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Combined Effect of Low-dose Nitric Oxide Gas Inhalation with Partial Liquid Ventilation on Hemodynamics, Pulmonary Function, and Gas Exchange in Acute Lung Injury of Newborn Piglets

We conducted a randomized animal study to determine whether there is a cumulative effect on hemodynamics, pulmonary function, and gas exchange when low-dose nitric oxide (NO) is added to partial liquid ventilation (PLV) in acute lung injury. Eighteen newborn piglets were saline-lavaged repeatedly, and randomly divided into two groups: PLV with perfluorocarbon group (n=8) and lavage only (control) group (n=10). Perfluorodecalin (30 mL/kg) was instilled into the endotracheal tube for 30 min, followed by 5-10 mL/kg/hr. Fifteen minutes after the completion of perfluorodecalin dosing, NO (10 ppm) was added to the inspiratory gas in an "on/off" manner. Perfluorodecalin instillation produced a significant improvement in gas exchange, pulmonary mechanics, shunt, and pulmonary arterial pressure (PAP). The addition of NO produced a further significant improvement in PaO_2 and PAP. The "on/off" response to NO was seen apparently in PAP, PaO_2, dynamic compliance, and shunt. All the variables in control group were remained at near the after-lavage levels without significant improvements until the end of the experiment. We concluded that NO might have a cumulative effect on gas exchange when combined with PLV, and this might be attributable to deceased PAP and V/Q mismatching.

Key Words : *Liquid Ventilation; Fluorocarbons; Nitric Oxide*

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INTRODUCTION

In acute respiratory distress syndrome, a disturbance of surfactant synthesis and function leads to an increased surface tension at the alveolar air-liquid interface. This increased surface tension leads to end-expiratory alveolar collapse, atelectasis, right-to-left shunt, and finally results in hypoxemia (1). The therapies to counterbalance the increased tendency for collapse may reverse above vicious sequence.

Partial liquid ventilation (PLV) is one of those therapies. PLV is performed by conventional gas ventilation of the perfluorocarbon-filled lung. Because of its high density and low surface tension, perfluorocarbon recruits the atelectatic lung region. A number of animal studies reported an improvement in gas exchange with this technique (2-9).

Inhaled nitric oxide (NO) is another therapeutic approach to reduce hypoxemia by selective vasodilation of the pulmonary vasculature of ventilated lung regions and resultant improvement in ventilation/perfusion matching (10). Administration of inhaled NO in adult patients with the acute respiratory distress syndrome has been shown to improve oxygenation by reducing pulmonary right-to-left shunt (10-12). Although the response to inhaled NO therapy is quite variable, and even doubtful especially in patients with severe lung injury (13, 14), it has been suggested that strategies to enhance alveolar recruitment may augment the responsiveness to inhaled NO therapy (15).

We did the present study to evaluate the effect of the addition of low-dose NO gas inhalation to PLV on hemodynamics, pulmonary function and gas exchange in newborn piglets with saline lavage-induced acute lung injury. We tested the hypothesis that the addition of low-dose NO gas inhalation to PLV, a novel modality to enhance alveolar recruitment, has a cumulative effects on oxygenation in acute lung injury.

MATERIALS AND METHODS

Animal Preparations and Surgical Procedures

The institutional Animal Care and Use Committee of the Samsung Biomedical Research Center, Seoul, Korea approved this investigation.

Studies were performed on 18, 7-9 day-old newborn piglets

of mixed strain (Yorkshire, conventional breed, purchased from Paju farm, Paju, Kyungki-Do, Korea). Surgical preparation of neonatal piglets was initiated by sedation with ketamine (20 mg/kg, intramuscular [IM]) and xylazine (2 mg/kg, IM), followed by thiopental anesthesia (5 mg/kg, intravenous [IV]). After local injection with lidocaine (1%), a tracheostomy was performed, and the piglet was paralyzed with the initial injection of pancuronium (0.1 mg/kg, IV), followed by hourly intravenous injections. Sedation was maintained with hourly doses of thiopental. The paralyzed piglet was placed on a mechanical ventilator (Sechrist Infant Ventilator, IV-100 V, Sechrist Industries, Anaheim, CA, U.S.A.) to attain an arterial O_2 tension of 80-100 mmHg and an arterial CO_2 tension of 35-45 mmHg. The right femoral artery was cannulated for arterial blood gas sampling and systemic arterial blood pressure monitoring. The right femoral vein was cannulated for administration of fluids and medications. A No. 5 Fr Swan-Ganz catheter (Baxter Health-care Corp., Irvine, CA, U.S.A.) was inserted into the right external jugular vein and was advanced into the pulmonary artery using direct-pressure and pressure wave monitoring. It was used for sampling of mixed venous blood and for the measurements of pulmonary artery and pulmonary arterial wedge pressures. An infusion of 0.9% saline containing 1U of heparin/mL was provided at 1-2 mL/hr through the arterial catheter and pulmonary arterial catheter, both of which were attached to a blood pressure transducer. A Hewlett Packard neonatal monitoring system (Hewlett-Packard Model M1276A, Andover, MA, U.S.A.) continuously monitored electrocardiogram, oxygen saturation, and systemic arterial and pulmonary arterial pressure. Animals were maintained supine with the head of the bed elevated 20 degrees throughout the study. Constant body temperature was maintained between 38-39℃ using a warmed operating table and servo-controlled overhead heater.

