Published in final edited form as:

Shock. 2013 January ; 39(1): 28–38. doi:10.1097/SHK.0b013e31827b47bb.

EARLY AIRWAY PRESSURE RELEASE VENTILATION PREVENTS ARDS- A NOVEL PREVENTIVE APPROACH TO LUNG INJURY

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INTRODUCTION

Acute Respiratory Distress Syndrome (ARDS) is a serious clinical problem with over 200,000 cases annually (1) which is resistant to treatment once the syndrome is fully clinically established (2, 3). ARDS has a mortality rate of 30–60% with significant costs of care and debilitating lifelong sequelae for survivors (3, 4). Despite decades of research only one therapeutic modality, low tidal volume ventilation (LTV), has been demonstrated to modestly improve ARDS-related mortality (9%) (5). LTV is a supportive therapy intended to limit the exacerbation of ARDS by mechanical ventilation (i.e. minimizing ventilator induced lung injury–VILI) rather than altering the pathophysiology of the primary disease. However since 1994 the mortality of ARDS has remained unchanged, calling into question the efficacy of LTV (3). The severity of established ARDS, the ineffectiveness of current therapy, and the long-term consequences of the disease suggest that preventative rather than supportive treatment strategies be sought.

Current therapy for lung injury is usually initiated when all of the clinical criteria for ARDS are met. This practice is consistent with the long-standing clinical concept that this disease is a binary phenomenon: ARDS is perceived as either present or absent (2, 5–7). However recent population data have challenged this concept, demonstrating ARDS is rarely present at the time of hospital admission but rather develops over a period of hours to days (8).

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Conflicts of Interest: None

This work will be presented in the New Investigator Award Competition at the 35th Annual Conference on Shock in June 2012

Disclosures: This work was partially funded by: NIH R33HL089076 & NIH R21HL092801-01

Collectively, these data imply that there is a temporal window of opportunity to intervene with preventive therapies such as protective ventilation targeting early drivers of ARDS pathophysiology. We hypothesize the preemptive application of protective ventilation with a sufficiently elevated airway pressure-time profile (P/T_p) will prevent ARDS by targeting prevention of alveolar edema, maintenance of alveolar stability throughout the ventilatory cycle (preventing atelectrauma) and maintenance of maximum alveolar recruitment (9, 10).

Airway pressure release ventilation (APRV) maintains a sustained airway pressure over a large proportion of the respiratory cycle, and therefore this ventilation strategy has a high P/T_p . We have previously shown the application of APRV before lung injury prevents ARDS in a translationally-applicable porcine model of sepsis and I/R-induced ARDS (9). We hypothesized that the primary mechanism operant in this type of protective ventilation was the prevention of alveolar flooding (9). Alveolar edema deactivates pulmonary surfactant triggering alveolar instability, which in turn causes mechanical injury via atelectrauma and subsequently propagates ARDS progression through the lung (11, 12). Thus, preventing pulmonary edema could block the pathologic cascade of surfactant deactivation and alveolar instability proximally and thereby avert the development of ARDS (9).

The present study builds on this prior work by comparing preemptive APRV to the standard of care ARDSnet low tidal volume LTV ventilation in a high-fidelity model of Peritoneal Sepsis + Ischemia/Reperfusion (PS+IR)-induced lung injury. APRV was applied to animals 1 h following injury according to current published guidelines (13). LTV was administered according to published ARDSnet guidelines once ALI criteria ($PaO_2/FiO_2 < 300$) are met (5). The goal of this study was to compare the effectiveness of APRV at preventing ARDS, against the effectiveness of the current standard of care LTV at treating ARDS. Our data show early application of APRV prevents clinical lung injury, whereas LTV allows the progression of disease to fulminant ARDS. These findings indicate ARDS is preventable and suggest a paradigm shift in the approach to management of patients at risk of developing ARDS from *treating* fully established ARDS to *preventing* it from developing.

MATERIALS AND METHODS

Animals

All techniques and procedures described reviewed and approved by the Committee for the Human Use of Animals at Upstate Medical University. Healthy female Yorkshire pigs (30–50 kg) were anesthetized using continuous infusion of ketamine/xylazine/propofol to maintain a surgical plane of anesthesia. Animals were continuously monitored and cared for by the investigators for the full 48 h duration of the experiment. Under sterile conditions, animals underwent tracheostomy, arterial and venous catheterization, and bladder catheterization. Baseline (BL) measurements were taken after surgical preparation and prior to injury.

Experimental Groups

Sham Group (n = 5)—Following surgical preparation, animals underwent laparotomy and dissection of the superior mesenteric artery without clamping, and cecotomy followed by closure of cecotomy. No fecal clot was placed in the peritoneum. These animals were then connected to a Hamilton G5 ventilator (Hamilton Reno, NV) and ventilator settings were: Tidal Volume (VT) = 10 cc/kg, positive end-expiratory pressure (PEEP) = 5, Respiratory Rate (RR) = 12 and $FiO_2 = 21\%$. RR was adjusted to maintain $PaCO_2$ within normal limits; no other ventilatory adjustments were made.

Following surgical instrumentation and Baseline measurements, secondary lung injury was induced using a “2-hit” model as previously described (14). Briefly: 1) *Ischemia/Reperfusion (IR)* The superior mesenteric artery (SMA) was clamped for 30 min to induce intestinal ischemia then released. 2) *Peritoneal Sepsis (PS)*: stool was harvested from a cecotomy and mixed with blood to create a fecal clot which was implanted in the abdomen prior to abdominal closure. Time Zero (T0) measurements were taken immediately after the induction of injury (i.e. removal of SMA clamp and placement of fecal clot) upon closure of the abdomen.

One hour following injury (T1), animals were randomized into two groups: Airway Pressure Release Ventilation (APRV, n = 4) or Low Tidal Volume (LTV) ventilation.

