The role of pendrin in acute lung injury (ALI) /acute respiratory distress syndrome (ARDS) caused by ventilator induced lung injury

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The role of pendrin in acute lung injury (ALI) /acute respiratory distress syndrome (ARDS) caused by ventilator induced lung injury

Directed by Professor Moo Suk Park

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Thank you.

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ABSTRACT

The role of pendrin in acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) caused by ventilator induced lung injury

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Purpose: Pendrin (SLC26A4) is a transmembrane protein that exchanges anions such as bicarbonate and iodide with chloride. Recent data indicate that the expression of this transporter is upregulated in inflammatory airway diseases. In this study, we analyzed the role of pendrin expression and the effect of prone positioning in a ventilator-induced acute lung injury (VILI) animal model.

Methods: VILI was induced in the supine or prone position by a high tidal volume (HTV) of 30 mL/kg with a frequency of 100 breaths/min for 5 hours in pendrin wild-type (WT) and knockout (KO) 129SVEV mice. Lung injury parameters were assessed by bronchoalveolar lavage fluid (BALF) analysis, inflammatory cytokine analysis by ELISA, and histopathological findings. Pendrin expression was determined by western blotting, immunofluorescence (IF) staining, and transmission electron microscopy (TEM) using immunogold labeling methods.

Results: The protein concentration and total cell count in BALF were significantly

increased in pendrin-WT mice after HTV ventilation compared with those in non-ventilation pendrin-WT (control group) and pendrin-KO mice with HTV ventilation. In lung pathology, pendrin-WT mice with VILI showed a significant increase in leukocyte infiltration and lung injury relative to pendrin-KO mice with VILI and control group mice. Pendrin expression was upregulated in pendrin-WT mice with VILI, as determined by western blotting, IF staining, and TEM-immunogold labeling. Prone positioning during ventilation attenuated lung inflammation, as determined by BALF analysis and lung histopathological findings; but, pendrin expression between supine and prone positioning did not significantly differ. Nevertheless, when HTV was administered in prone position to pendrin-KO mice, the least degree of lung injury was observed, and pendrin expression detected TEM-immunogold labeling was also the lowest.

Conclusions: Our results suggest that pendrin plays a critical role in VILI and could be a novel target for modulating VILI. Prone positioning and pendrin inhibition in VILI could be effective in managing these conditions.

Keywords: pendrin; SLC26A4; ventilator-induced lung injury; acute lung injury

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I. INTRODUCTION

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is characterized by the rapid onset of hypoxemia with diffuse bilateral pulmonary infiltrates, leading to a high mortality rate and significant financial burden on critically ill patients [1]. Mechanical ventilation is often necessary to manage ALI/ARDS; however, it can cause lung damage and aggravate ALI/ARDS [2]. This condition is known as "ventilator-induced lung injury" (VILI), which is due to the inhomogeneous distribution of lung damage and edema in ALI/ARDS [3]. The mechanism of VILI comprises initiation by induced tensile strain and direct tissue damage to the lungs, leading to increased permeability and disruption of the alveolar–capillary barrier [4]. This mechanical strain also interrupts the clearance of edema fluid from the airspaces and triggers the release of inflammatory mediators within the distal lung, inducing end-organ dysfunction by entering the systemic circulation [5]. Although a ventilator strategy with low tidal volume improves the prognosis of VILI in patients with

ALI/ARDS, some patients are inherently susceptible to VILI [6]. Prone positioning is effective in preventing VILI by reducing uneven lung injury [7]; however, limited research on the molecular mechanisms of prone position management is available [8].

Pendrin (SLC26A4) is a transmembrane anion exchanger protein located on the apical surface of epithelial cells in the kidney, thyroid, and inner ear [9]. This protein acts as a Cl^-/anion exchanger that transports Cl^- to bases, including iodide (I^-), bicarbonate (HCO_3^-), hydroxide (OH^-), and thiocyanate (SCN^-) [10]. The mutation of pendrin is associated with Pendred syndrome, a recessively inherited disorder presenting with congenital deafness, goiter, or thyroid hormone abnormalities [11]. Pendrin is also negligibly expressed in normal airway epithelia [12]. Respiratory diseases characterized by excessive mucus production, including bronchial asthma or chronic obstructive pulmonary disease (COPD), are identified by pendrin overexpression [13]. Pendrin significantly exacerbates airway diseases triggered by viral infections or allergen exposure [14]. In contrast, a previous animal model study showed that pendrin knockout mice exhibit significantly decreased lung inflammation caused by *Bordetella pertussis* infection [15].

Recent research has shown that pendrin plays a role in the pathology of lipopolysaccharide (LPS)-induced ALI, and airway epithelial cells treated with pendrin could be a therapeutic target for managing ALI/ARDS [16]. Previous *in vivo* and *in vitro* studies have identified that pendrin expression is increased in an ALI/ARDS animal model by LPS treatment and in the human bronchoalveolar fluid of patients with ARDS due to pneumonia. Additionally, researchers have demonstrated that a novel pendrin inhibitor reduces the inflammatory response in LPS-induced ALI, verifying that pendrin could be a target for ALI/ARDS treatment [17]. Alveolar epithelial cell damage also occurs and contributes to the lung inflammatory process in an animal model of lung injury by ventilator stretching [18]; however, the role of pendrin in VILI has remained unclear and has not been reported.

Based on these observations, we hypothesized that pendrin expression is associated with the pathogenesis of VILI-induced ALI/ARDS. Therefore, we constructed a VILI mouse model and observed an increase in pendrin expression. We also investigated whether

pendrin-null mice exhibit lower levels of inflammation during VILI. Furthermore, the protective effects of prone positioning were examined in a VILI mouse model.

II. MATERIALS AND METHODS

1. Experimental animals

The 129SVEV mice (weight 20–30 g, age 6–8 weeks) were generously donated by Prof. JY Choi at Yonsei University. Pendrin wild-type (WT) and knockout (KO) mice were prepared by mating. All experimental animals were fed a standard diet and water, and the environment was maintained with as little stress as possible for at least one week prior to the experiments. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Yonsei University College of Medicine (6-2018-0164) and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2. Ventilator-induced lung injury model in mice

The mice were subjected to intraperitoneal anesthetia using a combination of ketamine (100mg/kg) and xylazine (10mg/kg) before performing the tracheostomy. After initial anesthesia, the mice were placed in the supine position, after which the nuchal skin below the mouth was cut, and the muscles were separated under the skin. Thereafter, the trachea was exposed and gently cannulated using a 20-gauge flexible catheter. Next, the mice were ventilated in the supine or prone position at a high tidal volume (HTV) of 30 mL/kg and a rate of 100 breaths per minute during 5 hours to induce VILI [19,20]. The other ventilator settings were 0 cm H_2O end-expiratory pressure and 0.21 inspired oxygen fraction (Figure 1). To maintain anesthesia, half doses of the initial anesthetic were injected

intraperitoneally every 30 min during mechanical ventilation. The pulse rate and peripheral oxygen saturation were continuously measured to monitor the mice using MouseOx (Starr Life Sciences Corp., Oakmont, PA, USA) after the thigh hairs of the mice were removed. Mice were sacrificed after completing the HTV protocol.

