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Total Liquid Ventilation Provides Superior Respiratory Support to Conventional Mechanical Ventilation in a Large Animal Model of Severe Respiratory Failure

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Abstract

Total liquid ventilation (TLV) has the potential to provide respiratory support superior to conventional mechanical ventilation (CMV) in the acute respiratory distress syndrome (ARDS). However, laboratory studies are limited to trials in small animals for no longer than 4 hours. The objective of this study was to compare TLV and CMV in a large animal model of ARDS for 24 hours. Ten sheep weighing 53 ± 4 (SD) kg were anesthetized and ventilated with 100% oxygen. Oleic acid was injected into the pulmonary circulation until $\text{PaO}_2:\text{FiO}_2 \geq 60$ mmHg, followed by transition to a protective CMV protocol ($n=5$) or TLV ($n=5$) for 24 hours. Pathophysiology was recorded and the lungs were harvested for histological analysis. Animals treated with CMV became progressively hypoxic and hypercarbic despite maximum ventilatory support. Sheep treated with TLV maintained normal blood gases with statistically greater PO_2 ($p < 10^{-9}$) and lower PCO_2 ($p < 10^{-3}$) than the CMV group. Survival at 24 hours in the TLV and CMV groups were 100% and 40% respectively ($p < 0.05$). Thus, TLV provided gas exchange superior to CMV in this laboratory model of severe ARDS.

Keywords

respiratory distress; liquid ventilation; perfluorocarbons

INTRODUCTION

Despite improvements in management, the mortality of acute respiratory distress syndrome (ARDS) is 30–40%^{1–3}. Clinical strategies that hasten the resolution of lung injury may improve both survival and long-term outcomes for patients with ARDS⁴. Ventilation with liquid perfluorocarbon (PFC) has been investigated as a potential treatment for many respiratory diseases. Perfluorocarbon has high respiratory gas solubilities, low surface tension, and cytoprotective properties that allow for improved gas exchange at lower ventilation pressures while protecting the lung from inflammation. This may promote the

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resolution of lung injury and improve long-term outcomes. Partial liquid ventilation (partial filling followed by gas ventilation) supported this hypothesis in short-term (< 4 hr) laboratory experiments and clinical trials. Despite positive results, studies indicate that total liquid ventilation (TLV) may be a better approach.

In TLV, the lungs are both filled and ventilated with PFC using a ventilator designed specifically for delivery of liquid tidal volumes. Early laboratory studies of TLV identified safe and effective ventilation strategies and described the basic physiology in animal models of neonatal pulmonary support⁵. More recently, TLV has been evaluated in animals up to 25 kg with ARDS for as long as 4 hours^{6–9}. However, TLV is not yet sufficiently developed to be evaluated in clinical ARDS. Additional laboratory studies are needed to evaluate whether TLV can be successful in larger subjects with lung injury over a full course of treatment before progressing to clinical trials. We hypothesized that TLV could provide gas exchange superior to CMV and improve survival in a longer-term model of severe respiratory failure. Therefore, the purpose of the present study was to compare the performance of TLV and CMV in large animals with severe ARDS for 24 hours.

MATERIALS AND METHODS

Animal preparation and ARDS model

The experimental protocol and procedures were approved by the committee for the use and care of animals at the University of Michigan in accordance with the National Institutes of Health guidelines for ethical animal research. Ten sheep [weight 53 ± 4 (SD) kg] were anesthetized with sodium thiopental (15–20 mg/kg IV, Hospira Inc., Lake Forest, IL), placed supine, intubated, and gas ventilated with 100% oxygen. Anesthesia was maintained with propofol (6–12 mg/kg/hour, American Pharmaceuticals Partners, Schaumburg, IL) and ketamine hydrochloride (2–10 mg/kg/hour, Hospira, Inc., Lake Forest, IL) for the entire duration of ventilator support. An arterial line and pulmonary artery catheter (CCombo V777HF8, Edwards Lifesciences, Irvine, CA) were placed using aseptic technique. Paralysis was induced with 0.1 mg/kg IV pancuronium bromide (Hospira Inc., Lake Forest, IL) and the following baseline data were taken: arterial blood gases, mean arterial pressure (MAP), central venous pressure (CVP), cardiac output (CO), mean pulmonary artery pressure (PAP), and venous saturation of oxygen (SvO₂).

