Effect of dobutamine on lung aquaporin 5 in endotoxine shock-induced acute lung injury rabbit

Cai-Zhi Sun*, Hua Shen*, Xiao-Wei He, Lei Bao, Yang Song, Zheng Zhang, Hai-Dong Qin

Department of Emergency, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China
*These authors contributed equally to this work.
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Correspondence to: Zheng Zhang, MD. Department of Emergency, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China. Email: zzeicu@163.com; Hai-Dong Qin, MD. Department of Emergency, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China. Email: icuqhd@aliyun.com.

Background: Dobutamine, a commonly used vasoactive drug, has been reported to reduce pulmonary edema and protect against acute lung injury (ALI) by up-regulating aquaporin 5 (AQP_5) expressions. However, the underlying mechanism is still elusive.

Methods: ALI was induced by intravenous injection of LPS. Seventy male New Zealand white rabbits were randomly divided into seven groups: sham group, ALI group, dobutamine low-dose group [group ALI + Dob (L)], dobutamine medium-dose group [group ALI + Dob (M)], dobutamine high-dose group [group ALI + Dob (H)], ALI + Dob (H) + ICI group and sham + ICI group. ICI 118,551, a potent and specific beta-2 antagonist, could block the effect of dobutamine. The animals were sacrificed at 3 h after endotoxic shock and lungs were removed. The arterial blood gas was analyzed. The lung wet to dry (W/D) ratio was determined. The level of cyclic AMP (cAMP) in lung tissue was assessed by ELISA. The expression of AQP_5 protein was determined by western blotting and immunohistochemistry. The pathological alteration in lung tissue was evaluated by optical microscopy and electron microscope, and lung injury score was assessed.

Results: Dobutamine increased AQP_5 protein expression and cAMP level in a dose-dependent manner. Meanwhile, the degree of lung pathological and ultrastructural lesion was ameliorated and arterial blood gas was improved obviously. Additionally, W/D ratio and histological scores decreased significantly. However, the AQP_5 protein expression and cAMP level were significantly decreased in group ALI + Dob (H) + ICI than that in group ALI + Dob (H), the degree of lung pathological and ultrastructural lesion was more serious in group ALI + Dob (H) + ICI than that in group ALI + Dob (H) and the arterial blood gas was not obviously improved.

Conclusions: These results suggested that protective effect of dobutamine against endotoxin shock-induced ALI may be due to its ability of up-regulating AQP_5 protein expression via increasing intracellular cAMP concentration.

Keywords: Septic shock; acute lung injury (ALI); dobutamine; aquaporin 5 (AQP_5); cyclic AMP (cAMP)

Introduction

Acute lung injury (ALI) remains a major contributor to morbidity and mortality in critically ill patients. The frequency of ALI in the United States alone is ~190,000 cases/year with a result of 74,500 deaths (1). Unfortunately, the mortality remains at a high level in patients with ALI, despite that many studies aimed at investigating the mechanism of ALI have been performed. ALI is characterized by alveolar epithelial injury leading to increased permeability of the alveolar-capillary barrier, which often manifests with increased extravascular lung water and the formation of pulmonary edema. Recently, extravascular lung water, which is closely related to the formation of pulmonary edema, was shown to be an independent predictor of intensive care unit mortality in patients with septic shock (2). Accordingly, understanding the mechanisms of water transport across biological membranes and alleviating pulmonary edema during severe sepsis and septic shock may reverse the progression of lung injury.

The aquaporins (AQPs) are a family of small integral membrane proteins which are expressed in both prokaryotes and eukaryotes. Among the 13 isoforms in the mammalian AQP family identified to date (3), aquaporin 5 (AQP_5) is a water-selective AQP which is widely distributed in various tissues throughout the body such as granules of Brunner glands in the duodenum (4), salivary gland (5), cornea (6), lens (7) and apical membrane of type I alveolar epithelial cells (8,9). AQP_5 has been thought to be a pivotal participant in the fluid secretion of the submucosal serous gland. Studies in animal's models have shown that AQP_5 plays a key role in maintaining normal lung water homeostasis and changes in the amount of cell surface expression of AQP_5 may contribute to abnormal water metabolism in various lung diseases (10). A study examining the distribution of AQP_5 in lacrimal gland showed that a selective defect in the trafficking of AQP_5 in the lacrimal gland may be responsible for the pathogenesis of Sjogren syndrome (11). Furthermore, targeted deletion of AQP_5 in mice leads to significantly reduction of fluid secretion from submucosal glands (12) and a 10-fold decrease in osmotic water permeability of the alveolar-capillary barrier in distal lungs (13). These previous studies have showed that AQP_5 plays a pivotal role in maintaining normal tissues water homeostasis, and abnormal expression and/or dysfunction of AQP_5 may contribute to abnormal water metabolism in lung, resulting in the formation of pulmonary edema.

β-adrenergic receptors (β-ARs) are prototypic members of the G-protein-coupled receptors which participate in regulating the concentration of intracellular second messengers such as cyclic AMP (cAMP) (14). β-receptor binding activates trimeric G proteins, leading to the information of stimulatory G proteins (15). Stimulatory G proteins then activate adenyl cyclase, resulting in an increase in intracellular cAMP concentration, which subsequently leads to protein kinase A formation. Protein kinase A directly phosphorylates the specific sequence of AQP_5 to regulate its activity, which subsequently increase membrane permeability to water (15,16).

