Open-lung Protective Ventilation with Pressure Control Ventilation, High-frequency Oscillation, and Intratracheal Pulmonary Ventilation Results in Similar Gas Exchange, Hemodynamics, and Lung Mechanics

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Background: Pressure control ventilation (PCV), high-frequency oscillation (HFO), and intratracheal pulmonary ventilation (ITPV) may all be used to provide lung protective ventilation in acute respiratory distress syndrome, but the specific approach that is optimal remains controversial.

Methods: Saline lavage was used to produce acute respiratory distress syndrome in 21 sheep randomly assigned to receive PCV, HFO, or ITPV as follows: positive end-expiratory pressure (PCV and ITPV) and mean airway pressure (HFO) were set in a pressure-controlled manner after lung recruitment that achieved a ratio of PaO2/FIO2 > 400 mmHg. Respiratory rates were 30 breaths/min, 120 breaths/min, and 8 Hz, respectively, for PCV, ITPV, and HFO. Eucapnia was targeted with peak carinal pressure of no more than 35 cm H2O. Animals were then ventilated for 4 h.

Results: There were no differences among groups in gas exchange, lung mechanics, or hemodynamics. Tidal volume (PCV, 8.9 ± 2.1 ml/kg; ITPV, 2.7 ± 0.8 ml/kg; HFO, approximately 2.0 ml/kg) and peak carinal pressure (PCV, 30.6 ± 2.6 cm H2O; ITPV, 22.5 ± 4.8 cm H2O; HFO, approximately 24.3 cm H2O) were higher in PCV. Pilot histologic data showed greater interstitial hemorrhage and alveolar septal expansion in PCV than in ITPV, 22.3 cm H2O; HFO, approximately 24.3 cm H2O) were higher in PCV. Pilot histologic data showed greater interstitial hemorrhage and alveolar septal expansion in PCV than in ITPV and HFO.

Conclusion: These data indicate that HFO, ITPV, and PCV when applied with an open-lung protective ventilatory strategy results in the same gas exchange, lung mechanics, and hemodynamic response, but pilot data indicate that lung injury may be greater with PCV.

Despite recent clinical trials showing improved outcome in acute respiratory distress syndrome (ARDS) and acute lung injury, controversy still exists regarding the optimal approach to ventilate these patients. It is clear from animal studies, however, that lung injury occurs from large distending volume (volutrauma) and repetitive opening and closing of unstable lung units (atelectrauma). Studies by Amato et al.1 and the National Institutes of Health ARDSnet2 have established a low tidal volume (VT) strategy as the optimal approach for the management of ARDS and acute lung injury. However, this strategy does not address the issues of unstable lung units. Amato et al.1 and others9–14 have also proposed that in addition to a low VT, the lung should be recruited and end-expiratory pressure maintained at a level that avoids derecruitment.

An open lung can be achieved by a number of different approaches to ventilatory support. Conventional pressure control ventilation (PCV) can target an appropriate VT and maintain sufficient positive end-expiratory pressure (PEEP) to avoid derecruitment after a recruitment maneuver.1,14 High-frequency oscillation (HFO) has been used successfully for years in the treatment of neonates,15,16 and two recent case series17,18 and a randomized controlled trial19 suggest that new technology has allowed HFO to be applied successfully to adults. HFO is customarily applied after lung recruitment with a high mean airway pressure (Paw).20 Intratracheal pulmonary ventilation (ITPV) provides ventilation by maintaining a continuous flow of gas at the carina with phasic opening and closing of an exhalation valve, thus ensuring a small VT delivery and tracheal gas washout.21,22 As with PCV, during ITPV, sufficient PEEP can be applied after a recruitment maneuver. All three of these techniques use different mechanisms to establish a small VT. However, each can be applied with a similar open lung approach after lung recruitment by using a decreasing optimal PEEP or Paw setting approach as recently proposed by Hickling.23 However, of these three approaches, as described by Froese,20 HFO should provide the smallest VT delivery and as a result the lowest airway pressures.

In this study, we hypothesized that PCV, HFO, and ITPV would result in similar levels of gas exchange, hemodynamics, and lung mechanics, because all were applied with the same lung protective strategy. We tested this hypothesis in a large sheep, saline lavage, lung injury model.

Materials and Methods

This protocol was approved by the Research Animal Care Committee of the Massachusetts General Hospital.
NO DIFFERENCES AMONG PCV, HFO, AND ITPV

Fig. 1. Intratracheal pulmonary ventilation system. A continuous humidified bias gas flow enters the lung from one channel of a double-lumen prototype tracheal gas insufflation tube and exits through the secondary channel into the ventilator circuit of a Servo 900C ventilator. No gas flow was provided by the ventilator, but the exhalation valve opened and closed at a rate of 120 times per min with an inspiratory-to-expiratory ratio of 1:1.

Preparation
Twenty-one female Dorset sheep that weighed 28 ± 4.1 kg and fasted for 24 hr were studied. Anesthesia was induced with halothane by mask, and then orotracheal intubation was performed and mechanical ventilation started (Model 7200; Puritan Bennett Corporation, Carlsbad, CA). An 8-French catheter was inserted into the right external jugular vein, through which 25 mg/kg pentobarbital and 2 mg/kg ketamine were administered as initial loading doses followed by continuous infusion of pentobarbital (10 mg · kg⁻¹ · h⁻¹) and ketamine (0.5 mg · kg⁻¹ · h⁻¹). Paralysis was maintained with a loading dose of 0.1 mg/kg pancuronium followed by 0.1 mg · kg⁻¹ · h⁻¹.

