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Inhibition of Poly(Adenosine Diphosphate–Ribose) Polymerase Attenuates Ventilator-induced Lung Injury

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Background: **Mechanical ventilation can induce organ injury associated with overwhelming inflammatory responses. Excessive activation of poly(adenosine diphosphate–ribose) polymerase enzyme after massive DNA damage may aggravate inflammatory responses. Therefore, the authors hypothesized that the pharmacologic inhibition of poly(adenosine diphosphate–ribose) polymerase by PJ-34 would attenuate ventilator-induced lung injury.**

Methods: **Anesthetized rats were subjected to intratracheal instillation of lipopolysaccharide at a dose of 6 mg/kg. The animals were then randomly assigned to receive mechanical ventilation at either low tidal volume (6 ml/kg) with 5 cm H_2O positive end-expiratory pressure or high tidal volume (15 ml/kg) with zero positive end-expiratory pressure, in the presence and absence of intravenous administration of PJ-34.**

Results: **The high-tidal-volume ventilation resulted in an increase in poly(adenosine diphosphate–ribose) polymerase activity in the lung. The treatment with PJ-34 maintained a greater oxygenation and a lower airway plateau pressure than the vehicle control group. This was associated with a decreased level of interleukin 6, active plasminogen activator inhibitor 1 in the lung, attenuated leukocyte lung transmigration, and reduced pulmonary edema and apoptosis. The administration of PJ-34 also decreased the systemic levels of tumor necrosis factor α and interleukin 6, and attenuated the degree of apoptosis in the kidney.**

Conclusion: **The pharmacologic inhibition of poly(adenosine diphosphate–ribose) polymerase reduces ventilator-induced lung injury and protects kidney function.**

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INJURIOUS mechanical ventilation can lead to the development of an overwhelming inflammatory response and multiple organ dysfunction syndrome.[1–5] Acute renal failure is the most prevalent form of distal organ dysfunction associated with endothelial and epithelial cell death in patients with ventilator-induced lung injury (VILI).[2,6–8]

The clinical importance of VILI has been highlighted in a multicenter clinical trial demonstrating that mechanical ventilation with low tidal volume (V_T) significantly decreased cytokine responses, multiple organ dysfunction syndrome, and mortality rate compared with high V_T in patients with acute respiratory distress syndrome (ARDS).[9,10] However, in situations where a fully lung protective strategy is not possible, it would be necessary to use pharmacologic therapies to mitigate the consequences of VILI and multiple organ dysfunction syndrome.

Poly(adenosine diphosphate–ribose) polymerase (PARP) 1 is the most abundant member of PARP family,[11] whose primary role is to sense DNA damage, repair DNA, and maintain genomic stability.[12] However, when severe DNA injury occurs in response to oxidative stress, excessive up-regulation of PARP may be detrimental by depleting cellular adenosine triphosphate stores, resulting in cell dysfunction and death.[13–16] This cellular suicide mechanism has been implicated in the pathophysiology of acute lung injury,[17] acute renal failure secondary to ischemia–reperfusion,[18] and sepsis.[19] It has been reported that PARP-1 can directly interact with both subunits of p65 and p50 and synergistically coactivates nuclear factor κB (NF-κB).[20–23] The potent PARP inhibitor PJ-34 can decrease PARP-1 activity and thus NF-κB activation in animal models of endotoxic and hemorrhagic shock.[17–19,24–27]

In the current study, we tested the hypothesis that inhibition of PARP by PJ-34 would attenuate VILI and preserve kidney function by its antiinflammatory property. We demonstrated that high-V_T ventilation induced an increase in PARP activity in the lung associated with an enhanced inflammatory response. The treatment with PJ-34 attenuated the mechanical ventilation–induced cytokine responses, decreased the level of active plasminogen activator inhibitor 1 (PAI-1) in the lung, and reduced leukocyte infiltration and pulmonary edema. Furthermore, inhibition of PARP resulted in fewer kid-

ney apoptosis and thus preserved renal function during high-V_T ventilation.

Materials and Methods

Animal Preparation

The protocol was approved by the institutional animal care committee at St. Michael's Hospital, Toronto, Ontario, Canada. Thirty-six male Sprague-Dawley rats (Charles Rivers, St. Constan, Quebec, Canada) weighing 290 ± 10 g were anesthetized with intraperitoneal injection of 10 mg/kg xylazine (Bayer, Toronto, Ontario, Canada) and 100 mg/kg ketamine (Bimeda-MTC, Cambridge, Ontario, Canada). Anesthesia was maintained with 1 $mg \cdot kg^{-1} \cdot h^{-1}$, xylazine and 20 $mg \cdot kg^{-1} \cdot h^{-1}$ ketamine *via* a jugular vein; muscle relaxation was achieved by intravenous administration of 0.6 $mg \cdot kg^{-1} \cdot h^{-1}$ pancuronium bromide (Sabex Inc., Quebec, Canada). Rats were placed on a heating pad to maintain core temperature at 37°C. A tracheostomy was performed for intratracheal cannulation (14 gauge). The right carotid artery was catheterized for blood sampling and continuous arterial blood pressure measurements. The bladder was catheterized and sutured using a transabdominal approach for urine sampling.