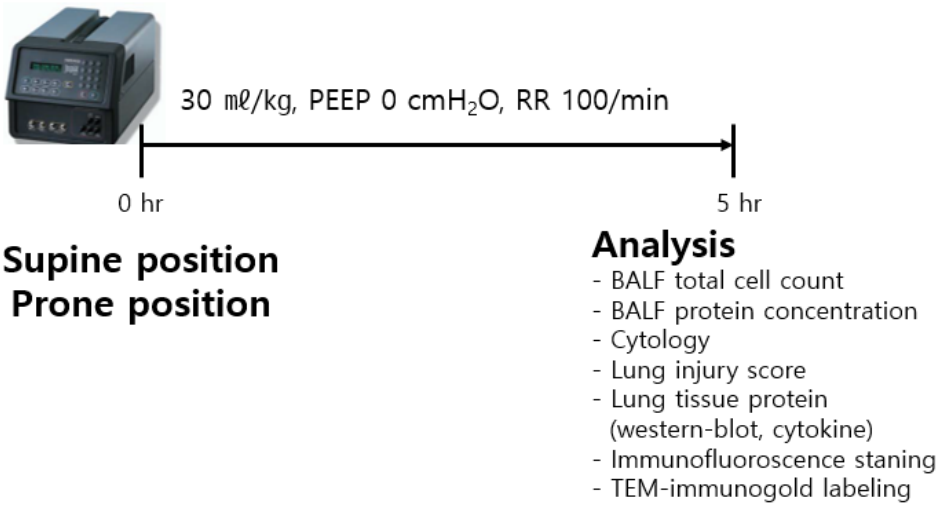


Figure 1. A ventilator-induced lung injury model in mice. Pendrin wild-type (WT) and knockout (KO) mice were tracheostomized and ventilated in the supine or prone position with a high tidal volume (HTV) of 30 mL/kg and a rate of 100 breaths/min for 5 h. The other ventilator settings were 0.21 inspired oxygen fraction and 0 cm H_2O end-expiratory pressure.

3. Study design and experimental protocol

Wild-type (WT) and knockout (KO) mice were randomly assigned to each HTV ventilation group in the supine and prone positions.

The experimental groups were as follows (n = 8–10 per group) :

1) non-ventilated group, pendrin WT (NVC, WT)

2) non-ventilated group, pendrin KO (NVC, KO)
3) supine HTV group, pendrin WT (supine_HTV, WT)
4) supine HTV group, pendrin KO (supine_HTV, KO)
5) prone HTV group, pendrin WT (prone_HTV, WT)
6) prone HTV group, pendrin KO (prone_HTV, KO).

4. Bronchoalveolar lavage fluid analysis

After mechanical ventilation with HTV for 5 hours, bronchoalveolar lavage (BAL) was performed through a tracheal catheter. The mice in the control group, which were not treated with mechanical ventilation, were humanely sacrificed through gas inhalation after being maintained for at least 7 days in the same environment as the experimental group, after which BAL was performed. BAL fluid (BALF) was directed into a tracheal catheter, gently retracted using 1 mL of sterile saline, and centrifuged (3000 rpm for 10 min at 4℃). The supernatant was stored at -80℃ to analyze the protein concentration and inflammatory cytokines. The protein concentration in the BALF supernatant was measured using a Bradford reagent (Coomassie Brilliant Blue G-250), and inflammatory cytokines were analyzed using ELISA. The remaining pellets were resuspended in 100 μL PBS to determine cell count. The sample was transferred to a slide chamber, which was placed into a cytocentrifuge (Shandon Cytospin 4 cytocentrifuge; Thermo Fisher Scientific, Waltham, MA, USA) facing outward. Afterward, the slides of each sample were centrifuged for 5 minutes at 800 rpm and dried at room temperature before staining. Subsequently, the slides of each sample were stained using a Diff-Quik Stain Set (Dade Behring, Newark, DE, USA) to determine cell count.

5. Lung tissue preparation and histological assessment

The bilateral diaphragms of the euthanized mice were cut, and the right ventricle of the heart was punctured and flushed with saline. After isolation, the right lung was immediately

stored at -80℃ for western blotting and PCR. The inflated left lung with low-melting 4% agarose was fixed in 10% formaldehyde for one day, after which it was cut to a 5-μm thickness using a Reichert microtome (Leica Microsystems, Wetzlar, Germany) after paraffin embedding. Subsequently, lung sections were conducted to hematoxylin and eosin staining and analyzed using bright-field microscopy. Histological evaluation of lung injury on each slide was performed using the weighted scoring scale described in the official American Thoracic Society Workshop Report [21].

6. Western blotting

The harvested right lung tissues were lysed and homogenized with homogenization buffer and then centrifuged for 30 minutes at 4℃. Protein concentrations in the supernatants were quantified using a BCA assay from Thermo Fisher Scientific. Subsequently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using an electrophorator with a running buffer. Thereafter, 20–30 μg of protein was loaded in each SDS-PAGE well, electrophoresed, and transferred to nitrocellulose membranes. The membranes were subjected to blocking for 1 hour at room temperature using a blocking buffer consisting of 5% skim milk, TBS-T, and 1% Tween 20 (Bio-Rad Laboratories, Hercules, CA, USA) for 1 hour at room temperature. Next, the blocked membranes were incubated with target protein-specific primary antibodies overnight at 4℃; the antibodies included rabbit SLC26A4 (ab98091; Abcam, Cambridge, UK) and mouse β-actin (sc47779; Santa Cruz Biotechnology, Dallas, TX, USA). Afterward, the membranes were washed with TBS-T to remove unbound antibodies and incubated with secondary antibodies in 5% skim milk and TBS-T for 1 hour at room temperature. Enhanced chemiluminescence substrates (SuperSignal West Pico Chemiluminescence Detection Kit; Thermo Fisher Scientific) were prepared to develop the protein, and ImageJ software (NIH, Bethesda, MD, USA) was used to quantify the western blots.

7. ELISA

Macrophage inflammatory protein (MIP-2), interleukin-6 (IL-6), interleukin-1β (IL-1ß), and tumor necrosis factor-α (TNF-α) levels in the lung lysates were measured using ELISA kits (MilliporeSigma, Burlington, MA, USA) according to the manufacturer's protocol.

8. Immunofluorescence staining

Pendrin immunofluorescence (IF) staining was performed using unstained mouse lung tissue slides from formalin-fixed paraffin-embedded blocks. Briefly, the formalin-fixed, paraffin-embedded tissue was deparaffinized at 65℃ for 30 minutes and dehydrated using xylene and ethanol (100, 95, and 70%). Thereafter, the slides were incubated with 0.1% Triton X in PBS for 50 minutes. Blocking in 1% BSA in PBS was performed before incubation with the antibody. The primary rabbit polyclonal antibody for pendrin mouse Pendrin R2 diluted in DAKO antibody dilution buffer (1:10,000; Agilent Technologies, Santa Clara, CA, USA) was incubated overnight at 4℃. After washing thrice with PBS, the secondary antibody fluorochrome-binding Alexa Fluor 488 goat anti-rabbit antibody diluted in DAKO antibody dilution buffer (1:500; Agilent Technologies) was incubated for 1 hour. Subsequently, the cell nuclei were stained using 1 μg/mL DAPI and phalloidin after removing the unbound secondary antibody by washing with PBS. Fluorescent images were acquired using a ZEISS LSM700 confocal microscope (ZEISS, Oberkochen, Germany). Morphometric analysis of captured images was performed using MetaMorph Microscopy Analysis Software (version 7.1; Molecular Devices, San Jose, CA, USA). The 24-bit confocal images, which included the red, blue, and green components, were transformed into three separate 8-bit monochannel images for quantification under each condition.