Each sheep then underwent a tracheostomy. A cuffed airway, ID 8 mm (Mallinkrodt Medical, Inc., St. Louis, MO) for PCV and HFO, and a prototype double-lumen tracheal gas insufflation tube24 for ITPV were placed (fig. 1). A small-bore catheter (ID 0.7 mm) was placed alongside the airway extending 1 cm beyond its tip for carinal pressure measurements. The left carotid artery was cannulated with a 14-gauge arterial catheter, through which a continuous blood gas probe was introduced (Trend Care; Diametric Medical, Inc., Roseville, MN) and a percutaneous gastrostomy was performed. Animal temperatures were maintained at 38–39°C by a heating blanket. A lactated Ringer’s infusion (8 mg · kg⁻¹ · h⁻¹) was maintained throughout the protocol.

Experimental Protocol
After the surgical procedures, animals were stabilized for 90 min in the supine position and mechanically ventilated in the volume control mode, with VT, 12 ml/kg; inspiratory-to-expiratory ratio (I:E) ratio, 1:2; FIO₂, 1.0; and PEEP, 5 cm H₂O, baseline ventilation. The respiratory rate was adjusted to achieve eucapnia (PaCO₂, 35–45 mmHg). After stabilization, baseline measurements were obtained.

Severe lung injury (PaO₂, 68.8 ± 10.5 mmHg) was then produced by bilateral lung lavage with 30 ml/kg isotonic (39°C) saline, repeated every 15 min until the PaO₂ decreased to 60–100 mmHg and was stable for more than 60 min at an FIO₂ of 1.0 and a PEEP of 5 cm H₂O. Then, injury measurements, including a pressure-volume (P-V) curve, were obtained.

Animals were then randomized to three treatment groups: PCV, HFO, and ITPV. Lung recruitment maneuvers (RMs) initially using a continuous positive airway pressure of 50 cm H₂O applied for 1 min were performed every 15 min until the PaO₂ was greater than 400 mmHg and stable for 15 min. In between each RM, animals were ventilated per treatment arm protocol with PEEP or PAW (HFO) set equal to the point of maximal compliance change on the deflation limb of the P-V curve. After achieving a PaO₂ greater than 400 mmHg, the FIO₂ was decreased until the PaO₂ was 60–100 mmHg (target level). Then the PEEP or PAW was decreased from the point of maximal curvature on the deflation limb of the P-V curve 2 cm H₂O every 15–20 min until the target PaO₂ decreased by more than 10% from the above target level. The PEEP or PAW preceding that causing the PaO₂ decrease was considered optimal. RMs were again performed after which the PEEP or PAW and FIO₂ were set at the optimal level determined.

Animals were ventilated in each of the three groups as per the following protocol (fig. 2):

- PCV (PB 7200 ventilator) rate was set at 30 breaths/min, I:E at 1:1, and pressure level to achieve a target PaCO₂ of 35–45 mmHg, but limited to a peak carinal pressure (PIPca) of 35 cm H₂O.
- HFO (3100B; Sensormedics Critical Care Corporation, Loma Linda, CA) rate was set at 8 Hz, I:E at 1:1, and pressure amplitude to achieve a target PaCO₂ of 35–45 mmHg. The sum of the PAW and 15% of the inspiratory pressure amplitude was not allowed to exceed 35 cm H₂O. With an 8-mm internal diameter airway, 15% of the inspiratory pressure amplitude is estimated to contribute to the peak alveolar pressure.17
- ITPV (Servo 900C Seimens Elema, Solna, Sweden) rate was set at 120 breaths/min, I:E at 1:1, and bias flow adjusted to maintain PaCO₂ 35–45 mmHg. PIPca was limited to 35 cm H₂O. The 900C ventilator was set in the pressure control mode at zero pressure. As a result, the exhalation valve opened and closed at a rate of 120 times per min; however, only the bias flow delivered by a separate humidified continuous gas flow was available for delivery to the animal.21,22 The bias flow entered the animal continuously through one channel of the prototype tracheal gas insufflation tube and exited the secondary channel during exhalation (fig. 1). During inspiration, the bias flow was directed toward the carina, whereas during exhalation, the flow was directed toward the ventilator yoke creating a

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negative pressure in the carinal area and establishing tracheal gas insufflation.\textsuperscript{21,22}

After all ventilator settings were established, post-RM measurements were obtained. Animals in all groups were then ventilated for 4 h and the FIO\textsubscript{2} was adjusted to maintain target Pa O\textsubscript{2} (60–100 mmHg).

At the end of the protocol, four sheep from each group were immediately killed for histologic, cytologic, and inflammatory mediator analysis. The remaining three sheep in each group were used to establish a final baseline. After completion of the protocol, these animals were returned to the initial volume control mode setting with a PEEP of 5 cm H\textsubscript{2}O (baseline ventilation) for 30 min, after which final baseline measurements were obtained.