Experimental Protocol

The rats were initially ventilated at V_T 6 ml/kg and positive end-expiratory pressure (PEEP) of 5 cm H_2O (Servo 300 ventilator; Siemens, Munich, Germany). After a baseline arterial blood gas measurement (Corning 248 blood gas analyzer; Ciba Corning, Medfield, MA) to confirm similar gas exchange conditions in all animals, lipopolysaccharide (055:B5; Sigma-Aldrich, St. Louis, MO) at a dose of 6 mg/kg in 0.5 ml normal saline was administered by using an intratracheal aerosolizer (PennCentury Inc., Philadelphia, PA). Five minutes later, a recruitment maneuver was performed by increasing PEEP level to 25 cm H_2O for five breaths, followed by 15 min of stabilization under the ventilator settings described above. The rats were then randomly allocated into four groups (n = 9 each) and ventilated for 4 h: group 1 (low V_T + PJ-34): V_T 6 ml/kg, PEEP 5 cm H_2O with infusion of PJ-34 (Alexis Biochemicals, Lausen, Switzerland); group 2 (low V_T + vehicle): V_T 6 ml/kg, PEEP 5 cm H_2O with the vehicle solution (normal saline); group 3 (high V_T + PJ-34): V_T 15 ml/kg, no PEEP with infusion of PJ-34; and group 4 (high V_T + vehicle): V_T 15 ml/kg, no PEEP with vehicle solution. Immediately after the randomization, PJ-34 was administered intravenously as a loading dose of 10 mg/kg over 30 min, followed by continuous infusion at 2 $mg \cdot kg^{-1} \cdot h^{-1}$ for the remainder of the experiments.[28] Arterial carbon dioxide tension ($Paco_2$) was maintained at 40 ± 5 mmHg by adjusting respiratory rate. Inspiration-to-expiration ratio was set to 1:2, and the fraction of inspired oxygen (Fio_2) was 0.45.

Measurements

Arterial blood gases were analyzed 30 min after randomization and hourly thereafter. Urine samples were collected during the last hour after emptying the urine tube. Upon completion of the mechanical ventilation, whole blood was collected for measurements of cytokines and creatinine, and the animals were killed with an overdose of anesthesia. Lungs and kidneys were harvested for histologic examination. Plasma and urine were stored at −80°C until assayed.

PARP Activity Assay

Poly(adenosine diphosphate–ribose) polymerase activity (PARP Universal Colorimetric Assay Kit; R&D Systems, Inc., Minneapolis, MN) was determined in lung homogenates by following the manufacturer's instruction, and the results were expressed as units of PARP per gram protein.

Bronchoalveolar Lavage and Wet-to-Dry Weight Ratio

The left upper lobe was excised for histologic examination. The right middle lobe was used to estimate wet-to-dry weight ratio, and the right lower lobe was snap frozen for cytokine measurements. The left lower and the right upper lobes were lavaged by intratracheal instillation of 2 ml cold phosphate-buffered saline (Sigma-Aldrich). After 5 s, the bronchoalveolar lavage fluid was obtained. This procedure was repeated twice.

After centrifugation, the bronchoalveolar lavage fluid was frozen at −80°C until further analysis. The cell pellet was resuspended in 1 ml phosphate-buffered saline for cell differentiation by using the Hemacolor Stain Set (EM Diagnostic System, Gibbstown, NJ).

Measurements of Cytokines, PAI-1 Activity, and Tissue Factor Activity

Analysis of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) in plasma, lung, and kidney homogenates was performed in a blinded fashion using rat-specific enzyme-linked immunosorbent assay kits (BioSource International, Camarillo, CA) at 450 nm (Multiskan Asscent microplate photometer; Thermo Lab Systems, Helsinki, Finland). PAI-1 activity (Innovative Research, Inc., Southfield, MI) and tissue factor activity (American Diagnostica Inc., Stamford, CT) were determined in plasma and lung homogenates. The tissue factor activity kit is specific for human but crossly reacts with rat tissue factor.[29] Total protein concentration in lung and kidney homogenates was determined by a Bradford assay (Bio-Rad Laboratories, Inc., Hercules, CA) using bovine serum albumin to construct a standard curve.

