Continuous tracheal gas insufflation during partial liquid ventilation in juvenile rabbits with acute lung injury

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Zhu, Guangfa, Thomas H. Shaffer, and Marla R. Wolfson. Continuous tracheal gas insufflation during partial liquid ventilation in juvenile rabbits with acute lung injury. J Appl Physiol 96: 1415–1424, 2004. First published December 19, 2003; 10.1152/japplphysiol.01121.2003.—To examine the hypothesis that combined treatment with tracheal gas insufflation (TGI) and partial liquid ventilation (PLV) may improve pulmonary outcome relative to either treatment alone in acute lung injury (ALI), saline lavage lung injury was induced in 24 anesthetized, ventilated juvenile rabbits that were then randomly assigned to receive (n = 6/group) 1) conventional mechanical ventilation (CMV) alone, 2) continuous TGI at 0.5 l/min, 3) PLV with perfluorochemical liquid, and 4) combined TGI and PLV (TGI + PLV), and subsequently ventilated with minimized pressures and tidal volume (VT) to keep arterial P O2 (PaO2) >100 Torr and arterial P CO2 (PaCO2) at 45–60 Torr for 4 h. Gas exchange, lung mechanics, myeloperoxidase, IL-8, and histomorphometry [including expansion index (EI)] were assessed. The CMV group showed no improvement in lung mechanics and gas exchange; all treated groups had significant increases in compliance, PaO2, ventilation efficacy index (VEI), and EI, and decreases in PaCO2, oxygenation index, physiological dead space-to-VT ratio (VD/VT), myeloperoxidase, and IL-8, relative to the CMV group. TGI resulted in lower peak inspiratory pressure, VT, VD/VT, and greater VEI vs. PLV group; PLV resulted in greater compliance, PaO2, and EI vs. TGI. TGI + PLV resulted in decreased peak inspiratory pressure, VT, VD/VT, and increased VEI compared with TGI, improved compliance and EI compared with PLV, and a further increase in PaO2 and oxygenation index and a decrease in PaCO2 vs. either treatment alone. These results indicate that combined treatment of TGI and PLV results in improved pulmonary outcome than either treatment alone in this animal model of ALI.

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Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are defined by hypoxemia, decreased pulmonary compliance, and bilateral infiltrates on chest radiograph (5a). ARDS continues to be associated with a relatively high mortality (38). Although conventional gas mechanical ventilation (CMV) remains the primary supportive therapy for this patient group, this intervention, even with relatively lower tidal volume (VT), has been shown to worsen preexisting lung injury [i.e., ventilator-induced lung injury (VILI)] because of uneven distribution of mechanical properties, regional overstretch due to atelectasis of the injured lung (i.e., baby lung concept), and increased shear stress in terminal units due to the repeated opening and closing of small airways and alveoli (13, 36). Therefore, various modifications of ventilation strategies aimed at lung protection and reducing VILI have been investigated to support ALI/ARDS patients (1, 5).

Tracheal gas insufflation (TGI) is a technique in which fresh gas is introduced into the trachea. It has been suggested to be a useful adjunct to CMV in patients with ALI/ARDS (32) and preterm infants with hyaline membrane disease (11). When combined with CMV, TGI can effectively augment ventilation by reducing the dead space of the patient-ventilator system, reducing ventilatory pressures and VT while maintaining constant arterial P CO2 (PaCO2) or promoting elimination of excessive CO2. However, TGI has not been shown to substantially improve oxygenation (17, 21).

Partial liquid ventilation (PLV), defined as a method of gas ventilation in the presence of perfluorochemical (PFC) liquid, has been proposed as a “lung protective” modality to support pulmonary gas exchange in ALI/ARDS. The mechanisms that support pulmonary gas exchange are associated with the physicochemical properties of the PFC liquids and biophysical effects of the liquid on lung mechanics. PFCs are liquids with high solubility for oxygen and CO2, which can facilitate respiratory gases to and from the alveoli. In addition, PFC liquid may facilitate opening of collapsed, noncompliant-dependent lung segments because it reduces interfascial tension and is incompressible. Consequently, the incompressible PFC liquid may prevent collapse of unstable terminal lung units (i.e., small airways and alveoli) even at low pressures, thereby improving lung compliance and oxygenation, decreasing disruptive forces acting on the small airway and parenchyma, and, therefore, minimizing the risk of VILI. The combination of improved lung volume and stability, thus reducing pulmonary shunt, has been shown to ameliorate hypoxemia. PLV has been shown to be effective in various animal models (2, 7, 19, 20, 26, 27, 31, 33, 35, 40, 41) and feasible in humans (3, 14–16, 22). Although results of small clinical studies are promising (14, 16, 22), improved outcome beyond optimized CMV could not be proven in a large trial of PLV using perfluoron in the adult (3, 15). Optimization of ventilatory settings, as well as maintenance of adequate PFC lung volumes during PLV in the clinical setting, has not been resolved. Although PFC fluids of
high CO₂ solubility may provide greater carrying capacity for CO₂, this same quality presents diffusional limitations during PLV at lung volumes sufficient to offer lung protection (24). As such, PaCO₂ could increase during PLV with instillation of larger volumes of PFC if a proportional increase in alveolar ventilation is not achieved. Thus, to eliminate CO₂, additional gas ventilation or a reduction in overall dead space is required to deplete CO₂ from the PFC reservoir in the lung.

In this study, we hypothesized that the combination of TGI and PLV in an animal model of ALI would result in improved pulmonary outcome relative to either treatment alone. Specifically, we reasoned that PLV may assist TGI to increase lung recruitment and improve arterial PO₂ (PaO₂) and that TGI may help PLV to offset the diffusion issues and remove CO₂.