**Measurements and Calculations**

Measurements were recorded at the following time points: baseline, injury, after RM, 30, 60, 120, 180, and 240 min of the protocol, and final baseline. Airway pressures were recorded for 30 s using pressure transducers calibrated at 20 cm H\textsubscript{2}O (Validyne, Northridge, CA). Driving pressure (P\textsubscript{D(R)}) pressure to deliver V\textsubscript{T} in PCV and ITPV, was determined as the difference between PIP\textsubscript{Ca} and total PEEP. In HFO, P\textsubscript{DR} was estimated as 15% of the inspiratory pressure amplitude.\textsuperscript{17} Expiratory V\textsubscript{T} was obtained from the ventilator during PCV and calculated from bias flow, ventilatory rate, and I:E ratio during ITPV. V\textsubscript{T} could not be measured during HFO but was estimated based on data using the same model.\textsuperscript{25} Mean arterial blood pressure, mean pulmonary artery pressure, central venous pressure, and pulmonary capillary wedge pressure were measured using calibrated pressure transducers (Argon; Maxim Medical, Athens, TX) zeroed at the midaxillary line. Cardiac output was measured by thermodilution technique three times and averaged (Baxter Corp., Deerfield, IL), with stroke volume calculated (stroke volume = cardiac output/heart rate). Body temperature was measured from the continuous blood gas monitor. Airway pressures and hemodynamic signals were amplified and continuously displayed on a six-channel computerized graphics program (Windaq; Dataq Instruments, Inc., Akron, OH) and recorded for later analysis.

Continuous arterial blood gas data were monitored by the Paratrend analyzer. Arterial and mixed venous blood samples were also drawn for blood gas analysis (Bayer Diagnostics Corp., Medfield, MA). Oxygen saturation and hemoglobin content were assessed by cooximeter (Radiometer, Copenhagen, Denmark). Shunt ratio (Q\textsubscript{S}/Q\textsubscript{T}) and oxygenation index (OI) were calculated using standard equations:

\[
\text{Q/S} = \frac{\text{capillary O}_2 \text{ content} - \text{arterial O}_2 \text{ content}}{\text{mixed venous O}_2 \text{ content}}
\]

\[
\text{OI} = \frac{(\text{P}_{aO2} \times 100 \times \text{F}_{O2})/\text{P}_{aO2})}}
\]

**Pressure-Volume Curve**

Quasistatic P-V curves using a 2-l super syringe were obtained at injury. Before the P-V curve, 1 min of PCV (peak inspiratory pressure, 50 cm H\textsubscript{2}O; PEEP, 5 cm H\textsubscript{2}O;
Table 1. Characteristics of Sheep in All Groups (n = 7)

<table>
<thead>
<tr>
<th>Item</th>
<th>PCV</th>
<th>HFO</th>
<th>ITPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>28.6 ± 4.1</td>
<td>26.9 ± 2.0</td>
<td>28.9 ± 3.3</td>
</tr>
<tr>
<td>BAL</td>
<td>2.9 ± 1.1</td>
<td>2.0 ± 0.8</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>RM initial</td>
<td>1.7 ± 0.8</td>
<td>2.0 ± 1.2</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>RM after</td>
<td>1.4 ± 0.8</td>
<td>1.3 ± 0.8</td>
<td>1.7 ± 1.5</td>
</tr>
<tr>
<td>P_{max}</td>
<td>21.2 ± 2.1</td>
<td>23.2 ± 2.2</td>
<td>22.3 ± 3.6</td>
</tr>
<tr>
<td>PMC</td>
<td>24.9 ± 2.0</td>
<td>26.9 ± 1.5</td>
<td>27.3 ± 1.0*</td>
</tr>
</tbody>
</table>

All figures are mean ± SD.

P < 0.05 vs. PCV.

BAL = number of saline lavages to cause injury; HFO = high-frequency ventilation; ITPV = intratracheal pulmonary ventilation; PCV = pressure control ventilation; P_{max} = lower inflection point on the inspiratory limb of P-V curve; PMC = point of maximal compliance change on deflation limb P-V curve; RM after = number of lung recruitment maneuvers needed to recruit the lung after identification of optimal positive end-expiratory pressure/mean airway pressure; RM initial = number of lung recruitment maneuvers needed to initially recruit the lung.

Respiratory rate, 6 breaths/min; I:E, 1:1) was applied to establish a volume history. Animals were then disconnected from the ventilator, and 6 s later, the lungs were inflated in steps of 50 ml for the first 200 ml and then steps of 100 ml with simultaneous measurement of airway pressure to a maximal pressure of 50 cm H₂O. Deflation of the lung in a similar manner established the deflation limb of the P-V curve. The lower inflection point was identified by the crossing of tangents applied manually to the varying slopes of the inspiratory P-V curve. The point of maximal compliance change on the deflation limb was identified by the crossing of tangents applied manually to the varying slopes of the deflation limb of the P-V curve.

Preparation for Histologic and Biologic Analysis

In four sheep in each group, after removal of the lungs with pleural surface intact, the lungs were separated into anterior order. From each slice, two blocks of 1 cm³, one from the apex and one from the base, were chosen at random, excised, and embedded in paraffin. From each block, three slices, 5 μm thick, were mounted on slides and stained with hematoxylin and eosin.