9. Transmission electron microscopy

Lung injury was assessed to visualize the target protein of pendrin in the tissue using gold-conjugated antibody labeling procedures for localization at the ultrastructural level through transmission electron microscopy (TEM). The harvested tissues obtained after euthanasia were trimmed into small pieces (1–5 mm^3) using a blade for TEM and washed with 1× PBS

until clear if needed. After rinsing, the tissue pieces were immediately placed in 2% paraformaldehyde and fixed for at least 2 hours. Afterward, the fixed tissue pieces were washed thrice for 10 min in 1× PBS using a low-speed shaker. The specimens were then placed on a cryostat chuck, which supported the tissue pieces with an optimal cutting temperature compound (OCT compound) surrounded by additional OCT compounds. Next, the tissue pieces in the OCT compound were cut with a cryostat to a thickness of 100 μm. The cut specimens were placed in Petri dishes or multi-well plates with 1× PBS and washed thrice with 1× PBS for 5 minutes. Thereafter, the washed specimens were put in a permeabilization solution (0.1% Triton X-100) for 3–5 minutes at room temperature. Afterward, the tissues were incubated in a Petri dish or multi-well plate containing 200 μL blocking buffer (1% BSA or 1% FBS in PBS) for 60 minutes at room temperature and washed five times for 5 minutes in 1× PBS in a low-speed shaker. Subsequently, 100 μL of a primary antibody (mouse R2 1:1000) diluted appropriately was added and incubated overnight at 4℃. After rinsing five times for 5 minutes in 1× PBS in a low-speed shaker to remove the remaining primary antibody, the section was placed in 100 μL of gold-conjugated secondary antibody with an antibody diluent for 1 hour at room temperature (at 4℃ if incubating for longer than 4 hours). The secondary antibody used was anti-rabbit IgG-conjugated gold nanoparticles, 5 nm, 1:25 (GRG-5; CD Bioparticles, Shirley, NY, USA). The TEM specimens were incubated in a solution including 2% glutaraldehyde and 2% paraformaldehyde with 0.1 M phosphate buffer (pH 7.4) for a fixation period of 12 hours. Following that, washing was conducted using 0.1 M phosphate buffer, post-fixed using 1% OsO_4 in 0.1 M phosphate buffer during 2 hours, and the specimens underwent dehydration through a series of ascending ethanol concentrations (50, 60, 70, 80, 90, 95, 100, 100%) for 10 minutes each and were then infiltrated with propylene oxide for 10 minutes. Afterward, the specimens were embedded with a Poly/Bed 812 kit (Polysciences, Warrington, PA, USA) and polymerized in an electron microscope oven (TD-700; Dosaka, Kyoto, Japan) at 65°C for 12 hours. The block was fitted with a diamond knife in an ultra-microtome (UC7; Leica Microsystems, Wetzlar, Germany), sliced to 200-nm semi-thin

sections. These sections were subsequently stained using toluidine blue and observed using an optical microscope. The specific region of interest was then sectioned into 80-nm thin sections with an ultra-microtome, placed on copper grids. Then, these sections were conducted to double-stained using 5% uranyl acetate during 20 minutes and 3% lead citrate for 7 minutes, and observed using a transmission electron microscope (HT7800; Hitachi, Tokyo, Japan) at an acceleration voltage of 80 kV. The transmission electron microscope was equipped with a Megaview III CCD camera (Soft Imaging System GmbH, NRW, Germany).

10. Statistical analysis

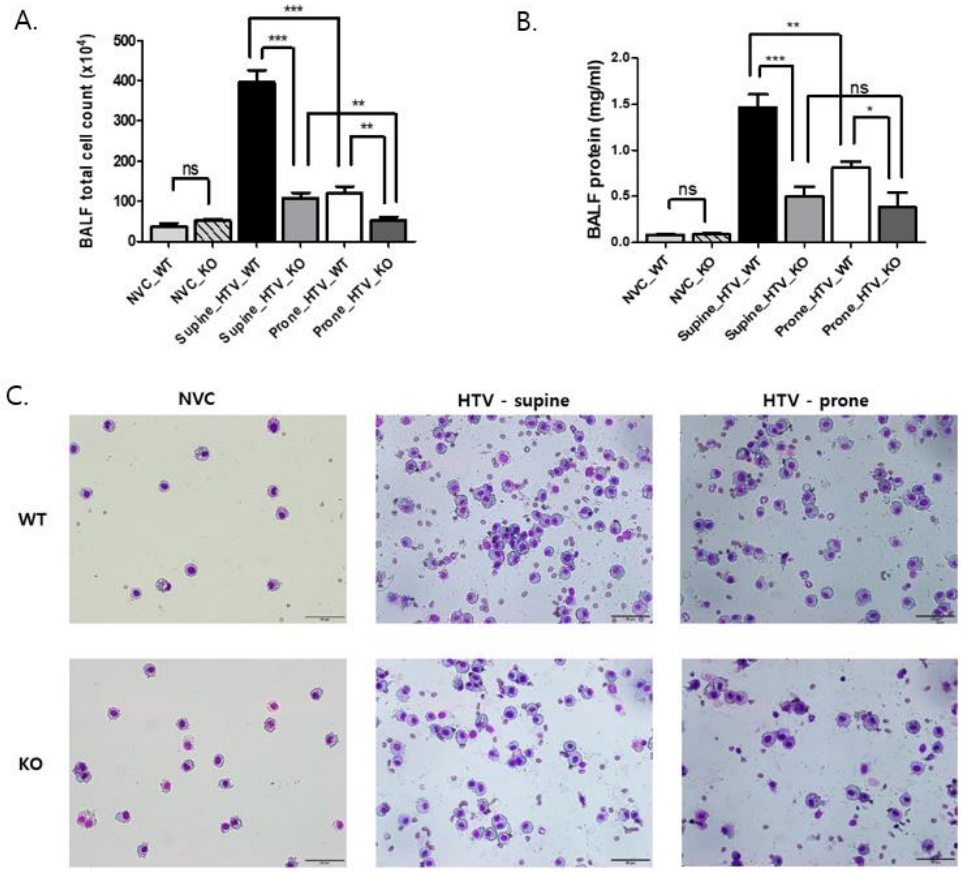
All statistical tests were performed using Student's unpaired two-tailed *t*-test for comparison of each group using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was considered at $p < 0.05$. Data are presented as the mean ± SEM. Lung injury score is presented as the median ± SE.