MATERIALS AND METHODS

TGI circuit. Continuous TGI was introduced by ancillary flow injected into the lower part of the endotracheal tube (ETT) through six capillaries, which are extruded into the wall and exit 1.5 cm above the distal end of the tube (inner diameter = 3.0 mm, tube volume = 2.8 ml; Vygon, 954-404 Encouen, France). Two other independent capillaries were used for collection of mixed expired gases and measurement of pressure at the tip of the ETT.

PFC. A mixture of two PFC liquids (F2 Chemicals; Preston, UK), perfluoromethylcyclohexane (PP2; density 1.79 g/ml, vapor pressure 141.0 mmHg, and viscosity 0.88 cSt at 25°C) and perfluoromethyldecalin (PP9; density 1.97 g/ml, vapor pressure 2.9 mmHg, and viscosity 3.32 cSt at 25°C) at a proportion of 25:75% (vapor pressure: 4.4 mmHg, viscosity: 2.21 cSt; density: 1.89 g/ml) was used in this study. This combination was chosen based on our laboratory’s previous study (20), which demonstrated comparable gas exchange, lung compliance, and histological findings for 4 h in the same animal model of ALI as that achieved with perfluorobenzene, a PFC used in both animal and clinical trials (19, 22). In addition, the relatively lower vapor pressure and higher viscosity of this combination compared with perfluorobenzene (5.2 mmHg and 1 cSt, respectively) reduced resuscitation requirements and enhance uniform and sustained distribution of the PFC during gas ventilation (19, 20, 27).

Animal preparation and measurements. The experimental protocol and procedures in this study were approved by the Institutional Animal Care Committee at Temple University. Twenty-four juvenile New Zealand White rabbits were studied [weight: 1.84 ± 0.06 (SE) kg, age: 4–6 wk]. After preanesthesia with an intramuscular injection of ketamine (23 mg/kg), acepromazine (0.58 mg/kg), and xylazine (0.78 mg/kg), a pulse oximeter probe (Nellcor, N-100), ECG leads, and a rectal thermistor probe were applied. Animals were tracheotomized, intubated (ETT; see TGI circuit), and mechanically ventilated with a time-cycled and pressure-limited mode (VIP Gold-Bird, ViaSys, Palm Springs, CA). The ventilator was initially set at a peak inspiratory pressure (PIP) of 10–14 cmH₂O positive-end-expiratory pressure (PEEP) of 5 cmH₂O, flow rate of 10 l/min to provide a V̅T of ~6 ml/kg, and respiratory frequency of 40 breaths/min to maintain PaCO₂ within normal range (35–45 Torr), with an inspiratory time (Ti) of 0.3 s and a fraction of inspired oxygen (FiO₂) of 1.0. Flow rate and FiO₂ were kept constant throughout the experiment. Catheters (5 Fr) were placed in a jugular vein and carotid artery. All animals were paralyzed with pancuronium bromide (0.1 mg/kg) and received metabolic substrate (5% glucose: 5 ml · kg⁻¹ · h⁻¹) and supplemental paralytic (pancuronium bromide: 0.1 mg · kg⁻¹ · h⁻¹) during the protocol. Sodium bicarbonate was given if the pH was ≤7.25 and PaCO₂ was ≥50 Torr; Tris was given if pH was ≤7.25 and PaCO₂ was ≥50 Torr. After instrumentation and stabilization, baseline assessment of arterial blood chemistry (Nova Statpro M, Waltham, MA; Radiometer OSM 3, Copenhagen, Denmark), V̅T by integrated pneumotachography signals (no. 00, Fleish, Eplings, Switzerland), airway manometry, calculated respiratory compliance (C; PeDS-LAB, MAS, Hatfield, PA), mean arterial pressure (MAP), and heart rate (HR) (Athena/Neoental 9040; S&W Medico Teknik, Albertslund, Denmark) were performed. In addition, mean expired PCO₂ was measured from a minimum of 20 ml of mixed expired gas collected manually from one of the independent capillaries of the ETT. The animals were then subjected to the experimental protocols.

Experimental protocols. Lung injury was induced by repeatedly lavaging the lung with 10 ml/kg of warm normal saline solution while adjusting the PIP to maintain V̅T at 6 ml/kg for all animals. Lung injury criteria were defined as PaO₂ < 100 Torr and C < 80% of baseline and <0.50 ml · cmH₂O⁻¹ · kg⁻¹, maintained for 45 min, defined as treatment time 0 (T₀). At T₀, animals were randomly assigned to one of four groups (n = 6/group): 1) control: CMV alone; 2) TGI alone: continuous insufflation rate of 0.5 (min; 3) PLV alone: 18 ml/kg of 25% PP2–75% PP9; and 4) combination of PLV and TGI described above (TGI + PLV). In all PFC-treated groups, room air-equilibrated PFC mixture (25% PP2–75% PP9) was instilled during inspiration over 10 min through the distal side port of the ETT as the animals were repositioned to optimize PFC distribution by using four equal increments of the total dose with Trendelenburg, reverse Trendelenburg, and left and right lateral decubitus positions. Pressure-volume loop monitoring was performed to guide the rate of PFC infusion so as to minimize opening pressure and prevent over-distension, as reflected by reduction in C and V̅T. For the TGI + PLV group, PFC liquid was supplemented (1 ml · kg⁻¹ · h⁻¹) via one of the independent capillaries of ETI. Continuous TGI was maintained at an insufflation rate of 0.5 l/min into the ETI wall capillaries. The humidified and warmed gas was derived from the continuous, circulating gas flow (FiO₂ = 1) in the inspiratory line of the conventional ventilation circuit and was regulated by a resistor in line with a calibrated rotometer. During the 4-h postinjury period in all groups, ventilator parameters, arterial blood gases, lung mechanics, mixed expired gas, and vital signs were measured and recorded at T₀ and then every 30 min; PIP, PEEP, frequency, and Ti were adjusted according to previous blood gas and lung mechanics to keep PaO₂ > 100 Torr and PaCO₂ at 45–60 Torr; PIP was kept below the high inflection point, and PEEP was kept above the low inflection point on the pressure-volume loop and limited to a maximum of 32/h cmH₂O; maximum frequency and Ti were limited to 60 breaths/min and 0.5 s, respectively, with the goal of minimizing ventilatory pressure and volume requirements. For animals who survived <4 h, measured values and physiological data recorded before deterioration of HR and blood pressure were included in the final analysis, and their lungs were processed immediately after death.