Dobutamine, as a synthetic β-AR agonist, is commonly administered to patients with low-output states secondary to ALI/ARDS to maintain tissue perfusion. In addition, dobutamine is usually used in patients with non-cardiac pulmonary edema and investigations have demonstrated that dobutamine has protective effect on ALI (17,18). However, the underlying mechanism is unclear. In the present study, we focused on the effect of dobutamine on AQP_5 expression in the lungs of New Zealand rabbits with ALI induced by endotoxin shock and further addressing the possible mechanism.

Materials and methods

Experimental animal

Seventy male New Zealand white rabbits, weighing approximately 2.0-2.5 kg, were purchased from the Laboratory Animal Center of Nanjing Hospital, Nanjing Medical University (Nanjing, China). The rabbits were housed in standard conditions with food and water ad libitum. All animal studies were approved by the Institutional Animal Use and Care Committee of Nanjing Hospital affiliated to Nanjing Medical University, and the care and handling of the animals were in accordance with the National Institutes of Health guidelines.

Experimental protocols

Rabbits were fasted for 12 h but allowed free access to water before the experiments. Animals were anesthetized with 20% urethane (5 mL/kg). Urethane was given through ear vein. The left carotid artery was cannulated to measure mean arterial pressure (MAP) continuously by using a Spacelab monitor instruments (USA). Left femoral vein was cannulated for medications and fluids. The animals were placed on a heating pad so that the body temperature

could be kept at 37.8-40.2 ℃. Lactated Ringer's solution was intravenously administered at 12.5 mL/kg/h by a LC-10ATvp plus Liquid Pump (Shimadzu, Japan).

Rabbits were randomly divided into seven groups (n=10 for each group): sham group, ALI group, dobutamine low-dose group [group ALI + Dob (L)], dobutamine medium-dose group [group ALI + Dob (M)], dobutamine high-dose group [group ALI + Dob (H)], ALI + Dob (H) + ICI (ICI 118,551, a specific beta-2 antagonist, Research Biochemicals International, Natick, MA) group and sham + ICI group. The rabbits in group ALI, group ALI + Dob (L), group ALI + Dob (M), group ALI + Dob (H) and group ALI + Dob (H) + ICI received intravenous LPS (600 µg/kg, Escherichia coli-serotype $O_{55}:B_5$, Sigma, USA) to induce endotoxic shock. Rabbits in group sham and sham + ICI received intravenous normal saline. Two hours after administration of LPS or normal saline, rabbits in group ALI, ALI + Dob (L), ALI + Dob (M), ALI + Dob (H) and ALI + Dob (H) + ICI were immediately resuscitated with Lactated Ringer's solution intravenously at 12.5 mL/kg/h. Rabbits in group ALI + Dob (L), ALI + Dob (M), ALI + Dob (H) continuously received dobutamine at 2.5, 5, 10 µg/kg/min, respectively, by a Liquid Pump (Shimadzu, Japan). Meanwhile, groups sham + ICI and ALI + Dob (H) + ICI were administrated with ICI 118,551. Ten rabbits from each group were sacrificed 3 h after septic shock respectively. ALI induced by endotoxic shock was confirmed by a lowered MAP (<60% of the baseline).

Sampling and storage

At the end of the experiment, rabbits were sacrificed and quickly perfused with phosphate-buffered saline (PBS) through the right ventricle after extracting the carotid artery blood for arterial blood gas analysis (BWD3-GEM Premier 3,000). And then lungs were removed for tissue analysis.

Measurement of cAMP level in lung

Lung tissue was treated with isobutyryl methylxanthine (Sigma-Aldrich) to inhibit phosphodiesterases. The amount of cAMP in lung tissue was evaluated using a commercial cAMP EIA kit (NewEast Biosciences; Malvern, PA, USA) according to the instructions of the manufacturer.

Wet to dry weight (W/D) ratio of lung

Left lung tissues were weighed and dried in an oven at 70 ℃ for 48 h to obtain pulmonary W/D ratio.

Pathological examination

Lung tissues were fixed by immersing into 4% paraformaldehyde and routinely processed into paraffin sections (5 µm). These sections were stained with hematoxylin and eosin. Six slices were selected from each group and six fields of each slice were visualized by light microscopy (×400) (Olympus, DP73). The degree of pathological injury was scored based on the following variables: hemorrhage, lung edema, inflammatory cell infiltration, hyaline membrane and atelectasis; The degree of each abnormality was graded numerically from 0 (normal) to 4 (diffuse injury) according to the following criteria (19): no injury =0; injury to 25% of the field =1; injury to 50% of the field =2; injury to 75% of the field =3; diffuse injury =4. Blind analysis was performed to determine the lesion degree of all samples.

Transmission electron microscope

Lung tissue samples were fixed with 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (PH 7.2) followed by 1% osmium tetroxide in the same buffer. The specimens were dehydrated through an upgraded acetone series at room temperature. Counterstaining was done in a saturated solution of uranyl acetate followed by lead citrate. Sections were examined with a transmissions electron microscope (×50,000) (Phillips, Netherlands).