For sampling of cells infiltrated into the alveolar space, one catheter was tied to the left upper bronchus and another catheter was tied to the left lower bronchus. Lung lavage was performed repetitively five times. Total leukocytes and neutrophils in sampled fluid from each bronchus were counted. Lavaged fluid from the apex and base were separately centrifuged at 3,000 rpm for 5 min. Isolation of mRNA was performed using special kits (QIAGEN, Inc., Valencia, CA). Isolated mRNA was frozen at −80°C.

Histologic Analysis

Light microscopic examination was performed by a pathologist (S.O.V.), masked to the experimental protocol and region of sampling. All tissues were examined microscopically at ×10 magnification. Areas of pathology were further evaluated at ×20–40. Pathologic features were recorded in the following manner:

1. Granulocytes: Granulocytic infiltration in alveolar spaces was graded on a scale of 0 (none) to 3+ (severe), with respect to the number of granulocytes and their distribution in the lung.

Table 2. Ventilatory Pattern and Gas Exchange in All Groups

<table>
<thead>
<tr>
<th>Measure/Group</th>
<th>BL</th>
<th>Injury</th>
<th>Post-RM 30 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>Final BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OI PCV</td>
<td>1.7 ± 0.2*</td>
<td>17.7 ± 7.2</td>
<td>5.3 ± 1.0*</td>
<td>9.4 ± 3.1</td>
<td>9.9 ± 3.0</td>
<td>10.7 ± 2.9</td>
<td>10.7 ± 3.1</td>
</tr>
<tr>
<td>OI HFO</td>
<td>1.6 ± 0.2*</td>
<td>18.7 ± 5.1</td>
<td>3.8 ± 1.4*</td>
<td>7.0 ± 0.8</td>
<td>7.0 ± 0.8</td>
<td>7.3 ± 1.2</td>
<td>7.9 ± 2.4</td>
</tr>
<tr>
<td>OI ITPV</td>
<td>1.5 ± 0.2*</td>
<td>18.7 ± 10.3</td>
<td>4.9 ± 0.9*</td>
<td>9.4 ± 2.0</td>
<td>9.5 ± 2.2</td>
<td>9.9 ± 2.0</td>
<td>11.2 ± 5.1</td>
</tr>
<tr>
<td>OI/Qt (%) PCV</td>
<td>0.1 ± 0.02*</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1*</td>
<td>0.1 ± 0.04</td>
<td>0.1 ± 0.04</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>OI/Qt (%) HFO</td>
<td>0.1 ± 0.04*</td>
<td>0.4 ± 0.1</td>
<td>0.1 ± 0.1*</td>
<td>0.1 ± 0.03</td>
<td>0.1 ± 0.03</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>OI/Qt (%) ITPV</td>
<td>0.1 ± 0.1*</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1*</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>P_{aw} (cm H₂O)</td>
<td>11.3 ± 1.7</td>
<td>22.8 ± 4.8</td>
<td>10.7 ± 2.2*</td>
<td>10.7 ± 2.4</td>
<td>11.3 ± 2.3</td>
<td>12.7 ± 2.5</td>
<td>12.5 ± 2.8</td>
</tr>
<tr>
<td>HFO</td>
<td>14.0 ± 3.1*</td>
<td>26.8 ± 6.7</td>
<td>3.8 ± 1.3*</td>
<td>3.6 ± 1.0†</td>
<td>3.5 ± 1.0†</td>
<td>3.74 ± 1.3† †</td>
<td>3.8 ± 1.3† †</td>
</tr>
<tr>
<td>ITPV</td>
<td>11.8 ± 2.3*</td>
<td>26.1 ± 5.6</td>
<td>5.3 ± 2.7*</td>
<td>5.8 ± 2.8†</td>
<td>6.2 ± 3.1†</td>
<td>6.6 ± 3.3†</td>
<td>7.2 ± 3.6†</td>
</tr>
</tbody>
</table>

All figures are mean ± SD.

P < 0.05 vs. injury; † P < 0.05 vs. PCV; ‡ P < 0.05 vs. ITPV.

BL = baseline; final BL = final baseline; HFO = high-frequency ventilation; ITPV = intratracheal pulmonary ventilation; OI = oxygenation index; PCV = pressure control ventilation; P_{aw} = driving pressure; Post-RM = immediately after recruitment; OI/Qt = intrapulmonary shunt fraction; 30 min, 60 min, 120 min, 180 min, 240 min = time after lung recruitment.

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2. Alveolar hemorrhage: Alveolar hemorrhage was defined as the presence of intraalveolar erythrocytes and was graded from 0 (none) to 3+ (severe), based on the number of cells and the extent of their distribution in the lung.

3. Interstitial hemorrhage: Interstitial hemorrhage was defined as the presence of erythrocytes in interstitial tissue in lobular septa or surrounding airways, arteries, and veins. It was graded from 0 (none) to 3+ (severe).

4. Alveolar septal expansion: Alveolar septal expansion was defined as septal walls thickened as a result of edema or cellular infiltrate and was graded from 0 (none) to 3+ (severe), based on the degree of thickening and the percentage of lung tissue involved.

5. Proteinaceous exudate: Proteinaceous exudate was defined as the presence of intraalveolar noncellular flocculent eosinophilic material. It was graded from 0 (none) to 3+ (severe), based on severity and extent of involvement.