Oxygenation index (OI), ventilation efficacy index (VEI), and physiological dead space-to-V̅T ratio (Vis/V̅T) were computed as previously described (17, 30): OI = [MAMW (cmH₂O) × Fio₂]/PaO₂ (Torr) × 100, where MAMW is mean airway pressure; VEI = 3,800 [respiratory rate × (PIP – PEEP) × PaCO₂ (Torr)]; Vis/V̅T = (PaCO₂ – mean expired Pco₂)/PaO₂.

To determine whether ventilatory pressures and delivered V̅T are altered directly by the low TGI flow rate, two bench experiments were conducted by using a lung model in which C, resistance, and ventilator settings were matched to the in vivo 4-h values of the TGI and TGI + PLV groups. Two pneumotachometer assemblies (see Animal preparation and measurements) with matched calibrations were used by placing one, proximally, between the ventilator and ETT and the other, distally, between the ETT the lung model. Airway pressures (PIP and PEEP) and V̅T transduced from each pneumotachometer assembly were recorded during conditions of TGI on (0.5 l/min) and off and compared between conditions.

Lung tissue collection and histological examination. At the end of the protocol, the animals were euthanized with an overdose of pentobarbital sodium and potassium chloride. With the ventilator cycling, the chest was opened to inspect for gross evidence of lung injury. The ventilator was then switched to the continuous positive airway pres-
sure set at the final PEEP value. The lungs were then perfused with cold Millonig’s buffer via the pulmonary arteries until the effluent ran clear, and the left and right lungs were dissected according to a consistent anatomic matrix across animals. The right main stem bronchi was clamped, and consistent regional samples were obtained from the dependent and nondependent regions of the apex and base of the right lung and placed in 10% buffered formalin for subsequent paraffin embedding and sectioning (5 μm). Hematoxylin and eosin-stained sections were examined by light microscopy for evidence of lung injury, as described elsewhere (41). Morphometric assessment was performed by using computerized image analysis software (Image Pro Plus, Silver Spring, MD) to measure alveolar diameter, area, and the proportions of gas exchange spaces per unit area and parenchyma per unit area to calculate the lung EI (gas exchange spaces per unit area/parenchyma per unit area) (12, 39, 41). Similarly, consistent anatomic matrix across animals. The right main stem artery and base of the left lung were obtained, snap frozen in liquid nitrogen, and stored at −80°C for subsequent analysis of inflammatory mediators (see Inflammatory mediator analysis).

**Inflammatory mediator analysis.** Lung tissue (200 mg) was homogenized in 2 ml of 0.05 M potassium phosphate buffer (pH = 6.0). The homogenate was centrifuged at 1,700 g for 30 min at 4°C. The supernatant was then incubated at 60°C for 2 h on a thermostated water bath and again centrifuged at 10,000 g for 5 min at 4°C to obtain supernatant for measurement of myeloperoxidase (MPO) activity by using a colorimetric bioassay described by Schierwagen et al. (34). Duplicate 10-μl aliquots of standard or sample were incubated in a 96-well plate with 10 μl of substrate buffer (0.1 M sodium citrate, 0.1% O-dianisidine, 1 mM hydrogen peroxide, pH = 5.5) for 1 min. The plate was read immediately at 560 nm in an automated plate reader (MRX Revelation, Thermo Labsystems, Franklin, MA). Human leukocyte MPO (ICN Biomedicals, Aurora, OH) was used as standard, detection limit was 0.0625 U/ml and interassay and intra-assay coefficient of variation were 9.8 and <6%, respectively.

**IL-8.** Lung tissue (200 mg) was washed twice with phosphate-buffered saline and homogenized in 1 ml of a buffer solution (50 mM Tris-HCl, 150 mM NaCl, 1% Igepal, 0.5% NaDOC, 0.1% SDS) containing Complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The homogenate was centrifuged at 12,000 g for 10 min at 4°C to obtain supernatant for measurement of IL-8 concentration by using a commercial rabbit-specific ELISA kit (OptEIA rabbit IL-8 set, PharMingen, San Diego, CA). The limit of detection was 3.1 pg/ml with interassay and intra-assay coefficient of variation of 8.4 and <5%, respectively.

Lung MPO and IL-8 were expressed per gram of tissue weight and corrected for the contribution of the PFC liquid to weight in the PLV groups.

**Statistical analysis.** All values are reported as means ± SE. ANOVA was used to determine significant differences in physiological data (2-way ANOVA; baseline vs. injury condition and group), lung morphometry, MPO, and lung IL-8 among groups (1-way ANOVA); Tukey’s multiple-comparison post hoc testing was used to determine significant differences as a function of group and/or condition. Two-way repeated-measures ANOVA followed by post hoc Tukey’s multiple-comparison test was used to determine significant differences in physiological data as a function of time postinjury and group. Significance was set at P < 0.05.