After collecting baseline data, lung injury was induced by injecting oleic acid (0.07 mL/kg/dose; C₁₈H₃₄O₂; Fisher Scientific, Pittsburgh, PA) into the right atrium until PaO₂/FiO₂ $\geq$ 60 mmHg using previously published methods⁹. Continuous trending of SvO₂ was used to indicate the onset and stabilization of lung injury followed by an arterial blood gas to quantify the severity. If PaO₂ was greater than 60 mmHg, additional doses were given. Once the criterion was met, animals were transitioned to a protective CMV protocol (n=5) or TLV (n=5) and supported for 24 hours.

Conventional Mechanical Ventilation

In the CMV group, the ventilator (Nellcor 7200, Puritan Bennett, Boulder, CO) was set to deliver a tidal volume (V_T) of 6 mL/kg, a frequency of 12 bpm, and FiO₂ of 1.0. Positive end-expiratory pressure (PEEP) was set between 5–13 to optimize PaO₂. Frequency and V_T were subsequently adjusted to maintain PaCO₂ between 35–50 mmHg, while limiting frequency and peak inspiratory pressure (PIP) to 40 bpm and 35 cmH₂O to minimize ventilator induced lung injury. Ventilator settings over the course of the experiment are shown in Table 1.

Total Liquid Ventilation

To initiate TLV, the gas ventilator was disconnected, the lungs were rapidly filled with warm pre-oxygenated perfluorocarbon (FC77, Fluorinert, 3M Corporation, St. Paul, MN) to a volume of 30 mL/kg. Liquid ventilation was initiated using a frequency of 5 bpm and V_T of 15 mL/kg, and then V_T was adjusted between 15 and 20 mL/kg to maintain PaCO_2 between 35–50 mmHg. Gas exchange was optimized during TLV by maintaining an end-expiratory lung volume (EELV) that was low to maximize liquid exchange in the lungs, yet great enough to prevent airway collapse during expiration. Supplemental PFC volume (8–10 mL/min) was continuously infused into the ventilator circuit to overcompensate for evaporative loss of PFC. This caused EELV to slowly increase with time. Lung volumes were then manually adjusted every 1–2 hours by withdrawing volume from the system until the airway pressure curve indicated early signs of airway collapse. Airway collapse is characterized by a sudden decrease in endotracheal tube pressures with a decrease or no change in flow rate.¹⁰ Once identified, PFC volume was then returned until the airway pressure waveform indicated resolution of the airway collapse. Ventilator settings over the course of the experiment are shown in Table 1.

The study applied a modular liquid ventilator configuration constructed by MC3, Inc. (Ann Arbor, MI) using a combination of custom-built and off the shelf components. It was designed to be modular with two parallel circuits for providing tidal PFC flow to the subject and continuous PFC flow to the oxygenators to supply O_2 to and remove CO_2 from the PFC. One module is illustrated in Figure 1. The tidal flow portion of the circuit consists of a custom-built piston with a commercial linear actuator (Exlar SR21, Chanhassen, MN) for controlling piston movement. The linear actuator is controlled by a custom Labview program (National Instruments, Austin, TX, USA). The continuous flow PFC refreshing circuit includes a spiral-wound, silicone membrane oxygenator with integral heat exchanger (I-4500-2A, Medtronic, Inc. Minneapolis, MN), an adult bubble trap (Quest Medical, Allen, TX), and a roller pump (Cobe Perfusion System, Cobe BCT Inc., Lakewood, CO). In this study, four modules were used. PFC outflow from each piston converged into a single connection near the endotracheal tube (ETT) to provide adequate tidal volumes to support 60 kg subjects. Circuit temperature was maintained at 37°C.

Data collection and animal management

The following parameters were recorded hourly: arterial blood gases, CO, MAP, CVP, PAP, and ventilator parameters RR, V_T , FiO_2 , PEEP, and PIP. A maintenance infusion of 5% dextrose solution was given intravenously at 2.5–4.0 mL/kg/hour. Gentamicin sulfate (120 mg IM, Abraxis, Schaumburg, IL) and nafcillin sodium (500 mg IV, Sandoz, Inc., Broomfield, CO) were given at 8 and 6-hour intervals respectively. Phenylephrine (0–20 mg/kg/hr IV, American Regent, Inc., Shirley, NY) was given as needed to treat hypotension unresponsive to volume administration and supplemental bicarbonate (Hospira, Inc., Lake Forest, IL) was given as needed.