Immunohistochemistry

Lung tissue samples were fixed in 4% paraformaldehyde and cut into 5 µm sections. Immunohistochemistry was performed after blocking endogenous peroxidase activity with 3% H_2O_2 and methanol for 10 min, and non-specific protein binding with 10% sheep serum for 15 min. Sections were then incubated with anti-AQP_5 antibody (1:200 dilution; SantaCruz Biotechnology, CA, USA) overnight at 4 ℃. After three rinses of 5 min with PBS, sections were incubated with peroxidase-conjugated IgG antibody. Following three rinses of 5 min with PBS, the slides were then stained utilizing the biotin-avidin peroxidase method. After development, slides were counterstained with hematoxylin. The immunoreaction was evaluated with the Imagepro-Plus 12.0 which had been programmed to determine the mean optical density (MOD) of positive areas.
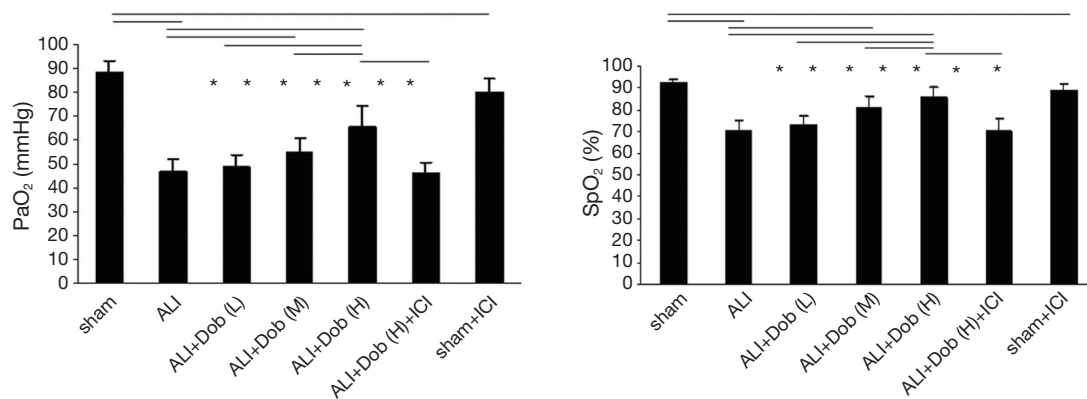
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Figure 1 Changes of PaO$_2$ and SpO$_2$ level in different groups. The values are expressed as mean ± SD, n=10 per group, *, P<0.05. ALI, acute lung injury.
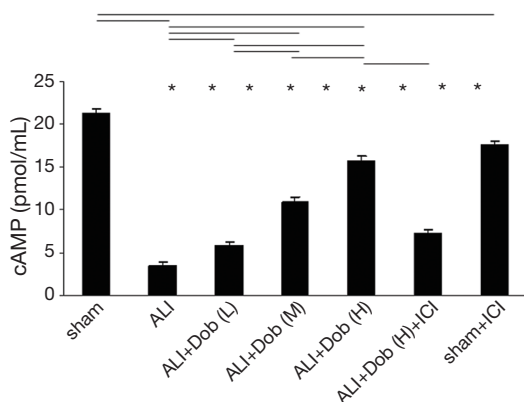

Figure 2 The lung tissue cAMP concentration was measured by ELISA in different groups. The values are expressed as mean ± SD, n=10 per group. *, P<0.05. ALI, acute lung injury.

Western blotting analysis

Samples were homogenized in cold RIPA lysis buffer (150 mM NaCl, 25 mM Trise-HCl PH 7.6, 1% NP-40, 1% sodium deoxycholate, 1% SDS, and 1% protease inhibitor cocktail) and lysed 30 min on ice. Total lysate proteins were resolved using 15% SDS-PAGE and transferred to a PVDF-membrane. Membrane was blocked with blocking solution (5% nonfat dry milk in TBS-T) at room temperature for 2 h and then incubated overnight at 4 ℃ with anti-AQP$_5$ polyclonal antibody (1:400, SantaCruz biotechnology, CA, USA). After washing in TBS-T, the membrane was incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase at room temperature for 2 h, then washed in TBS-T. Immunoblots were visualized on photographic films using Enhanced Chemical

Luminescence reagents. Bands were quantified using Gel-Pro32 software. The relative expression of AQP$_5$ was normalized to that of GAPDH.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed using SPSS 17.0 statistic software (SPSS Inc., USA). The differences among the different groups were analyzed by one-way ANOVA (LSD t-test). P<0.05 was considered statistically significant.

Results

Changes of PaO$_2$ and SpO$_2$ level in different groups

As demonstrated in *Figure 1A,B*, LPS produced significant decrease of PaO$_2$ and SpO$_2$ in group ALI compared with group sham (P<0.05). PaO$_2$ and SpO$_2$ in group ALI + Dob (M) and ALI + Dob (H) were significantly improved by dobutamine when compared with group ALI (P<0.05). Compared with group ALI + Dob (L), SpO$_2$ in group ALI + Dob (M) and ALI + Dob (H) was also increased (P<0.05) and PO$_2$ in group ALI + Dob (H) increased significantly (P<0.05). However, SpO$_2$ and PaO$_2$ in group ALI + Dob (H) + ICI lowered obviously than that in group ALI + Dob (H).

