ORIGINAL ARTICLES

Experimental study on the protective effect of ulinastatin on lung tissue in rats with severe scalded

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Abstract

Objective: To explore the potential protective effects of ulinastatin on ventilation-induced lung injuries of severe burned rats.
Methods: Ninety Wistar rats were randomly divided into three experimental groups: the control group (n = 30), the ventilation group (n = 30) and the ventilation-ulinastatin group (n = 30). After establishing the severe burn model, the rats of latter two groups were mechanically ventilated for 1 hour with or without the pre-treatment of ulinastatin. After severe scald, the protective effect of ulinastatin on lung injury caused by mechanical ventilation was estimated through the observation of the tissues samples, and evaluation of the pathological changes of lung tissue by HE staining, ultrastructure change by electron microscopy, lung coefficient, and the expression levels of lung tissue cytokines TNF-α, IFN-γ, IL-2 by immunohistochemical staining.
Results: Edema in lung tissues of the control group and the ventilation group was obvious, the hemorrhagic focus could be seen, and the cut surface was observed to be scattered and swelling; Edema in lung tissues of the ventilation-ulinastatin group was mild. HE staining revealed that the pathological changes of the ventilation-ulinastatin group were milder than the ventilation group. Under the electron microscope, the lung tissue organelles of the control group and the ventilation group were seriously damaged; the corresponding changes in the ventilation-ulinastatin group were lighter. The lung coefficient of the ventilation-ulinastatin group was significantly lower than that in the ventilation group. The immunohistochemical results showed that the intensity of TNF-α, IL-2 and IFN-γ in lung tissue of the ventilation-ulinastatin group was significantly lower than that in the ventilation group.
Conclusions: Ulinastatin has protective effects on lung injury caused by mechanical ventilation in severe scalded rats, whose mechanism may be related to the capacity of ulinastatin to reduce the expression of cytokines including TNF-α, IL-2 and IFN-γ.

Key Words: Anesthesia, Ulinastatin, Burns, Ventilator-induced lung injury

One of the important subjects in anesthesia is the treatment of lung protection to moderate to severe burn patients with secondary pulmonary complications. Lung injury in the pathophysiology process of large area burn, especially in the early stage of severe burn and infection, could be observed. Surgical removal of necrotic tissue and closure of wounds at this time is required.

During anesthesia, mechanical ventilation is necessary. Mechanical ventilation provides respiratory support on one hand. On the other hand, it may lead to severe lung injury, that is, lung injury caused by mechanical ventilation. On the other hand, it can lead to severe lung injury, that is,

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ventilator-induced lung injury (VILI).[1–3] Pulmonary complications are often found in patients with large area burns after scab operation, while ulinastatin can be used to protect lung tissue.[4] In this study, we used ulinastatin in the treatment of severe burned rats during anesthesia mechanical ventilation in order to explore the mechanism of ulinastatin in relieving acute lung injury/acute respiratory distress syndrome caused by mechanical ventilation.

1 Materials and methods

1.1 Source of animals

90 adult healthy Wistar rats, male and female, weighing (230 $\pm$ 20) g, according to the random number table, were randomly divided into the control group, the ventilation group and the ventilation-ulinastatin group (each group of 30). In the control group, 10% chloral hydrate (0.3 ml/100 g) was intraperitoneally injected 24 hours after scald without any treatment. At 24 h after scald, the anesthesia tracheostomy intubation was maintained for 1 h. After scalded for 24 h in the ventilation-ulinastatin group, it was intraperitoneally injected with ulinastatin (10^4 U/kg) after anesthesia, tracheotomy intubation and mechanical ventilation were maintained for 1 h.