6. Hyaline membranes: Hyaline membranes were defined as rims of intraalveolar dense homogeneous eosinophilic material. Their presence or absence was recorded.

7. Other disease: The presence or absence of preexist-
ing chronic lung disease was noted and animals with such disease were excluded from analysis.

During analysis of results, we considered high-grade injuries to be those rated 2 and 3 and low-grade injuries to be those rated 0 and 1.

**Biologic Analysis**

Quantitative examination was performed by one of the authors (M.S.) masked to the experimental protocol and region of sampling. Isolated mRNA aliquots were used to determine mRNA level of interleukin-1β (IL-1β) and interleukin-8 (IL-8). Reverse transcription polymerase chain reaction enzyme-linked immunosorbent assay was used, as previously described, with minor modifications.26,27 First-strand complementary DNA was synthesized and polymerase chain reaction amplification of complementary DNA was performed using polymerase chain reaction enzyme-linked immunosorbent assay (Boehringer Mannheim Corp., Indianapolis, IN) with target-specific sense primer and a target-specific biotinylated antisense primer in the presence of digoxigenin-labeled 2-deoxyuridine 5’-triphosphate. The reaction protocol used a programmed thermal cycler (Perkin Elmer Corp., Emervile, CA) with a sequence of 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. After being purified by polymerase chain reaction purification (QIAGEN, Inc.), the polymerase chain reaction products were immobilized onto streptavidin-coated 96-well enzyme-linked immunosorbent assay plates and detected by antidigoxigenin antibody conjugated with peroxidase and the substrate 2,2’-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid] (Boehringer Mannheim Corp.). The colorimetric signal at 450 nm was recorded by microplate reader. The absorbance values of mRNA for IL-1β and IL-8 were normalized by those for glyceraldehyde-3-phosphate dehydrogenase, a housekeeping gene product, as a control.

**Statistical Analysis**

Data were expressed as mean ± SD. Injury characteristics for the three groups were compared by one-way analysis of variance. Ventilatory and circulatory parameters over time were compared using repeated-measures two-way analysis of variance. If statistical significance was reached, a Tukey post hoc analysis (honest significant difference) was performed. Analysis of biologic and histologic data for six combinations of three groups and two regions were performed by noncontinuous analysis using chi-square test. If statistical significance was reached, a post hoc analysis (Fisher exact test) was used to obtain two-tailed P values between each set of two groups. A statistics software package (STATISTICA 5.1; StatSoft, Inc., Tulsa, OK) was used, with P < 0.05 as statistically significant.

**Results**

**Lung Injury**

A similar number of lavages were required in all groups (table 1), and there were no differences in Pflex across groups at injury, but the point of maximal compliance change was higher in ITPV than in PCV or HFO (P < 0.05) (table 1). PaO2/FIO2 at injury was significantly lower (P < 0.05) in all groups than at baseline, whereas Qs/QT, OI, PIPca, and PDR increased in all groups at injury (P < 0.05) (table 2 and figs. 3 and 4). All hemodynamic variables were similar in all groups before and after injury (table 3).

**Setting of PEEP and Paw**

The optimal PEEP and Paw maintaining recruitment differed among the three groups (P < 0.05) (fig. 4). Paw was higher during HFO, and PEEP was lowest during ITPV (P < 0.05) (fig. 3). Similar numbers of RMs were required in all three groups before the determination of PEEP and Paw setting and after its determination (table 1).

**Post-RMs.** PaO2/FIO2 was significantly higher than at injury (P < 0.05) in all groups, (fig. 3), whereas Qs/QT, OI, and PDR decreased in all groups compared with the injury level (P < 0.05) (table 2). PIPca significantly increased in PCV and decreased in ITPV compared with injury levels and were lower after RM (P < 0.05) in ITPV and HFO than in PCV (fig. 4).

Hemodynamics were similar in all groups at injury and after RM, except for an increase (P < 0.05) in pulmonary capillary wedge pressure in the ITPV group after RM compared with injury and a decrease in pulmonary capillary wedge pressure (P < 0.05) in the PCV group after RM compared with baseline (P < 0.05) (table 3).

**During 4-h Ventilation**

**Gas Exchange.** In all groups, a stable and equivalent PaO2/FIO2 was reached within 30 min after RM, and by 30 min, the PaCO2 was within target range and there were no differences among groups (fig. 3).

**Lung Mechanics.** Compliance increased significantly (P < 0.05) after RM in PCV and ITPV (unable to measure in HFO). Higher PIPca and PDR were measured during PCV compared with ITPV and HFO throughout the 4-h protocol (P < 0.05). In HFO, pressure amplitude was 48.2 ± 13 cm H2O at the start of ventilation and 52.8 ± 18.1 cm H2O at the end of the protocol. VT was markedly larger during PCV compared with ITPV and HFO throughout the protocol (P < 0.005), but was unchanged in each group throughout the 4-h ventilation period (fig. 4). An average bias flow of 16.8 ± 4.4 l/min was required during ITPV.