The cAMP concentration of lung tissue in different groups

As shown in *Figure 2*, the cAMP concentration was markedly lower at 3 h (P<0.01) in group ALI than that in group sham and it was up-regulated in a dose-dependent manner by dobutamine treatment in group ALI + Dob (L).
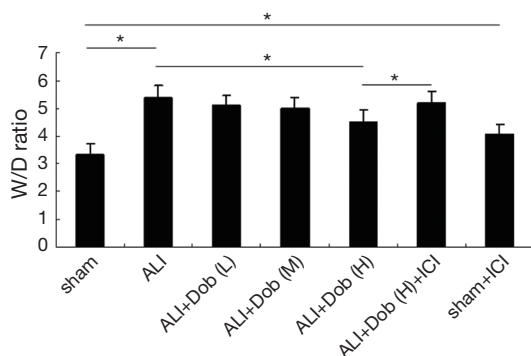
Figure 3 Change on W/D ratio in different groups. Lung permeability was evaluated by W/D ratio and the values are expressed as mean ± SD, n=10 per group. *, P<0.05. W/D, wet to dry; ALI, acute lung injury.

ALI + Dob (M) and ALI + Dob (H). But the level of cAMP in group ALI + Dob (H) + ICI was decreased that that in group ALI + Dob (H) (P<0.05), and it was lessened in group sham + ICI compared with that in group sham (P<0.05).

Changes of lung tissue W/D ratio in different groups

Figure 3 showed that the W/D ratio was significantly higher in group ALI than that in group sham at 3 h after shock (P<0.01). Compared with group ALI, the W/D ratio in group ALI + Dob (L) and ALI + Dob (M) did not have statistically significant differences (P>0.05), while the W/D ratio in group ALI + Dob (H) was decreased obviously (P<0.01); additionally, the W/D ratio in group ALI + Dob (H) + ICI was higher than that in group ALI + Dob (H) and it was the same in group sham + ICI than that in group sham.

The lung histological alteration in different groups

Figure 4 demonstrated the results of lung tissue pathological examination. (A) Lungs were collected 3 h after shock and subject to histological staining; (B) the lung injury scores in different groups.

As demonstrated in *Figure 4A*, only few inflammatory cells were observed in group sham, however, the lung injury in group ALI was very serious after LPS treatment. After administration of dobutamine, lung injury had been improved in different degrees. Interestingly, the lung damage had not been significantly alleviated in group ALI + Dob (H) + ICI compared with that even in group

ALI + Dob (L). In addition, lung histological alteration in group sham + ICI was nearly the same as that in group sham. *Figure 4B* showed that the histological scores were significantly higher in group ALI than that in group sham (P<0.01). Administration of dobutamine reversed the lung injury in a dose-dependent manner as indicated by decreasing histological scores of lung tissues in different groups. Nevertheless, the total histological scores in group ALI + Dob (H) + ICI were increased significantly compared with that in group ALI + Dob (H) when administration of ICI 188,551, a specific beta-2 antagonist.

The lung ultrastructural alteration in different groups

As shown in *Figure 5*, group sham revealed that alveolar type I epithelial cells (AT I) had only mild injury. However, group ALI exhibited that mitochondria were swelled, most of mitochondrial cristae were broke off or even disappeared in vacuoles degeneration; endoplasmic reticulum expanded significantly, forming different sizes vacuoles degeneration. Dobutamine administrated in group ALI + Dob (L) showed no significant effect on the improvement of AT I, but ultrastructural injury of AT I in group ALI + Dob (M) and ALI + Dob (H) improved obviously. However, the degree of ultrastructural lesion in group ALI + Dob (H) + ICI did not get significantly improved compared with that in group ALI + Dob (H).

The distribution and expression of lung tissue AQP_5 protein in different groups

Immunohistochemistry demonstrated AQP_5 was located in the cell membrane (indicated by black arrows, *Figure 6A*). As seen in *Figure 6B*, group ALI revealed a significant decrease in the MOD of the immunohistochemically positive areas of AQP_5 (P<0.05) compared with group sham and it was up-regulated by dobutamine in a dose-dependent manner. The results were further confirmed by Western blot (*Figure 7A,B*). Nevertheless, the distribution and expression of AQP_5 protein decreased obviously in group ALI + Dob (H) + ICI compared with that in group ALI + Dob (H) when effected by ICI 118,551 (P<0.05).