Low Tidal Volume Group (LTV n = 3).—Animals were connected to a Dräger™ Evita XL ventilator (Dräger, Lubeck, Germany), with initial settings identical to Sham group. Animals were transitioned to LTV (VT= 6 cc/kg) when they met ARDSnet clinical criteria of $\text{PaO}_2/\text{FiO}_2 < 300$ per ARDSnet protocol. Appropriate adjustments in RR were made to maintain adequate minute volume. PEEP and FiO_2 were adjusted in response to changes in SaO_2 along the “High PEEP, Low FiO_2 ” scale(5). If airway Plateau Pressure (P_{plat}) rose above 30cm H_2O VT was further reduced by 1cc/kg increments to 4cc/kg with appropriate adjustments in RR to maintain equivalent minute volume per ARDSnet guidelines. The upper limit for respiratory rate was 35/min with titrations made in VT if respiratory acidosis was detected ($\text{pH} < 7.15$) according to the ARDSnet protocol.

APRV Group (n = 4)—The technique of administering APRV in this study has been described previously (13). Animals were connected to a Dräger™ Evita XL ventilator (Dräger, Lubeck, Germany) with the same pre-injury settings as Sham. At one hour post-injury (T1), the ventilator mode was switched to APRV with the following initial settings: High Pressure (P_{high}) was initially set at the Plateau Pressure from the volume-cycled setting used for Baseline measurements. Low Pressure (P_{low}) was set at 0 cm H_2O for the entire 48 h to minimize expiratory resistance and maximize Peak Expiratory Flow Rate (PEFR). Duration of P_{high} (T_{high}) was set at 3.9–6.0 seconds to equal 90–95 % of the respiratory cycle. Duration of release phase was set using T_{low} and ranged between 0.40–0.55 seconds in order to achieve a Termination of Peak Expiratory Flow Rate (T-PEFR) equal to 75% of PEFR. This parameter was calculated and monitored for adjustment based on the angle of deceleration noted on the expiratory flow waveform (13). Adjusting the T_{low} to maintain 75% of PEFR generated release volumes in a range of 10cc/kg. Adjustments to P_{high} and T_{high} were made to ensure that the APRV animals were not inadvertently receiving low-tidal volume ventilation. The P_{high} , T_{high} , T_{low} and FiO_2 were titrated throughout the study to minimize lung de-recruitment, limit airway pressures, optimize the efficiency of CO_2 clearance, and minimize dead space ventilation. These adjustments were based on interpretation of expiratory flow waveform, static compliance, calculated resistance, tidal volumes and arterial blood gases (13).

Clinical Management

Broad-spectrum antibiotics (Ampicillin 2 grams IV [Bristol Myers Squibb, Princeton, NJ] and Metronidazole 500mg IV [Baxter, Deerfield, IL]) given following abdominal closure and every 12 hours until the end of the study. Animals were treated with intravenous fluid resuscitation and vasopressors in a protocol adapted from the Early Goal Directed Therapy (EGDT) strategy (15). This strategy is considered standard-of-care for management of hemodynamic collapse in sepsis. Maintenance intravenous fluid requirements were calculated by body weight and given via continuous infusion. Ringer's Lactate was used for maintenance and resuscitation. Hemodynamic parameters were assessed to determine the

need for resuscitative fluid bolus according to parameters described by Rivers et al. According to EGD guidelines, continuous infusion of Norepinephrine was started when the animal was no longer responsive to fluid bolus. Vasopressin and epinephrine were added when Norepinephrine was no longer effective.

Physiologic Measurements

Hemodynamic parameters were measured (Agilent, CMS-2001™ System M1176A, with Monitor M1094B Böbingen, Germany) using Edwards transducers (Pressure Monitoring Kit [PXMK1183], Edwards Lifesciences, Irvine, CA). Pulmonary parameters were measured or calculated by the Dräger™ ventilator (Lubeck, Germany). Blood was drawn every 6 hours. Measurement of blood gases and chemistries were made with Roche Blood gas analyzer (Cobas b221, Basel, Switzerland). Clinical pathology and blood cultures were performed by the Upstate Medical University pathology laboratory facility.

Systemic Inflammation

Plasma was frozen every 6 h for ELISA quantification of IL-6 levels in systemic circulation.

Calculation of P/T_P

We have developed a novel method to analyze the force imparted on the lung by mechanical ventilation, incorporating the pressure and time over which pressure is sustained during each breath. This relationship, termed the *Pressure/Time Profile (P/T_P)*, is defined as the area under the airway pressure waveform for each breath and is therefore the integral of airway pressure over time during each breath, Equation 1 (Eq 1). P/T_P is a unique characteristic of any given ventilatory mode.

$$P/T_P = \int_{T_{\text{insp}}}^{T_{\text{exp}}} P dt \quad (\text{Equation 1})$$

where P=airway pressure (cmH₂O); t=Time (sec); T_{insp}=the time at the beginning of inspiration; and T_{exp}=the time at end expiration. This calculation was performed hourly for each experimental group.

Necropsy

After 48 h the experimental protocol was terminated, animals were euthanized and necropsy was performed. The lungs, liver, spleen kidney and small intestine were removed and preserved in formalin. Lungs were inflated to 25 cmH₂O using stepwise increases in PEEP to standardized lung volume history and grossly photographed. The left lung was filled with 10% formalin to a height of ~25cm, clamped and immersed in formalin. This technique standardized the inflation pressure necessary for quantitative histologic measurements

Bronchoalveolar Lavage Fluid (BALF) and Lung Tissue

The right middle lobe of the lung was lavaged with 60mL of normal saline, spun at 3500rpm at 4°C, and snap-frozen for later analysis. The concentration of IL-6 was determined in the plasma and BALF using ELISA according to the manufacturer's recommendations. Western blot analyses of surfactant protein A (SP-A) abundance in the BALF as well as of Epithelial Cadherin (E-Cadherin) in lung tissue homogenates were performed (16, 17).

Quantitative Histology

The quantitative histological assessment of the lung was based on image analysis of 120 photomicrographs (10 per animal), made at high-dry magnification following a validated, unbiased, systematic sampling protocol (14). Each photomicrograph was scored using a 4-point scale for each of 5 parameters: atelectasis, fibrinous deposits, and blood in air space, vessel congestion, alveolar wall thickness, and leukocytes (14).

Statistics

Data are mean $\pm$ SEM. Repeated Measures ANOVA with pig number and treatment as random effects were performed to compare differences within and between treatment groups for continuous parameters; probability values <0.001 were considered significant. *Post hoc* Tukey's tests were performed on continuous data at specific time points only if significance was found in the group*time effect using Repeated Measures ANOVA. Categorical data were compared using an unpaired Student's *t*-test. Quantitative histology data was analyzed using Mann-Whitney U test after testing for normality. Probability values <0.05 were considered significant. All analyses were performed using JMP version 5.1.1 software (Cary, NC).