Perfluorodecalin

Perfluorodecalin (Fluka, Switzerland), a kind of perfluorocarbon, is insoluble in water, and is too stable at room temperature to react with air or water, and has the following characteristics; density, 1.95 g/mL at 25℃; kinematic viscosity, 2.9 centistokes at 25℃; boiling point, 142℃; vapor pressure, 14 mmHg at 37℃; surface tension, 15 dyne/cm at 25℃; O_2 solubility, 49 mL of gas/100 mL of liquid at 25℃; and CO_2 solubility, 140 mL of gas/100 mL of liquid at 37℃.

Nitric Oxide Delivery System

Nitric oxide (NO) was obtained as 800 ppm in nitrogen in 47-liter stainless steel cylinder that was authenticated and supplied by Korea Industrial Gases Co. The concentration of nitrogen dioxide was less than 1%, and the range of error in the concentration of nitric oxide was within the range of 1%. NO was decompressed by using a tri-staged regulator and a rotameter that were designed and assembled by medical engineering team of our hospital, and was injected into the inspiratory limb of mechanical ventilator through a teflon tube. The injection site of NO was between humidifier and the connecting site to endotracheal tube, and was made by using a part of NO delivery system by Drager Co. The distance between the site of the NO injection and the connecting site to endotracheal tube was over 50 cm. The concentration of NO and NO_2 in the inspiratory limb of the ventilatory circuit just before the entry to the endotracheal tube were monitored by portable electrochemical analyzer (Drager Co.). The adjustment of NO concentration was done by manipulation of the regulator and the rotameter. The exhaled gas was eliminated by the scavenging system installed at the exhalation outlet of mechanical ventilator. The flow rate of mechanical ventilator was maintained over 10 L/min to reduce the retention time of NO in ventilatory circuit, and thus to diminish the conversion of NO into NO_2 and N_2O_4.

Experiment Protocols

After a period of stabilization at about 30 min after anesthetic and surgical procedures, the ventilator settings were adjusted as follows: FiO_2 1.0, rate 25/min, PIP 30 cmH_2O, PEEP 4 cmH_2O, and iT 0.7 sec. These adjusted settings were maintained until the end of the experiment. At this point (BASE), baseline measurements of arterial blood gases, hemodynamic variables, and pulmonary mechanics were done. Thereafter, induction of acute lung injury was done by repetitive lung lavage with warm saline. A dose of 30 mL/kg of 37℃ saline was instilled into trachea and was removed repeatedly 5-10 times until an arterial O_2 tension of 60 mmHg was reached at the ventilator settings of FiO_2 1.0, rate 25/min, PIP 30 cmH_2O, PEEP 4 cmH_2O, and iT 0.7 sec. Lavage baseline point, when stabilization was reached, was appointed as after about an hour after lung lavage. Second measurement of arterial blood gases, hemodynamic variables, and pulmonary mechanics were done at this point (LAVAGE). Then, newborn piglets were randomly divided into two groups: partial liquid ventilation with perfluorocarbon administration group (PFC+NO group, n=8) and lavage only group (control group, n=10). Newborn piglets in PFC+NO group received perfluorodecalin. An hour after lung lavage, a dose of 30 mL/kg of perfluorodecalin was instilled continuously via the side-port of an adapter that connects ventilator circuit with endotracheal tube for 30 min. Supplemental instillation of perfluorodecalin was done once in every one hour with a dose of 5-10 mL/kg after checking arterial blood gases. Positional change was not done during and after perfluorodecalin instillation. Fifteen minutes after the completion of PFC dosing, NO in a concentration of 10 ppm was added to the inspiratory gas in an 'on/off' manner: on for 75 min (*first NO on*); off for an hour (*first NO off*); on again for an hour (*second NO on*); and finally off for an hour until the end of the experiment (*second NO off*).