**Hemodynamics.** There were no significant changes in hemodynamics during the 4-h ventilation period (table 3).
Table 3. Hemodynamics during the Study in All Three Groups

<table>
<thead>
<tr>
<th>Measure/Group</th>
<th>BL</th>
<th>Injury</th>
<th>Post-RM</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>Final BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAB (mmHg)</td>
<td>127.6 ± 17.7*</td>
<td>123.9 ± 10.6</td>
<td>100.7 ± 17.6</td>
<td>106.4 ± 12.2</td>
<td>103.7 ± 15.9</td>
<td>101.4 ± 15.2</td>
<td>105.7 ± 15.4</td>
<td>102.4 ± 13.9</td>
<td>98.3 ± 28.4</td>
</tr>
<tr>
<td>HFO</td>
<td>126.9 ± 6.9</td>
<td>115.7 ± 11.3</td>
<td>106.1 ± 14.6</td>
<td>107.9 ± 13.8</td>
<td>112.1 ± 12.9</td>
<td>108.6 ± 8.5</td>
<td>107.1 ± 13.2</td>
<td>104.3 ± 14.6</td>
<td>113.1 ± 12.7</td>
</tr>
<tr>
<td>ITPV</td>
<td>121.4 ± 16.5</td>
<td>120.7 ± 12.7</td>
<td>106.1 ± 22.2</td>
<td>105.7 ± 22.3</td>
<td>107.9 ± 22.5</td>
<td>110.0 ± 25.8</td>
<td>104.3 ± 20.7</td>
<td>105.6 ± 22.9</td>
<td>103.3 ± 20.2</td>
</tr>
<tr>
<td>CVP (cmH2O)</td>
<td>5.1 ± 2.3</td>
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<td>11.3 ± 3.5</td>
<td>11.4 ± 3.7</td>
<td>11.6 ± 3.9</td>
<td>11.3 ± 3.5</td>
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<td>11.9 ± 3.3</td>
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<tr>
<td>HFO</td>
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<td>9.9 ± 3.8</td>
<td>10.1 ± 4.3</td>
<td>9.9 ± 4.5</td>
<td>9.9 ± 3.2</td>
<td>10.0 ± 3.4</td>
<td>10.0 ± 4.1</td>
<td>12 ± 1</td>
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<td>6.4 ± 2.8</td>
<td>9.7 ± 2.2</td>
<td>8.9 ± 2.8</td>
<td>8.4 ± 2.8</td>
<td>8.4 ± 2.2</td>
<td>8.4 ± 2.6</td>
<td>8.7 ± 4.3</td>
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<tr>
<td>PCWP (cm H2O)</td>
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<td>8.9 ± 3.9</td>
<td>13.6 ± 3.6</td>
<td>14.1 ± 3.8</td>
<td>15 ± 2.9</td>
<td>14.6 ± 4.1</td>
<td>13.4 ± 3.6</td>
<td>13.4 ± 3.9</td>
<td>18.3 ± 5.9†</td>
</tr>
<tr>
<td>CO (l/min)</td>
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<td>5.9 ± 1.8</td>
<td>3.7 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>4.5 ± 0.7</td>
<td>4.3 ± 0.7</td>
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<td>SV (ml)</td>
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<td>36.6 ± 7.0</td>
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<tr>
<td>HR (bpm)</td>
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<td>148.1 ± 20.5</td>
<td>132.7 ± 18.5</td>
<td>157.9 ± 35.6</td>
<td>154.7 ± 28.9</td>
<td>140.3 ± 25.1</td>
<td>149.6 ± 18.4</td>
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<td>151.9 ± 28.8</td>
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<tr>
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<td>150.9 ± 41.6</td>
<td>164.8 ± 38.6</td>
<td>156.2 ± 26.1</td>
<td>153.8 ± 26.2</td>
<td>138.1 ± 14.5</td>
</tr>
</tbody>
</table>

All figures are mean ± SD.
* P < 0.05 vs. Post-RM; † P < 0.05 vs. Injury.
BL = baseline; CO = cardiac output; CVP = central venous pressure; Final BL = final baseline; HFO = high-frequency ventilation; HR = heart rate; Injury = lavage injury; ITPV = intrathoracic pulmonary ventilation; mAB = mean arterial blood pressure; PCV = pressure control ventilation; PCWP = pulmonary capillary wedge pressure; Post-RM = immediately after recruitment; SV = stroke volume; 30 min, 60 min, 120 min, 180 min, 240 min = time after lung recruitment.

**Injury versus Final Baseline**

No significant differences were observed for any variable in any group except for pulmonary capillary wedge pressure in the PCV group between injury and final baseline (8.9 ± 3.9 mmHg at injury and 18.3 ± 5.9 mmHg at final baseline, P < 0.05).

**Biologic Evaluation**

Leukocyte and neutrophil counts obtained by lavage from the dependent and nondependent left lung immediately after the experiment were similar in PCV, HFO, and ITPV (fig. 4). Expression of mRNA for IL-1β and IL-8 showed no significant differences among groups (fig. 5).

**Histologic Examination**

The main histologic findings are summarized in table 4. Hyaline membranes were not seen in any case. One slide originating from an ITPV-ventilated animal was excluded from all histologic analyses because of the presence of underlying disease (chronic follicular bronchitis and bronchiolitis).