1.2 Methods

After anesthesia by intraperitoneally injected with 10% chloral hydrate (0.3 ml/100 g), the back region of the rats was shaved. The rats were placed in a self-made scald mold and immersed in 85°C for 15 s, resulting in a deep second degree 30% burn wound, 30% S (cm^2) = 30% K $\times$ $W^{2/3}$, the coefficient K = 9.1 (W: weight [g]).[5] After injury, injection of Ringer's fluid by 40 ml $\cdot$ kg^{-1} $\cdot$ $(cm^2)^{-1}$ intraperitoneal injection was carried out. The local tissue specimens were taken immediately after the injury, fixed with 10% formaldehyde, paraffin embedded and sliced. HE staining was used to determine the depth of the burn. After the operation, the rats were raised in a single cage and were free to have water. After 24 h of successful model, the rats were anesthetized again by intraperitoneal injection. The mechanical ventilation of tracheotomy was maintained for 1 h. The rats were sacrificed at 2 h, 6 h, and 12 h after 1 hour of mechanical ventilation. After the death of the abdominal aorta, the thoracic cavity was immediately removed, and the proportion of lung was measured by electronic balance to calculate the lung coefficient: Lung weight (g)/Body weight (kg). After switching to a non anticoagulant syringe, 4 ml blood was taken from abdominal aorta, centrifuged at 4°C, 600 $\times$ g, and then the supernate was stored at -80°C for further use. After the collection was complete, the samples were thawing at room temperature. The enzyme linked immunosorbent assay (ELISA) was used to determine the expression of IL-2, IL-10, TNF-α and IFN-γ. Immunohistochemical detection of TNF-α, IL-2 expression in lung tissue samples was performed using streptomycin-resistant peroxidase (SP method). The lung tissue specimens were soaked with 4% paraformaldehyde, paraffin embedded, sectioning, HE staining, and optical microscope observation at the final time point. The tissues detected by electron microscopy were fixed with 25% glutaraldehyde (purchased from Inner Mongolia Medical College).

1.3 Statistical analysis

SPSS13.0 statistical software was used for statistical analysis. The data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm$ s). Multiple groups were used to analyze the variance of repeated measurements, $p < .05$ means statistically significant.

2 Results

The gross lung tissue was pink, smooth, soft and elastic (see Figure 1). In the control group and the ventilation group, the manifestation of the acute lung injury was presented along with the swelling of the lung tissue, and the dark and the dark red sections were seen, accompanied with massive bleeding on the surface and the atelectasis of pulmonary margin. The change of lung tissue at 12 h was the most significant in the ventilation group. The edge of the lung was swollen. There were atelectasis and massive bleeding points and hemorrhagic foci. At the same time, there was obvious pleural effusion in the chest (see Figure 1). There was no obvious atelectasis and massive hemorrhage in the ventilation-ulinastatin group (see Figure 1).

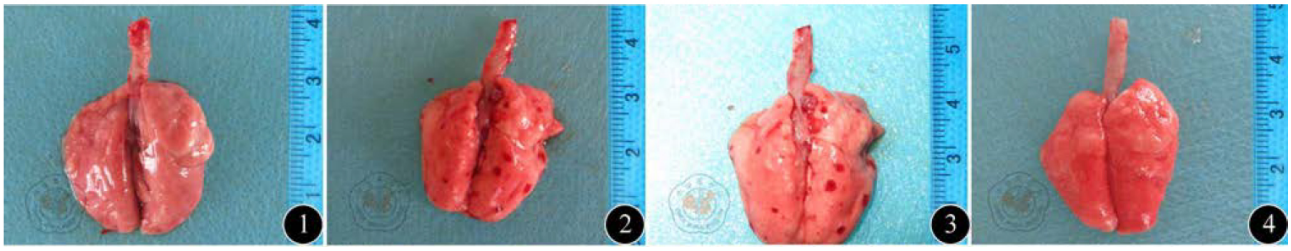

Figure 1: (1) The lung of normal rat; (2) The lung of rat in the control group at 12 h; (3) The lung of rat in the ventilation group at 12 h; (4) The lung of rat in the ventilation-ulinastatin group at 12 h

Table 1: Comparison of lung coefficient of rats in each group ($\bar{x} \pm s$, n = 10)

Groups	2 h	6 h	12 h	*F*-value	*p*-value
Control Group	4.51 ±0.16	4.96 ±0.33	5.21 ±0.21	12.714	.001
Ventilation Group	4.79 ±0.12*	5.56 ±0.19*	6.09 ±0.32*	48.269	< .001
Ventilation-Ulinastatin Group	4.48 ±0.20**	5.10 ±0.21**	5.46 ±0.37**	20.618	< .001
F-value	6.518	9.722	12.943		
p-value	.009	.002	.001		

Note. Compared with the control group, * $p < .05$; Compared with the ventilation group, ** $p < .05$

The lung coefficient (lung weight [g]/body weight [kg]) in the ventilation group was significantly higher than that in the control group ($p < .05$). Compared with the ventilation group, the lung coefficient decreased in the ventilation-ulinastatin group, the difference was statistically significant ($p < .05$) (see Table 1). The lung proportion of the three groups increased gradually and reached the maximum at 12 h.

