Lung volume recruitment in a preterm pig model of lung immaturity

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Arrindell EL Jr, Krishnan R, van der Merwe M, Caminita F, Howard SC, Zhang J, Buddington RK. Lung volume recruitment in a preterm pig model of lung immaturity. Am J Physiol Lung Cell Mol Physiol 309: L1088–L1092, 2015. First published September 25, 2015; doi:10.1152/ajplung.00292.2015.—A translational preterm pig model analogous to infants born at 28 wk of gestation revealed that continuous positive airway pressure results in limited lung recruitment but does not prevent respiratory distress syndrome, whereas assist-control + volume guarantee (AC+VG) ventilation improves recruitment but can cause injury, highlighting the need for improved ventilation strategies. We determined whether airway pressure release ventilation (APRV) can be used to recruit the immature lungs of preterm pigs without injury. Spontaneously breathing pigs delivered at 89% of term (model for 28-wk infants) were randomized to 24 h of APRV (n = 9) vs. AC+VG with a tidal volume of 5 ml/kg (n = 10). Control pigs (n = 36) were provided with supplemental oxygen by an open mask. Nutrition and fluid support was provided throughout the 24-h period. All pigs supported with APRV and AC+VG survived 24 h, compared with 62% of control pigs. APRV resulted in improved lung volume recruitment compared with AC+VG based on radiographs, lower PCO2 levels (44 ± 2.9 vs. 53 ± 2.7 mmHg, P = 0.009) and lower inspired oxygen fraction requirements (36 ± 6 vs. 44 ± 11%, P < 0.001), and higher oxygenation index (5.1 ± 1.5 vs. 2.9 ± 1.1, P = 0.001). There were no differences between APRV and AC+VG pigs for heart rate, ratio of wet to dry lung mass, proinflammatory cytokines, or histopathological markers of lung injury. Lung protective ventilation with APRV improved recruitment of alveoli of preterm lungs, enhanced development and maintenance of functional residual capacity without injury, and improved clinical outcomes relative to AC+VG. Long-term consequences of lung volume recruitment by using APRV should be evaluated.

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METHODS

Source of preterm pigs. The caesarian section, care, and sampling of preterm pigs followed our established protocol (2) and were approved by the Institutional Animal Care and Use Committees of the University of Tennessee Health Science Center (location of caesarian section) and the University of Memphis (site of critical care). Preterm pigs were delivered from four specific pathogen-free, artificially inseminated sows of a defined genetic lineage without antenatal steroids on gestation day 102 (89% of 115-day term), when lung development is similar to that of 28-wk preterm infants. After the airway was suctioned and cleared and spontaneous breathing was established the pigs were transported to a facility established for chronic intensive care.

Processing and intensive care. Pigs with birth weight >600 g were intubated with 2.5-French endotracheal tubes but were not provided surfactant. Within each litter those of similar body weights were randomized to either APRV (n = 9) or AC+VG (n = 10) using Dräger VNS500 ventilators (Dräger Medical, Dräger, Telford, PA). Pigs with significant tracheal trauma (e.g., perforation) caused by placement of the endotracheal tube were removed from the study.
Panel (EMD, Millipore, Billerica, MA).

IFN-\(\gamma\)/H9251 selected level of significance was a two-sided categorical variables were compared by Fischer's exact test. The MV. Pigs were repositioned each hour to avoid dependent edema and to be lung protective (18). All pigs were allowed to breathe spontaneously. Caffeine or doxapram were not provided during the 24 h of MV. Pigs were repositioned each hour to avoid dependent edema and lung atelectasis and sedated as needed (ketamine, Bioniche Teoranta, Galway, Ireland; 2 mg·kg\(^{-1}\)·dose\(^{-1}\)) via an umbilical catheter (3.5-Fr Argyle, Coviden), which was also used for arterial blood sampling, parenteral nutrition, single prophylactic doses of maternal plasma (5 ml/kg) for passive immunity, antibiotic (cefazolin; 50 mg·kg\(^{-1}\)·dose\(^{-1}\)), and supplemental lactated Ringer solution as needed to maintain tissue perfusion (usually 3–4 ml·kg\(^{-1}\)·h\(^{-1}\)).