After 24 hours, animals were euthanized with Pentobarbital Sodium (85 mg/kg IV, Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) and the heart and lungs were removed en bloc. Consistent regional samples were obtained from the dependent and nondependent regions of the apex, middle, and base of the right lung. Hematoxylin and Eosin stained slides were prepared and evaluated.

Statistical Methods

All data are presented as the mean and standard error of the mean (SEM) unless indicated otherwise. A t-test was used to compare baseline and post-oleic acid injury data between the CMV and TLV groups. To determine differences between treatment modes (CMV or TLV),

all longitudinal data was compared using a mixed model analysis with repeated measures within SPSS (Chicago, IL). Lastly, survival between CMV and TLV groups was compared using the Kaplan-Meier technique within SPSS.

RESULTS

Mortality

Survival at 24 hours in the TLV and CMV groups were 100% and 40% respectively. Three out of five animals in the CMV group died (asystole) while on maximum ventilator support. These deaths all featured marked hypoxia and hypercapnia and occurred during the ninth hour ($\text{PaO}_2 = 38 \text{ mmHg}$, $\text{PaCO}_2 = 116 \text{ mmHg}$), the twelfth hour ($\text{PaO}_2 = 41 \text{ mmHg}$, $\text{PaCO}_2 = 216 \text{ mmHg}$), and the 24th hour ($\text{PaO}_2 = 64 \text{ mmHg}$, $\text{PaCO}_2 = 89 \text{ mmHg}$). Survival was statistically longer in the TLV group than the CMV group ($p < 0.05$).

Gas Exchange

The average ventilation settings for the CMV and TLV groups are shown in Table 1. Gas exchange data for PaO_2 and PaCO_2 are shown in Figure 2. There was no significant difference between groups in PaO_2 or PaCO_2 at baseline ($p = 0.12$ and 0.29 , respectively) or after oleic acid injury ($p = 0.92$ and 0.84 , respectively). Over 24 hours of support, however, PaO_2 was significantly higher ($p < 10^{-9}$) and PaCO_2 was significantly lower in the TLV group ($p < 10^{-3}$).

Hemodynamics

The systemic hemodynamic variables of MAP and CO are shown in Figure 3. The average MAP decreased upon lung injury and throughout the course of the study while cardiac output increased slightly throughout the course of the study. There was no significant difference in MAP or CO between groups at baseline ($p = 0.37, 0.78$). Upon injection of oleic acid, no significant difference in cardiac output was observed between groups ($p = 0.45$), but MAP was significantly lower in the TLV group ($p < 0.05$). During 24 hours of ventilator support, there was no significant difference in MAP or CO between groups ($p = 0.94, 0.99$). Average phenylephrine doses are shown in Figure 4. There was no significant differences between the groups ($p = 0.80$).

Mean PAP and CVP data are shown in Figure 5. There was no significant difference between groups with respect to PAP at baseline ($p = 0.68$), upon stabilization of lung injury ($p = 0.15$), and throughout the 24 hours of ventilatory support ($p = 0.78$). There was no significant difference in CVP between groups at baseline ($p = 0.57$). Upon injection of oleic acid, CVP approached a significantly lower value in the TLV group when compared to the CMV group ($p = 0.056$), and was significantly lower in the TLV group for the remainder of the study ($p < 0.05$).

Gross Pathology and Histology

The gross appearance of the lungs from the CMV and TLV groups are shown in Figure 6. The lungs from TLV treated animals showed less evidence of injury than CMV treated animals. In the CMV group, consolidation and edema was evident in the dependent lung regions as manifested by the dark red coloration in Figure 6. On cut section, these regions were relatively airless and filled with fluid. By comparison, the lungs of the TLV treated animals showed much less consolidation and edema.