HE staining of lung tissue was observed under light microscope. In the control group, the lung tissue was clear in structure, interstitial vascular dilatation, inflammatory infiltration, alveolar cavity exudation, alveolar wall thickening and bronchial epithelial destruction (see Figure 2). More diffuse alveolar collapse and telangiectasia could be seen in the lung tissue of rats under general anesthesia. Intravascular erythrocyte destruction caused by erythrocyte destruction, focal alveolar hemorrhage, and interstitial neutrophils in the lungs were found in large quantities. Pulmonary interstitial edema, alveolar septum to varying degrees of expansion and neutrophil infiltration were also observed (see Figure 2). In the ventilation-ulinastatin group, the pathological changes of lung tissue were less than those of the lung injury group. The alveolar hemorrhage was rare and the interstitial edema was reduced (see Figure 2).

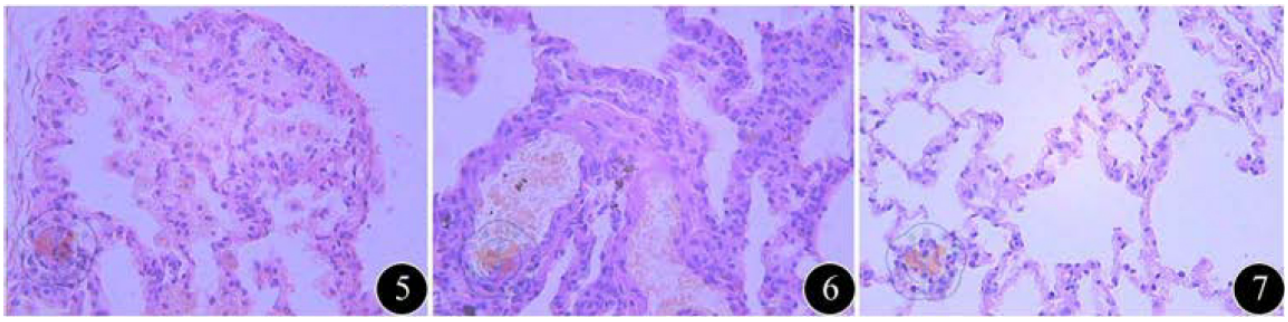

Figure 2: HE staining of lung tissue
(5) The control group at 6 h (HE × 400); (6) The ventilation group at 6 h (HE × 400); (7) The ventilation-ulinastatin group at 6 h (HE × 400)

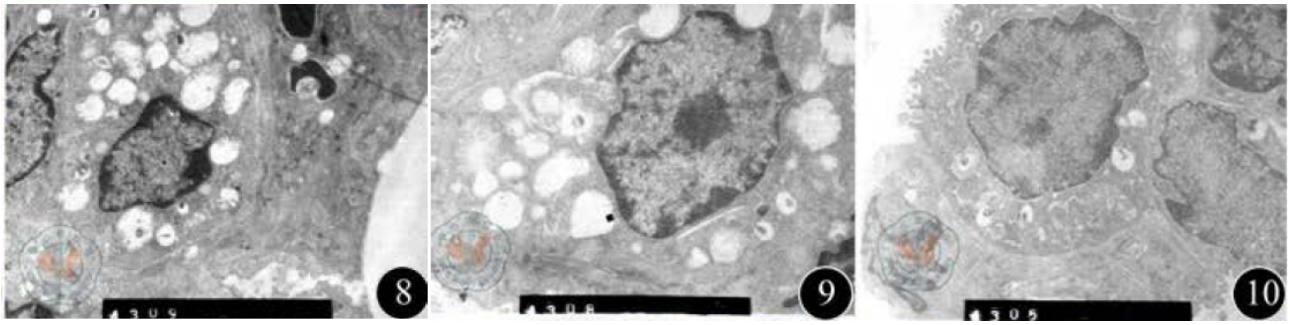

Figure 3: Electron microscopy observation
(8) The control group (× 7,000); (9) The ventilation group (× 9,000); (10) The ventilation-ulinastatin group (× 7,000)

Electron microscopy showed that in the control group and the ventilation group, the type I epithelial cells swelled the mitochondria cavitation and the crista disappeared. In type II epithelial cells, most of the lamellar bodies were emptying, forming larger vacuoles, swelling of capillary endothelial cells, falling off microvilli and fusion along with continuous damage of endothelial cells, widening of cell gap, loosening and widening of capillaries and alveolar basement

membrane, irregular thickening and uneven thickness (see Figure 3). In the ventilation-ulinastatin group: type I epithelial cell swelling was not obvious, and the lamellar corpuscle of type II epithelial cells was relieved. The number of vacuoles was less and the volume was small. The connection between capillary endothelial cells was normal, and the basement membrane was mildly loose and irregular thickening (see Figure 3).