Heart rate and oxygen saturation were monitored continuously (Masimo Radical 7, Masimo). Arterial blood gas measurements (iSTAT and CG 4 cartridges; Abaxis, Union City, CA) were performed every 3 h or after adjustment of ventilator settings to maintain a pH of 7.25–7.35, \(\text{PO}_2\) of 40–55 mmHg, and oxygen levels within a range of 90–95% saturation.

Radiography. A chest X-ray (CXR) was obtained after insertion of the endotracheal tube to confirm and if necessary adjust placement and as a baseline measure of lung volume recruitment (Duoview High Resolution Digital Radiography System, Revo\(^2\), Kennesaw, GA). A second CXR after 24 h was used to assess lung volume recruitment.

 Necropsy. After 24 h of MV the pigs were euthanized (Euthasol; Virbac, Fort Worth, TX; 1 ml/kg iv); the lungs were removed en bloc, inflated by using the endotracheal tube and a NeoPuff (Fisher & Paykel Healthcare, Irvine, CA) to a pressure of 20 cmH\(_2\)O; and the lungs were removed en bloc, submerged in formalin for histopathology. Bronchoalveolar lavage fluid (BALF) was collected from the right middle lobe for analysis of cytokines. The weight of the left lung was recorded before and after fluid (BALF) was collected from the right middle lobe for analysis of cytokines. The weight of the left lung was recorded before and after drying (50°C for 48 h) to determine the ratio of dry weight relative to wet weight as an indicator of pulmonary edema.

Cytokine analysis. Interleukin (IL)-1\(\alpha\), IL-1\(\beta\), IL-4, IL-6, IL-8, IFN-\(\gamma\), and TNF-\(\alpha\) were measured in the BALF by using Millipore Milliplex Map Porcine Cytokine and Chemokine Magnetic Bead Panel (EMD, Millipore, Billerica, MA).

Statistical analysis. Data were analyzed by Student’s \(t\)-test and \(\chi^2\)-test using a Likert scale that ranged from 0 (no injury), 1 (1–25% injury), 2 (26–50% injury), 3 (51–75% injury), to 4 (76–100% injury).

RESULTS

Preterm pigs are challenging to intubate and seven pigs were excluded because of substantial trauma or damage to the trachea or a bronchus. The APRV and AC+VG pigs within each litter had similar birth weights, with both larger than controls (Table 1). Litters 1 and 2 provided six pigs each that were large enough for successful intubation. Litters 3 and 4 provided fewer pigs >600 g. All 19 of the successfully intubated pigs provided MV survived the 24 h period of mechanical ventilation, whereas 12/36 (33%) of the control pigs died suddenly or required euthanasia prior to 24-h after developing symptoms consistent with RDS. Risk of death for control pigs <600 g was similar to pigs >600 g (\(P = 0.75\)).

Physiology. During the first 60 min of APRV, the \(P_{\text{high}}\) (18.7 ± 0.2 cmH\(_2\)O) exceeded the peak inspiratory pressure (PIP) required to achieve the targeted 5 ml/kg volume of the AC+VG protocol (13.7 ± 1.1 ml/kg; \(P < 0.01\)) and yielded an average ventilation volume (\(V_t\)) of 6.3 ml (±0.4). At 3 h the \(V_t\) had increased to 7.7 (±0.4; \(P = 0.008\)) despite gradually decreasing \(P_{\text{high}}\) in response to blood gases and oxygenation (Fig. 1). Even though \(P_{\text{high}}\) remained relatively stable after 3 h, lung recruitment continued and the \(V_t\) for the last hour of the study (9.1 ± 0.4) was higher compared with 3 h (\(P = 0.02\)). As expected, adjusting \(P_{\text{high}}\) changed ventilation volumes.