III. RESULTS

1. Deletion of the pendrin decreases the inflammatory response in VILI

To evaluate the role of pendrin in VILI, we performed BALF and histological assessments by comparing pendrin-WT and pendrin-KO mice (n = 8–10 mice per group). The total cell count and protein concentration in BALF were markedly increased in pendrin-WT mice after HTV ventilation compared with those in the non-ventilation control group (Figure 2A and 2B). After HTV ventilation, pendrin-KO mice had a lower total cell count and protein concentration than those of WT mice (Figure 2A and 2B). And, cell differentiation in pendrin-WT mice with HTV ventilation predominantly consisted of macrophages, with a few numbers of neutrophils and lymphocytes (Figure 2C and 2D). In addition, pendrin-KO

mice showed significantly reduced leukocyte infiltration and lung injury scores compared with those of WT mice after HTV ventilation (Figure 3A and 3B).



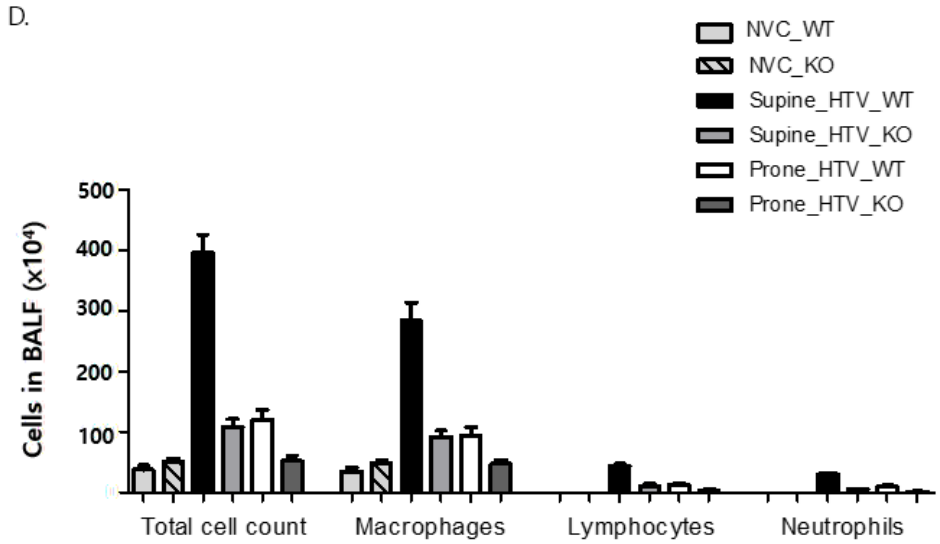


Figure 2. Deletion of pendrin and prone positioning attenuated the VILI in a mice model, as demonstrated by bronchoalveolar lavage (BAL) fluid and histologic findings. Pendrin wild-type (WT) and knockout (KO) mice were conducted to a tracheostomy and ventilation with a supine or prone position at a high tidal volume (HTV) of 30 mL/kg. (*A*) Total cell count in BAL fluid. (*B*) Protein concentration of BAL fluid. (*C*) BAL fluid cytology presented cell differentiation with visual fields. (*D*) Cell differential count of BAL fluid. NVC, non-ventilation control group; supine HTV, HTV ventilation in supine position; prone HTV, HTV ventilation in prone position. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ analyzed by Student′s unpaired two-tailed *t*-test.

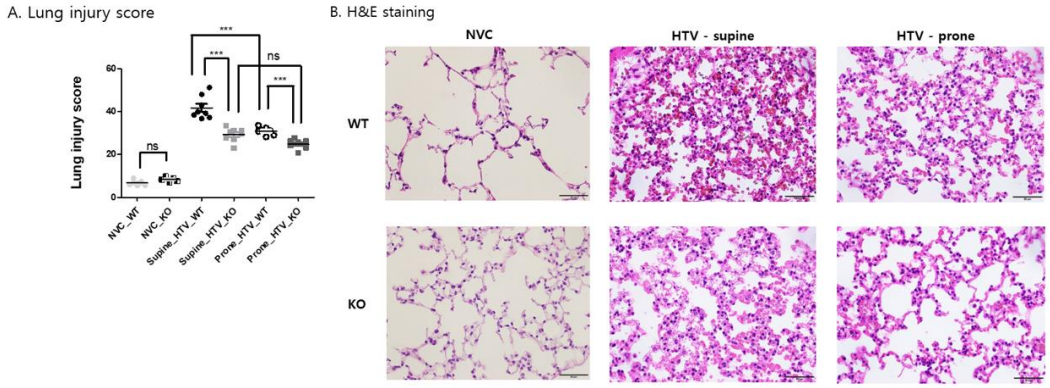


Figure 3. Lung histology in pendrin-KO mice after 5-h HTV ventilation showed a significantly lower degree of leukocyte infiltrations and lung injury score. Pendrin wild-type (WT) and knockout (KO) mice were conducted to a tracheostomy and ventilation with a supine or prone position at a high tidal volume (HTV) of 30 mL/kg. (*A*) Lung injury scoring of each group. (*B*) Histopathologic image of hematoxylin and eosin (H&E) staining (×400). NVC, non-ventilation control group; supine HTV, HTV ventilation in supine position; prone HTV, HTV ventilation in prone position. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ analyzed by Student′s unpaired two-tailed *t*-test.

2. Prone positioning during ventilation with HTV attenuates the VILI

To investigate the protective effect of prone positioning during HTV ventilation, we compared BALF and lung histology results between supine and prone HTV mice. In the BALF, the total cell count and protein concentration were significantly decreased in prone HTV mice relative to supine HTV pendrin-WT mice (Figure 2A and 2B). Assessment of lung histology in prone HTV mice revealed a significantly lower degree of leukocyte infiltration and lung injury scores relative to supine HTV pendrin-WT mice (Figure 2A and 2B). However, supine and prone HTV pendrin-KO mice did not significantly differ in terms of BALF and lung injury scores of histological findings (Figure 2A and 2B).

3. Pendrin deletion and prone positioning suppress inflammatory cytokine release in the VILI mice model

We further investigated the levels of inflammatory cytokines using ELISA to determine the effects of pendrin deletion and prone positioning in a mouse model of VILI. After HTV ventilation, the levels of cytokines, including MIP-2, IL-6, IL-1β, and TNF-α, were remarkably elevated than those in non-ventilation (Figure 4). In the supine HTV group, pendrin-KO mice showed significantly lower levels of all inflammatory cytokines, whereas WT and pendrin-KO mice in the prone HTV group did not significantly differ (Figure 4).