After immunohistochemical staining, the absorbance was analyzed (see Tables 2-4 and Figures 4-6). The expression intensity of TNF-α, IL-2 and IFN-γ in the ventilation-ulinastatin group showed no significant difference compared with the ventilation group in the first phase (2 h). The intensity of IL-2 and IFN-γ expression was significantly lower in the second phase (6 h) than that in the ventilation group ($p < .05$), and in the third phase (12 h), both three cytokines were significantly lower than those in the ventilation group ($p < .05$).

Table 2: Comparison of optical density of TNF-α in each group ($\bar{x} \pm$ s, n = 10)

Groups	2 h	6 h	12 h	*F*-value	*p*-value
Control Group	0.201 ±0.045	0.413 ±0.093	0.614 ±0.141	25.031	< .001
Ventilation Group	0.228 ±0.052	0.631 ±0.127*	0.946 ±0.238*	30.866	< .001
Ventilation-Ulinastatin Group	0.214 ±0.063	0.452 ±0.138	0.634 ±0.157**	16.768	< .001
F-value	0.371	5.560	6.152		
p-value	.696	.016	.011		

Note. Compared with the control group, * $p < .05$; Compared with the ventilation group, ** $p < .05$

Table 3: Comparison of optical density of IL-2 in each group ($\bar{x} \pm$ s, n = 10)

Groups	2 h	6 h	12 h	*F*-value	*p*-value
Control Group	0.256 ±0.063	0.488 ±0.099	0.722 ±0.202	17.915	< .001
Ventilation Group	0.349 ±0.103	0.743 ±0.151*	1.178 ±0.225*	36.826	< .001
Ventilation-Ulinastatin Group	0.328 ±0.083	0.515 ±0.121**	0.834 ±0.221**	16.761	< .001
F-value	1.978	7.443	7.276		
p-value	.173	.006	.006		

Note. Compared with the control group, * $p < .05$; Compared with the ventilation group, ** $p < .05$

Table 4: Comparison of optical density of IFN-γ in each group ($\bar{x} \pm$ s, n = 10)

Groups	2 h	6 h	12 h	*F*-value	*p*-value
Control Group	0.229 ±0.053	0.490 ±0.072	0.677 ±0.114	43.208	< .001
Ventilation Group	0.273 ±0.059	0.603 ±0.073	0.887 ±0.157*	50.383	< .001
Ventilation-Ulinastatin Group	0.259 ±0.057	0.467 ±0.093**	0.669 ±0.109**	31.621	< .001
F-value	0.973	4.983	5.479		
p-value	.401	.022	.016		

Note. Compared with the control group, * $p < .05$; Compared with the ventilation group, ** $p < .05$

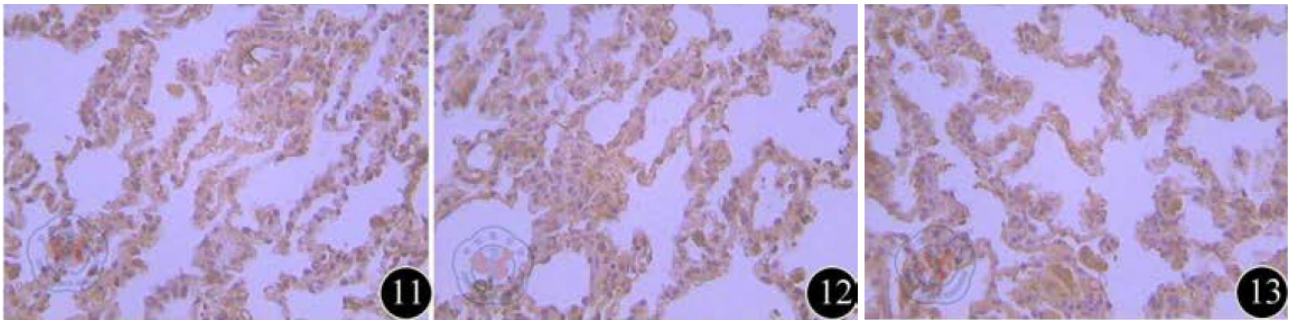

Figure 4: Expression of TNF-α in each group
(11) The control group at 12 h (immunohistochemical × 400); (12) The ventilation group at 12 h (immunohistochemical × 400); (13) The ventilation-ulinastatin group at 12 h (immunohistochemical × 400)