The PIP declined from 13.6 cmH\(_2\)O (±0.4) for the first 60 min to 11.6 cmH\(_2\)O (±0.3) for the third hour of AC+VG (Fig. 1; \(P = 0.002\)). During the last 2 h of ventilation the PIP increased in 3 of the 10 AC+VG pigs and averaged >25 cmH\(_2\)O and \(P_{\text{O}_2}\) was increased to as high as 0.9 to maintain targeted oxygen saturation. At necropsy, examination of the lungs revealed findings consistent with early RDS.

Pigs provided APRV required a lower \(P_{\text{O}_2}\) (\(P < 0.001\)) compared with pigs assigned to AC+VG (Table 2). \(P_{\text{CO}_2}\) was in the normal range for both groups but was higher among AC+VG pigs. There were no differences for pH, \(P_{\text{O}_2}\), and oxygen saturation, as expected since ventilator settings were adjusted to maintain these values within the targeted range. The oxygenation index (\(P_{\text{O}_2}\) \(\times\) mean airway pressure/\(P_{\text{O}_2}\)) was higher for the APRV pigs (\(P < 0.001\)), indicating improved gas exchange. The heart rate was similar for both groups of ventilated pigs.

Radiography and gross pathology. The lungs were consoliated at the start of MV. After 24 h, lung expansion was significantly improved with APRV compared with AC+VG (Fig. 2). Inflation of the lungs to a pressure of 20 cmH\(_2\)O revealed the AC+VG pigs had extensive areas of atelectasis, patchy expansion, and focal sites of hemorrhage that were not evident in the APRV pigs (Fig. 2). As before (2),

Table 1. Birth weights of preterm pigs assigned for APRV, AC+VG, and as controls

<table>
<thead>
<tr>
<th>Litter</th>
<th>All (n females, intubation failures)</th>
<th>APRV (n females)</th>
<th>AC-VG (n females)</th>
<th>Control (n females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>917 ± 68(14,9,0)</td>
<td>1.076 ± 33* (3.2)</td>
<td>1.177 ± 49* (3.1)</td>
<td>760 ± 77* (8,6)</td>
</tr>
<tr>
<td>2</td>
<td>895 ± 36(16,9,0)</td>
<td>1.029 ± 18* (3.2)</td>
<td>1.038 ± 20* (3.3)</td>
<td>813 ± 38* (10,4)</td>
</tr>
<tr>
<td>3</td>
<td>513 ± 42(15,9,3)</td>
<td>644 ± 190* (1.1)</td>
<td>655 ± 83* (2.2)</td>
<td>422 ± 40* (9,6)</td>
</tr>
<tr>
<td>4</td>
<td>882 ± 73(15,4,2)</td>
<td>991 ± 25* (2.0)</td>
<td>1.093 ± 101* (2.1)</td>
<td>780 ± 112* (9,3)</td>
</tr>
<tr>
<td>All</td>
<td>802 ± 35(60,31,5)</td>
<td>1.019 ± 27* (9.5)</td>
<td>1.005 ± 78* (10.7)</td>
<td>695 ± 42* (36,19)</td>
</tr>
</tbody>
</table>

Values are means ± SE, in grams. Treatment groups with different superscripts are significantly different (\(P < 0.05\)). APRV, airway pressure release ventilation; AC+VG, assist-control + volume guarantee.
lung volume recruitment was limited among control pigs that survived for 24 h.

The wet/dry lung ratio did not differ between the APRV and AC+VG groups (86.1 ± 0.6 vs. 86.2 ± 0.4% wet mass, respectively, P = 0.8).

Histopathology. There were no significant differences in hemorrhage, atelectasis, edema, necrosis, or inflammation, with all scores averaging <1 for both groups (all P > 0.1). The lungs of APRV pigs had regions with alveolar septal thinning whereas the lungs of AC+VG pigs exhibited areas of microatelectasis (Fig. 2).

Inflammation. None of the proinflammatory cytokines IL-1α, IL-1β, IL-6, and IL-8 measured in the lung lavage samples differed between the groups of ventilated pigs. IL-10, IFN-γ, and TNF-α were below the limits of detection and IL-4 was detected at low levels in 1 of the 9 APRV and 1 of the 10 AC+VG pigs.