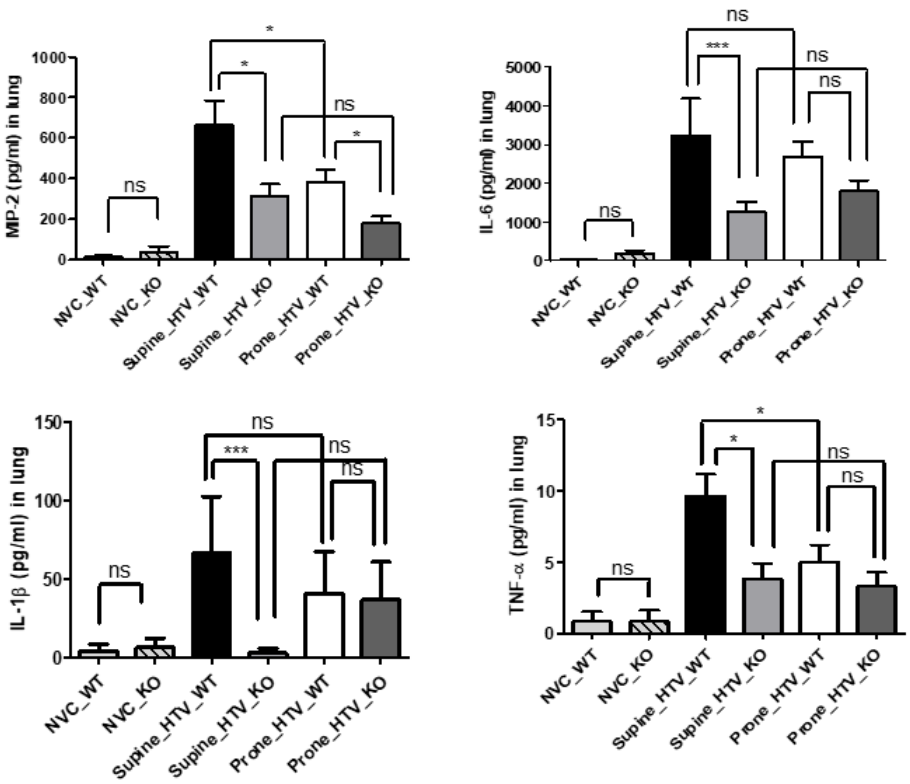


Figure 4. Inflammatory cytokines were significantly decreased in the pendrin-KO mice group than those in pendrin-WT mice in the supine HTV group, analyzed using ELISA. NVC, non-ventilation control group; supine HTV, HTV ventilation in supine position; prone HTV, HTV

ventilation in prone position; HTV, high tidal volume; WT, wild-type; KO, knockout. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ analyzed by Student′s unpaired two-tailed *t*-test.

4. Pendrin expression increases in HTV-ventilated mice

To confirm the increase in pendrin expression, we performed western blot analysis, IF staining, and TEM-immunogold labeling for pendrin (SLC26A4). Western blotting showed that pendrin expression was higher in pendrin-WT mice with VILI than that in pendrin-KO mice with VILI and the control non-ventilation groups (Figure 5). IF staining for pendrin R2 in WT mice revealed the upregulation of pendrin expression relative to that in KO mice after HTV ventilation (Figure 6A and 6B). Pendrin was also detected in the pendrin-WT mice after HTV ventilation in the prone position (Figure 6C).

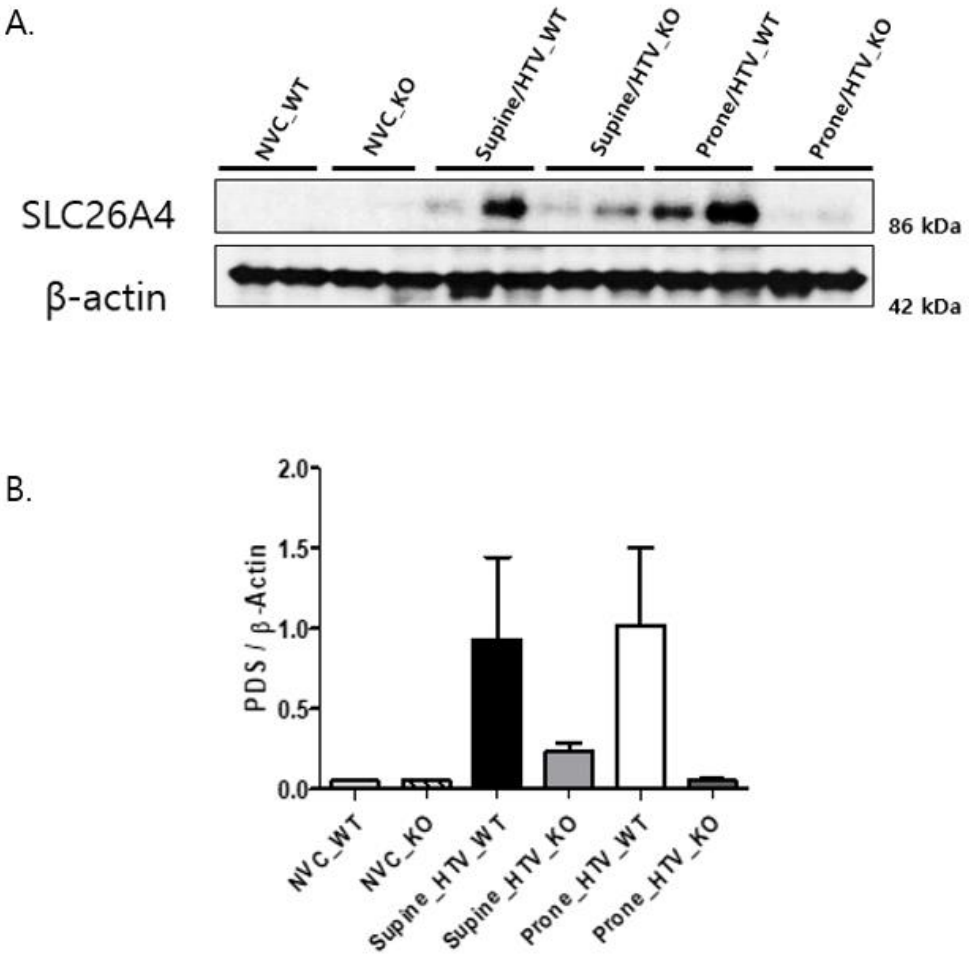


Figure 5. Pendrin expression increases in HTV ventilation mice. Pendrin wild-type (WT) and knockout (KO) mice were conducted to a tracheostomy and ventilation with a supine or prone position at a high tidal volume (HTV) of 30 mL/kg. (*A*) Western blot and (*B*) densitometry analyses of pendrin bands. NVC, non-ventilation control group; supine HTV, HTV ventilation in supine position; prone HTV, HTV ventilation in prone position; PDS, pendrin. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ analyzed by Student′s unpaired two-tailed *t*-test.