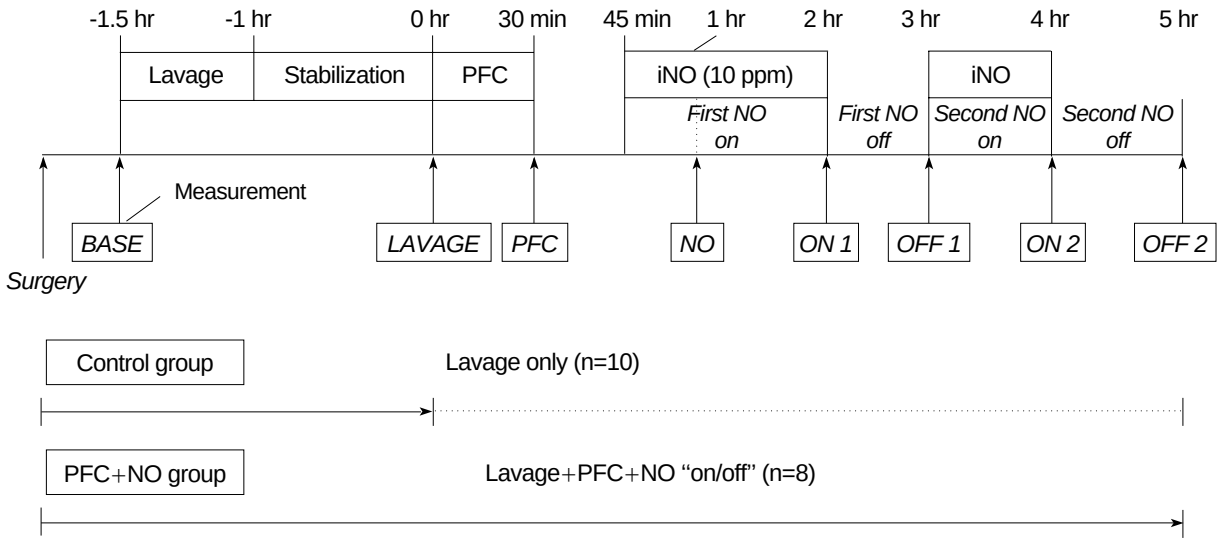

Fig. 1. Experimental protocol. PFC, perfluorocarbon; iNO, inhaled nitric oxide.

The control animals were kept being mechanically ventilated without any interventions during a 5 hr-protocol. Following measurements of arterial blood gases, hemodynamic variables, and pulmonary mechanics were done at the end of PFC dosing period (*PFC*), at 15 min after the beginning of first NO on (*NO*), at the end of the first NO on (*ON 1*), at the beginning of second NO on (*OFF 1*), at the end of second NO on (*ON 2*), and finally at the end of second NO off (*OFF 2*) in both groups (Fig. 1).

Measurements of Hemodynamic Variables, Gas Exchange, Pulmonary Mechanics, and Intrapulmonary Right-to-Left Shunt

Heart rate (HR), mean arterial pressure (MAP, in mmHg), and mean pulmonary arterial pressure (MPAP, in mmHg) were measured by using femoral arterial and pulmonary arterial catheter. Arterial blood pH, PaO_2, and $PaCO_2$ were measured from arterial blood samples taken from the femoral arterial catheter, and were analyzed by using Ciba-Corning blood gas analyzer. Dynamic compliance, tidal volume, airway resistance, and minute ventilation were measured continuously by Bicore CP-100R. Intrapulmonary right-to-left shunt was calculated by Fick equation (Ca-Cc/Cv-Cc). CcO_2, CaO_2, and CvO_2 are the oxygen content in pulmonary capillary blood, in arterial blood and in mixed venous blood. Alveolar capillary O_2 content (CcO_2) was calculated by using PAO_2 (partial pressure of oxygen in the alveoli) from the alveolar gas equation with $PAO_2=[(BP-47)\times(FiO_2)]-PaO_2$.

Data Analysis and Statistics

All numerical values measured were described as mean±standard deviation. Arterial blood gases, hemodynamic variables, pulmonary mechanics, and intrapulmonary shunt were assessed by repeated measures analysis of variance with the group as the main effect and time as the within-subject factor for the analysis of group-by-time interaction and intragroup differences, taking the Bonferroni principles into account. Intergroup difference at each time point was evaluated by one-way analysis of variance. Intragroup comparions with the previous value were analyzed with paired t-test. We used SPSS (version 10.0) and StatView (version 5.0) for these analyses. *p*-value lower than 0.05 was regarded significant.

RESULTS

Changes in Hemodynamic Variables

Heart rate (HR) showed a significant increase from 148±16/min to 189±32/min ($p<0.05$) in PFC+NO group, and from 131±16/min to 179±39/min ($p<0.01$) in control group after lavage. Thereafter, there were no significant changes in HR in control group. In PFC+NO group, HR demonstrated a tendency to decrease during *NO on* periods, and to increase during *NO off* periods. Only the latter was statistically significant ($p<0.05$). There were no significant differences in HR over time between both groups (Fig. 2).

Mean arterial pressure (MAP) did not show a significant change over time in control group. In PFC+NO group, MAP increased significantly from 65±11 mmHg to 81±15 mmHg after lavage ($p<0.01$), and decreased significantly from 84±23 mmHg to 75±26 mmHg during *second NO* on period ($p<0.05$). Intergroup comparison did not demonstrate significant differences between both groups (Fig. 2).