RESULTS

Hemodynamics and Laboratory Data

The APRV and LTV groups demonstrated hemodynamic compromise consistent with shock: tachycardia, progressive hypotension, and high resuscitative fluid and vasopressor requirements. As expected, the Sham group did not exhibit any of these hemodynamic derangements (Table 1). MAP decreased more rapidly in the LTV group than in the APRV group with significant differences appearing by 24 h ($p<0.05$; Table 1). Consistent with this trend in hemodynamic deterioration, lactate levels and norepinephrine requirements increased more rapidly in the LTV group as compared with the APRV group, with significant differences appearing by 24 h ($p<0.05$).

All animals in both APRV and LTV groups developed polymicrobial bacteremia in blood cultures, typical of this animal model (9). There were no differences between the APRV and LTV groups in hemoglobin/hematocrit, white blood cell count, platelets, or coagulation parameters (data not shown). There was a significant time trend towards leukopenia, thrombocytopenia, anemia and coagulopathy in both APRV and LTV groups. Plasma IL-6 levels were equivalent between APRV and LTV at baseline, and increased for both groups by 48 h however they were significantly higher in LTV ($p<0.05$ vs APRV).

Fluid Balance

The Sham group animals did not develop significantly positive fluid balance ($p<0.05$ vs. APRV and LTV), nor did they require bolus fluids above the maintenance intravenous fluid requirements. All animals in APRV and LTV groups experienced severe shock requiring aggressive intravenous fluid resuscitation and exhibited similarly positive fluid balances that were not statistically different between the APRV and LTV groups.

Sequential Organ Failure Assessment (SOFA) Scores

To estimate shock severity, SOFA scores were calculated. The SOFA score of the Sham group was normal for 48 h, reflecting neither critical illness nor instability and was significantly different from the other two groups ($p<0.05$ vs. APRV and LTV, respectively). The SOFA score of the APRV group was 6.75 ± 1.31 at 12 h and 9.75 ± 1.79 at 48-h reflecting severe, progressive critical illness. The SOFA score for the LTV group at 12 h was

10.33±2.33 and 16.5±0.96 at 48 h reflecting severe, progressive critical illness which progressed more rapidly than in the APRV group.

Organ Injury

The animals in both the APRV and LTV groups exhibited nonpulmonary organ injury. Three out of four animals in the APRV group and all animals in the LTV group developed the abdominal compartment syndrome (ACS), with elevated bladder pressure, acute increases in airway pressure, reduction in MAP, and acute decrease in urine output. These animals underwent urgent decompressive laparotomy as clinically indicated. All animals in both groups developed both gross and microscopic intestinal inflammation with mucosal necrosis, villous sloughing, and inflammatory cellular infiltration (data not shown). All animals in the both APRV group LTV groups developed gastric stress ulceration. Liver histology in animals from both groups demonstrated paracentral hepatonecrosis characteristic of 'shock liver.' All animals in both LTV and APRV groups developed oliguric renal failure (<0.5cc/kg/hr urine output) for the final 2–3 hours of life.

Pulmonary Data

The development of clinical lung injury was determined by $\text{PaO}_2/\text{FiO}_2$ (P/F) ratio, and static compliance (C_{stat}) (Figure 1 A and B). The APRV and Sham groups had normal P/F ratios throughout the 48 h experiment. There were no significant differences in P/F ratio between these two groups (Figure 1A). The APRV group had a significant increase in C_{stat} after being transitioned from volume-cycled ventilation to APRV (Figure 1B) As compared to the Sham group, the APRV group maintained a 'supranormal' C_{stat} level throughout the course of the study. In contrast the LTV group developed progressively severe lung injury, with declining P/F ratio and C_{stat} over the course of the study (Figure 1A and B). The P/F ratio in the LTV group decreased to meet AECC criteria for Acute Lung Injury (ALI) (<300) by 19 h and ARDS criteria (<200) by 33 h (Figure 1A) (11). This progression to lung injury in the LTV group was corroborated by steadily declining C_{stat} to < 50% of baseline by the end of the study, consistent with the stiff, noncompliant lungs characteristic of ARDS (Figure 1B).

The Sham group maintained normal mean airway pressures (P_{mean}) throughout the 48-h study ($p<0.001$ vs APRV and LTV Groups; Fig 1C). P_{mean} was significantly higher in APRV than both Sham and LTV after transition from conventional ventilation at 1 h (Fig 1C). Due to stepwise increases in PEEP per the ARDSnet protocol the P_{mean} were identical from 39 h to 48 h for LTV and APRV (Fig 1C). Tidal Volume (VT) was initially ~10cc/kg for all groups at baseline and remained at this level for 48 h in the Sham and APRV groups. In contrast, the VT in the LTV group was lowered in response to development of ALI (Table 2). PEEP was initially at 5 cmH₂O for all groups at BL and remained at this level for the Sham group throughout the study (Table 2). The LTV group required progressive increases in PEEP in response to refractory hypoxemia throughout the study (Table 2).

Figure 1D shows that the APRV group had a significantly higher P/T_P than both other groups as soon as the transition was made from volume cycled ventilation (Fig 1D). Furthermore in spite of progressive increases in PEEP (Table 2) in the LTV group P/T_P remained low and didn't change over the 48-h course of the study (Fig 1D). Sham group animals also had low P/T_P , which was not significantly different from the LTV group throughout the course of the study. P/T_P in both the Sham and LTV groups remained similarly low and did not change over the course of the study, despite substantial increases in PEEP (Table 2). This is explained by the quantities that contribute to P/T_P . As lung injury progressed in the LTV group respiratory rate was increased to clear CO₂. However in order to maintain an Inspiratory to Expiratory ratio (I:E) of 1:2 as required by LTV protocol, the inspiratory time was decreased simultaneously. Since time and pressure are equally

weighted in P/T_p (Equation 1), this decrease in inspiratory time had the effect of maintaining a low P/T_p in LTV in spite of increases in PEEP.