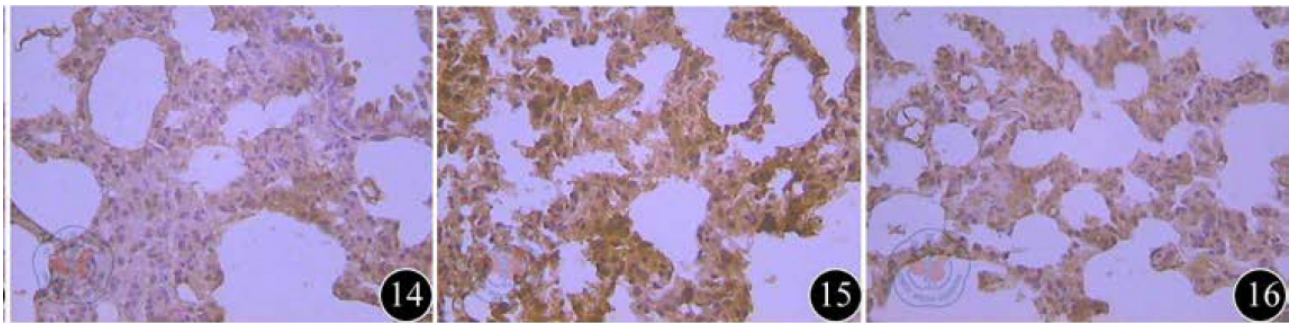

Figure 5: Expression of IL-2 in each group
(14) The control group at 12 h (immunohistochemical × 400); (15) The ventilation group at 12 h (immunohistochemical × 400); (16) The ventilation-ulinastatin group at 12 h (immunohistochemical × 400)

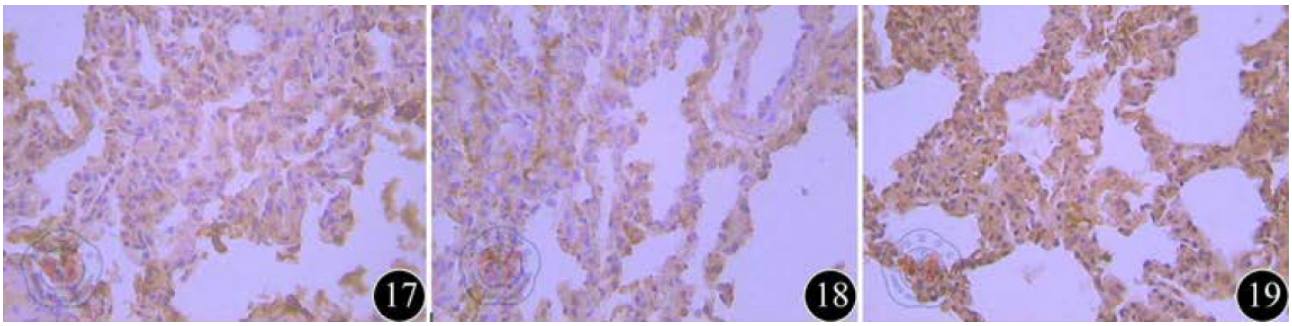

Figure 6: Expression of IFN-γ in each group
(17) The control group at 12 h (immunohistochemical × 400); (18) The ventilation group at 12 h (immunohistochemical × 400); (19) The ventilation-ulinastatin group at 12 h (immunohistochemical × 400)

3 Discussion

Perioperative mechanical ventilation after burns is more prone to acute lung injury, leading to acute respiratory distress syndrome. To find effective treatment methods has become a hot issue in today's medical research. Van der Heijden et al.[6] believed that the inflammatory response in the pathogenesis of lung injury after burns plays a key role. Basu et al.[7,8] stated that systemic inflammatory response disorder, runaway, and aggravation of body and tissue damage are the results from the development of acute lung injury or multiple organ dysfunction syndrome after burn. In this experiment, the rate of respiratory frequency was observed to increase and the bloody secretions at the mouth and nose were found in rats after 24 h, suggesting that there was a serious lung injury. It indicates that moderate to severe burns are mostly caused by the activation of disseminated inflammatory cells, and the injury of tissue in the distant site may occur.