Pulmonary arterial pressure (PAP) showed a significant increase from 13±1 mmHg to 36±6 mmHg ($p<0.01$) in PFC+NO group, and from 14±1 mmHg to 28±3 mmHg ($p<0.01$) in control group after lavage. Thereafter, there were

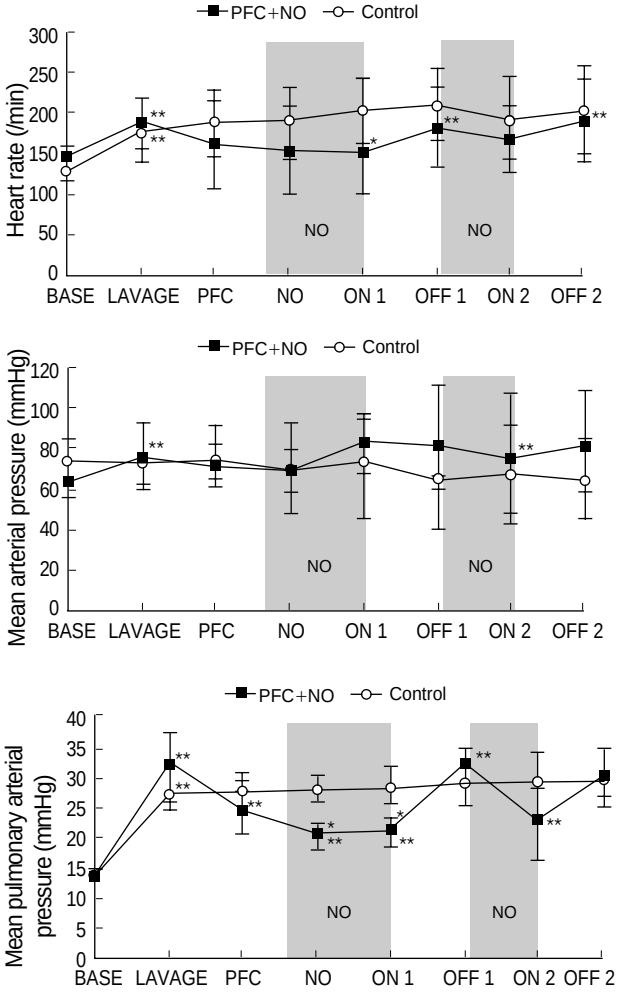

Fig. 2. Plots representing hemodynamic variables for PFC+NO group and control group: Top, heart rate; middle, mean arterial pressure; bottom, pulmonary arterial pressure at baseline (BASE), after lavage (LAVAGE), at the end of PFC dosing period (PFC), at 15 minutes after the beginning of first NO on (NO), at the end of the first NO on (ON 1), at the beginning of second NO on (OFF 1), at the end of second NO on (ON 2), and at the end of second NO off (OFF 2). Shaded area in figure designates inhaled NO administration in PFC+NO group. PFC, perfluorocarbon; *, $p<0.05$ compared to the value of control group; **, $p<0.05$ compared to the previous value within the group.