Lungs ventilated with PCV showed significantly more injury than HFO, as indicated by greater interstitial hemorrhage (P = 0.002) and alveolar septal expansion (P = 0.0001), and ITPV, as indicated by greater alveolar septal expansion (P = 0.04). In addition, the sum of parameters showed significantly (P = 0.01) greater injury in PCV (fig. 6, table 4). No significant differences were observed between HFO and ITPV or between the dependent and nondependent regions in any group.

**Discussion**

Our hypothesis that there would be no differences in gas exchange, hemodynamics, or lung mechanics during PCV, HFO, and ITPV when applied with a similar open-lung protective strategy was proven correct. This occurred despite differences in V̇1, PDR, PIPca, and Paw among the groups. However, pilot histologic data indicate that lung injury may be greater with PCV.

This study clearly shows that mode of ventilation does not affect gas exchange, lung mechanics, or hemodynamics in ARDS if all modes are applied with a lung protective ventilatory strategy. That is a strategy that opens the lung and keeps it open but also avoids high peak alveolar pressures and large V̇1s. The trend toward greater lung injury in PCV may have been related to the actual V̇1s, peak alveolar pressures, and Paw established. V̇1 in PCV was 8.9 ± 2.1 ml/kg during the 4-h ventilation period, which was much greater than that provided by ITPV (2.7 ± 0.8 ml/kg) or HFO (estimated 2.0 ml/kg).25 More importantly, the peak alveolar pressure in PCV (equal to PIPca) was 30.6 ± 2.6 cm H2O, versus 22.3 ± 4.8 cm H2O in ITPV and an estimated 24.2 cm H2O in HFO.17 In PCV, PIPca equaled the peak alveolar pressure.
because the flow was zero at the end of inspiration. In ITPV, the peak alveolar pressure could be no higher than PIPca, and in HFO, the peak alveolar pressure was estimated.\textsuperscript{17} As discussed by Tobin\textsuperscript{28} and shown by Amato \textit{et al.}\textsuperscript{1} and the ARDSnet,\textsuperscript{2} a peak alveolar pressure of less than 30 cm H\textsubscript{2}O may be the critical variable associated with improved survival in ARDS in humans. In all groups, peak alveolar pressure was less than 30 cm H\textsubscript{2}O, except in PCV, in which it was 30.6 cm H\textsubscript{2}O. The P\textsubscript{a} (difference between assumed peak alveolar pressure and end-expiratory pressure) at the alveolar level was higher with PCV (10.7 ± 2.4 cm H\textsubscript{2}O) than with HFO (approximately 3.6 ± 0.98 cm H\textsubscript{2}O; 15% of inspiratory pressure amplitude)\textsuperscript{17} or ITPV (5.8 ± 2.8 cm H\textsubscript{2}O). Finally, P\textsubscript{aw} during PCV (24.75 ± 1.84 cm H\textsubscript{2}O) was higher than that during HFO (20.03 ± 2.98 cm H\textsubscript{2}O) or ITPV (22.48 ± 6.3 cm H\textsubscript{2}O).

\textbf{Histology}

The pathogenesis of ventilator-induced lung injury is still poorly understood.\textsuperscript{29} The pilot histologic data from this study showed that lungs ventilated with PCV developed significantly greater interstitial hemorrhage than those ventilated with HFO. It is noteworthy that the degree of interstitial hemorrhage was greatest during the approach with the greatest peak alveolar pressure and V\textsubscript{T} and lowest in the approach with the lowest peak alveolar pressure and V\textsubscript{T}. These data would suggest that alveolar pressure and V\textsubscript{T} should be decreased to the lowest level possible to avoid injury. A significantly higher degree of alveolar septal expansion was seen with PCV versus ITPV and HFO, suggesting that high peak pressures cause this injury. This suggests that the primary injury occurs in the alveolar walls; perhaps the low peak alveolar pressures generated with HFO and ITPV interfered the least with the lung’s efficiency in recruiting neutrophils.

The absence of hyaline membranes in all cases suggests that hyaline membrane formation is not a feature of ventilator-induced lung injury in sheep. Alternatively, it may be a late-stage phenomenon and occur more than 4 h after the onset of mechanical ventilation. Hyaline membranes are thought to represent a coalescence of intraalveolar proteinaceous fluid. The degree of fluffy noncoalesced intraalveolar fluid was greatest with PCV and least with HFO; the lack of statistical significance among the groups may be the result of the lack of

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
 & Granulocytes & Alveolar Hemorrhage & Interstitial Hemorrhage & Alveolar Septal Expansion & Proteinaceous Exudate & Hyaline Membrane & Sum of Parameters \\
\hline
PCV & 62.5 & 16.7 & 33.3 & 62.5 & 8.3 & 0 & 30.6 \\
HFO & 37.5 & 0 & 0\textsuperscript{*} & 20.8\textsuperscript{*} & 0 & 0 & 11.1\textsuperscript{*} \\
ITPV & 33.3 & 4.2 & 20.8 & 29.1\textsuperscript{*} & 12.5 & 0 & 16.7\textsuperscript{*} \\
\hline
\end{tabular}
\caption{Percentage of Slides Showing High Grade of Histologic Injury}
\end{table}

Values are expressed as percentages.

\textsuperscript{*} \( P < 0.05 \) vs. PCV.