Discussion

LPS is a glycolipid which is the major constituent of the outermost membrane of gram-negative bacteria (20) and it has been thought to be responsible for ALI/ARDS. Previous
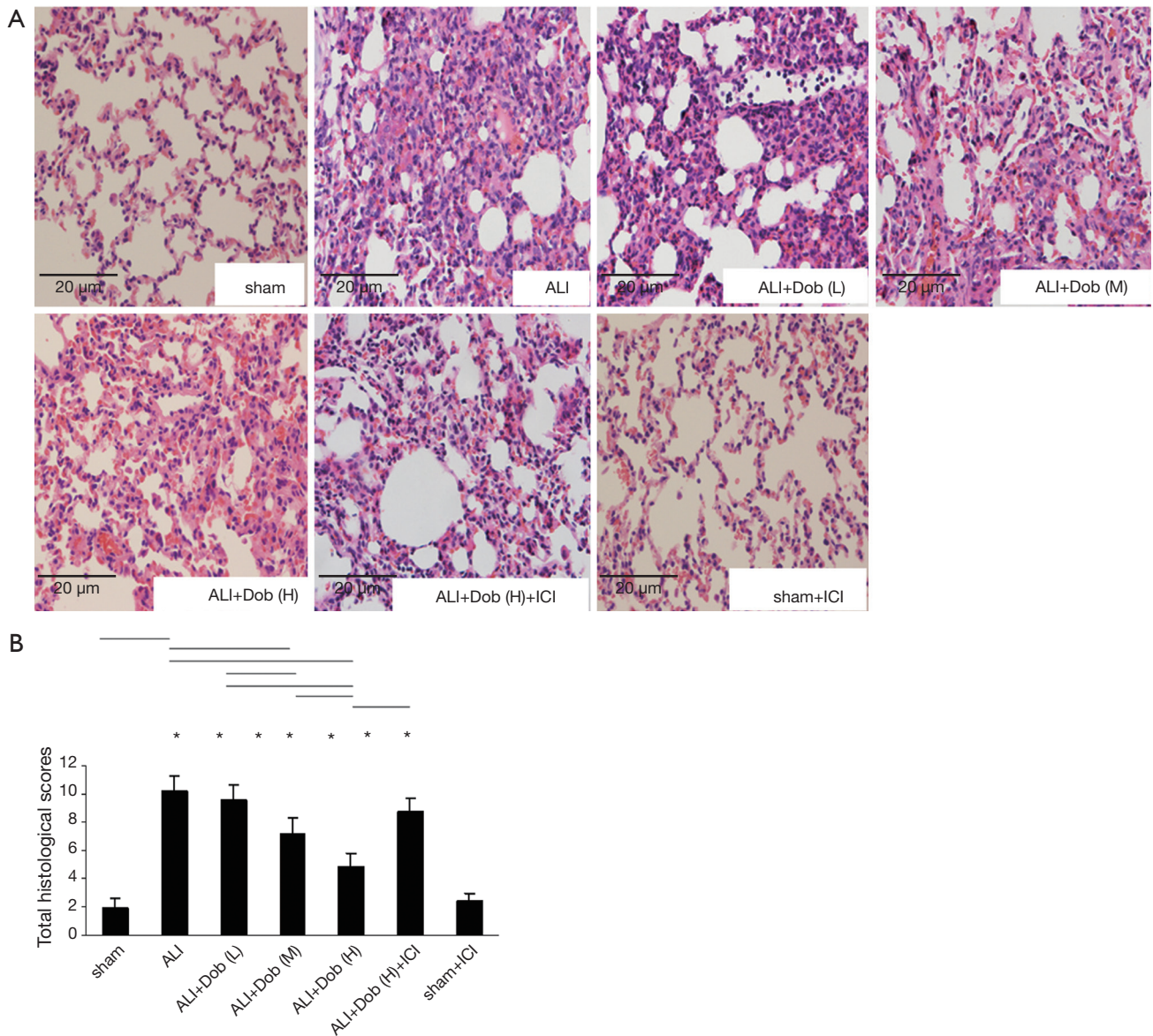
Figure 4 The lung tissue pathological examination in different groups. (A) The lung tissues were histologically analyzed by hematoxylin and eosin in different groups (scale bar =20 µm); (B) lung injury was assessed by histological scores in different groups. The values are expressed as mean ± SD, n=10 per group. *, P<0.05. ALI, acute lung injury.

ALI models have been established by intratracheal/ intranasal LPS instillation (21,22). However, local LPS exposure in lungs may not lead to systemic inflammation and multi-organ failure (23). Accordingly, we established lung injury model induced by endotoxic shock by injection of LPS intravenously and studied the protective effect of dobutamine. Microscopy studies of lung tissue in group ALI showed typical ALI. Dobutamine could mitigate ALI in a dose-dependent manner as indicated by histological

and ultrastructural studies and decreased pulmonary edema as well as the arterial blood gas analysis. In addition, the result of inflammatory cells in lung tissue decreased after the treatment of dobutamine further supported that β-AR agonist had significant anti-inflammatory effect (15,24).