Mechanical ventilation should achieve the most appropriate gas exchange with minimal ventilation value and emphasize the protection of vital organs and avoid ventilator-related lung injury occurred, namely "lung protective ventilation strategy": to maintain low airway pressure,[9] permissible hypercapnia,[10] moderate expiratory pressure plus low tidal volume[11] and so on. In this study, the injury of the ventilation group was significantly greater than that of the ventilation-ulinastatin group, although we followed the above aspects, respiratory support could also cause damage to the lungs in varying degrees.

Monocyte-macrophages in vivo are the main cells that produce IFN-γ,[12] which has an important role in triggering further inflammatory responses. In this study, we showed that the content of IFN-γ in lung tissue immunohistochemistry was significantly higher than that in control group ($p < .05$), which indicated that IFN-γ played an important role in the pathophysiological process of acute lung injury/acute respiratory distress syndrome. This conclusion is consistent with the literature at home and abroad.[13] In the ventilation-ulinastatin group, it was observed that the content of IFN-γ was significantly lower than that of the ventilation group, which indicated that ulinastatin could inhibit the expression of IFN-γ and reduce the lung injury caused by IFN-γ. In the ventilation-ulinastatin group, it was observed that the content of IL-2 was significantly lower than that of the ventilation group, indicating that ulinastatin could inhibit the expression of IL-2 and reduce the lung injury caused by IL-2. TNF-α is one of the earliest cytokines produced after burns. It is the most important inflammatory cytokine and the ultimate promoter of inflammatory response.[14] Ye et al.[15,16] suggested that TNF-α led to a

decrease in the formation of surfactants, reduced the physiological function and caused acute respiratory distress syndrome. This experiment showed that, the expressions of inflammatory cytokines IL-2, IFN-γ were significantly higher, and the lung injury was relatively severer when TNF-α was relatively high in the ventilation group. Chen et al.[17] also showed that the increase of IL-2 and other inflammatory factors was significantly inhibited after the use of ulinastatin in perioperative patients. With the achievement of monoclonal anti symptomatic treatment of TNF-α, we affirmed the inflammatory promoting effect of TNF-α in acute lung injury/acute respiratory distress syndrome after moderate to severe burns.[18] In the experiment, the symptoms of dysuria, lips and cyanosis were found significantly improved in the ventilation-ulinastatin group, and the lung coefficient was significantly slighter than that in the ventilation group, TNF-α, IL-2 and IFN-γ decreased at the same time. We could conclude that ulinastatin is able to improve the body's anoxia, inhibit the formation of pulmonary edema and pulmonary alveolar exudation.

In our study, the effect of ulinastatin was observed to be better than that of the ventilation group. It indicates that in the clinical stage, ulinastatin is effective to prevent ventilator related acute lung injury in the perioperative period of high-risk patients with acute lung injury.[19] The pharmacological effects of ulinastatin are consistent with some of the major principles of treatment of lung injury, systemic inflammatory response syndrome, acute respiratory distress syndrome, and multiple organ dysfunction syndrome due to mechanical ventilation.[20] It can reduce the damage of cell tissue, improve blood circulation and tissue perfusion, inhibit excessive inflammatory reaction, especially in burn operation, and it also has protective effect on organ function.[21] Therefore, the effect of ulinastatin in the prevention and treatment of acute lung injury during the perioperative period has attracted wide attention of clinical workers and experimental researchers.

The study revealed that ulinastatin can significantly alleviate lung injury and reduce lung pathological changes, which may be related to the inhibiting effect on the level of proinflammatory factors and excessive inflammatory reaction in rats. The rational application of ulinastatin can significantly relieve the degree of lung injury in rats after burns. By studying the mechanism of ulinastatin on various cytokines, it provides a new therapeutic idea and experimental basis for the clinical treatment of lung injury after burns. Early prevention and treatment of lung injury caused by mechanical ventilation in burn patients may be of important clinical significance in preventing the development of acute respiratory distress syndrome.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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