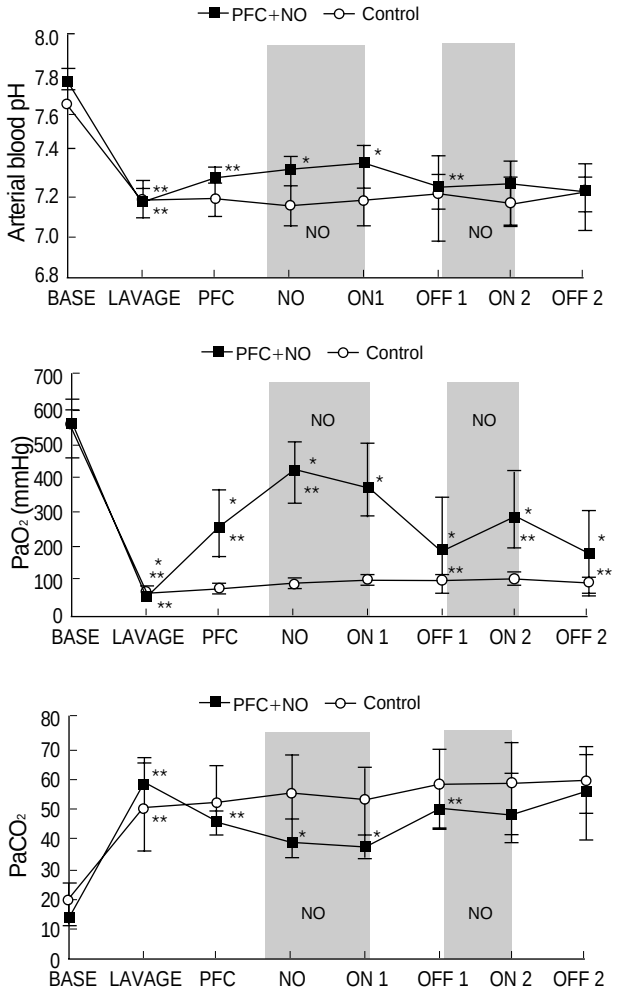

Fig. 3. Plots representing gas exchange for PFC+NO group and control group: Top, arterial blood pH; middle, PaO_2; bottom, $PaCO_2$ at baseline (BASE), after lavage (LAVAGE), at the end of PFC dosing period (PFC), at 15 minutes after the beginning of first NO on (NO), at the end of the first NO on (ON 1), at the beginning of second NO on (OFF 1), at the end of second NO on (ON 2), and at the end of second NO off (OFF 2). Shaded area in figure designates inhaled NO administration in PFC+NO group. PFC, perfluorocarbon; *, $p<0.05$ compared to the value of control group; **, $p<0.05$ compared to the previous value within the group.

no significant alterations in PAP in control group until the end of the protocol. In PFC+NO group, PAP decreased significantly to 26±4 mmHg ($p<0.01$) after PFC was added, and further significantly decreased to 20±3 mmHg ($p<0.01$) shortly after the beginning of *first NO on* period. However, as NO inhalation continued, PAP slowly increased to 22±3 mmHg ($p<0.05$) at the end of *first NO on* period. Then, PAP repeated to rise and fall according to the NO inhalation. PAP increased abruptly to 34±2 mmHg ($p<0.01$) at the end of *first NO off* period, decreased rapidly back to 24±6 mmHg ($p<0.05$) at the end of *second NO on* period, and finally increased once more at the end of *second NO off* period (p=NS). The PAP values at *NO* and *ON 1* time point in PFC+NO group were significantly less than those of control group (20±3 mmHg vs. 28±3 mmHg at NO, $p<0.01$; 22±3 mmHg vs. 30±4 mmHg at ON1, $p<0.01$), otherwise there were no intergroup differences (Fig. 2).

Changes in Gas Exchange

Arterial blood pH (pH), PaO_2, and $PaCO_2$ were all significantly aggravated after lavage in both groups ($p<0.01$). In control group, all of above three variables of gas exchange remained in low levels without significant improvements until

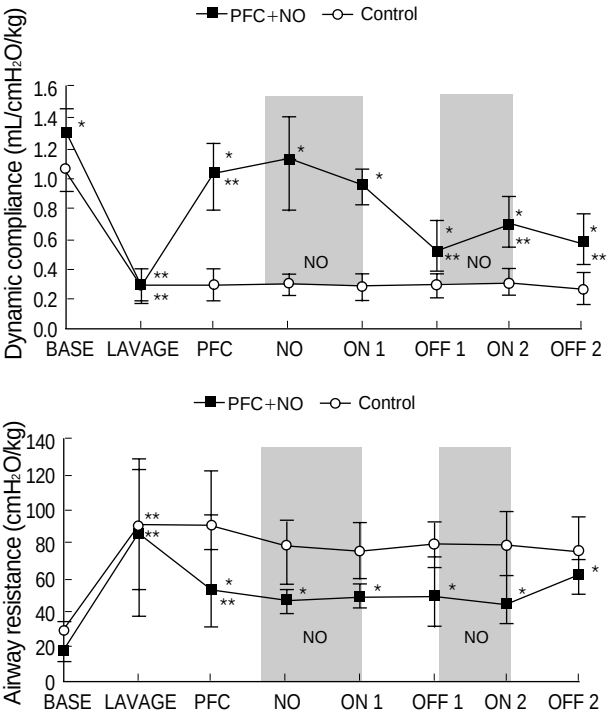

Fig. 4. Plots representing pulmonary mechanics for PFC+NO group and control group: Top, dynamic compliance; bottom, airway resistance at baseline (BASE), after lavage (LAVAGE), at the end of PFC dosing period (PFC), at 15 min after the beginning of first NO on (NO), at the end of the first NO on (ON 1), at the beginning of second NO on (OFF 1), at the end of second NO on (ON 2), and at the end of second NO off (OFF 2). Shaded area in figure designates inhaled NO administration in PFC+NO group. PFC, perfluorocarbon; *, $p<0.05$ compared to the value of control group; **, $p<0.05$ compared to the previous value within the group.