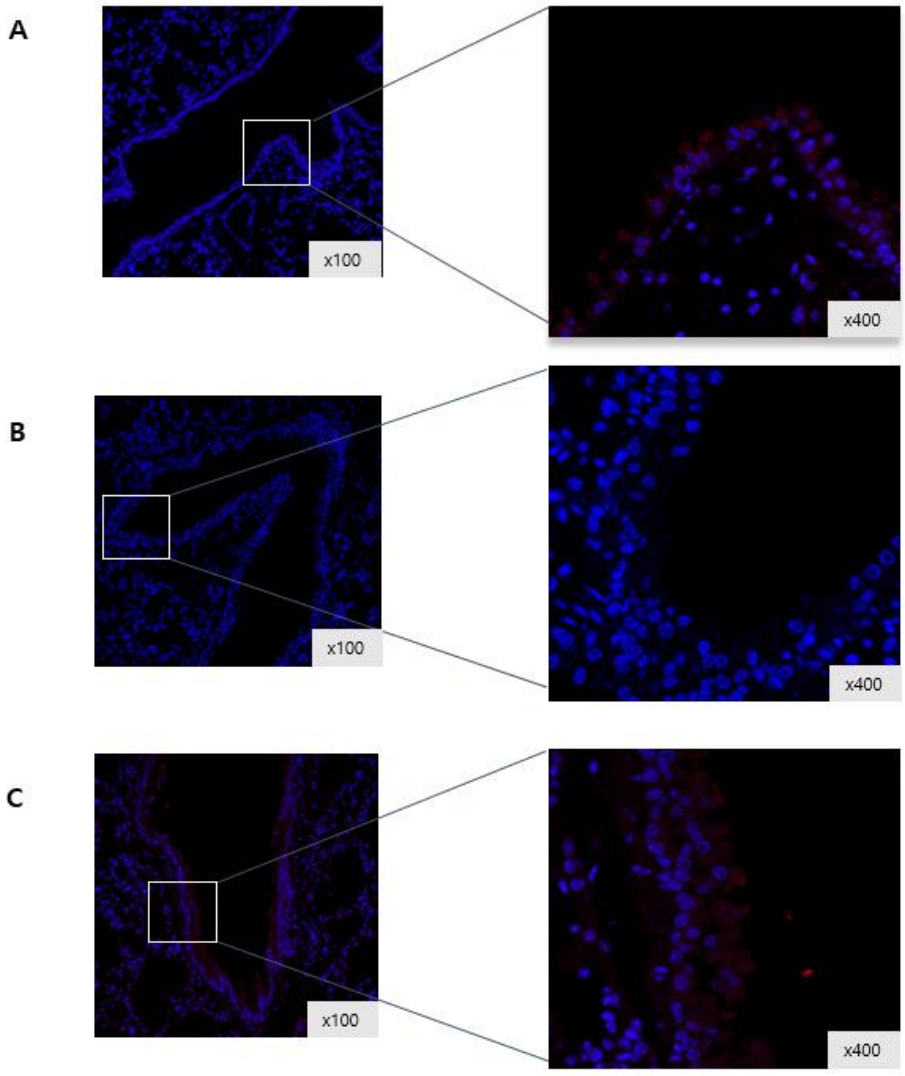
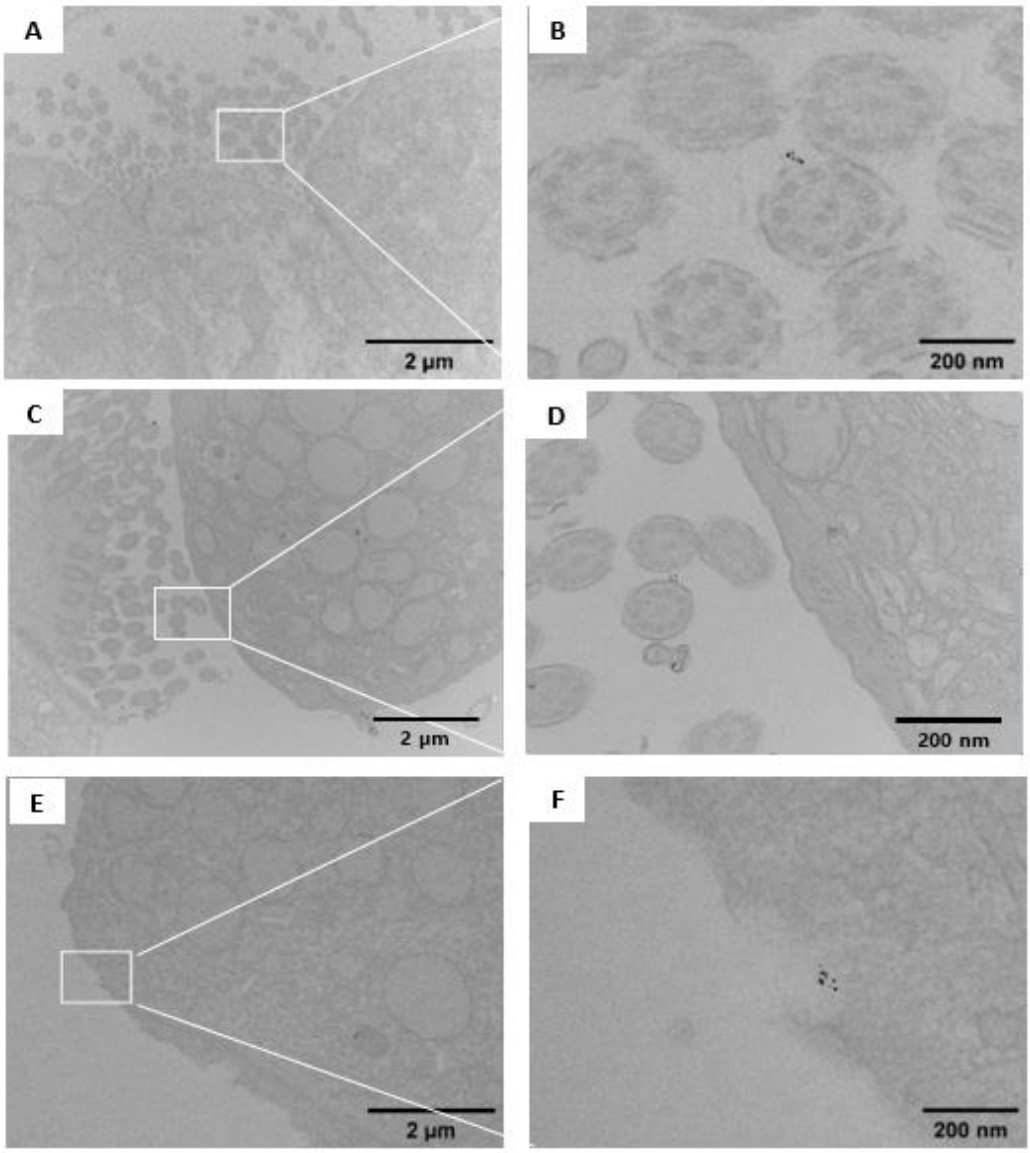
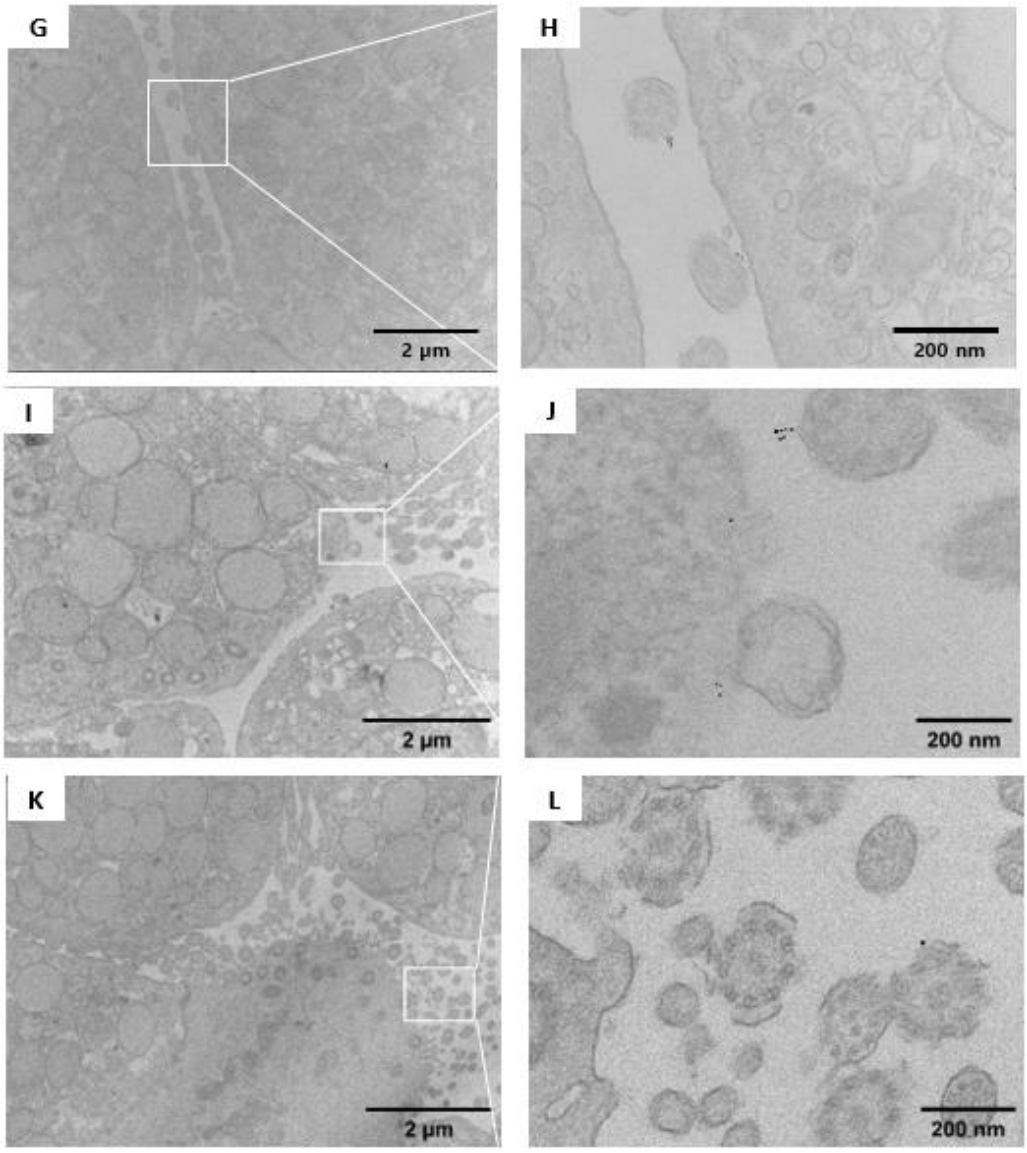