In the present study, pulmonary edema was detected by W/D ratio analyses in rabbits' lungs after administration of LPS. We found that endotoxin shock-induced ALI led to a significant increased W/D ratio compared with
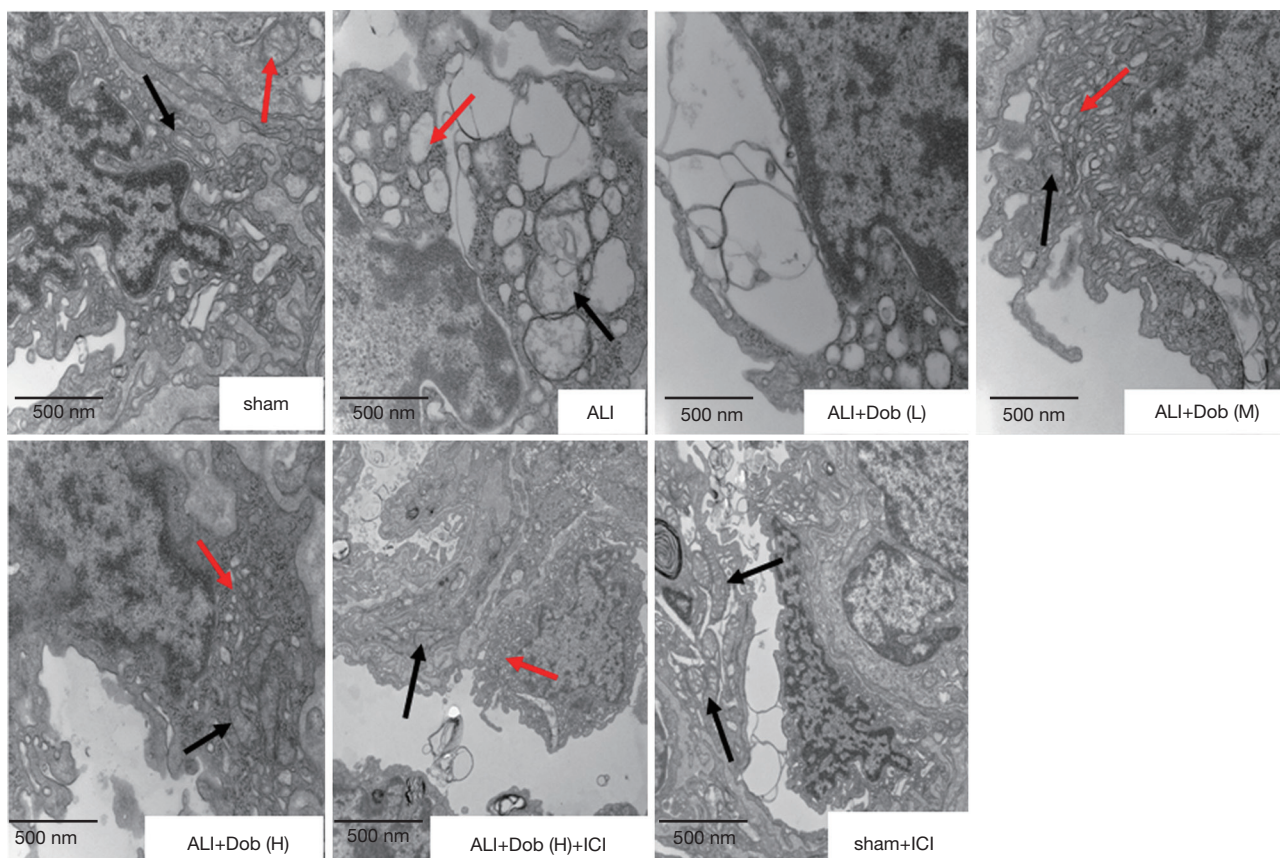
Figure 5 The ultrastructural alteration of lung tissue in different groups. Black arrows indicate mitochondria; red arrows indicate endoplasmic reticulum; the lung tissues were stained by uranium acetate in different groups, scale bar =500 nm. ALI, acute lung injury.

that of control group and the increase in W/D ratio was highly consistent with the presence of pulmonary edema. In addition, the decreased AQP_5 protein expression was demonstrated in the rabbits lungs after ALI induced by LPS besides the level of cAMP and serious pathology damage of lung tissue. The result of decreased AQP_5 expression was in line with the findings from Hasan and colleagues (25) that the expression of AQP_5 was significantly decreased at 12 h and 24 h after treated with LPS in the mice model though the AQP_5 expression was higher at 24 h than that at 12 h. However, in contrast to our results, Ohinata *et al.* (20) reported that the exposure of mouse lung epithelial cell line (MLE-12) to LPS at 0.5-2 h led to increased expression of AQP_5 in the plasma membrane and increased osmotic water permeability. Whether the discrepancy is species-related and time-related deserves further studies. In addition, in the process of the development of ALI induced by LPS, we found that the down-regulation of AQP_5 protein expression may be closely related to a large number of inflammatory

mediators releasing after administration of LPS and those cytokines activating the NF-κB and/or MAPK signal pathway (8,26,27).