HFO = high-frequency ventilation; ITPV = intratracheal pulmonary ventilation; PCV = pressure control ventilation.
statistical power, given the small numbers of animals studied. These pilot histologic data indicate a trend toward greater injury with PCV, but this study must be repeated with a greater number of animals providing histologic information before conclusions can be established.

**Inflammatory Mediator Response**

The lack of pilot data difference in mRNA expression for IL-1β and IL-8 in lavage fluid may be the result of the number of animals studied, timing, or length of the experiment. However, as is obvious from figure 4, there is a trend in mRNA expression for IL-1β and IL-8, indicating a difference. The lowest levels were in the HFO group but were not significantly different from the other groups. Similarly, insignificant trends also existed for leukocyte and neutrophil counts. Large standard deviations in all these variables were observed, and this aspect of the study was underpowered to prevent identification of significant difference in this area and establish any conclusions.

**High-frequency Oscillation**

High-frequency ventilation was applied in a manner consistent with that of other groups. All variables among sheep were maintained constant, except pressure amplitude, which averaged 50 cm H₂O across the 4-h ventilation period. Alveolar PDR during HFO was determined based on the approach outlined by Fort et al. with an 8-mm internal diameter airway, approximately 15% of the inspiratory pressure amplitude is transmitted to the distal lung. Because pressure amplitude represents the total range of pressure change around the Paw, 50% of the pressure amplitude represents the inspiratory excursion, 15% of which equaled approximately 3.6 ± 1.0 cm H₂O (alveolar driving pressure, PDR). This PDR base on our previous data in this model resulted in a VT of approximately 2.0 ml/kg. As a result, peak alveolar pressure (approximately 24.2 cm H₂O) and VT were low in this mode.

**Intratracheal Pressure Ventilation**

Intratracheal pressure ventilation was first described by Kolobow et al. Our setup was similar to that used by their group. In essence, a continuous bias flow through a two-channel endotracheal tube was maintained. The design of the prototype tracheal gas insufflation tube (fig. 1) used in this protocol directed gasflow up the secondary channel toward the ventilator circuit. The Servo 900C was used simply as an exhalation value, opening and closing at a rate of 120 times per min with an I:E of 1:1. The only factor that varied among animals was the bias flow (16.8 ± 4.4 l/min).

As with all continuous-flow tracheal gas insufflation systems, static measurement of end-inspiratory or end-expiratory pressure is difficult. Because of the reverse direction of the bias flow (toward the ventilator circuit), end-expiratory pressures at the carina are reduced by the high velocity of the gas flow. Although we did not quantify the effect on actual PEEP in this study, previously with this tube, PEEP levels measured at the carina were reduced compared with actual alveolar PEEP level by the reverse flow. As with HFO, ITPV resulted in low peak alveolar pressure and PDR.

**Pressure Control Violation**

The reason that PCV may have shown a trend toward greater lung injury was mostly probably the VT used in this mode. As indicated, VT was 8.9 ± 2.1 ml and end-inspiratory plateau pressure was 30.6 ± 2.6 cm H₂O. As a result, injury may have been induced despite our open-lung strategy of recruitment with optimal PEEP. It is interesting to speculate that our results would have been different if a VT of 6 ml/kg and a plateau pressure less than 30 cm H₂O, as is currently recommended, were applied. Future study of lung protection using different approaches to ventilatory support must ensure that overdistension does not occur in any group.

**Comparison with the Literature**

There are two studies in small animal models in which HFO and conventional ventilation were provided in a lung protective manner. In these comparisons, no difference on any monitored variable was observed between groups. Ventilatory strategies in these studies and ours were similar. The lungs were all recruited, prevented from derecruitment and ventilated at low peak alveolar pressures and VTs. Both studies showed no differences in gas exchange, hemodynamic, lung mechanics, or indices of inflammatory response. However,
only one performed histologic analysis. In this study, 2.5- to 3.5-kg rabbits, as opposed to 30-kg sheep, were studied. It may be that species differences or the peak alveolar pressure and $V_T$ we used during PCV accounted for the trend in histologic outcome we observed, but in none of these studies is peak alveolar pressure or $V_T$ listed. As far as we are aware, this is the first comparison of ITPV with HFO or PCV.

**Limitations**

The main limitation of this study is that it was performed on an animal model, not patients. In addition, it can be argued that our lung model (saline lavage) may not reflect the same pathophysiology as adult ARDS. However, no lung model can be expected to perfectly match the pathophysiology of all the multiple causes of adult ARDS. Our choice of a protective strategy during PCV may not have been ideal because $V_T$ was greater than 6 ml/kg (8.9 ± 2.1 ml/kg) and peak alveolar pressure exceeded 30 cm H$_2$O (30.6 ± 2.6 cm H$_2$O). The 4-h study period may have been inadequate to identify differences among ventilatory approaches because all approaches were designed to protect from overdistension and repetitive opening and closing of unstable lung. A lengthy study period may be necessary to show outcome differences. The data on histology must be considered underpowered because of the small number of animals evaluated. Clearly, additional data evaluating lung injury from a large series of animals is required before conclusions on lung injury can be established.

**Conclusion**

In this saline lavage, lung injury, sheep model of ARDS, approach to an open lung protective ventilatory strategy did not have an effect on gas exchange, hemodynamics, or lung mechanics. Pilot histologic data indicate that lung injury may be greater with PCV.

**References**