Figure 6. Pendrin expression in alveolar epithelial cells determined using immunofluorescence staining. Pendrin wild-type (WT) and knockout (KO) mice were conduted to a tracheostomy and ventilation with a supine or prone position at a high tidal volume (HTV) of 30 mL/kg. (*A*) Pendrin-WT mice with HTV ventilation in the supine position. (*B*) Pendrin-KO mice with HTV ventilation in the supine position. (*C*) Pendrin-WT mice with HTV ventilation in the prone position.

We conducted TEM-immunogold labeling for pendrin to compare the expression of pendrin in each group at the ultrastructural level. The HTV WT mice in the supine and prone positions revealed that pendrin expression was increased on the cell membrane and in vesicles compared with that on the apical side of epithelial cells in the non-ventilation control and pendrin-KO mice groups (Figure 7A–7L). The number of gold particles labeled with pendrin was higher in pendrin-WT mice with HTV ventilation than that in pendrin-KO and non-ventilation mice. However, HTV in the supine and prone positions did not significantly differ. (Figure 7M).





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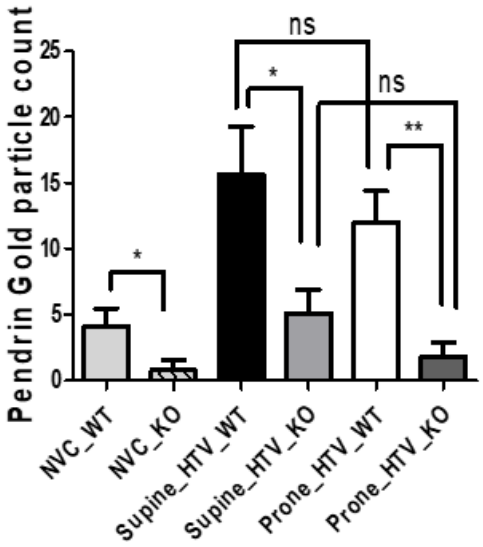

Figure 7. Effect of pendrin deletion on VILI as demonstrated by transmission electron micrographic immune-gold labeling. Pendrin wild-type (WT) and knockout (KO) mice were conduted to a tracheostomy and ventilation with a supine or prone position at a high tidal volume (HTV) of 30 mL/kg. (*A* and *B*) Non-ventilated group, pendrin-WT mice. (*C* and *D*) Non-ventilated group, pendrin-KO mice. (*E* and *F*) HTV ventilation in the supine position, pendrin-WT mice. (*G* and *H*) HTV ventilation in the supine position, pendrin-KO mice. (*I* and *J*) HTV ventilation in the prone position, pendrin-WT mice. (*K* and *L*) HTV ventilation in the prone position, pendrin-KO mice. (*M*) Comparisons of gold particle count labeled with pendrin. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ analyzed by Student′s unpaired two-tailed *t*-test.

IV. DISCUSSION

The clinical outcomes of ALI/ARDS have significantly improved owing to therapeutic strategies such as low tidal volume with low plateau pressure; however, it remains a challenging condition to manage [22]. Mechanical ventilation is an important treatment option for ALI/ARDS [23]; notwithstanding, it may paradoxically lead to further lung injury, known as VILI, which can result in poor prognosis [24]. In this study, we identified that pendrin expression was increased in response to HTV ventilation, and increased pendrin expression was associated with the inflammatory cascade. And, pendrin deletion resulted in a decreased inflammatory response in VILI. Furthermore, we demonstrated that prone positioning during HTV attenuated lung injury in WT mice.

The transmembrane anion exchanger protein pendrin (SLC26A4) is associated with various disorders in related organs [25]. Recent research has provided evidence supporting the role of pendrin as a pivotal protein in airway diseases, such as asthma, COPD, and rhinitis [12]. The involvement of pendrin in ALI/ARDS has been suggested in previous studies, although its exact mechanism remains unclear. Lee et al. [17] confirmed that pendrin expression increased in both human BAL samples with pneumonia and an LPS-induced ALI mouse model, and pendrin-null mice presented reduced lung damage by LPS. Moreover, they identified that the pendrin inhibitor (YS-01) could attenuate LPS-induced lung injury by inhibiting the pendrin/$OSCN^-$/NF-κB-mediated pathway, indicating the possibility of developing a novel treatment of ALI. Jia et al. [16] showed that the distal airway plays a role in ALI/ARDS, and pendrin in alveolar epithelial cells is involved in the inflammatory process of LPS-induced ALI. Furthermore, they demonstrated that the treatment of LPS-induced ALI mice with methazolamide, a carbonic anhydrase inhibitor, significantly reduced lung inflammation. They concluded that airway epithelial cells could be a therapeutic target for developing new drugs and strategies for ALI/ARDS treatment. However, additional research is necessary to explore the molecular mechanism of pendrin upregulation and its therapeutic potential in ALI/ARDS.