The histologic changes in the lungs of these animals are shown in Figure 7. As expected based on the gross appearance of the lungs, animals treated with TLV showed much less injury than the animals treated with CMV. The dependent lobes are shown as they represent

the regions with the greatest degree of lung injury. The histologic appearance of the CMV treated animals is shown in panel A. There is a prominent degree of leukocyte infiltration with edema, hemorrhage and focal infarction (arrow). The inset demonstrates the focal hemorrhage (arrow). By comparison the lung injury seen in the TLV treated animals is shown in panel B. There was some inflammation present with neutrophils, but inflammation, edema, and hemorrhage were much less severe in the TLV group. The inset shows preservation of the lung architecture with some edema and congestion.

DISCUSSION

Gas Exchange

This study demonstrates superior gas exchange and survival using TLV in this animal model of severe ARDS. In the TLV group, arterial PO_2 is significantly greater and PCO_2 is significantly lower. This difference appears to be the main cause of the mortality difference between the groups. There are several explanations for improved gas exchange using TLV in the setting of respiratory failure. During the onset of ARDS, hemorrhagic edema fluid floods the airspace, presenting a barrier to oxygen delivery and creating regional lung consolidation, especially in the dependent regions of the lung where perfusion is greatest. Mechanical ventilation of these lungs causes the cyclic opening and closing of unstable alveoli and over-distension of functional alveoli, which can further induce lung injury^{11, 12}. In the present study, approximately 100–300 ml of hemorrhagic, edema fluid was collected in bubble traps and removed from the circuit during the initial hours of TLV. In contrast to CMV, which ventilates gas against the edema fluid, ventilation with immiscible PFC provides pulmonary lavage, replacing edema fluid with a dense, low surface tension liquid that can recruit or stabilize injured lung while supporting gas exchange. In the PFC-breathing lung, the air-liquid interface is completely removed, thus ventilation occurs at lower peak inspiratory pressures, and the density of PFC causes a redistribution of pulmonary blood flow to less injured, non-dependent lung regions. The cumulative result is better V:Q matching compared to gas ventilation.

Hemodynamics

All sheep demonstrated decreasing arterial pressure over time that is unrelated to ventilation technique. After an initial drop in MAP associated with the oleic acid lung injury, MAP continued to decline despite increasing CO (see Figure 3). The cause of the decrease in MAP, therefore, is marked systemic vasodilation in both groups. This is likely an effect caused by the use of propofol anesthesia. Propofol has been shown to cause MAP decreases of 13–17 mmHg when utilized over 10–30 minute periods of sedation or anesthesia.^{13,14} The vasodilation was likely worse in this experiment due to the longer duration of the protocol. For optimization of long-term TLV and improvements in future comparative studies, a better method of sedation must be employed. In this study, a surgical plane of anesthesia was employed for the entire experiment. It may be possible to employ a lower dose of propofol and achieve more stable arterial pressures, but other means of sedation such as a ketamine and propofol with diazepam should be explored. To compensate for the arterial hypotension, phenylephrine and intravenous fluids were given. The intravenous fluids lead to a progressive increase in CVP over the course of the experiment but neither were sufficient to maintain MAP.

The average mean PAP rose significantly in all ten animals during lung injury with no effect on cardiac output. Mean PAP was slightly higher after injury in the TLV group, perhaps related to the higher average dose of oleic acid needed to meet the injury criteria in this group. However, the transition to either ventilation strategy did not further affect the PA pressure in this study.

Limitations of TLV and CMV protocols

The CMV protocol used in the present study was unable to provide adequate respiratory support in this model of severe lung injury model. Based on ARDSnet recommendations, a high frequency, low tidal volume, peak pressure and rate limited strategy was used to minimize ventilator induced lung injury. Adjustments in PEEP were allowed, but increases in PEEP were not associated with increased oxygenation, perhaps because no recruitment maneuvers preceded the increase. Furthermore, since a pressure limited ventilation mode was used in the face of severely impaired lung compliance, any increase to PEEP caused a reduction in the tidal volume achieved with each breath. Therefore, since all animals required maximum respiratory rate, increases in PEEP caused decreased minute ventilation and thus accelerated hypercapnia. This problem may be attributed to severely impaired lung compliance associated with a severe lung injury.