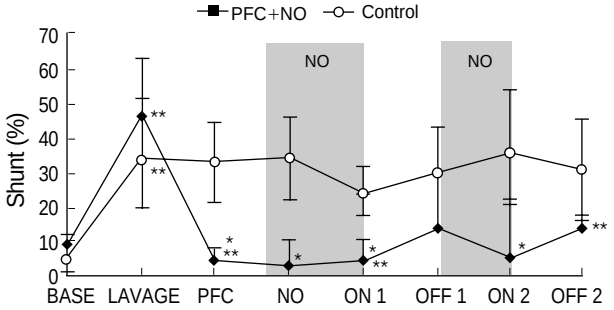

Fig. 5. Plots representing intrapulmonary right-to-left shunt for PFC+NO group and control group at the end of PFC dosing period (PFC), at 15 min after the beginning of first NO on (NO), at the end of the first NO on (ON 1), at the beginning of second NO on (OFF 1), at the end of second NO on (ON 2), and at the end of second NO off (OFF 2). Shaded area in figure designates inhaled NO administration in PFC+NO group. PFC, perfluorocarbon; *, $p<0.05$ compared to the value of control group; **, $p<0.05$ compared to the previous value within the group.

the end of the protocol after *LAVAGE* (Fig. 3).

In PFC+NO group, pH increased significantly from 7.16 ±0.07 to 7.26±0.06 ($p<0.05$), and $PaCO_2$ decreased significantly from 59±9 mmHg to 41±6 mmHg ($p<0.01$) after PFC was administered. However, both pH and $PaCO_2$ did not change significantly during and after *N0 on* periods. At the end of *first N0 off*, however, pH decreased significantly back to 7.19±0.11 ($p<0.01$), and $PaCO_2$ increased significantly back to 48±7 mmHg ($p<0.01$). At the end of *second N0 off* period, there were no such effects. In intergroup comparisons, the pH values in PFC+NO group at *BASE*, *N0*, and *0N 1* time point were significantly greater than those of control group (7.78±0.06 vs. 7.65±0.14 at *BASE*, $p<0.05$; 7.29±0.07 vs. 7.14±0.13 at *N0*, $p<0.05$; 7.30±0.10 vs. 7.14±0.13 at *0N1*, $p<0.05$), and the $PaCO_2$ values in PFC+NO group at *N0* and *0N 1* time point were significantly less than those of control group (39±7 mmHg vs. 54±15 mmHg at *N0*, $p<0.05$; 37±4 mmHg *vs.* 52±13 mmHg at *0N1*, $p<0.01$) (Fig. 3). After *LAVAGE*, PaO_2 increased significantly from 45±10 mmHg to 281±99 mmHg ($p<0.01$) after PFC was added. Subsequent NO administration further increased PaO_2 significantly to 378±75 mmHg ($p<0.05$), and the interruption of NO administration rapidly decreased PaO_2 back to 174±133 mmHg ($p<0.01$). This phenomenon was also observed during the next NO 'on/off' cycle (increased to 304±119 mmHg, $p<0.01$; decreased to 162±124 mmHg, $p<0.01$). In intergroup comparison, the PaO_2 values in PFC+NO group were significantly greater than those of control group at all time points ($p<0.05$) (Fig. 3).

Changes in Pulmonary Mechanics

Dynamic compliance decreased significantly and the airway resistance increased significantly after lavage in both groups ($p<0.01$). In control group, both of above two variables of pulmonary mechanics did not change significantly over a 5-hr protocol after *LAVAGE* (Fig. 4).

In PFC+NO group, dynamic compliance significantly increased from 0.30±0.09 mL/cmH_2O/kg to 1.01±0.25 mL/cmH_2O/kg ($p<0.01$) after PFC was administered, and from 0.56±0.19 mL/cmH_2O/kg to 0.79±0.22 mL/cmH_2O/kg ($p<0.01$) during *second N0 on*-but it did not change significantly during *first N0 on*. Dynamic compliance significantly decreased back from 0.91±0.12 mL/cmH_2O/kg to 0.56±0.19 mL/cmH_2O/kg ($p<0.01$) during *first N0 off*, and from 0.79±0.22 mL/cmH_2O/kg to 0.60±0.17 mL/cmH_2O/kg ($p<0.01$) during *second N0 off*. Intergroup comparison demonstrated that all the values of dynamic compliance in PFC+NO group, except the value at *LAVAGE*, were significantly greater than those of control group ($p<0.01$) (Fig. 4). After a significant decrease from 84±48 cmH_2O/kg to 49±20 cmH_2O/kg ($p<0.05$) following an addition of PFC, airway resistance did not change significantly over time until the end of the protocol. Intergroup comparison showed that all the values of airway resistance in PFC+NO group after *LAVAGE* were

significantly less than those of control group ($p<0.05$) (Fig. 4).

Changes in Intrapulmonary Right-to-Left Shunt

After lavage, intrapulmonary right-to-left shunt (shunt) increased significantly from 9.8±3.8% to 47.0±16.0% in PFC+NO group ($p<0.01$), and from 5.2±3.8% to 35.5±16.0% in control group ($p<0.01$). In control group, shunt did not change significantly over a 5-hr protocol after *LAVAGE* (Fig. 5).