Gross Pathology

The APRV treated animals exhibited normal, pink, homogeneously well-inflated lung tissue with no evidence of inflammation and no evidence of atelectasis, and appeared to be inflated nearly to total lung capacity (Figure 2A). The cut surface of the representative APRV lung specimen shows neither bronchial nor septal edema (Figure 2B). The lungs of LTV group were predominantly atelectatic with heterogeneous parenchymal inflammation (Fig 2C). In addition gel-like edema occupied the interlobular septae of the lung and airway edema was present in the bronchial openings (Figure 2D) in the LTV group.

Quantitative and Descriptive Histology

The quantitative histological technique utilized in this study allows for an unbiased comparison of degree of tissue injury to the lungs between the LTV and APRV groups. The histological lesions analyzed are considered pathognomonic for ARDS when the clinical context is appropriate (11). These lesions include: atelectasis, fibrinous deposits, capillary congestion, leukocyte infiltration, intra-alveolar hemorrhage, and alveolar wall thickness. APRV resulted significantly reduced fibrinous exudates, capillary congestion, leukocyte infiltration, and alveolar wall thickness ($p<0.05$ vs. LTV and Sham; Table 4). Sham animals exhibited more severe histological injury than LTV and APRV animals in the categories of atelectasis, fibrin deposits, leukocyte infiltration, and intra-alveolar hemorrhage ($p<0.05$; Table 3).

Histological section of the lung of a representative LTV animal (Fig 3B) shows stigmata of lung injury including atelectasis, fibrinous exudates, intra-alveolar hemorrhage, congested capillaries, thickened alveolar walls and leukocytic infiltrates. A histological section of the lung from the APRV Group (Figure 3C) shows preservation of normal pulmonary architecture and none of the stigmata of lung injury described. Notably, APRV animals exhibited prominent perivascular edema cuffs and dilated lymphatics as compared to LTV animals. Furthermore, APRV animals exhibited normal alveolar patency whereas LTV animals exhibited intra-alveolar fibrin as well as alveolar collapse. The Sham group exhibited signs of histological lung injury (although there had been no clinical evidence of this throughout the 48-h experiment) including: mild to moderate atelectasis, fibrinous exudates, intra-alveolar hemorrhage, congested capillaries, thickened alveolar walls and leukocytic infiltrates (Fig 3 A).

Wet: Dry (W:D) Weight ratio

In order to assess the degree of pulmonary edema we assessed W:D weight ratio. The LTV group had significantly higher W:D weight ratio (10.63 ± 1.72) than the APRV group (7.18 ± 1.12) ($p<0.05$). Both ARDSnet and APRV had higher W:D weight than Sham group (5.63 ± 0.51). There were no differences between groups in W:D weight ratios for liver, kidney, intestine or spleen.

Bronchoalveolar lavage fluid (BALF) and Lung Tissue

Surfactant composition was assessed by quantifying surfactant protein-A (SP-A) in BALF by western blot. SP-A was significantly lower in the LTV group ($p<0.05$ vs. APRV; Figure 4B). All LTV animals exhibited total degradation of the SP-A protein monomer, whereas SP-A monomer bands were expressed at higher levels in the APRV group ($p<0.05$) (Figure 4B). Interleukin-6 levels in BALF were analyzed by ELISA (Figure 4C) and showed that APRV was associated with significantly lower pulmonary IL-6 than LTV ($p<0.05$ vs APRV).

and Sham, respectively). The APRV group expressed significantly higher levels of Epithelial Cadherin in lung tissue homogenates than the LTV group ($p < 0.05$; Figure 4A) as measured by Western Blot.

DISCUSSION

The present study strongly suggests early intervention using a ventilator mode with a high P/P_T may successfully prevent ARDS, based on studies in our clinically-relevant porcine model of lung injury. APRV applied 1 h after injury preserved oxygenation (P/F ratio), lung compliance, surfactant protein abundance, histological lung architecture and alveolar epithelial integrity (E-Cadherin) compared with standard-of-care LTV treatment. APRV also reduced pulmonary edema and lung inflammation (BALF IL-6) compared to LTV. In fact P/F ratio, pulmonary mechanics, lung permeability and inflammation in the APRV group were indistinguishable from the Sham group, which received mechanical ventilation alone without PS+IR injury. LTV ventilation resulted in significant gross and histologic lung injury with very high histologic lung injury scores. APRV ventilation preserved normal pulmonary histology in spite of significant systemic inflammation (Plasma IL-6) and various indicators of critical illness (MAP, Lactate, SOFA). Histological lung injury scores for the APRV group were even lower than that of the Sham group. The implications of these findings are both clinically and mechanistically significant to the understanding and management of ARDS.

The Case for Early Intervention

Since established ARDS is accompanied by high mortality despite LTV (3) and ARDS survivors exhibit significant morbidities and limited quality of life (4) preventive approaches for this disease have been advocated in the literature (18). The current study describes a novel, preventive strategy of mechanical ventilation using high P/P_T which effectively averts lung injury by targeting key elements in early ARDS pathophysiology. Preventive management of disease rests on a clear understanding of pathologic progression and identification of early drivers of pathophysiology, which may be targeted by therapy to block development of the fulminant disease (19). The shift in management of colorectal malignancy over the last 3 decades is exemplary of this strategy (19). As pre-malignant colonic mucosal lesions were identified and targeted, the management of colorectal cancer radically shifted from treatment of clinically evident disease to prevention at early asymptomatic stages in high-risk patients (19). The current study demonstrates that ARDS may have reversible pre-clinical stages during which targeted intervention prevents the development of established ARDS. The prevention of all stigmata of lung injury by APRV applied immediately post-injury in our model suggests that blocking the key pathologic drivers of ARDS onset targeted by high P/P_T ventilation can halt the progression of pathology in the lung. Successful application of this preventive strategy will necessitate departure from the binary paradigm of understanding ARDS as either present or absent so that patients at risk may be selected early for preventive ventilation with APRV.