Once the excess water gathered in the lung tissue, the alveolar fluid needs to be reabsorbed for the epithelium to heal. Investigations had shown that β-agonists could promote the edema clearance in normal and injured lungs (28,29). In the present study, dobutamine was shown to decrease the W/D weight ratio and relieve the pulmonary edema. Additionally, the AQP_5 protein expression and the cAMP level were elevated in a dose-dependent manner and the pathological damage of lung tissue was obviously improved. The above results were the evidence that dobutamine could alleviate pulmonary edema, increase AQP_5 protein expression and cAMP level, which the protective effects of dobutamine on ALI were in accordance with the recent studies. Wu *et al.* (18) have previously reported that dobutamine may enhance alveolar fluid reabsorption by increasing the expression of AQP_5 in a rat
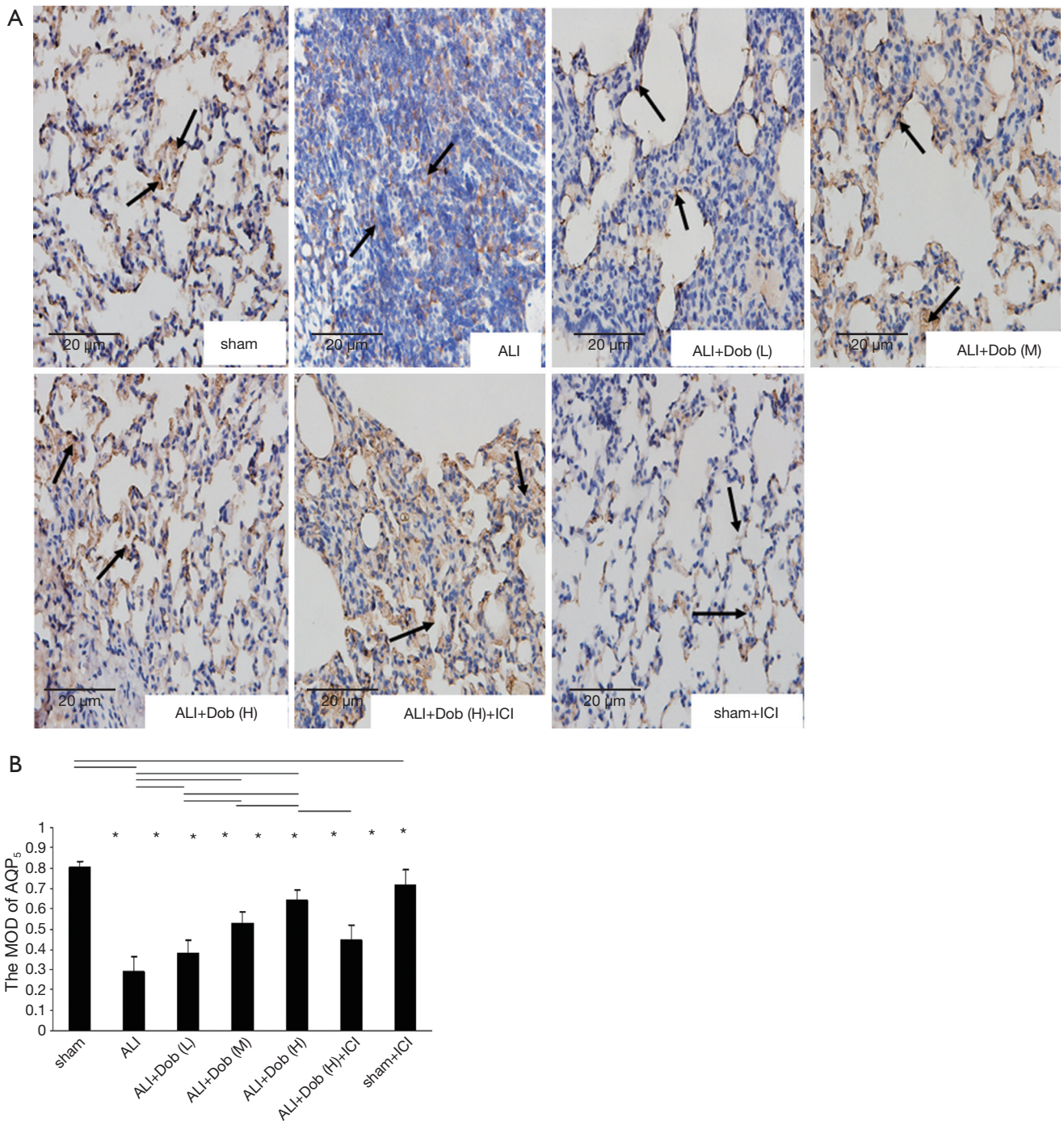
Figure 6 The distribution of lung AQP$_5$ protein expression in different groups. (A) The distribution of lung AQP$_5$ protein expression detected by immunohistochemistry (black arrows indicate the distribution of AQP$_5$ protein in lung tissue; the lung tissues were addressed by avidin biotin-enzyme compound staining method in different groups, scale bar =20 μm); (B) the mean optical density (MOD) of AQP$_5$ immunohistochemically positive areas in different groups. The values are expressed as mean ± SD, n=10 per group. AQP$_5$, aquaporin 5; ALI, acute lung injury.
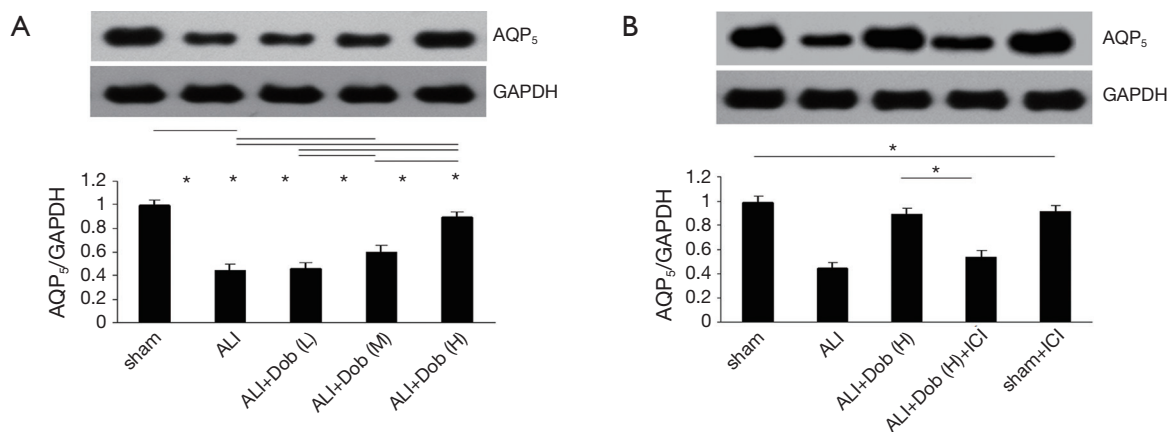
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Figure 7 Expression of AQP_5 protein in lung tissue detected by western-blot in different groups. (A) The effect of dobutamine on lung AQP_5 protein expression in rabbits ALI model induced by septic shock; (B) ICI 118,551 blocked the dobutamine effect on lung AQP_5 protein expression. The values are expressed as mean ± SD, n=10 per group. *, $P<0.05$. AQP_5, aquaporin 5; ALI, acute lung injury.