In PFC+NO group, shunt decreased significantly to 3.9±2.7% ($p<0.01$) after PFC was administered. Shortly after the beginning of *first NO on* period, shunt decreased further to 1.1±10.3% (p=NS), but as NO inhalation continued, shunt slowly increased to 1.9±7.1% ($p<0.05$) at the end of *first NO on*. During *First NO off*, shunt increased rapidly, but the increase was not statistically significant. During *Second NO on*, shunt decreased back again, but the decrease also was not significant, either. However, during *Second NO off*, shunt increased significantly from 3.6±18.8% to 13.1±5.0% ($p<0.05$). Intergroup comparison showed that the shunt values at *PFC*, *NO*, *ON 1*, and *ON 2* in PFC+NO group were significantly less than those of control group (3.9±2.7% vs. 29.2±10.2% at *PFC*, $p<0.01$; 1.1±10.3% vs. 29.2±10.3% at *NO*, $p<0.01$; 1.9±7.1% vs. 21.8±7.1% at *ON1*, $p<0.01$; 3.6±18.8% vs. 37.8±18.8% at *ON2*, $p<0.01$) (Fig. 5).

DISCUSSION

The results of our study showed that PLV with PFC improved gas exchange, pulmonary mechanics, and ventilation/perfusion (V/Q) matching in a lavage-induced acute lung injury model. Concerning the hemodynamic effects of PFC administration, PAP reduction was the only one statistically significant change in the study. The improvement of pulmonary gas exchange during PLV is attributable to the enhancement of alveolar recruitment of atelectatic lung regions, especially in the dependent zone, through the low surface tension and noncompressible nature of PFC in the alveolus (16, 18, 19). Moreover, the redistributing effect of the weight of PFC on the pulmonary blood flow may contribute to the improvement of gas exchange (20). By this mechanism, pulmonary blood flow to the relatively well-ventilated zone increases and thus, V/Q matching is improved. Our findings support this mechanism because PFC administration resulted in a significant decrease in intrapulmonary shunt. PFC administration also improved dynamic compliance and airway resistance in our study. These findings are compatible with the previous studies that demonstrated that PFC increased pulmonary compliance and reduced airway pressure (3-5, 19, 21-23). The improvements in pulmonary mechanics might have further improved gas exchange during PLV in our study. Our results confirmed that there were no significant effects on systemic circulation by PLV, and these results were also compatible with previous studies (8, 24). However, regarding pulmonary circulation, PLV decreased PAP significantly. Uchida et al. and Wilcox et al. also reported similar findings as ours (22, 24). The reduction of PAP might have improved pulmonary blood flow and reduced intrapulmonary right-to-left shunting, but further study is necessary to confirm this speculation. The mechanism for the reduction of PAP by PLV is not clear, but may have been caused by a reduction in hypoxic pulmonary vasoconstriction around the recruited alveoli.

The addition of 10 ppm dose of NO inhalation to PLV further improved PaO_2, and improved dynamic compliance partly (only during *second NO on* period). In terms of hemodynamics, NO inhalation further decreased PAP significantly, and decreased systemic arterial pressure partly (only during *second NO on* period). Because NO inhalation was administered in an 'on/off' manner, we could observe the rebound phenomenon according to the discontinuation of NO inhalation. In addition to PaO_2 and PAP that showed an honest 'on/off' response according to NO inhalation, arterial blood pH, $PaCO_2$, dynamic compliance, airway resistance, and intrapulmonary shunt showed rebound phenomenon when NO was withdrawn, although their responses to the administration of NO inhalation were absent or incomplete (responded during only one of two *NO on* periods). Our results are compatible with the several previous studies of combination of PLV and NO inhalation (9, 22, 25-27). All of these studies demonstrated an improvement in oxygenation and PAP when short-term (10 to 15 min) inhalation of NO was added to PLV as our study did.

Inhaled NO has shown to increase oxygenation in injured lung by decreasing pulmonary right-to-left shunt through its selective vasodilating effect on pulmonary vessels of ventilated areas (10). However, Uchida et al., in their study of combination of PLV with NO inhalation in the severe oleic acid lung injury model, observed that NO inhalation did not improve pulmonary gas exchange during conventional ventilation, while it improved gas exchange during PLV (24). This observation was supported by several clinical studies, in which the pulmonary vasodilating effect was not necessarily accompanied by the improvement of gas exchange in severe lung injury (13, 28). With regard to this phenomenon, Puybasset et al. speculated that pulmonary vasodilation and improvement of gas exchange are linked but occur at different anatomic levels in the lung (28). If pulmonary arteries, alveolar territories of which own are poorly ventilated, were dilated by NO that entered from surrounding well-ventilated alveolar space, ventilation/perfusion mismatching would be aggravated. Therefore, recruitment of poorly ventilated alveolar unit is essential to improve arterial oxygenation caused by NO inhalation. They proved their speculation by demonstrating that peak end expiratory pressure (PEEP) induced alveolar recruitment potentiated the NO-induced improvements in arterial oxygenation in ARDS patients. In our current study, NO inhalation was added after gas exchange was already improved