Identification of targets for early preventive therapy requires examination of the pathologic changes in the lung *prior* to the onset of clinical ARDS. The pathophysiology of ARDS onset follows from an insult (trauma/hemorrhage/sepsis) triggering systemic inflammation, which results in increased permeability causing pulmonary edema. Proteinaceous edema fluid floods alveoli, deactivating surfactant (11) and promoting alveolar instability with tidal ventilation (20). Mechanical ventilation in the context of unstable alveoli results in atelectrauma (21), which further propagates tissue injury and propels the lung towards ARDS (12). The pathologic tetrad of permeability, edema, surfactant deactivation and alveolar instability are therefore the early drivers of ARDS onset (11). We hypothesized early mechanical ventilation with the appropriate P/P_T would specifically target these key

elements of ARDS pathophysiology and prevent ARDS. The effect of sustaining pressure over time (high P/T_P) has three effects interrupting the pathologic tetrad, which are mechanisms for the success of APRV demonstrated in the present study. Sustained airway pressure: 1) alters lung edema handling, preventing alveolar flooding, 2) maintains alveolar stability thereby blocking atelectrauma, and 3) recruits collapsed alveoli and prevents derecruitment. Each of these effects of P/T_P will be discussed in detail.

Pressure Time Profile- Targeting Early Drivers of ARDS Pathophysiology

Since the only difference in management between APRV and LTV animals was the ventilatory strategy, the marked differences in outcome between these groups were likely related to the ventilatory forces applied at the alveolar surface, measured as P/T_P . The force exerted on the lung by mechanical ventilation is dependent on both pressure (P) and the duration of time (T) that this pressure is applied over the ventilatory cycle (22). We developed a novel method to quantify this concept by integrating the airway pressure curve over time (Equation 1). The Pressure Time Profile (P/T_P) therefore reflects the combined pressure-time force effect exerted by mechanical ventilation on the lung tissue and represents a unique characteristic of any given ventilatory mode. Since P/T_P incorporates the temporal effect of pressure, this parameter is responsive to the dynamic forces of tidal respiration and differs significantly from static pressure measurements. The defining characteristic of APRV in the current study is the unique shape of the airway pressure curve during the entire breath as visualized by the airway pressure tracing (9, 13). APRV sustains a relatively constant airway pressure (P_{High}) for > 90% of the duration of the breath (T_{High}) with a brief sub-second release (T_{Low}) to allow for CO_2 clearance (13). Thus APRV is effectively continuous positive airway pressure (CPAP) with a release phase for ventilation (13). We postulate that it is this sustained pressure on the pulmonary interstitium that is the key mechanism of reduced pulmonary edema.

The current study demonstrates differences in outcome correlating closely with differences in P/T_P between the 3 separate ventilatory strategies examined. The highest P/T_P mode (APRV) had the best lung histology outcome, as well as reduction in edema, and preservation of alveolar epithelial integrity. Both low- P/T_P modes (LTV and Sham) had measurable tissue injury at the histological level demonstrating that mechanical ventilation with low P/T_P is injurious, even in a normal lung (18). Low P/T_P combined with systemic inflammation in the LTV group was associated with significant lung injury, as assessed by clinical, molecular and histological parameters. These data suggest both systemic inflammation and mechanical ventilation with low P/T_P are necessary for the development of ARDS. Our finding that injury to the normal lung can be caused by mechanical ventilation is consistent with recent literature (18). The most proximal event in the pathologic tetrad of ARDS onset is increased permeability resulting in alveolar edema. High P/T_P may attenuate edema formation to preempt ARDS as a potential mechanism of the success of APRV.

Pressure Time Profile - Attenuation of Permeability And Alveolar Edema

The Systemic inflammatory response syndrome (SIRS) disrupts the delicate alveolar-capillary fluid balance by increasing the vascular permeability (σ), which increases capillary filtration rate (J_v) as expressed in the Starling relationship (23). Ventilation with the appropriate P/T_P may shift the balance of the Starling equation from high capillary filtration to a significantly reduced filtration rate, even in the presence of increased σ (9). In the present study, the high P/T_P APRV group had lower lung edema (W:D ratio) and greater alveolar epithelial integrity (E-Cadherin) than the LTV group. Thus, mechanical ventilation with sustained airway pressure over time may alter the fluid flux relationship to prevent edema flow into the alveolus.

There are several potential mechanisms by which sustained airway pressure (high P/T_P) might lead to reduced edema. Elevating and sustaining airway pressure by early increases in PEEP causes reduction in lung water in high-permeability edema (23), high alveolar surface tension edema (24) and barotraumatic edema (25). Sustained airway pressure over time increases the vascular transmural pressure secondary to an increase in the interstitial hydrostatic pressure (P_i) thereby opposing fluid movement out of the capillaries (23). While these prior mechanistic studies were designed to prevent edema with airway pressurization the current study was designed to efficaciously prevent ARDS in a translational ARDS model. The precise mechanisms by which high P/T_P delivered by APRV reduces lung edema and maintains alveolar epithelial integrity in this model remain to be determined.

P/T_P stabilizes alveoli preventing cyclic recruitment & derecruitment, which is known to increase capillary permeability (26) and cause pulmonary edema (27). It is also possible that P/T_P normalizes σ by preventing the cyclic stretch of the alveolar endothelium (28). Lastly, P/T_P may act to support the integrity of the interstitial matrix. An intact interstitial matrix comparable to a low compliance glove surrounding the capillary and plays a key role in restricting capillary fluid filtration (29). As long as the extracellular matrix is intact edema is maintained within the interstitial space. Severe edema develops rapidly once damage to the extracellular matrix reaches a critical 'tipping point' when the fluid restrictive component of the matrix is lost, allowing rapid efflux of fluid from the capillaries into the interstitial and alveolar space (30). The sustained pressure transmitted to the interstitial space (P_i) with P/T_P would prevent edema swelling-induced injury to the extracellular matrix, maintaining this important 'edema safety factor', preventing the rapid influx of edema and alveolar flooding.

Pressure Time Profile - Alveolar Stability

Alveolar instability, the dynamic opening and closure of alveoli during tidal ventilation, has been shown to result in atelectrauma which is a key driver of ARDS (12) and represents a target of our High P/T_P preventive ventilation strategy. Although we did not directly measure alveolar stability in this study we did measure the relative abundance of Surfactant Protein A. Surfactant proteins convey functionality to alveolar surfactant, which stabilizes alveoli during tidal ventilation (11). If a ventilator mode induces or propagates alveolar instability SP-A could be expected to be degraded in the BALF (9). The present study shows that APRV clearly preserved SP-A while LTV clearly degraded this protein. These data suggest APRV is capable of alveolar stabilization as a result of its high P/T_P -induced protection of surfactant function.