model of LPS induced lung injury. Susa and colleagues had also demonstrated that isoproterenol, a β-AR stimulant, could increase significantly AQP_5 expression in rat salivary glands (30).

In the present study, we also noticed the opposite trend of AQP_5 and W/D ratio both in rabbit ALI model and after the intervention of dobutamine, which we may speculate that decreased AQP_5 protein expression may lead to decreased water efflux from the lung and form the pulmonary edema and eventually aggravate lung injury. Surprisingly, the above speculation was consistent with those of Zhang *et al.* (31), reporting that AQP_5 deficiency blocked the excess water transport and damaged the pulmonary barrier function and, thus, aggravated the ALI induced by pseudomonas aeruginosa in mice model. Moreover, it is noteworthy that PaO_2 and SpO_2 level decreased in ALI model induced by LPS while that were improved significantly after administration of dobutamine. The variation tendency of PaO_2 and SpO_2 level was consistent with the pathological process of ALI. The alveolar epithelial was damaged and the permeability of alveolar-capillary barrier increased after the occurrence of ALI induced by LPS, resulting in large amounts of fluid exuding into alveolus, which affected the gas exchange of lung and finally decreased the level of PaO_2 and SpO_2. When the pulmonary edema was mitigated and pulmonary ventilation function was improved significantly with the administration of dobutamine, we found that the PaO_2 and SpO_2 level was improved obviously.

cAMP, as an intracellular second messenger, has been reported to modulate the expression of AQP_5 in series

of researches. Yang *et al.* (32) showed that the elevation of endogenous cAMP could evoke the increase in AQP_5 expression level in murine lung epithelial cell line and mouse lung tissue slices. Sidhaye *et al.* (33) demonstrated that AQP_5 expression in lung epithelial cells increased after sustained exposure to cAMP (8 h). In addition, it has been reported that cAMP-PKA signal pathway played an important role in regulating the AQP_5 expression in rat cultured nasal epithelial cells (34). Indeed, PKA may rapidly induce AQP_5 phosphorylation at Ser156 in BEAS-2B human bronchial epithelial cells (35) and Thr259 in human salivary gland cells and mouse salivary glands rapidly through intracellular cAMP signaling pathways (36). Similarly, β-AR agonist may increase AQP_5 expression via cAMP (10,32,33). The question of why dobutamine increases the AQP_5 level and decreases the pulmonary edema is undoubtedly an important consideration. Taken together with the above previous findings, our present data suggest that the regulation of AQP_5 protein expression in lung tissue of rabbit ALI model may be induced via cAMP pathway.

In order to further confirm the role of cAMP pathway in regulating AQP_5 protein expression in lung tissue, we examine the ICI 118,551, a specific β-2 AR antagonist, blocking the effect of dobutamine on expression of AQP_5 protein and cAMP level. Surprisingly, we found that the expression of AQP_5 protein and the level of cAMP in lung tissue both decreased with ICI 118,551 blocking the effect of dobutamine along with increased pulmonary edema. Therefore, we may speculate that the protective effect

of dobutamine on ALI by attenuating lung edema and improving the pathological process may be dependent on the up-regulation of AQP_5 via increasing intracellular cAMP level.

However, the present study has some limitations. First, we only investigated the acute effects of dobutamine on ALI within 3 h the chronic effects remain to be elucidated. Second, intravascular blood in lung may affect the accuracy of lung W/D ratio which is widely used to assess pulmonary edema. Nevertheless, in the experiment, we exsanguinated the animals carefully in all groups and did not note significant amounts of blood in the pulmonary vessels, suggested that the results could truly reflect pulmonary edema.

Conclusions

In summary, the present study has demonstrated that treatment with dobutamine significantly increase AQP_5 protein expression and intracellular cAMP concentration, attenuate lung tissue W/D ratio, improve arterial blood gas analysis and the pathological process of ALI induced by endotoxin shock in rabbits in a dose-dependent manner. And ICI 118,551 could block the protective effect of dobutamine on ALI. Those results suggest that dobutamine is beneficial in the treatment of ALI and the mechanism of the protective effects of dobutamine in lung injury model may be dependent on up-regulation of AQP_5 protein expression via increasing intracellular cAMP concentration. However, the exact mechanism of dobutamine on ALI needs to be further investigated before clinical application is expected.

Acknowledgements

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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