**RESULTS**

Twenty-four juvenile New Zealand White rabbits were anesthetized and prepared as described. A mean of 6.7 ± 0.3 saline lavages (range 5–10) was required to reach the physiological defined end point for ALI. There were no significant differences in the number of lavages needed to achieve the desired lung injury between groups. One animal in the CMV group died of severe hypoxemia and acidosis at 2.5 h after treatment.

Baseline gas exchange, C, Vt, and physiological Vd/Vt are summarized in Table 1. The temporal profiles of these parameters are shown in Figs. 1 and 2. Acid base, MAP, and HR are summarized in Table 2. Saline lavage injury resulted in significant (all at least P < 0.01) decreases in Pao2, C, VEi, and pH, and increases in OI, Paco2, and Vd/Vt compared with respective baseline values. There were no significant differences in MAP or HR between baseline and injury, independent of group. During treatment, MAP remained stable in all treated groups but decreased significantly (P < 0.01) in the CMV group. There were no significant differences in HR as a function of group or time.

Figure 1, A and B, illustrates oxygenation data as a function of group and time. ANOVA demonstrated significant differences in Pao2 and OI (P < 0.0001) as a function of group and time. Post hoc testing demonstrated that Pao2 was greater (P < 0.001 or P < 0.01) and OI was lower (P < 0.001) in all treated groups compared with CMV alone. Pao2 in the PLV group was greater than that in the TGI group (P < 0.01). Further analyses demonstrated that Pao2 was greater and OI was lower in the TGI + PLV group compared with either the TGI (P < 0.001) or PLV (P < 0.01) group. Additionally, there was a significant increase in Pao2, and decrease in OI in the TGI + PLV (P < 0.01) and PLV (P < 0.05) groups as a function of time with improvement occurring by 0.5 h after treatment.

Figure 1, C and D, shows ventilation data as a function of group and time. ANOVA demonstrated significant differences in Paco2 (group: P < 0.0001; time: P < 0.05) and VEi (group and time: P < 0.001) as a function of group and time. Post hoc testing demonstrated that Paco2 in all treated groups was lower (P < 0.001 vs. TGI + PLV; P < 0.05 vs. TGI or PLV) and VEi was greater (P < 0.001) compared with CMV alone. Further analyses demonstrated that Paco2 was lower in the TGI + PLV group compared with either the TGI (P < 0.05) or PLV (P < 0.01) group and that VEi in both TGI-treated groups (TGI and TGI + PLV) was greater (P < 0.01) than that in PLV group. Additionally, there was a significant decrease in Paco2, and increase in VEi in the TGI + PLV group (P < 0.01) as a function of time with improvement occurring 0.5 h after treatment. In contrast, there was a significant increase in Paco2, and decrease in VEi in the CMV group (P < 0.05) as a function of time, with deterioration occurring by 1.0 h and thereafter. Two-way ANOVA demonstrated that, during the 4-h experiment, pH values in TGI + PLV and TGI groups

<p>| Table 1. Summarized baseline values for gas exchange, respiratory compliance, Vt, and Vo/Vt |</p>
<table>
<thead>
<tr>
<th>CMV</th>
<th>TGI</th>
<th>PLV</th>
<th>TGI + PLV</th>
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<tbody>
<tr>
<td>Pao2, Torr</td>
<td>405±20</td>
<td>388±27</td>
<td>432±17</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>36.8±3.4</td>
<td>39.2±1.9</td>
<td>35.5±3.8</td>
</tr>
<tr>
<td>OI</td>
<td>1.59±0.12</td>
<td>1.78±0.17</td>
<td>1.66±0.17</td>
</tr>
<tr>
<td>VEI</td>
<td>0.36±0.05</td>
<td>0.32±0.02</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td>C, ml·cmH2O−1·kg−1</td>
<td>0.91±0.11</td>
<td>0.91±0.05</td>
<td>0.92±0.06</td>
</tr>
<tr>
<td>Vt, ml/kg</td>
<td>5.92±0.68</td>
<td>5.93±0.31</td>
<td>6.12±0.62</td>
</tr>
<tr>
<td>Vo/Vt</td>
<td>0.13±0.01</td>
<td>0.17±0.04</td>
<td>0.19±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. OI, oxygenation index; VEI, ventilation efficiency index; C, respiratory compliance; Vt, tidal volume; Vo/Vt, physiological dead space-to-Vt ratio; CMV, conventional mechanical ventilation; TGI, tracheal gas insufflation; PLV, partial liquid ventilation; Pao2, arterial P02; Paco2, arterial Pco2. P = no significant difference between groups in all parameters.
were significantly higher ($P < 0.05$ or $P < 0.01$) than in CMV and PLV groups, although base excess values in all groups were kept within normal range (Table 2). There was no significant difference in pH as a function of treatment time for any group.

As shown in Fig. 2A, C in all treated groups increased significantly ($P < 0.05$) from injury (T0). ANOVA demonstrated a significant difference in C as a function of group and no significant difference as a function of time during the treatment protocol. Post hoc analysis demonstrated that C in all treated groups was significantly ($P < 0.001$) greater than CMV alone. Further analysis showed that C was significantly ($P < 0.001$) greater in PLV and PLV + TGI, and both groups > TGI > CMV. There were no significant differences in C between the PLV and TGI + PLV groups. The improvement in C was sustained over the treatment time course.

Figure 2, B and C, illustrates Vt and physiological Vd/Vt as a function of group and time. ANOVA demonstrated significant differences in Vt and Vd/Vt as a function of group ($P < 0.0001$) and time ($P < 0.05$). Post hoc testing demonstrated that both Vt and Vd/Vt were lower ($P < 0.001$) in both TGI-treated groups (TGI and TGI + PLV) compared with those in CMV and PLV.