Pressure Time Profile- Lung Recruitment

Sustained pressure over time takes advantage of the temporal component of recruitment (22) leading to progressive recruitment with each tidal breath. Over a short period of time this pattern of ventilation results in near-total recruitment resulting in the phenomenon that the lung is ventilated at near total lung capacity (TLC). Examination of the gross lung specimens reveals the striking finding that APRV lungs appeared to be inflated to near TLC with no atelectatic segments (Figure 2a) whereas a majority of lung parenchyma of LTV lungs were atelectatic with only apical segments inflated (Figure 2b). Recruitment of alveolar units is known to be a function of both pressure and time (22). It appears that the high P/T_P of APRV maintains recruitment of the entire lung ventilating very near TLC for the majority of the respiratory cycle. When the *ex vivo* lung was left connected to the ventilator at the same APRV settings it was noted that the change in lung volume during release phase was nearly imperceptible (data not shown). Atelectasis with heterogeneously inflated lung parenchyma results in pressure gradients and stress-strain forces acting injuriously on lung tissue (31). Therefore there is a clear advantage to ventilating near TLC where the lung is homogeneously recruited eliminating these injurious mechanical forces.

The current study utilizes a model in which peritoneal sepsis is allowed to progress without source control leading to escalating systemic inflammation, severe shock physiology and organ failure. In the setting of this model resuscitative fluid requirements increase throughout the course of the study leading to extensive edema and Abdominal Compartment Syndrome. Edema-induced extrapulmonary force vectors compress the lung from the abdominal contents and from the chest wall. We postulate high P/T_p represents an intrapulmonary counterforce to these pro-atelectatic extrapulmonary forces and resists lung derecruitment and development of heterogeneity. APRV and LTV both had high fluid requirements, increasingly elevated intra-peritoneal pressures and eventual ACS. In response to this compression from the abdominal compartment the LTV animals developed grossly evident atelectasis as the whole lung specimens demonstrate. In contrast APRV animals displayed fully recruited gross lung specimens in spite of similar extrapulmonary forces.

CLINICAL IMPLICATIONS

The present study was designed to detect a difference in ARDS incidence between preemptively applied APRV vs. LTV applied according to present standards of care. In current clinical practice LTV is typically not applied before respiratory failure but rather at the onset of hypoxemia ($PF < 300$) and stepwise increases in PEEP are made in response to oxygen desaturation. We showed that early, sustained airway pressurization (high P/T_p) delivered before the onset of lung injury prevents edema and the downstream sequelae (surfactant degradation, alveolar instability, atelectrauma & parenchymal damage) associated with ARDS.

It is possible that early application of LTV with high PEEP could achieve similar effects provided that the P/T_p was equivalent to that of APRV. However there are several reasons why we believe early application of APRV would be superior to high PEEP+Low V_t . First, APRV has a significantly higher P/T_p than high PEEP+Low V_t due to the extended time at P_{high} . For example, we calculated P/T_p for a high PEEP+Low V_t breath from a preliminary study in the same 2 hit porcine model, where PEEP = 20 cm H₂O, P_{plat} = 29 cmH₂O, T_{insp} = 1.3s and T_{exp} = 1.69s. Inserting these numbers into Equation 1, we find the calculated P/T_p is 71 cm H₂O *s. Thus, the P/T_p in the high PEEP+Low V_t animal is much higher than in LTV or Sham animals (range: 30–60 cm H₂O*s) but still significantly lower than the P/T_p for APRV (range: 130–190 cm H₂O*s) (Fig 1D). This suggests that a substantially higher P/T_p than delivered by high PEEP+Low V_t is necessary to achieve lung protection. This inadequate P/T_p is with a PEEP of 20 cm H₂O, which is significantly higher than typical clinical practice (6). We have not yet conclusively demonstrated that a higher P/T_p correlates with reduced lung injury but the present study as well as unpublished data from our lab suggests that this relationship is true.

Second, application of APRV is more practical than applying a high PEEP strategy to a relatively normal lung. High PEEP requires delivery of tidal volume above the PEEP level in order to ventilate, which can lead to barotrauma since additional mechanical force is applied to pulmonary tissue with tidal volume delivery. In contrast APRV, described as CPAP with a brief release phase, releases pressure from P_{high} in order to generate a ventilatory volume. APRV therefore harnesses the potential energy contained within the elastic properties of the respiratory system causing the lung to recoil naturally to a lower lung volume to generate the tidal volume.

Clinically APRV may be the most practical method to apply early high- P/T_p . First, there are no prohibitive negative side effects from APRV, such that APRV can be applied to all patients at risk of developing ALI/ARDS. Second, because APRV is a form of CPAP and differs only in the added release phase, patients can comfortably breathe spontaneously at

any point throughout the respiratory cycle. As a result of the CPAP element, APRV fulfills the spectrum from weaning to full support of patients on mechanical ventilation (13, 32). Additionally, APRV is associated with increased patient comfort and less sedation.

APRV has been a standard of care mode of mechanical ventilation at the largest freestanding trauma center in the USA (R Adams Cowley Shock Trauma Center - STC). At the STC, APRV is applied as the initial and sole mode of ventilation up to and through extubation including non-invasive applications (13, 32–35). Thus, STC employs a non-selective approach of early APRV application for nearly all patients with trauma and sepsis. Although not all individuals who are at risk actually develop ARDS, many of these patients do with serious morbidity and mortality. Early universal application of APRV to patients at risk is a preemptive measure to counter a potentially catastrophic outcome similar to the use of seatbelts, thromboprophylaxis, or stress ulcer prophylaxis.

CONCLUSIONS

The current study demonstrates that SIRS-induced ARDS can be prevented with high airway P/T_P when APRV is used early in the course of mechanical ventilation in a clinically relevant translational porcine model of lung injury. APRV prevented clinical and histological lung injury by preserving alveolar epithelial integrity, reducing lung edema, preserving surfactant, and maintaining alveolar stability. In summary, these data suggest ARDS development involves a close interplay of both systemic inflammation as well as mechanical ventilation with low P/T_P. Future studies are needed to elucidate the mechanical ventilation strategies that will offer the appropriate P/T_P for prevention of ARDS.

