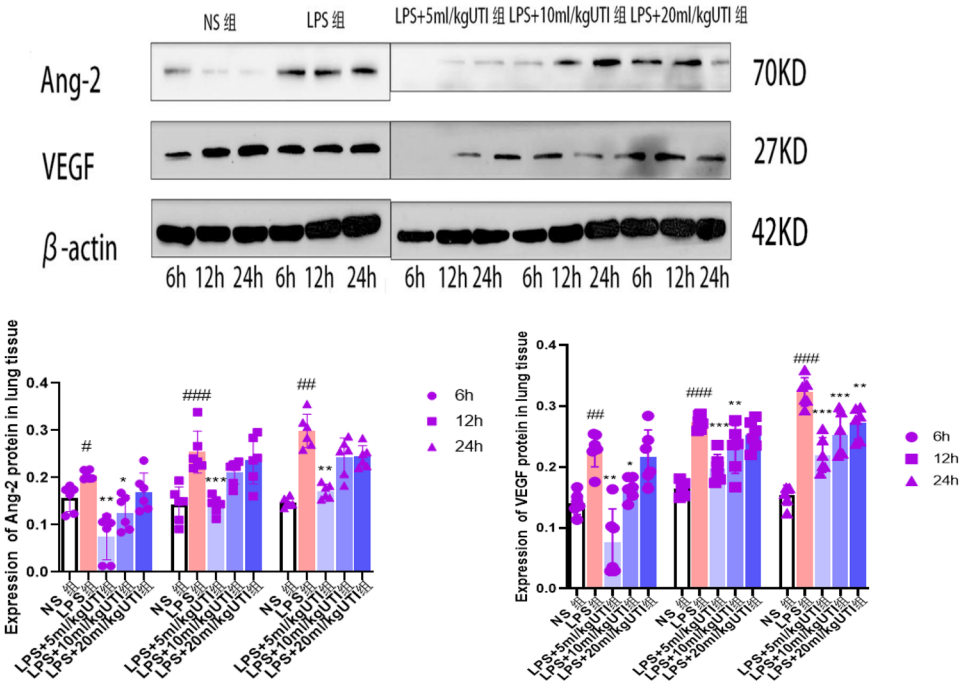

Fig. 7 The expression of Ang-2 and VEGF mRNA in different groups. #Compared with the control group: #$P<0.05$; ##$P<0.01$; ###$P<0.001$. *Compared with the LPS group: *$P<0.05$; **$P<0.01$; ***$P<0.001$

Table 3 Comparison of Ang-2 protein expression in lung tissue of each group ($\bar{x}\pm s$)

	6 h	12 h	24 h	*F* value	*P* value
NS group	0.16 ± 0.03	0.14 ± 0.04	0.15 ± 0.01	0.361	0.703
LPS group	0.21 ± 0.01[a]	0.25 ± 0.04[a]	0.30 ± 0.04[a]	11.984	0.001
LPS + 5 ml/kgUTI group	0.07 ± 0.05[b]	0.14 ± 0.02[b]	0.17 ± 0.02[b]	15.296	0.000
LPS + 10 ml/kgUTI group	0.13 ± 0.04[b]	0.21 ± 0.02[a]	0.24 ± 0.04[a]	18.866	0.000
LPS + 20 ml/kgUTI group	0.17 ± 0.04	0.24 ± 0.05[a]	0.24 ± 0.02[a]	6.598	0.009
F	11.774	12.162	30.304		
P	0.000	0.000	0.000		

At the same point in time: [a]compared with the control group, $P<0.05$. [b]Compared with the LPS group, $P<0.05$

Table 4 Comparison of VEGF protein expression in lung tissue of each group ($\bar{x}\pm s$)

	6 h	12 h	24 h	*F* value	*P* value
NS group	0.14 ± 0.02	0.17 ± 0.01	0.15 ± 0.02	3.627	0.052
LPS group	0.23 ± 0.03[a]	0.27 ± 0.01[a]	0.32 ± 0.02[a]	26.662	0.000
LPS + 5 ml/kg UTI group	0.08 ± 0.05[b]	0.20 ± 0.02[a,b]	0.22 ± 0.03[a,b]	24.850	0.000
LPS + 10 ml/kg UTI group	0.16 ± 0.02[b]	0.23 ± 0.04[a,b]	0.25 ± 0.04[a,b]	11.855	0.001
LPS + 20 ml/kg UTI group	0.22 ± 0.04[a]	0.25 ± 0.02[a]	0.27 ± 0.03[a,b]	4.508	0.029
F	17.870	19.312	33.253		
P	0.000	0.000	0.000		

At the same point in time: [a]compared with the control group, $P<0.05$. [b]compared with the LPS group, $P<0.05$

mainly expressed in bronchial epithelial cells and vascular endothelial cells. At different time points, the expression of Ang-2 protein in the UTI intervention groups was lower than that in the model group, among which the effect of the low dose of UTI was obvious, and the effect of the high dose of UTI was the worst. We considered that the high dose of UTI might aggravate lung inflammation and damage of bronchial epithelial cells and vascular endothelial cells. RT-PCR