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### Table 2. Summarized acid-base and vital signs of saline-injured animals during CMV, TGI, PLV, and TGI + PLV

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
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<tbody>
<tr>
<td></td>
<td>BL</td>
<td>T0</td>
<td>1 h</td>
<td>2 h</td>
<td>3 h</td>
</tr>
<tr>
<td>CMV</td>
<td>7.38 ± 0.01</td>
<td>7.27 ± 0.04</td>
<td>7.18 ± 0.17</td>
<td>7.27 ± 0.07</td>
<td>7.18 ± 0.03</td>
</tr>
<tr>
<td>TGI</td>
<td>7.42 ± 0.02</td>
<td>7.29 ± 0.02</td>
<td>7.32 ± 0.03</td>
<td>7.32 ± 0.03</td>
<td>7.31 ± 0.02</td>
</tr>
<tr>
<td>PLV</td>
<td>7.40 ± 0.03</td>
<td>7.28 ± 0.05</td>
<td>7.21 ± 0.06</td>
<td>7.28 ± 0.04</td>
<td>7.26 ± 0.04</td>
</tr>
<tr>
<td>TGI + PLV</td>
<td>7.44 ± 0.02</td>
<td>7.28 ± 0.02</td>
<td>7.34 ± 0.01</td>
<td>7.28 ± 0.02</td>
<td>7.29 ± 0.01</td>
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- **BE, mM**
  - CMV: 3.9 ± 2.0
  - TGI: 2.3 ± 1.1
  - PLV: 1.1 ± 2.0
  - TGI + PLV: 0.9 ± 1.1

- **MAP, mmHg**
  - CMV: 79 ± 15
  - TGI: 62 ± 4
  - PLV: 68 ± 8
  - TGI + PLV: 68 ± 4

- **HR, beats/min**
  - CMV: 186 ± 16
  - TGI: 196 ± 7
  - PLV: 194 ± 14
  - TGI + PLV: 208 ± 18

Values are means ± SE; $n = 6$ group. BL, baseline before saline lavage; T0, saline lavage injury value; −BE, base excess; MAP, mean arterial blood pressure; HR, heart rate. *$P < 0.01$ vs. treated groups; †$P < 0.05$ and ‡$P < 0.01$ vs. CMV and PLV groups.
groups; neither VT nor Vd/Vt was significantly different between TGI and TGI + PLV groups or between CMV and PLV groups. Additionally, both VT and Vd/Vt in TGI-treated groups (TGI and TGI + PLV) decreased significantly ($P < 0.05$) as a function of time starting from 0.5 h after treatment.

Ventilator parameters are summarized in Table 3. Saline lavage injury resulted in significant increases in PIP ($P < 0.001$) from 14.19 ± 0.6 to 24.1 ± 0.7 cmH2O and MAWP ($P < 0.01$) from 7.0 ± 0.4 to 9.1 ± 0.4 cmH2O, but no significant differences in PEEP and MV compared with corresponding baseline values in all groups. There were no significant differences in PIP, PEEP, MAWP, or MV between groups at baseline and injury ($T_0$). During the treatment time, PIP ($P < 0.01$) and MAWP ($P < 0.05$) increased further from the injury value in the CMV group. PIP was significantly lower in all treated groups than in the CMV group (30.1 ± 1.1 cmH2O; $P < 0.01$) and was further lower in TGI-treated groups (TGI: 21.3 ± 0.9 cmH2O and TGI + PLV: 21.8 ± 0.4 cmH2O) ($P < 0.05$) than in PLV group (23.7 ± 1.0 cmH2O). MAWP and MV were also significantly lower ($P < 0.05$) in TGI (9.2 ± 0.2 cmH2O; 165 ± 15 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$) and TGI + PLV (9.9 ± 0.3 cmH2O; 159 ± 19 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$) groups than those in PLV (10.9 ± 1.1 cmH2O; 272 ± 18 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$) and CMV (11.4 ± 1.3 cmH2O; 264 ± 30 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$) groups; there was no significant difference between the TGI vs. TGI + PLV or PLV vs. CMV groups. PEEP was not significantly different as a function of group over the treatment time course.

Differences in airway pressure and VT between conditions of TGI on (0.5 l/min) and off from the bench experiments, which were performed to evaluate the direct effect of the TGI flow rate alone, are shown in Table 4. These data demonstrate that <1 cmH2O pressure and 0.5 ml VT are attributable to TGI flow rate alone.

Lung histological (Fig. 3) findings included prominent atelectasis, hyaline membranes, edema, intra-alveolar and interstitial patchy hemorrhage, and infiltration of neutrophils in the lungs of animals in the CMV group, especially in the dependent lung regions. These changes were reduced and the lungs were more homogenously expanded in all treated groups; both PLV and TGI + PLV groups seemed to be better than the TGI group. Morphometric analysis (Fig. 4A) demonstrated improvement in the EI in all lung regions in the treated groups compared with the CMV group ($P < 0.05$). Analysis of regional differences (Table 5) in EI demonstrated that the improvement in EI was greater in the dependent than nondependent regions in all treated groups and greater ($P < 0.05$) in the PLV and TGI + PLV groups than in TGI alone. Importantly, in contrast to the CMV and TGI (dependent vs. nondependent, $P = 0.057$) groups, there were no significant regional differences in EI in either PLV or TGI + PLV groups. These group and regional differences were further confirmed by indexes of alveolar morphometry (Table 5).