Lung and Kidney Apoptosis

Apoptosis was quantified from paraffin sections of lung and kidney by terminal deoxynucleotidyltransferase-me-

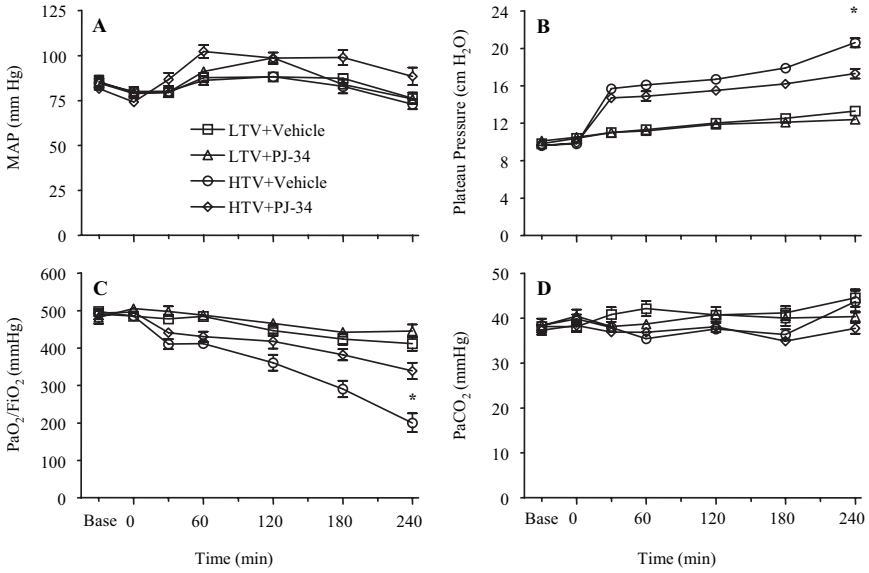

Fig. 1. Effects of PJ-34 on arterial pressure and respiratory variables during mechanical ventilation. The rats received lipopolysaccharide at time 0, followed by mechanical ventilation. n = 9/group. (*A*) Mean arterial pressure (MAP) over time. (*B*) Plateau pressure over time. HTV = high tidal volume; LTV = low tidal volume. (*C*) Arterial oxygen tension (Pa_{O_2})/fraction of inspired oxygen (F_{IO_2}) ratio over time. (*D*) Arterial carbon dioxide tension (Pa_{CO_2}) over time. *$P < 0.05$, LTV + vehicle *versus* HTV + vehicle and HTV + vehicle *versus* HTV + PJ-34 at time 240 min.

diated dUTP nick end labeling (TUNEL) assay. Hematoxylin staining for nucleus was also performed to identify individual cell. Twelve fields randomly chosen in each section were read in a blinded fashion. An apoptotic index was calculated as [100% × (TUNEL-positive cells)/(total cells)].

Caspase-3 Enzymatic Activity

Caspase-3 activity was determined in lung and kidney homogenates (Caspase-3 Colorimetric Assay kit; R&D Systems, Inc.). Recombinant human caspase-3 enzyme was used to construct a standard curve (R&D Systems, Inc.). Results were normalized to protein levels.

Lactate Dehydrogenase Assay

The lactate dehydrogenase assay (Cytotoxicity Detection Kit; Roche Applied Science, Mannheim, Germany) was performed at 492 nm.

Histology

The lung injury scores, including alveolar collapse, perivascular hemorrhage, alveolar hemorrhage, perivascular edema, vascular congestion, alveolar polymorphonuclear leukocytes, membranes, alveolar edema, macrophages, and bronchial epithelial lesions, were performed by a pathologist who was unaware of the experimental groups. Five regions from each specimen were examined, and an injury score of 0–3 (0 = normal; 1 = mild; 2 = moderate; 3 = severe) was assigned and then calculated for a total score of lung injury.

Creatinine Clearance

Creatinine clearance was calculated over the last hour of experiments using the formula $CC = U_{Cr} \times V/P_{Cr}$, where U_{Cr} represents the creatinine concentration in urine (mM), V represents the urine flow (ml/min), and P_{Cr} represents the creatinine concentration in plasma (mM).

Statistics

Results are reported as mean ± SEM. Data were analyzed in nonparametric tests by using the Prism Graphpad 4.0 software package (Prism, San Diego, CA). Comparison among groups was performed using the Kruskal-Wallis test. When an overall *P* value was less than 0.05, a Dunn multiple-comparison *post hoc* analysis was conducted. A *P* value less than 0.05 was considered statistically significant.

Results

Effects of PJ-34 on Hemodynamics, Gas Exchange, and Respiratory Mechanics

Mean arterial pressures were similar at baseline and during the experiments among groups (fig. 1A), as was fluid administration (low V_T + vehicle: 1.4 ± 0.1 ml/h; low V_T + PJ-34: 1.5 ± 0.1 ml/h; high V_T + vehicle: 1.7 ± 0.1 ml/h; high V_T + PJ-34: 1.4 ± 0.1 ml/h; *P* = not significant). Airway plateau pressure was higher in the high-V_T groups, which was attenuated by the treatment with PJ-34 (fig. 1B). Mean values of arterial carbon dioxide tension (Pa_{O_2})/F_{IO_2} ratio were similar in all animals until the second hour of mechanical ventilation, when the Pa_{O_2}/F_{IO_2} ratio decreased in the high-V_T group without PJ-34 treatment compared with the other groups (fig. 1C). There were no differences in the levels of Pa_{CO_2} (fig. 1D), pH, and bicarbonate among groups (data not shown).