**DISCUSSION**

Concern for VILI underlies the preference for noninvasive ventilation such as CPAP to provide the positive-pressure ventilation support recommended to recruit FRC after preterm delivery (23). Using lung protective modes of MV proactively as an alternative is novel and clinically relevant. The improved recruitment of FRC for the APRV pigs compared with AC+VG based on CXR, gross pathology, lower FIO2, to meet targeted oxygen saturation, and higher oxygenation index are consistent with the limited clinical data that suggest APRV as a rescue mode for preterm infants with RDS results in improved gas exchange without causing excessive lung injury (8, 14).

The prolonged Thigh and short Tlow of APRV results in a higher mean airway pressure without wide pressure swings that effectively provides a continuous distending pressure that enhances the replacement of fluid with air without allowing derecruitment (9, 21) and causes a progressive and even distribution of lung recruitment in both the compliant and noncompliant regions of the lung. The continuous but lower pressure provided by CPAP results in minimal lung recruitment and lower survival (2) and is consistent with concerns of efficacy for use with preterm infants <28 wk gestation (3). Conventional tidal ventilation such as AC+VG causes wider pressure fluctuations with fluid expulsion limited to the inspiration phase (12). Pressure during the expiration phase must remain high enough to prevent the collapse of the distal airways and derecruitment (11). Even though the 5 cmH2O pressure used for the PEEP is a common setting in clinical practice (18), the longer expiratory period of AC+VG results in a larger magnitude of pressure fluctuations and may increase the risk of alveolar collapse and derecruitment of lung volume, contributing to regional variability in lung recruitment (1) and requiring more time and a larger number of inflations to reach a full FRC. It is possible that a higher PEEP would enhance the efficacy of the AC+VG and decreased the risk of alveolar collapse (11).

The similar wet-to-dry lung mass ratio for AC+VG and APRV pigs is not surprising based on the limited differences in lung recruitment after 24 h. The proactive use of either model of MV for 24 h did not result in VILI by histological criteria, even with the APRV protocol causing lung expansion to the 11th rib, which is indicative of overdistension. However, the heterogeneous, patchy pattern of lung recruitment and hemorrhage seen in the AC+VG pigs and the increasing PIP values recorded during the final hours for 30% of the AC+VG pigs are suggestive of a decrease in lung compliance and derecruitment and coincided with increased FIO2 to maintain targeted oxygen saturation levels.
The APRV protocol is a gentler method than sustained lung inflation protocols that use high pressure or volume for neonatal resuscitation and rapid lung recruitment (6, 19, 27). Rapid, large-scale lung distension can cause histopathological damage, increase BALF protein concentrations, trigger rapid (<30 min) inflammatory responses (29), increase the expression of inflammatory cytokines and acute phase proteins (10), and alter expression of genes associated with alveolar development and vascularization of newborn lungs (4, 15, 28).

One concern is the potential impact of APRV on cardiac function in neonates due to increased intrathoracic pressure that could impede venous return. A small case series in children demonstrated that APRV does not decrease blood pressure or urine output (13), but this needs to be investigated in preterm neonates. Though not specifically addressed in this study, there were no gross signs at necropsy of intracranial hemorrhage or compromised bowel. The possible increased translocation of bacteria from the lungs to the blood caused by the effectively higher PEEP of APRV (5) needs to be evaluated.

**PERSPECTIVES**

The present study is limited by the use of preterm pigs that are relevant to 28-wk preterm infants that with surfactant often do well without intense ventilation support (2). The potential benefits and limitations of APRV need to be evaluated at earlier stages of preterm lung development, and the optimal initial P_{high} to effectively and safely recruit lung volume must be determined. Although the short-term benefits of proactive...
APRV are evident, the longer-term pulmonary outcomes, including assessing the impact on survival, time to extubation, and subsequent lung development need to be evaluated.

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GRANTS

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