The preemptive ventilation strategy presented in our study has the potential to change the current clinical practice paradigm from treating ARDS once it manifests to preventing it from ever developing. Since preemptive ventilation strategies such as Airway Pressure Release Ventilation are already approved for use, clinical application can begin without delay with an immediate impact on patient care. If successful, our ventilation strategy would be the first prophylactic intervention of any kind to prevent ARDS.

Acknowledgments

The investigators would like to thank our patients for all they have taught us, and Robert Cooney for his painstaking guidance.

ABBREVIATIONS

ARDS	Acute Respiratory Distress Syndrome
LTV	Low Tidal Volume Ventilation
APRV	Airway Pressure Release Ventilation
PS	Peritoneal Sepsis
I/R	Ischemia Reperfusion
PEEP	Positive End Expiratory Pressure
IL-6	Interleukin 6
EGDT	Early Goal Directed Therapy
ELISA	Enzyme-linked immunosorbent assay
BALF	Bronchoalveolar Lavage Fluid

SP-A	Surfactant Protein A
ANOVA	Analysis of Variance
SOFA	Sequential Organ Failure Assessment
P_{peak}	Peak Pressure
C_{stat}	static compliance
P_{mean}	Mean Airway Pressure
P/T_p	Pressure Time Profile
SIRS	Systemic Inflammatory Response Syndrome
ARDSnet	Acute Respiratory Distress Syndrome Network
VILI	Ventilator Induced Lung Injury
RR	Respiratory Rate
PEFR	Peak Expiratory Flow Rate
T-PEFR	Termination of Peak Expiratory Flow Rate
RM-ANOVA	Repeated Measures Analysis of Variance

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Figure 1. Pulmonary Data

A. PaO₂/FiO₂ Ratio- APRV maintains a normal P/F ratio throughout 48 h study with no significant difference from uninjured Sham animals. LTV develop ALI (P/F < 300) by 19 h and ARDS (PF<250) by 33 h, ventilation strategy does not alter steady progression of increasing hypoxemia (p<0.001 vs APRV & Sham). **B. Static Compliance (C_{stat})-** The APRV shows significant increase in C_{stat} after transition from volume-cycled mode to APRV (p<0.001 vs Sham & LTV). Sham maintained a normal C_{stat} level throughout the course of the study. In contrast the LTV group developed progressive decreases in C_{stat} to less than 50% of baseline. **C. Mean Airway Pressure (P_{mean})-** Sham group maintained normal P_{mean} throughout 48-h significantly different from both APRV and LTV (p<0.001). P_{mean} was significantly higher in APRV than both Sham and LTV after transition from conventional ventilation at 1-h. Due to stepwise increases in PEEP per the ARDSnet protocol the P_{mean} were identical from 39-h to 48-h for LTV & APRV. **D. Pressure Time Profile (P/T_P)-** APRV group had significantly higher P/T_P than both other groups as soon as the transition was made from volume cycled ventilation (p<0.001 vs Sham & LTV). In the LTV group P/T_P remained low and didn't change over the 48-h course of the study. Sham group animals also had low P/T_P, which was not significantly different from the LTV group throughout the study.

Figure 2. Gross Pathology

Representative specimens of gross lungs from LTV and APRV groups are shown.

A. APRV Whole Lung- animals exhibited normal, pink, homogenously well-inflated lung tissue with no evidence of inflammation, no evidence of atelectasis and appeared to be inflated nearly to total lung capacity.

B. APRV Cut Surface- The cut surface of the representative APRV lung specimen shows no bronchial nor septal edema.

C. LTV Whole Lung- The lungs were predominantly atelectatic with heterogenous parenchymal inflammation.

D. LTV Cut Surface- The cut surface shows gel-like edema filling the interlobular septae of the lung in the LTV group and airway edema in the bronchial openings.

Figure 3. Histology

Photomicrographs of representative lung sections of specimens from each treatment group at 40x magnification. **F** = fibrinous deposit in the air compartment. **Arrow** = blood in alveolus. **Arrowhead** = congested alveolar capillary. **Bracket** = thickened alveolar wall.

A. Sham- animals received 48 hours of mechanical ventilation without PS+I/R injury.

Specimen exhibits stigmata of lung injury including fibrinous deposits, blood in alveolus, congested capillaries, and thickened alveolar walls.

B. LTV- animals received PS+I/R injury and LTV ventilation after onset of ALI. Specimen exhibits stigmata of lung injury including fibrinous deposits, blood in alveolus, congested capillaries, leukocyte infiltration, and thickened alveolar walls.

C. APRV- Animals received APRV one hour following PS+I/R injury. Specimen shows normal pulmonary architecture, alveoli are well expanded, thin walled and there are no exudates.