Effect of PJ-34 on PARP Activity

Poly(adenosine diphosphate–ribose) polymerase activity was increased in the high-V_T group compared with the low-V_T group. The treatment with PJ-34 decreased the PARP activity (fig. 2A).

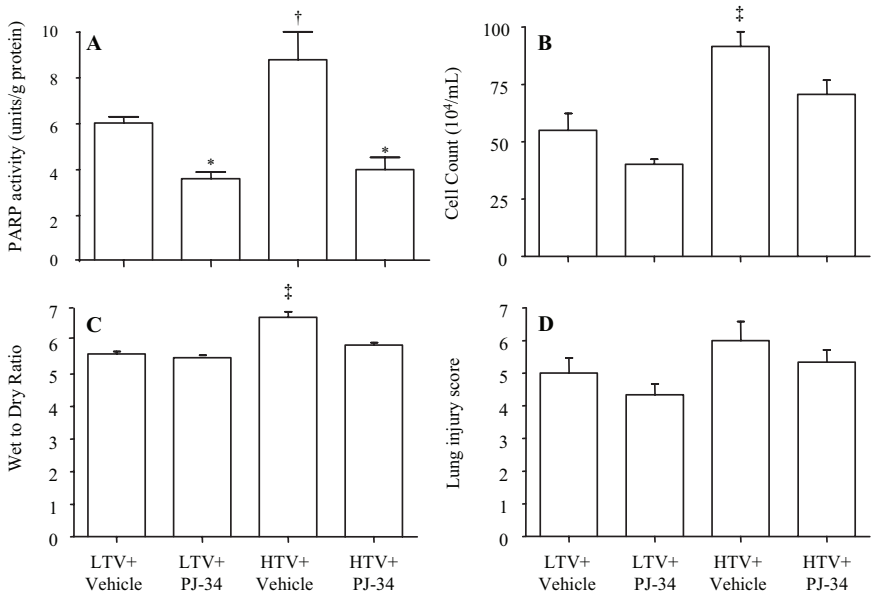

Fig. 2. Effects of PJ-34 on poly(adenosine diphosphate–ribose) polymerase (PARP) activity, inflammatory cell counts, and lung injury. (*A*) PARP activity (U/g protein) in lung homogenate after 4 h of ventilation. HTV = high tidal volume; LTV = low tidal volume. (*B*) Leukocyte cell counts in lung lavage after 4 h of ventilation. (*C*) Lung wet-to-dry weight ratio. (*D*) Lung injury score. * $P < 0.05$, LTV + vehicle *versus* LTV + PJ-34, and HTV + vehicle *versus* HTV + PJ-34. † $P < 0.05$, LTV + vehicle *versus* HTV + vehicle. ‡ $P < 0.05$, HTV + vehicle *versus* others.

Effect of PJ-34 on Leukocyte Migration and Lung Injury

The leukocyte count in bronchoalveolar lavage fluid and the mean value of the lung wet-to-dry weight ratio were greater in the high-V_T than in the low-V_T group, and the treatment with PJ-34 attenuated leukocyte migration in the lung and lung edema (figs. 2B and C). Although the lung injury score had a similar pattern as the wet-to-dry weight ratio, the differences did not statistical reach significance (fig. 2D).

Effect of PJ-34 on Production of Cytokines and Coagulation Variables

Lung Tissue. Tumor necrosis factor α is an early and central cytokine in response to tissue injury.[30,31] IL-6 has been used to guide therapeutic intervention in clinical trials.[32,33] We found no differences in TNF-α among groups, but IL-6 levels were higher in the high-V_T group than in the other groups, and the treatment with PJ-34 decreased IL-6 level to control levels (figs. 3A and B).

Previous studies demonstrated that ARDS was associated with increased coagulation and decreased fibrinolysis.[34,35] PAI-1 is a main component in the antifibrinolytic system, and tissue factor may initiate the extrinsic coagulation pathway. We observed that the PAI-1 activity of the lung increased in the high-V_T group than in the other groups, and the treatment with PJ-34 normalized PAI-1 levels at a control level (fig. 3C). There was no significant difference in tissue factor activity among the groups (fig. 3D).