considerably by preceding PFC administration; in other words, after PLV-induced alveolar recruitment was obtained, and we confirmed the additional significant improvement in oxygenation by NO inhalation. Moreover, we demonstrated a reduction of intrapulmonary shunt by adding NO inhalation, although the reduction was not significant. These findings illustrate that alveolar recruitment was so sufficient that pulmonary vasodilating effect of inhaled NO went in a beneficial way toward the reduction of intrapulmonary shunt, and support the speculation of Puybasset et al. Furthermore, like abovementioned studies (9, 22, 25-27), PAP was significantly decreased by adding NO inhalation. This result reflects that the improvement in oxygenation by adding of NO inhalation may be attributable to pulmonary vasodilating effect of inhaled NO.

In our current study, the addition of NO inhalation to PLV affected dynamic compliance. Dynamic compliance significantly increased during *second NO on* period, and decreased during *first and second NO off* periods. These findings were incompatible with several studies of combined therapy of PLV and NO inhalation (22, 25, 29). In their studies, there were no changes of dynamic compliance by adding NO to PLV. Further study may be necessary to be able to explain these findings.

According to the study by Houmes et al., there was a sharp 'on/off' effect of inhaled NO when added to PLV (26). This finding suggested a similar kinetic of NO in the PFC-filled lung compared to conventional gas ventilation. They supposed that the total amount of NO dissolved in PFC must be low. Our current results showed a similar rapid 'on/off' effect of inhaled NO on PAP, PaO_2, dynamic compliance, and intrapulmonary shunt in PLV setting, although we did not set a conventional gas ventilation control. In the explanation of the 'on/off' effect of inhaled NO in PLV setting, Kaisers and Rossaint suggested a possibility that inhaled NO is mainly effective in the air-filled compartment of injured lung (30). Their speculation is that due to gravitational force, PFC may preferentially pooled in dependent lung regions, filling and distending alveoli; whereas in non-dependent, well-ventilated lung regions, PFC may form thin layers and reduce the surface tension at liquid-gas interfaces, resulting in an improvement in lung mechanics. However, the same gravitational force may redistribute pulmonary blood flow toward non-dependent, well-ventilated alveoli, which are stabilized by the surface tension-reducing properties of PFC, therefore, inhaled NO in air-filled compartment may play a more important role on improving V/Q matching than NO dissolved in PFC-filled compartment. Recent study by Uchida et al., in which the addition of NO inhalation to PLV has shown to significantly increase the pulmonary blood flow to the non-dependent region and improve pulmonary gas exchange, supports Kaisers and Rossaint's speculation (30).

In our current study, we set the control group as a conventional gas ventilation alone group. Therefore, we did not evaluate the time-response patterns of PLV and NO inhalation, respectively. We examined the response of inhaled NO only in PFC-filled lung. Therefore, it may be complicated to differentiate the response of inhaled NO from that of PLV itself. However, in our pilot study with the same experimental settings (unpublished), three newborn piglets that were given PFC administration alone were gradually aggravated in terms of pulmonary hemodynamics, gas exchange, pulmonary mechanics, and V/Q matching as time passed, after they showed a brisk improvement in above outcome measures as soon as PFC was administered. Moreover, we added NO in an 'on/off' manner, not a continuous inhalation, and we could observe the relevant 'on/off' responses in the outcome measures. Therefore, the problem of the lack of control in our study design seems to be lessened.

Moreover, we used a fixed dose of PFC (30 mL/kg) and NO (10 ppm). We adopted these doses from our previous studies, in which these doses accomplished optimal responses without significant hemodynamic compromises (32, 33). However, those studies evaluated the effect of PLV and the effect of NO inhalation, respectively. Therefore, it may be necessary to evaluate the combined effect of PLV and NO inhalation by using varying doses of PFC and NO, to find the optimal doses of PFC and NO. According to the study of the combining effect of PLV and NO inhalation by Houmes et al., in which varying doses of PFC and NO were used, additional NO inhalation resulted in a maximal increase in PaO_2 at 30 ± 10 ppm NO at each PFC dose, and maximal effect of additional NO inhalation on PaO_2 occurred at 5 mL/kg perflubron dose (26).

In summary, we confirmed that the PLV with PFC improved gas exchange, and this may be attributable to improved pulmonary mechanics and V/Q matching. When NO inhalation was combined to PLV, there were further improvements in gas exchange, and this may be attributable to deceased PAP and improved V/Q matching. There were no hemodynamic compromises during the combination therapy. The combination of these two novel therapies could be investigated for the infants with severe lung injury.

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