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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.

respiratory distress syndrome; animal model; development; prematurity; pulmonary; positive airway pressure (CPAP), despite concerns about its efficacy, safety (3), and limited lung recruitment (2).

We hypothesized that lung-protective modes of mechanical ventilation (MV) can be adapted to recruit functional reserve capacity (FRC) immediately after birth without surfactant. We compared the commonly used assist-control + volume guarantee (AC+VG) mode with airway pressure release ventilation (APRV), also known as biphasic positive airway pressure. APRV is a pressure-limited, time-cycled, time-triggered mode of ventilation (19) that recruits FRC using a defined peak pressure (P_{high}) that is extended over a prolonged period (T_{high}). Ventilation occurs when P_{high} is reduced (P_{low}) for a period of time (T_{low}) that allows for expiration but is short enough to prevent alveolar collapse and derecruitment. Although APRV improves oxygenation, decreases physiological dead space ventilation, and reduces alveolar edema, surfactant inactivation, histopathological damage, and the incidence and severity of RDS (17, 21), the use of APRV for preterm neonates has not been systematically studied, except for a few case studies after onset of RDS (8, 14). To determine the efficacy of proactive APRV initiated soon after delivery, we used a preterm pig model of lung immaturity and surfactant deficiency, since it closely mimics respiratory issues in preterm humans and therefore provides information that can readily be translated to clinical care (2).

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.

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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_{high} (18.7 ± 0.2 cmH2O) exceeded the peak inspiratory pressure (P_{IPI}) 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_{high} in response to blood gases and oxygenation (Fig. 1). Even though P_{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_{high} changed ventilation volumes.

The PIP declined from 13.6 cmH2O (±0.4) for the first 60 min to 11.6 cmH2O (±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 cmH2O and FiO2 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 FiO2 (P < 0.001) compared with pigs assigned to AC+VG (Table 2). P_{CO2} was in the normal range for both groups but was higher among AC+VG pigs. There were no differences for pH, PaO2, and oxygen saturation, as expected since ventilator settings were adjusted to maintain these values within the targeted range. The oxygenation index (FiO2 × mean airway pressure/Pao2) 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 consolidated 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 cmH2O 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 ± 77b (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 significantly 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 *FIO*₂, 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 Tₘₚ and short Tₗ 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, 25). Even though the 5 cmH₂O pressure provided 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 decrease 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.

**Table 2. Responses of preterm pigs to 24 h of APRV or AC+VG**

<table>
<thead>
<tr>
<th>Variable</th>
<th>APRV</th>
<th>AC+VG</th>
<th><em>P</em> Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>9 of 9</td>
<td>10 of 10</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 (7.1–7.5)</td>
<td>7.3 (7.0–7.5)</td>
<td>NS</td>
</tr>
<tr>
<td><em>P</em>co₂, mmHg</td>
<td>44 (31–74)</td>
<td>53 (30–79)</td>
<td>0.009</td>
</tr>
<tr>
<td><em>P</em>aO₂, mmHg</td>
<td>105 (54–173)</td>
<td>100 (56–180)</td>
<td>NS</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>5.1 (1.9–12.9)</td>
<td>2.9 (1.2–7.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fraction of inspired oxygen, %</td>
<td>36 (30–50)</td>
<td>44 (30–90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dynamic compliance, ml/cmH₂O</td>
<td>1.3 (1.1–1.5)</td>
<td>1.5 (0.9–1.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Oxygen saturation, %</td>
<td>97 (75–100)</td>
<td>96 (73–100)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>160 (129–196)</td>
<td>167 (109–199)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean airway pressure, cmH₂O</td>
<td>13 (9.4–18)</td>
<td>7.4 (4.4–20)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means (ranges). *Result of Student’s *t*-test for continuous variables and Fishers exact test for categorical variables. NS, not significant.
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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s). Although F. Caminita is an employee of Dräger Medical, the manufacturer of the ventilators used for this study, there is no conflict of interest. Ventilators from other suppliers include the same modes. Therefore the findings from this study will also benefit competing companies.

AUTHOR CONTRIBUTIONS


REFERENCES

3. Committee on Fetus and Newborn; American Academy of Pediatrics. Pregnancy and subsequent lung development need to be evaluated.