Plasma. Plasma levels of TNF-α and IL-6 increased in the high-V_T group compared with the low-V_T group,

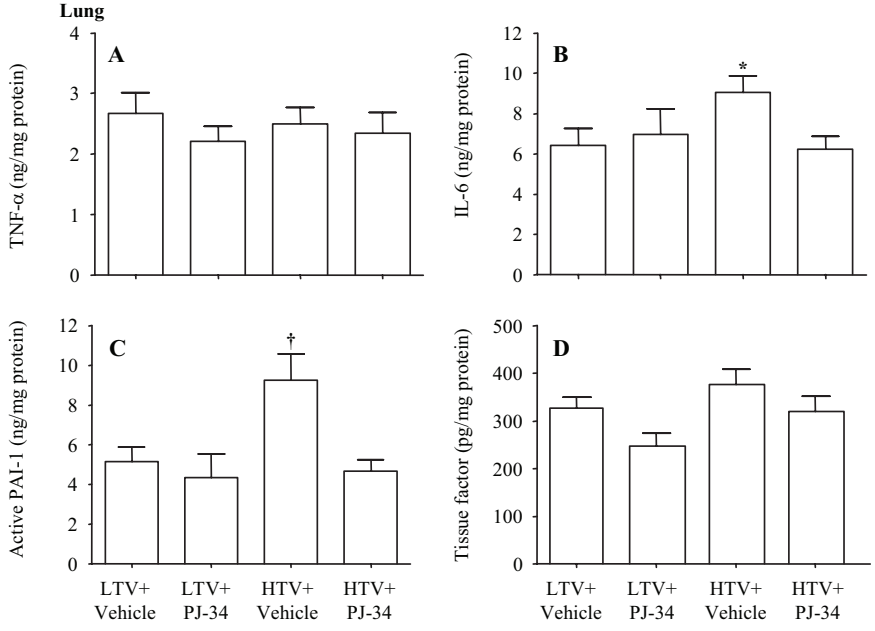

Fig. 3. Effects of PJ-34 on the cytokine levels and coagulation variables in lung. The levels of tumor necrosis factor α (TNF-α; *A*), interleukin 6 (IL-6; *B*), active plasminogen activator inhibitor 1 (PAI-1; *C*), and tissue factor (*D*) were measured in lung homogenate after 4 h of ventilation. HTV = high tidal volume; LTV = low tidal volume. * $P < 0.05$, HTV + vehicle *versus* HTV + PJ-34 or LTV + vehicle. † $P < 0.05$, HTV + vehicle *versus* others.

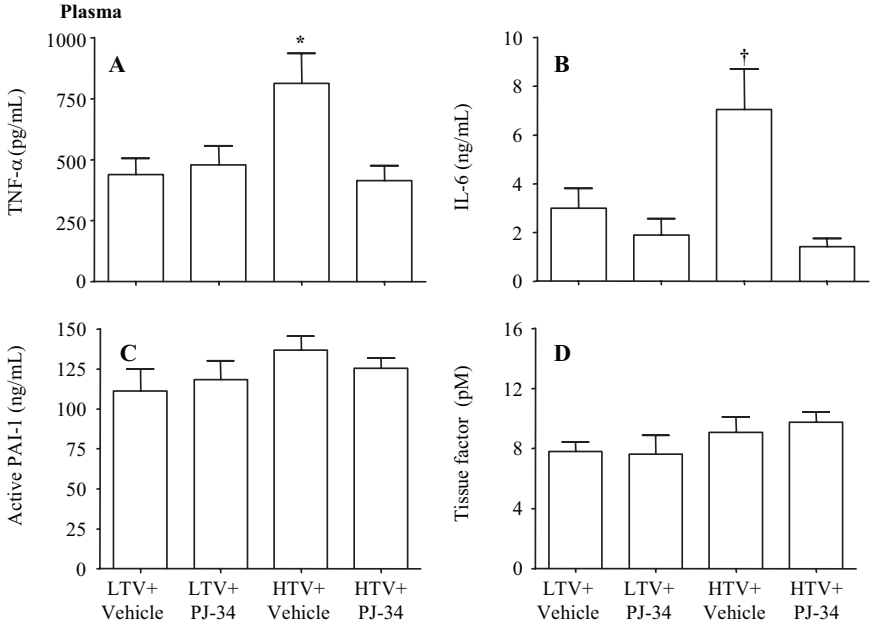

Fig. 4. Effects of PJ-34 on the cytokine levels and coagulation variables in plasma. The levels of tumor necrosis factor α (TNF-α; *A*), interleukin 6 (IL-6; *B*), active plasminogen activator inhibitor 1 (PAI-1; *C*), and tissue factor (*D*) were measured in plasma after 4 h of ventilation. HTV = high tidal volume; LTV = low tidal volume. * $P < 0.05$, HTV + vehicle *versus* HTV + PJ-34 or LTV + vehicle. † $P < 0.05$, HTV + vehicle *versus* others.

which was blunted by the administration of PJ-34 (figs. 4A and B). The expression of PAI-1 and tissue factor activity was similar in all of the groups (figs. 4C and D).