Total lung MPO and IL-8 data are shown in Fig. 4, B and C. ANOVA demonstrated that the concentrations of these mediators in all treated groups were significantly lower ($P < 0.05$) than those in the CMV group with no significant differences as a function of treatment groups. Further analyses demonstrated group $\times$ regional differences in these mediators (Table 6) where lung MPO in the dependent region of the CMV group was greater ($P < 0.05$) than that in the nondependent region, whereas no significant regional differences in lung MPO and IL-8 were noted within or between any of the treated groups.

**DISCUSSION**

The principal finding of this study on the effects of TGI and PLV in saline-induced ALI is that the combinational effects of TGI and PLV provided a more favorable pulmonary outcome profile than either modality alone.

ALI/ARDS lung is a nonuniform process with more aerated and relatively healthy units in the nondependent region (20–30%) and more collapsed and consolidated units in the dependent region, which renders the ALI/ARDS lung to be vulnerable to mechanical stretch during CMV and results in VILI (1, 5, 13, 36). The main mechanical factors proposed as responsible for VILI are 1) uneven distribution of mechanical properties of the lung with alveolar overstretch due to high distention pressure and/or relatively large VT; 2) increased shear stress in terminal units due to repeated alveolar collapse and

![Image of respiratory compliance, tidal volume, and physiological dead space-to-tidal volume ratio](image-url)
reopening when these units are ventilated at inappropriate PEEP; and 3) mechanical stimuli generated by different ventilatory modalities that trigger signal-transduction processes, leading to release of inflammatory mediators that can cause pulmonary and systemic injury. On the basis of these mechanisms of injury, present efforts to minimize the potential risk of VIILI emphasize lung protective ventilatory strategies aimed at limiting volume and/or pressure and keeping the lung open (1, 4, 23).

TGI has been proposed as an adjunct to CMV to reduce ventilatory pressures and $V_t$ while maintaining constant $P_aC_O_2$, or to minimize excessive CO$_2$ in the presence of hypercapnia. Up to now, TGI has been investigated with regard to the phase of the respiratory cycle (continuous flow or expiratory phasic flow) (17, 32), flow rates (0.5–25 l/min) (6, 11, 21), direction of flow (straight or reverse) (28), and catheter (separate catheters advanced into the ETT or built into the wall of ETT) and ventilator designs (11, 29) in various animal models and humans. In general, these studies reported that TGI was effective in reducing anatomic dead space and improving CO$_2$ removal. In a sheep model with ALI, Kirmse et al. (21) confirmed that continuous TGI, at 10 l/min achieved by advancing a catheter into the main lumen of ETT, decreased the carinal pressure and $V_t$, by 28 and 34%, respectively, compared with the postinjured values, and decreased mean physiological dead space (Vd/Vt) by ~45% while maintaining eucapnia. However, intraluminal TGI is problematic in the neonate due to the significantly smaller tracheal dimensions, relatively greater contribution of the ETT to anatomic dead space in the neonate, and heightened vulnerability of the relatively more compliant neonatal airways to higher driving pressures to overcome obstructive loads associated with an intraluminal catheter.

To address these limitations, a novel ETT was designed to introduce flow within the walls of the ETT (10, 32). In a clinical study of human neonates with hyaline membrane disease, Dassieu et al. (10) confirmed that low-flow continuous TGI delivered with this tube design decreased mean $P_aC_O_2$ from 45 to 35 Torr while the mean pressure gradient (PIP–PEEP) was either maintained or decreased from 20.8 to 14.7 cmH$_2$O with constant $P_aC_O_2$. In addition, they also confirmed through bench testing that the reduction in $P_aC_O_2$ induced by TGI correlated well with the ratio of instrument dead space. In agreement with these authors, our results showed that animals in the TGI group had significantly improved ventilation, as indicated by decreased $P_aC_O_2$, Vd/Vt, $V_t$, and PIP, and increased pH and VEi compared with the CMV group. The direct mechanism by which TGI affects $P_aC_O_2$ is reduction in anatomic dead space ventilation attributable to the prosthetic dead space of ETT and anatomic dead space of the large airways. In this regard, TGI acts to wash out CO$_2$-rich gas, which accumulates in the ETT and proximal airways at the end of

### Table 3. Summarized ventilation parameters of saline-injured animals during CMV, TGI, PLV, and TGI + PLV

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIP, cmH$_2$O</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CMV</td>
<td>14.1±0.9</td>
<td>24.7±0.6</td>
<td>20.7±0.9*</td>
<td>30.7±0.7*</td>
<td>30.1±1.4*</td>
</tr>
<tr>
<td>TGI</td>
<td>14.4±0.5</td>
<td>24.0±0.5</td>
<td>21.4±0.9</td>
<td>21.0±0.9</td>
<td>21.1±0.9</td>
</tr>
<tr>
<td>PLV</td>
<td>14.1±0.3</td>
<td>23.5±1.3</td>
<td>22.9±1.3†</td>
<td>22.7±1.1†</td>
<td>23.4±1.3†</td>
</tr>
<tr>
<td>TGI + PLV</td>
<td>13.7±0.5</td>
<td>24.0±0.3</td>
<td>22.1±0.4</td>
<td>21.8±0.3</td>
<td>21.5±0.3</td>
</tr>
</tbody>
</table>

| **PEEP, cmH$_2$O** |    |     |     |     |     |
| CMV    | 5.5±0.2 | 5.7±0.3 | 5.7±0.6 | 5.8±0.6 | 5.8±0.7 | 5.8±0.7 |
| TGI    | 5.2±0.2 | 6.0±0.3 | 6.6±0.4 | 6.0±0.2 | 6.0±0.3 | 6.4±0.2 |
| PLV    | 5.2±0.4 | 5.2±0.4 | 6.0±0.7 | 6.0±0.7 | 6.7±0.8 | 6.7±0.8 |
| TGI + PLV | 5.0±0.2 | 6.0±0.3 | 6.0±0.3 | 6.8±0.3 | 6.8±0.4 | 6.7±0.3 |