Likewise, the performance of TLV was not optimal in this study. The PFC used (FC77) is a more economical alternative to perflubron with a much higher vapor pressure, which requires more careful monitoring of lung volumes. Secondly, TLV was performed with a respiratory rate of only five/min and expiratory flows were simple square waves. Unlike CMV, expiration is not passive during TLV. The ventilator drives the rate of expiration and must withdraw liquid slow enough to not exceed a critical negative airway pressure (approximately $-15 \text{ cmH}_2\text{O}$)^{10,15,16} at which large airways begin to close and expiration becomes restricted. Despite being non-injurious,¹⁷ more complex, “sculpted” expiratory flow patterns have been developed to optimize expiration and increase minute ventilation. These advances were not applied or needed in the present study. Finally, lung volume measurements are traditionally derived indirectly by measuring body weight as it varies cyclically during TLV, or by maintaining a desired end expiratory pause pressure. Techniques exist to monitor lung volumes and the rate PFC is lost from the ventilator during TLV which may be practical in future clinical applications.^{18,19} No such system was used in the current study and lung volumes were adequately maintained as described. Despite these limitations, TLV was effective in providing gas exchange in the setting of a severe lung injury.

CONCLUSIONS

Total liquid ventilation demonstrated superior gas exchange and reduced mortality when compared to CMV in a sheep model of severe ARDS. Future studies will prolong the duration of TLV to provide support through the reparative phase of ARDS to allow survival and long term outcomes studies.

Acknowledgments

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Figure 1. Liquid Ventilator Schematic

Roller pump (E) provides continuous flow through the recirculation loop (yellow) containing the heater / oxygenator (D) and mixing chamber (C). Inspiration and expiration are driven by the piston (A) through one-way valves (B). Tidal volume is ejected from the piston through the mixing chamber to the subject along the inspiratory pathway (dark blue). Tidal volume is returned from the subject to the piston along the expiratory pathway (light blue) through a bubble trap (F) where exudate from the lungs was sequestered and removed.

Figure 2. Gas Exchange

PaO₂ and PaCO₂ data at baseline (BL), after stabilization of oleic acid injury (OA), and hourly after transition to CMV or TLV. FiO₂ was 1.0 throughout. Asterisks indicate deaths in the CMV group during the 9th, 12th, and 24th hour. All 5 animals in the TLV group survived to 24 hours.

Figure 3. Systemic Hemodynamics

Mean arterial pressure (MAP) and cardiac output data at baseline (BL), after stabilization of oleic acid injury (OA), and hourly after transition to CMV or TLV.

Figure 4. Phenylephrine dose
at baseline (BL), after stabilization of oleic acid injury (OA), and hourly after transition to CMV or TLV.

Figure 5. Pulmonary Hemodynamics

Mean pulmonary artery and central venous pressures are shown at baseline (BL), after stabilization of oleic acid injury (OA), and hourly after transition to CMV or TLV.

Figure 6. Gross Pathology

Representative images of anterior and posterior view of the heart-lung bloc following CMV and TLV.

Figure 7. Lung Histology

Histologic appearance of the lungs of the CMV treated animals (Panel A) as compared to the TLV treated animals (Panel B). Less injury was observed in the TLV treated animals with a reduction in necrosis and hemorrhage (arrows). Hematoxylin and Eosin 200 $\times$ and 400 $\times$.

Table 1
Ventilator settings during total liquid ventilation (TLV) and conventional mechanical ventilation (CMV)

Hour	Respiratory Rate (min^{-1})		Tidal Volume (mL)		Peak Inspiratory Pressure (cm H ₂ O)		Positive End Expiratory Pressure (cm H ₂ O)	
	TLV	CMV	TLV	CMV	TLV	CMV	TLV	CMV
2	5±0	28±4	996±45	348±39	Data not taken	31±2	Data not taken	9±2
4	5±0	32±4	1018±33	300±57		31±2		9±2
6	5±0	32±4	1018±33	313±41		32±1		9±2
8	5±0	36±3	1048±31	297±29		33±1		10±1
10	5±0	35±4	1048±31	301±39		32±1		10±2
12	5±0	32±6	1048±31	342±56		32±1		9±2
14	5±0	33±7	1048±31	307±47		32±1		9±2
16	5±0	33±7	1048±31	300±40		32±1		9±2
18	5±0	40±0	1068±43	250±12		33±1		9±2
20	5±0	40±0	1068±43	260±6		32±1		9±2
22	5±0	40±0	1078±51	253±9		31±1		9±2
24	5±0	40±0	1100±57	295±45		33±3		9±1