Kidney Tissue. There were no significant differences in the levels of TNF-α, IL-6, PAI-1, and tissue factor activity between the high-V_T and low-V_T groups irrespective of PJ-34 treatment (data not shown).

Organ Apoptosis

Lung Tissue. Figure 5A shows a representative image of the TUNEL staining to detect apoptosis. The apoptotic index (defined as percentage of TUNEL-positive cells divided by the total cells) was higher in the high-V_T group than in the other groups, and the treatment with PJ-34 reduced the apoptotic index (fig. 5B). This observation was in agreement with a decreased caspase-3 activity in the high-V_T group treated with PJ-34 (fig. 5C). This observation was further confirmed by an increased level of lactate dehydrogenase activity as an index of cell death in the high-V_T group compared with the low-V_T group, and treatment of PJ-34 decreased lactate dehydrogenase activity (fig. 5D).

Kidney Tissue. The degree of apoptosis was greater in the high-V_T group than in the other groups, and there seemed to be more apoptotic cells in the medulla compared with the cortex (figs. 6A–C). The greater number

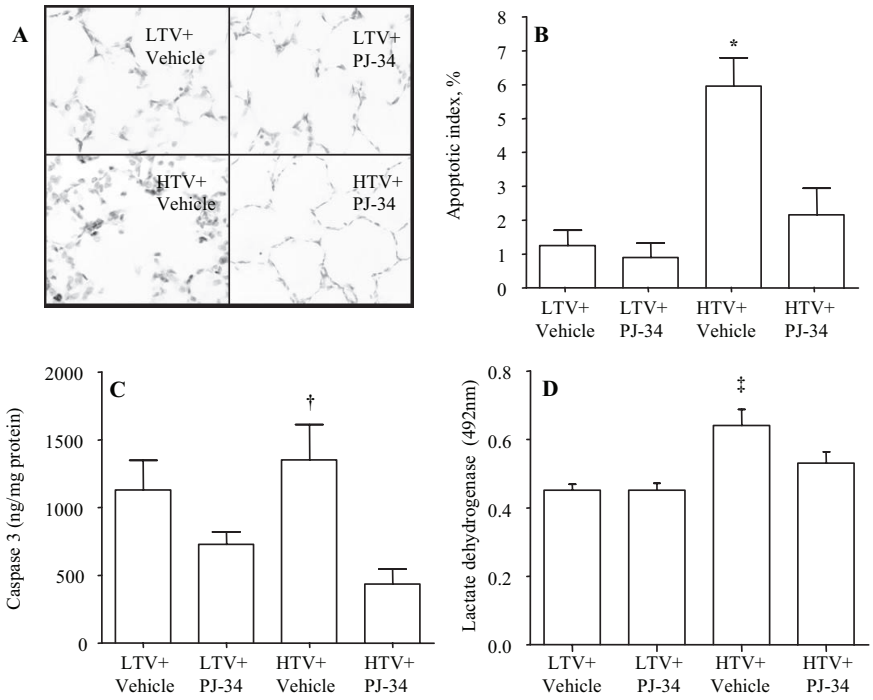

Fig. 5. Effects of PJ-34 on lung apoptosis. (*A*) Representative terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling staining in the lung. Apoptotic cells are identified by the presence of brown staining (*dark dots*). Hematoxylin staining was also performed to identify individual nuclei. HTV = high tidal volume; LTV = low tidal volume. (*B*) Apoptotic index. The apoptotic index was expressed as the percentage of positive nuclei. * $P < 0.05$, HTV + vehicle *versus* others. (*C*) Caspase-3 activity. The lung homogenate was clarified by centrifugation at 5,000*g* for 5 min, and the supernatant was incubated with caspase-3 colorimetric substrate (DEVD-pNA) at 37°C for 30 min. Concentrations of released chromophore pNA were measured spectrophotometrically at a wavelength of 405 nm. † $P < 0.05$, HTV + vehicle *versus* HTV + PJ-34. (*D*) Lactate dehydrogenase levels were measured in bronchoalveolar lavage fluid after 4 h mechanical ventilation. ‡ $P < 0.05$, HTV + vehicle *versus* LTV + vehicle.