| **MAWP, cmH$_2$O** |    |     |     |     |     |
| CMV    | 7.3±0.4 | 9.8±0.6 | 11.3±0.6 | 11.9±1.5 | 11.1±1.4 | 11.4±1.4 |
| TGI    | 6.8±0.2 | 9.5±0.1 | 9.3±0.3 | 9.0±0.2‡ | 9.1±0.2† | 9.5±0.2‡ |
| PLV    | 7.1±0.6 | 8.8±0.5 | 9.6±1.0 | 10.4±0.2 | 11.2±1.4 | 12.4±1.8 |
| TGI + PLV | 6.6±0.2 | 9.0±0.3 | 9.8±0.2 | 9.8±0.3‡ | 9.4±0.3‡ | 10.6±0.2‡ |

Values are means ± SE; $n = 6$/group. PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; MAWP, mean airway pressure; $V_t$, minute ventilation. *P < 0.01 vs. all treated group. †P < 0.05 vs. TGI and TGI + PLV groups. ‡P < 0.05 vs. PLV and CMV groups.

### Table 4. Pressure and volume differences associated with TGI flow from bench experiments

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
<th>Proximal</th>
<th>Distal</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIP, cmH$_2$O</strong></td>
<td></td>
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</tr>
<tr>
<td>TGI</td>
<td>0.75±0.02</td>
<td>0.94±0.02</td>
<td>0.40±0.03</td>
<td>0.86±0.01</td>
<td>-0.22±0.02</td>
<td>-0.14±0.04</td>
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<tr>
<td>TGI + PLV</td>
<td>0.44±0.01</td>
<td>0.69±0.01</td>
<td>0.44±0.02</td>
<td>0.84±0.02</td>
<td>-0.31±0.03</td>
<td>-0.63±0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
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<tbody>
<tr>
<td><strong>PEEP, cmH$_2$O</strong></td>
<td></td>
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<tr>
<td>TGI</td>
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<tr>
<td>TGI + PLV</td>
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</table>

Values are means ± SE. Proximal, between the ventilator and endotracheal tube; Distal, between endotracheal tube and lung model.

*J Appl Physiol • VOL 96 • APRIL 2004 • www.jap.org*
expiration. In addition, turbulence around the TGI catheter tip, due to the high-velocity gas from the catheter, may enhance gas mixing in the airway and contribute to a reduction in alveolar dead space as well (29).

Using the results obtained in this study, we also explored the contribution of PLV to the Vd/Vt and the effect of TGI-augmented PLV compared with TGI alone on Vd/Vt in an injured lung. In contrast to tidal liquid ventilation in which CO2 in the PFC is eliminated through the ventilator circuit, physiological ventilation during PLV is dependent on alveolar ventilation (39, 41). Although PFC fluids of high CO2 solubility may provide greater carrying capacity for CO2, this same quality presents diffusional limitations and risk of pneumothorax with PLV at lung volumes sufficient to offer lung protection (8, 39). As such, PaCO2 could increase during PLV with instillation of larger volumes of PFC if a proportional increase in ventilation is not achieved. Thus, to eliminate CO2, additional gas ventilation or a reduction in overall dead space is required to deplete CO2 from the PFC reservoir in the lung. In this regard, similar to CMV, PLV alone did not reduce the near fourfold elevation in Vd/Vt due to injury. In contrast, although still elevated by 4 h, both TGI alone or in combination with PLV remediated the injury-induced elevation in Vd/Vt to within twofold of preinjury values. On further analysis in absolute terms, we found that the reduction in Vd/Vt was near equal to the tube volume during TGI (2.77 ml) and, interestingly, greater than tube volume (3.64 ml) during TGI + PLV. These findings further substantiate the effectiveness of TGI to eliminate prosthetic dead space and suggest that, when used in conjunction with PLV, TGI may further reduce the contribution of anatomic and alveolar dead space.

Although the TGI effects on CO2 have been established, the effects on oxygenation have been less clear (11, 17, 21, 32). Because oxygenation is related to the alveolar recruitment and ventilation-perfusion match, when TGI is used with a volume- and/or pressure-limited ventilation strategy, there is some concern that decreased Vt and/or pressures may result in alveolar instability and progressive decruitment (9, 23), and thus deterioration in oxygenation. It has been suggested that improvement in oxygenation during TGI could be due to an increase in functional residual capacity if TGI flow is excessively high (≥10 l/min) due to an associated increased distal pressure in the ETT by high expiratory flow through the ETT (29). However, both of these mechanisms are suggested to be negligible when a low rate of 0.5 l/min is used (18). To exclude the potential contribution of TGI to ventilatory pressures and resultant volumes, we performed bench testing with a lung model adjusted to the in vivo lung mechanics of both TGI and TGI + PLV groups. The results demonstrated minimal alterations in ventilatory pressure and volumes attributable to the low TGI flow alone. These data confirm previous suggestions that 0.5 l/min TGI flow will not create excessive, unexpected pressures (10, 11). As such, although pressures distal to the TGI flow were not measured in vivo, these data indicate that it is unlikely that these small differences contributed to the improvement in alveolar recruitment and thus oxygenation observed in the poorly compliant lung of all TGI-treated animals in this study.