The mechanisms of VILI, including conventional barotrauma, volutrauma, and atelectrauma, can be explained by the mechanical forces that lead to barrier disruption, local inflammation, and decreased airspace edema clearance [5]. Among them, an increased inflammation response due to mechanical stretching during HTV ventilation, including elevation of TNF-α, IL-1β, and IL-6, has been observed not only in the BALF of patients with ARDS but also in lung injury mice models [26-28]. In the present study, we observed increased inflammatory pathology by developing HTV-induced lung injury, consistent with previous studies.

Recent research has focused on elucidating the molecular mechanism of the mechanotransduction pathway [29]. For example, Held et al. [30] reported that mechanical stimuli were found to trigger NF-κB activation, leading to the release of inflammatory cytokines. Notably, they also demonstrated the possibility of a therapeutic strategy in which NF-κB activation by mechanical stimuli is inhibited using corticosteroids. In addition, Lee et al. [31] reported that NOX4 and Eph/ephrin signaling are implicated in VILI, and a NOX4 inhibitor could potentially serve as a therapeutic intervention for VILI. Furthermore, Park et al. [8] reported that the lung-protective effects of altered EphA2/ephrinA1 signaling through prone positioning or EphA2 antagonism were observed during injurious mechanical ventilation. Prone positioning during invasive mechanical ventilation has beneficial effects in improving the outcome in patients with ARDS [32]. The protective mechanism of prone positioning in ARDS includes improvement of hypoxemia, hemodynamic effects, respiratory mechanics, and prevention of VILI [33]. In a previous study, Broccard et al. [34] confirmed that a significant reduction in lung injury and homogeneous distribution of VILI were present in normal dogs with HTV ventilation for 6 h in the prone position. Moreover, a recent study by Park [35] reported that prone positioning could have an impact on VILI at the molecular level by influencing the activation of mitogen protein kinases in rodents exposed to HTV ventilation. However, the molecular mechanisms underlying prone positioning also remain largely unknown. There has been no prior research on the relevance of pendrin in VILI caused by mechanical strain. Our data show

that pendrin expression was elevated in the VILI mouse model, demonstrating an increased response to lung inflammation. Additionally, we revealed that pendrin deletion has a preventive effect in a VILI mouse model using pendrin-null mice. Taken together, our data suggest that pendrin expression is associated with lung injury due to mechanical stretching, and the modulation of pendrin might be a novel strategy for preventing VILI.

Our study had several limitations. First, we were unable to identify the exact mechanism of pendrin expression or its downstream signaling cascades. Second, we did not confirm a significant correlation between pendrin expression and prone positioning. However, in the results of pendrin expression through TEM-immunogold labeling, we observed a lower gold particle count labeled with pendrin in the prone than in the supine position, although the difference was not statistically significant. In this respect, it is possible that the reduction in pendrin expression in airway epithelial cells plays a partial role in the decreased VILI in prone positioning but is not directly involved. Nevertheless, our study is the first to investigate the potential protective role of pendrin against VILI. Additional research on the downstream signaling of pendrin in VILI and the association between prone positioning and pendrin expression, with an increasing number of mice per group, is required.

V. CONCLUSION

In conclusion, we suggested that increased pendrin expression in response to HTV ventilation is related to the inflammatory cascade induced by mechanical strain and tissue injury. And, our results showed that modulation of pendrin resulted in decreased inflammatory response in VILI, indicating its protective role against lung injury. In addition, we showed that prone positioning during HTV attenuated lung injury in WT mice. But, we could not identify a direct correlation between prone position and pendrin expression in

VILI. Instead, we observed the least lung injury and pendrin expression in both modulations of pendrin through pendrin KO mice and prone position group. In conclusion, we identified pendrin as a potential therapeutic target for managing VILI and improving the outcomes of patients with VILI. Further studies are needed to investigate the relationship between pendrin expression and prone position, and to explore therapeutic strategies for modulating the expression and activity of pendrin.

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ABSTRACT(IN KOREAN)

인공호흡기에 의한 급성 폐손상에서 'pendrin' 역할에 대한 규명

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최지수

목적: 펜드린(SLC26A4)은 중탄산염, 요오드화물 및 기타 음이온을 염화물로 교환하는 막관통 단백질이다. 최근 데이터에 따르면 염증성 기도 질환에서 펜드린의 발현이 증가되어 있는 것으로 나타났다. 본 연구에서는 인공호흡기 유발 급성 폐손상(Ventilator induced lung injury, VILI) 동물 모델에서 펜드린 발현의 역할과 복와위 자세의 효과를 분석해보고자 한다.

방법: 인공호흡기를 통한 급성 폐손상은 펜드린 Wild-Type (WT) 과 Knock-Out (KO) 129SVEV 마우스에게 5시간 동안 30 ml/kg의 높은 일회 호흡량(Hgih Tidal Volume, HTV)과 0 cm H_2O 호기말 압력 및 0.21의 흡기 산소 분율로 분당 100회의 빈도로 앙와위 자세 또는 복와위 자세로 유발하였다. 폐손상의 정도는 기관지폐포세척액 분석(bronchoalveolar lavage fluid, BALF), 염증성 사이토카인 및 조직병리학적 소견을 통해 평가하였다. 펜드린의 발현은 Western-blotting, 면역 형광 염색 및 투과전자현미경을 통해 확인하였다.

결과: 기관지폐포세척액의 총 세포수와 단백질 농도는 인공호흡기 치료를 하지 않은 그룹과 HTV 호흡을 시행한 펜드린 KO 그룹에 비해 HTV 호흡을 시행한 펜드린 WT 그룹에서 유의하게 증가하였다. 그리고 조직병리학적

평가에서도 VILI 가 있는 펜드린 WT 그룹이 VILI 가 있는 펜드린 KO 그룹과 인공호흡기 치료를 하지 않은 그룹에 비해 백혈구 침윤 및 폐손상이 크게 증가한 것으로 나타났다. 펜드린의 발현은 VILI 가 있는 펜드린 WT 그룹에서 발현이 높게 나타난 것을 확인하였다. 인공호흡기 치료 중 복와위 자세는 VILI를 적게 유발함을 확인하였으나, 앙와위 자세와 복와위 자세 사이의 펜드린 발현에는 유의한 차이가 없었다. 그럼에도 불구하고, 펜드린 KO 그룹에서 복와위 자세로 VIILI를 유발하였을 때, 폐손상이 가장 적게 나타났을 뿐만 아니라, 펜드린 발현 또한 가장 적게 나타남을 확인하였다.

결론: 본 연구 결과는 펜드린이 인공호흡기로 인한 폐 손상에 중요한 역할을 하며 펜드린이 VILI 조절을 위한 새로운 치료 표적이 될 수 있음을 시사한다. 그리고 인공호흡기 치료 시 복와위 자세와 펜드린의 억제는 VILI를 예방하는 데 효과적일 수 있다.

핵심되는 말: 펜드린, SLC26A4, 인공호흡기 유발 폐손상, 급성 폐손상