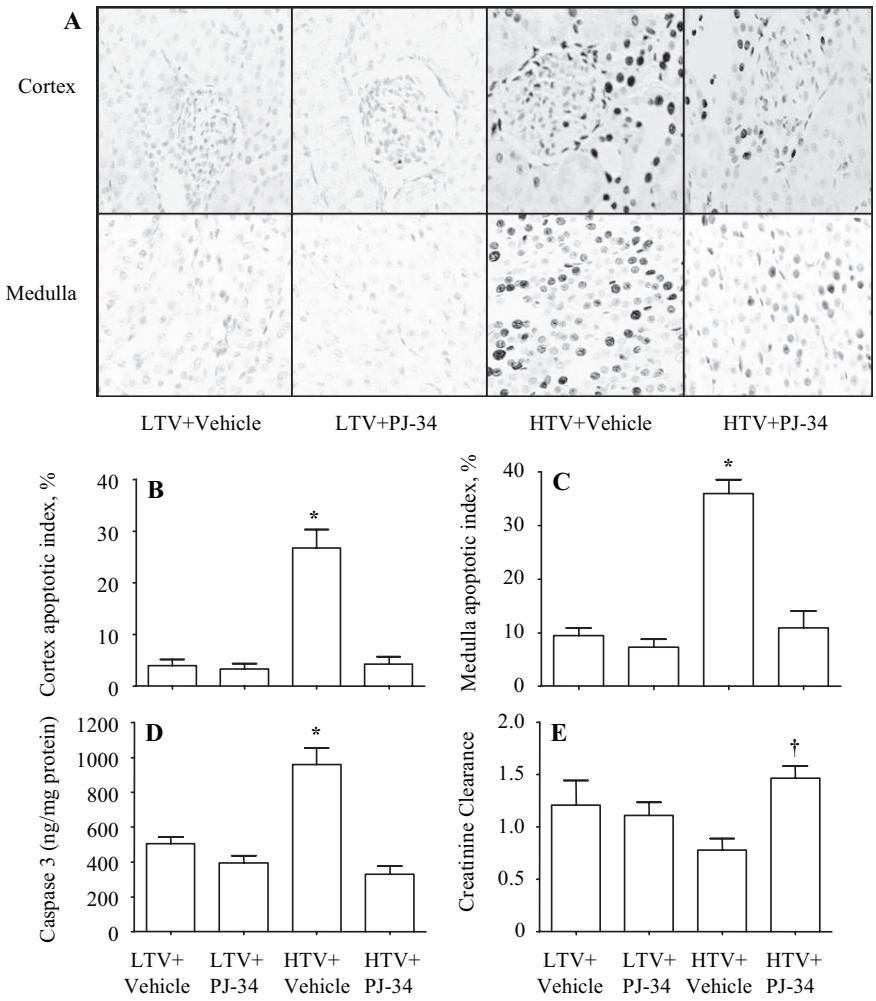

Fig. 6. Effects of PJ-34 on kidney apoptosis and function. (*A*) Representative terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling staining in kidney cortex and medulla. Apoptotic cells are identified by the presence of brown staining (*dark dots*). Hematoxylin staining was also performed to identify individual nuclei. HTV = high tidal volume; LTV = low tidal volume. (*B*) Apoptotic index in cortex. The apoptotic index was expressed as the percentage of positive nuclei. (*C*) Apoptotic index in medulla. (*D*) Caspase-3 activity in kidney homogenate. (*E*) Creatinine clearance (CC) was measured over the last hour of mechanical ventilation. $CC = U_{Cr} \times V/P_{Cr}$, where U_{Cr} represents the creatinine concentration in urine (mM), V represents the urine flow (ml/min), and P_{Cr} represents the creatinine concentration in plasma (mM). * $P < 0.05$, HTV + vehicle *versus* others. † $P < 0.05$, HTV + vehicle *versus* HTV + PJ-34.

of apoptotic cells was associated with higher levels of caspase-3 activity (fig. 6D). The administration of PJ-34 reduced the apoptotic index as well as the caspase-3 activity (figs. 6A–D). The decreased apoptotic index was associated with an increased creatinine clearance (fig. 6E).

Discussion

The current study provides evidence that PARP activation plays an important role in the development of VILI and inflammatory responses during mechanical ventilation after lipopolysaccharide priming. Inhibition of PARP with PJ-34 reduced lung injury and inflammatory responses and preserved kidney function.

Sepsis-associated ARDS shows the highest mortality rate in the ARDS population[36,37]; mortality is lower when ARDS occurs after gastric aspiration, trauma, or fat embolism.[38] A higher incidence of ARDS is present in patients with sepsis where overwhelming inflammatory responses have taken place.[36,37] To portray this clinical situation, we used a two-hit model combining an initial lipopolysaccharide instillation to induce pulmonary inflammation, followed by mechanical ventilation. The choice of V_T was based on certain clinical applications, *i.e.*, a V_T of 6 ml/kg has been suggested to ventilate patients with ARDS,[9] and a V_T of 15 ml/kg is reportedly used in patients without previous lung injury subjected to a short-term mechanical ventilation.[39] Similar to other two-hit models such as acid aspiration and ischemia–reperfusion followed by high-V_T ventilation,[2,6] we observed an increased plateau pressure, a lower Pa_{O_2}/F_{IO_2} ratio and an enhanced pulmonary and systemic inflammatory response, and distal organ dysfunction. Because ARDS is implicated with inflammatory responses, we believe that the results observed in the current two-hit model may also apply to a single-hit of ARDS resulting from pulmonary source.