As an alternative explanation for the improvement in oxygenation noted, the results from the histological profile support that TGI facilitated distribution of ventilation in our animal model. In this regard, TGI resulted in improved expansion in all lung regions compared with CMV, with the greatest improvement in the relatively atelectatic-dependent lung. As would be expected by the presence of an incompressible fluid, expansion was further enhanced during PLV to reflect a pattern of more homogenous lung recruitment, and this profile was improved further with TGI-augmented PLV. The pattern of progressive improvement in oxygenation from CMV, to TGI, to PLV, to TGI + PLV is consistent with this histological profile.

Indexes of lung inflammation demonstrated that both MPO and IL-8 in the lung were significantly lower in all treated groups compared with CMV. Because high PIP and Vt and inappropriate PEEP are thought to contribute to the structural as well as inflammatory profile of VILI, we monitored pres-

Fig. 3. Micrographs of hematoxylin- and eosin-stained lung sections from nondependent (NDep) and dependent (Dep) regions in CMV (A and B), TGI (C and D), PLV (E and F), and in combination (TGI + PLV; G and H) groups. Magnification: ×100 (inset: ×400). (See online supplemental data figure 1, A–H, at http://jap.physiology.org/cgi/content/full/01121.2003/DC1 for full-page color images of each panel.)
sures and VT for all animals carefully. Despite this effort to maintain a protective strategy, significant lung injury resulted with CMV. The inflammation profile during CMV is consistent with the atelectatic histological profile and disruptive forces associated with the relatively higher PIP and VT, which might cause alveolar overstretching in this group.

Although lung histology and inflammation in all treated groups were significantly improved compared with the CMV group, we speculate that the mechanisms for these improvements differ. In this regard, during TGI alone, reduced lung inflammation is likely associated with reduced disruptive forces of the lower PIP and VT and less atelectasis, all of which would reduce the magnitude of cyclic recruitment and collapse (i.e., lung resting concept) and, therefore, minimize the risk of shear stress acting on already injured lung tissue. It is noteworthy that, although expansion was greater than CMV alone, regional differences in expansion still persisted with TGI (Table 5). Thus, during TGI alone, it is likely that much of the dependent lung remained poorly expanded (i.e., rested) and thus was perhaps not exposed to ventilator injury. During PLV alone, although VT was comparable to CMV and greater than TGI alone, the lung was relatively more recruited and homogeneously expanded at lower inspiratory pressures. These factors could minimize the risk of VILI. These results are in accordance with previous studies with similar animal models (2, 20, 33). The mechanisms that support pulmonary gas exchange are associated with the physicochemical properties of the PFC liquids and biophysical effects of the liquid on lung mechanics (40). In addition, it has been suggested that PFC modifies alveolar macrophage function, clears airway debris and inflammatory mediators, and reduces pathological fluid filtration in injured lung (26, 37, 40). PFC liquids are synthetic, fluorinated hydrocarbons that are biologically inert, not metabolized, non-transformable, and immiscible in both aqueous and lipid media. PFC liquids have high respiratory gases (O2, CO2) solubility and relatively low surface tension, which supports spreading through the lung. On PFC instillation, a liquid-liquid interface replaces the gas-liquid interface at the lung surface; surface tension from gas is eliminated, and interfacial tension is reduced. In this way, lung volume is recruited; compliance is increased, requiring less ventilator pressures to support gas exchange. Furthermore, the high density of PFC liquid may

### Table 5. Quantitative histomorphometry

<table>
<thead>
<tr>
<th>Group</th>
<th>ND</th>
<th>DT</th>
<th>Area</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>0.30±0.03</td>
<td>0.26±0.06</td>
<td>17.8±2.5</td>
</tr>
<tr>
<td>TGI</td>
<td>0.91±0.10*</td>
<td>0.68±0.09*</td>
<td>22.8±1.1*</td>
</tr>
<tr>
<td>PLV</td>
<td>1.10±0.08*</td>
<td>1.00±0.03*</td>
<td>21.0±0.6*</td>
</tr>
<tr>
<td>TGI + PLV</td>
<td>1.10±0.10*</td>
<td>1.00±0.14*</td>
<td>22.9±0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 in the CMV group and n = 6 in all treated groups. ND, nondependent; DT, dependent; Area, μm². *P < 0.05 and †P < 0.01 vs. CMV group. ‡P < 0.05 vs. TGI group. §P < 0.05 vs. ND.
favor distribution to the dependent areas (25, 27), which would preferentially enhance recruitment and protection of those dependent alveolar units that are at greatest risk of detrimental shear stress. As such, ventilation with PFC liquids may offer both mechanoprotection and cytoprotection. Our findings are consistent with these mechanisms of protection. After PLV alone, lung MPO and IL-8 were significantly decreased, and overall lung expansion was homogenous. Therefore, the combined effect of PFC liquids to provide low-pressure mechanical support to the atelectatic lung and to offer cytoprotection may have offset lung inflammation associated with the relatively higher Vt. When PLV was augmented with TGI, Vt could be reduced to that observed with TGI alone, thus further removing potentially injurious mechanical insult.

In summary, this study demonstrates that the combination of TGI and PLV is more effective than either modality alone in this ALI animal model. In this regard, TGI helps PLV to offset the atelectatic lung and to offer cytoprotection. Our findings are consistent with these mechanisms of protection. After PLV alone, lung MPO and IL-8 were significantly decreased, and overall lung expansion was homogenous. Therefore, the combined effect of PFC liquids to provide low-pressure mechanical support to the atelectatic lung and to offer cytoprotection may have offset lung inflammation associated with the relatively higher Vt. When PLV was augmented with TGI, Vt could be reduced to that observed with TGI alone, thus further removing potentially injurious mechanical insult.

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GRANTS

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