Table 1

Hemodynamic Parameters

	Group	BL	6h	12h	18h	24h	30h	36h	42h	48h	p>0.05
HR (bpm)	SHAM	132.0 ± 13.5	106.8 ± 3.8	93.0 ± 5.5	91.0 ± 3.7	90.2 ± 4.3	93.0 ± 5.2	83.8 ± 5.9	78.6 ± 6.2	81.6 ± 6.2	
	APRV	98.7 ± 2.4	125.3 ± 9.5	108.0 ± 7.1	104.0 ± 7.9	108.0 ± 9.4	116.5 ± 10.6	125.5 ± 16.0	110.3 ± 14.3	126.3 ± 25.2	
	LTV	96.3 ± 12.8	106.3 ± 20.9	102.0 ± 21.0	92.0 ± 12.7	96.0 ± 5.0	105.7 ± 11.3	103.0 ± 6.9	99.0 ± 13.9	91.7 ± 6.9	
MAP (mmHg)	SHAM	124.8 ± 8.2	84.2 ± 9.8	85.6 ± 5.3	76.0 ± 6.2	67.0 ± 4.6	68.6 ± 4.5	69.6 ± 3.8	66.2 ± 4.4	65.8 ± 5.1	§
	APRV	112 ± 2.7	70.3 ± 7.8	83.5 ± 7.7	79.3 ± 5.3	80.0 ± 7.1 [†]	81.5 ± 16.6	82.8 ± 12.2	73.8 ± 4.1	76 ± 7.2 [†]	
	LTV	125.0 ± 5.9	71.0 ± 9.5	68.3 ± 9.0	58.0 ± 4.9	53.3 ± 7.0 [†]	48.7 ± 4.3	57.0 ± 10.0	41.3 ± 2.2 [*]	46.0 ± 7.5 [†]	
Lactate (mmol/L)	SHAM	2.0 ± 0.3	4.0 ± 1.2	1.5 ± 0.4		0.9 ± 0.1		1.0 ± 0.3		0.7 ± 0.1	
	APRV	1.5 ± 0.3	6.7 ± 2.6	3.6 ± 1.8		2.0 ± 0.7		2.0 ± 1.4		6.0 ± 3.1	
	LTV	1.6 ± 0.2	5.0 ± 1.8	5.2 ± 2.8		7.6 ± 5.4		11.6 ± 4.1 [*]		14.0 ± 2.3 [*]	
Norepi Dose (mcg/min)	SHAM	0	0	0	0	0	0	0	0	0	§
	APRV	0	1.3 ± 1.3	1.8 ± 1.2	2.0 ± 1.2	2.0 ± 1.2	4.5 ± 1.5	5.2 ± 1.7	8.0 ± 3.7	6.3 ± 4.3	
	LTV	0	2.0 ± 2.0	5.3 ± 5.3	3.3 ± 3.3	8.0 ± 6.1 [*]	11.5 ± 4.3 [*]	20.3 ± 3.2 [*]	21.7 ± 2.2 [*]	23.7 ± 1.9 [*]	
Bladder Pressure	SHAM	3.0 ± 1.3	4.0 ± 1.6	4.2 ± 1.5 [*]	6.8 ± 2.4	3.0 ± 1.6 [*]	2.4 ± 0.5 [*]	7.6 ± 2.6 [†]	7.0 ± 2.2	2.6 ± 7.0 [†]	
	APRV	5.3 ± 2.0	10.0 ± 2.7	13.5 ± 3.2	14.0 ± 3.5	11.8 ± 3.4	14.0 ± 2.0	17.0 ± 4.3	15.5 ± 2.8	15.8 ± 3.2 [†]	
	LTV	3.0 ± 1.6	14.3 ± 7.4	17.0 ± 5.6	15.7 ± 6.2	17.0 ± 5.5	27.3 ± 8.1	24.7 ± 2.8 [†]	26.7 ± 0.9 [*]	26.7 ± 0.9 [†]	

§ p>0.05 following Repeated Measures ANOVA

* p<0.05 following post-hoc analysis with Tukey's Test,

† p<0.05 between groups following post-hoc analysis with Tukey's Test

HR= Heart Rate in beats per minute, MAP= Mean Arterial Pressure in millimeters of mercury, Norepinephrine Dose in mcg/min

Table 2

Pulmonary Data

	Group	BL	6h	12h	24h	36h	48h	p>0.05
Vt	SHAM	10.2 ± 0.1	10.2 ± 0.2	10.0 ± 0.2	9.9 ± 0.2	10.2 ± 0.3	10.0 ± 0.2 [‡]	§
	APRV	9.9 ± 0.2	10.3 ± 0.4	10.3 ± 0.5	10.3 ± 0.4	11.8 ± 0.8	12.0 ± 0.8 [‡]	
	LTV	10.0 ± 0.1	9.8 ± 0.2	8.5 ± 1.2	7.4 ± 1.4	5.7 ± 0.3 [*]	5.8 ± 0.4 [‡]	
PEEP	SHAM	5.0 ± 0.0	5.0 ± 0.0 [‡]	5.0 ± 0.0	5.0 ± 0.0 [‡]	5.0 ± 0.0	5.0 ± 0.0	§
	LTV	5.0 ± 0.0	9.3 ± 3.4	7.3 ± 2.3	8.7 ± 3.7	15.3 ± 2.9 [*]	20.0 ± 2.3 [*]	
RR	SHAM	13.6 ± 0.9	18.7 ± 2.7 [‡]	14.9 ± 2.3 [‡]	12.6 ± 1.4	9.8 ± 0.6	10.1 ± 0.8	§
	APRV	18.0 ± 4.7	10.8 ± 0.6 [‡]	11.0 ± 1.5 [‡]	10.0 ± 0.7	11.8 ± 2.5	11.3 ± 2.3	
	LTV	17.0 ± 2.1	17.0 ± 2.9	18.0 ± 4.0	22.0 ± 3.8 [*]	29.7 ± 0.9 [*]	32.0 ± 3.1 [*]	
PaCO ₂	SHAM	36.1 ± 2.2	34.6 ± 2.9	37.0 ± 2.2	37.7 ± 1.7	36.6 ± 1.3	34.7 ± 2.5	
	APRV	39.6 ± 1.1	42.5 ± 4.6	38.8 ± 2.0	43.3 ± 2.2	43.9 ± 3.0	38.6 ± 1.2	
	LTV	34.6 ± 1.4	40.6 ± 1.7	38.6 ± 1.9	42.9 ± 3.0	57.0 ± 9.4	64.0 ± 8.8	

§ p<0.05 following RM ANOVA.

* p<0.05 following post-hoc analysis with Tukey's Test.

‡ p<0.05 between groups following post-hoc analysis with Tukey's Test.

Vt=Tidal Volume in Liters, PEEP=Positive End Expiratory Pressure in cmH₂O, RR=Respiratory Rate in breaths per minute, PaCO₂ = partial pressure of carbon dioxide in arterial blood

Table 3

Quantitative Histological Scoring of Lung Injury

	Histopathology Scores *		
	Sham Operation	LTV (2 hit)	APRV (2 hit)
Atelectasis	0.28 ± 0.07 ‡	0.05 ± 0.07	0.00 ± 0.07
Fibrinous Deposits	1.30 ± 0.13 ‡	0.68 ± 0.13	0.38 ± 0.13 ‡
Blood in Alveoli	0.23 ± 0.13 ‡	0.20 ± 0.13	0.30 ± 0.13
Capillary Congestion	2.15 ± 0.13	1.90 ± 0.13	0.83 ± 0.13 ‡
Thick Alveolar Wall	1.75 ± 0.17	1.70 ± 0.17	0.25 ± 0.17 ‡
Leukocyte Infiltration	1.18 ± 0.13 ‡	2.10 ± 0.13 ‡	1.80 ± 0.13 ‡

* Values are Mean ± SEM (N=40)

‡ Significantly different from the other two treatment conditions ($P<0.05$)