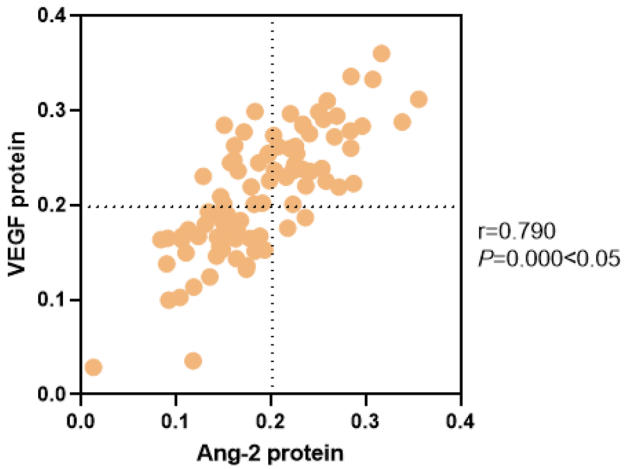

Fig. 8 The correlation between VEGF and Ang-2 protein. $P<0.05$: $0.3<|r|<0.5$, weak correlation; $0.5<|r|<0.7$, moderate correlation; $0.7<|r|<0.9$, strong correlation

showed that the expressions of Ang-2 mRNA in the low-, medium- and high-dose groups of UTI were lower than those in the model group at different time points ($P<0.05$). In addition, Western blot showed that the expression of Ang-2 protein in the UTI low-dose group was lower than that in the model group at different time points ($P<0.05$). At 6 h, the expression of Ang-2 protein in the UTI medium-dose group was significantly lower than that in the model group ($P<0.05$). At different time points, the expression of Ang-2 protein in the UTI low-dose group was lower than that in UTI high-dose group ($P<0.05$). The expressions of Ang-2 protein in the UTI low-dose group at 12 h and 24 h were significantly lower than those in the UTI middle-dose group ($P<0.05$). The results suggested that UTI might attenuate LPS-induced ALI by inhibiting the expression of Ang-2 in lung tissues, and the low dose of UTI (25,000 U/kg) was the appropriate dosage for treatment.

VEGF is a glycosylated mitogen, which promotes the proliferation and survival rate of endothelial cells, mediates endothelial cell migration, and inhibits apoptosis. VEGF is also known as vascular permeability factor and can increase vascular permeability. The overexpression of VEGF in lung tissue can increase microvascular permeability in the lung, and the increase of pulmonary vascular permeability is one of the pathological features of ALI. A study had shown that serum VEGF levels increased while the expression of VEGF in lung tissue decreased in the mouse model of ALI, and the upregulation of VEGF activity and function in lung tissue reduced alveolar-capillary permeability, thereby reducing ALI (Guo et al. 2011). We considered that VEGF in lung tissue might be involved in the occurrence and development of ALI, and the repair process of ALI. VEGF might also be other target for the treatment of ALI. Immunohistochemistry showed that the expression of VEGF protein in the model group was higher than that in the control group, which indicated that the expression of VEGF protein in lung tissue increased in ALI. The expression of VEGF protein in the UTI intervention group was lower than that in the model group, which indicated that UTI could reduce the expression of VEGF protein in lung tissue. RT-PCR showed that the expression of VEGF mRNA in the UTI low-dose group was lower than that in the model group at different time points ($P<0.05$). At 6 h and 12 h, the expression of VEGF mRNA in the UTI middle-dose group was lower than that in the model group ($P<0.05$). At 6 h, the expression of VEGF mRNA in the UTI high-dose group was lower than that in the model group ($P<0.05$). Western blot showed that the expressions of VEGF protein in the low- and middle-dose groups of UTI were lower than those in model group at different time points ($P<0.05$). At 24 h, the expression of VEGF protein in the UTI high-dose group was significantly lower than that in model group ($P<0.05$). At different time points, the expression of VEGF protein in the UTI low-dose group was lower than that in high-dose UTI group ($P<0.05$). At 12 h and 24 h, the expression of VEGF protein in the UTI low-dose group was significantly lower than that in the UTI medium-dose group ($P<0.05$). All the above results indicated that VEGF might be another potential target for the treatment of ALI, and UTI might also improve LPS-induced ALI in juvenile male SD rats by decreasing the expression of VEGF in lung tissue, and the low-dose of UTI (25,000 U/kg) was the appropriate dose for the treatment.

We also found that the expressions of Ang-2 and VEGF mRNA in the UTI low-dose group at 12 h and 24 h both were higher than that at 6 h ($P<0.05$), but there was no statistical significance between 12 and 24 h ($P>0.05$). We considered that it might be related to the half-life and drug concentration of UTI.

At the same time, we also realized that this experiment had certain limitations, such as small sample size and individual differences of rats, which led to data dispersion and affected statistical results. In the future, we need more animal experiments and more sufficient data to verify the experimental result.

Conclusion

To sum up, the expressions of Ang-2 and VEGF in lung tissue of juvenile SD rats with LPS-induced ALI were increased, and which were related to the degree of lung injury and increased with the aggravation of lung injury. Ang-2 is the target of treating ALI, UTI might improve LPS-induced ALI in juvenile male SD rats by decreasing the expression of Ang-2 in lung tissue. VEGF may be another target of UTI in improving ALI. Therefore, UTI could be

used as an alternative drug for the treatment of ALI, and the half-life and dosage of the drug should be considered. The low dose of UTI (25,000 U/kg) was more suitable for the treatment of ALI in juvenile SD rats.

Funding This work was supported by the Henan Province Science and Technology Research Project (222102310191).

Data availability The data are free access to available upon request.

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