We demonstrated in the current model an increased PARP activity in the lung of the animals ventilated with high V_T compared with the low-V_T group. PARP inhibition by PJ-34 attenuated inflammatory responses and protected lung and kidney function. We believe that the mechanisms by which PJ-34 exerted beneficial effects in our model are through inhibition of both PARP and NF-κB activity. It has been shown that pharmacologic inhibition of PARP attenuated the DNA-binding capacity and subsequent reduction of NF-κB transcriptional activ-

ity.[40–43] The expression of NF-κB–dependent proinflammatory mediators was decreased in PARP-1–deficient mice.[20,23] It has been suggested that PARP binds NF-κB after the translocation of the κB heterodimer in the nucleus at the stage of the formation of the transcription complex, altering DNA binding affinity to NF-κB.[23,44] These studies suggest that NF-κB could be a downstream pathway of PARP-1. Interestingly, other studies reported that neither enzymatic activity nor the DNA-binding activity of PARP-1 was required for NF-kB–dependent transcriptional activation.[21] We did not measure NF-κB activation in the current study, but we and others have previously shown that mechanical ventilation resulted in NF-κB translocation in the lung of animal models of acute lung injury and ARDS.[45,46] It has been demonstrated that inhibition of NF-κB translocation resulted in a reduction in VILI by using other pharmacologic interventions, such as phosphoinositide 3-OH kinase inhibitor and genistein.[46–49]

Pharmacologic inhibition of PARP has been investigated in a variety of experimental conditions of acute lung injury and lipopolysaccharide-induced organ injury.[17,28,50–52] When mice were subjected to intratracheal injection of lipopolysaccharide for 24 h, the treatment with PJ-34 attenuated lung injury by reducing leukocyte extravasation and pulmonary inflammation.[50] In an ovine pneumonia model, treatment with PARP inhibitor INO-1001 preserved lung histology after intrabronchial injection of *Pseudomonas aeruginosa* bacteria associated with an increased oxygenation and a better respiratory mechanic.[51] The administration of the PARP inhibitor 3-aminobenzamide protected against endothelial dysfunction in a rat model of endotoxic shock.[52] Moreover, it has been reported that PJ-34 improved survival rate and cardiovascular function in a pig model of sepsis induced by *Escherichia coli*.[28] Our results are in accord with the previous studies to support the concept that PARP plays an important role in the development of inflammation. We further expand the previous studies by demonstrating that inhibition of PARP can attenuate mechanical ventilation–associated biotrauma in the context of VILI.

It has been shown that lung parenchymal cells produce proinflammatory cytokines in response to tissue stretch contributing to VILI.[53] Damage to the alveolar–capillary barrier in combination with release of inflammatory cytokines is thought to be a major contributor to the development of multiple organ dysfunction syndrome and death.[54] Our data demonstrate that PARP inhibition can attenuate IL-6 release in the lung, and attenuate concentrations of both TNF-α and IL-6 in the circulation. These results are consistent with previous reports demonstrating that inhibition of PARP resulted in a down-regulation of chemokines and cytokines in several animal models of lung injury.[50,55,56] A decreased level of IL-6 might have led to an attenuated expression of PAI-1 in the lungs after PJ-34 treatment.[57]

Leukocyte transmigration is an important feature of diffused alveolar damage characterizing VILI.[58] We find that PARP inhibition reduced leukocyte infiltration in the lung, decreased permeability, and improved oxygenation and respiratory mechanics. Other studies have reported a role of PARP in the inhibition of leukocyte trafficking in conditions such as inflammation, shock, and ischemia–reperfusion injury.[50,59,60]

We have previously observed some degree of lung epithelial apoptosis with dominant expression of necrosis in an acid-induced acute lung injury model in rabbits undergoing ventilation with a high V_T.[2] In the current study, our results show higher levels of apoptosis than of necrosis in the lungs. We also noted that in the kidneys, the baseline apoptosis rate was approximately 10%, and increased to 30–40% with high V_T, which is higher than that observed in the acid aspiration model in rabbits.[7] The differences are likely due to the different priming stimuli, because acid aspiration resulted in a more severe and direct lung injury, whereas lipopolysaccharide induced more systemic effects. Also, the ventilatory strategies were somewhat different where higher PEEP levels were used in the low-V_T group and some PEEP level was applied in the high-V_T group in the previous study,[7] compared with the current study. Finally, the species difference might have a role with respect to organ sensitivity in response to mechanical ventilation. Of interest, we observed that the administration of PJ-34 reduced apoptosis in the kidney. The exact mechanisms remain to be elucidated, but PARP-deficient mice are protected against ischemic renal injury.[18,61]

In conclusion, we demonstrated that mechanical ventilation can induce PARP activation, and the pharmacologic inhibition of PARP reduced inflammatory responses and VILI and preserved kidney function in the rat model of lipopolysaccharide priming followed by mechanical ventilation.

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