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Segmental hemodynamics during partial liquid ventilation in isolated rat lungs

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Abstract

Partial liquid ventilation (PLV) is a means of ventilatory support in which gas ventilation is carried out in a lung partially filled with a perfluorocarbon liquid capable of supporting gas exchange. Recently, this technique has been proposed as an adjunctive therapy for cardiac arrest, during which PLV with cold perfluorocarbons might rapidly cool the intrathoracic contents and promote cerebral protective hypothermia while not interfering with gas exchange. A concern during such therapy will be the effect of PLV on pulmonary hemodynamics during very low blood flow conditions. In the current study, segmental (i.e. precapillary, capillary, and postcapillary) hemodynamics were studied in the rat lung using a standard isolated lung perfusion system at a flow rate of 6 ml/min (~5% normal cardiac output). Lungs received either gas ventilation or 5 or 10 ml/kg PLV. Segmental pressures and vascular resistances were determined, as was transcapillary fluid flux. The relationship between individual hemodynamic parameters and PLV dose was examined using linear regression, with $n = 5$ in each study group. PLV at both the 5 and 10 ml/kg dose produced no detectable changes in pulmonary blood flow or in transcapillary fluid flux (all R^2 values < 0.20). Conclusion: In an isolated perfused lung model of low flow conditions, normal segmental hemodynamic behavior was preserved during liquid ventilation. These data support further investigation of this technique as an adjunct to cardiopulmonary resuscitation.

Keywords

Partial liquid ventilation; Rat lungs; Perfluorocarbon liquid

1. Introduction

Partial liquid ventilation (PLV) is a method of ventilatory support during which mechanical ventilation is carried out in a partially liquid-filled lung. The material most thoroughly studied for this application is perflubron (C₈F₁₇Br, perfluorooctylbromide), a perfluorocarbon liquid capable of carrying enough dissolved oxygen and carbon dioxide to support gas exchange when administered intratracheally in volumes approximating the lung's functional residual capacity. PLV has been the focus of considerable attention as a potential therapy for acute lung injury (ALI) and acute respiratory distress syndrome

(ARDS) because of its ability in laboratory models to improve arterial oxygenation and lung mechanics during acute injury and pulmonary edema [1]. Preclinical evidence also strongly suggests that perfluorocarbons can alter the inflammatory processes underlying ALI and ARDS, in part through interference with transmembrane signaling in inflammatory cells [2,3]. Most recently, this technique has been proposed as an adjunctive therapy for cardiac arrest, during which PLV with cold perfluorocarbons might be given to rapidly cool intrathoracic contents, promoting central and cerebral protective hypothermia[4,5].

A potentially important issue in the delivery of PLV, particularly in the setting of cardiopulmonary resuscitation, is its effect on pulmonary blood flow. In normo-tensive animals, the effects of PLV are complex. The high density of this material (1.92 g/ml) is known to compress dependent pulmonary vessels, leading to a regional change in vascular resistance and redistribution of blood flow in normal sheep [6]. Pulmonary veins would be predicted to be particularly susceptible to this effect, given their thinner vascular walls and lower distending pressures. PLV has been shown by our group to also affect pulmonary blood flow distribution in a model of unilateral lung injury in dogs, a phenomenon which in part may be mediated by regional changes in pulmonary arterial vascular tone [7].

The relative contributions of arterial and venous pulmonary vascular resistance have not been previously reported. There is also little published information about the hemodynamic consequences of PLV during low-flow conditions, although our group has demonstrated that PLV can be tolerated when initiated simultaneously with fluid resuscitation in a model of profound hemorrhage in rats [3].

In addition to segmental hemodynamics, the effect of PLV on transcapillary fluid flux previously has not been reported. PLV is known to diminish the accumulation of radiolabeled, intravascularly delivered albumin in the lung [3,8], which likely results from a general decrease in inflammation during injury. The flux of water into the lung, a phenomenon more closely tied to the development of lung edema than extravasation of relatively large albumin molecules, can be measured in isolated lungs by measuring the rate of weight gain (i.e. extra-vascular fluid accumulation) that occurs following a step increase in pulmonary capillary pressure [9].

If PLV is to further develop as a means of inducing intra-arrest hypothermia, better understanding of its effects on pulmonary blood flow is needed. We therefore examined the effect of PLV on segmental pulmonary vascular hemodynamics using an isolated perfused rat lung system. Specifically interested in the behavior of this system in a low-flow state likely to be encountered during resuscitation from cardiac arrest, we asked the following: Does PLV alter pulmonary vascular resistances in the setting of low cardiac output, and if so, does the effect occur chiefly in the pulmonary arterial or pulmonary venous circulation? If there is a change in vascular resistance during liquid ventilation, is it dose-dependent in the range of PLV doses likely to be employed clinically? Lastly, does PLV impact the rate of transcapillary fluid flux across the pulmonary vascular bed?

2. Methods

The protocol was approved by the institution's committee on the use and care of animals and complied with federally published guidelines. All reagents were purchased from Sigma, St. Louis, MO, unless otherwise stated. Perflubron was purchased from F2 Chemicals, Preston, Lancashire, UK.

2.1. Preparation of isolated lungs

Specific pathogen free male Sprague Dawley rats (300–350 g, Harlan Industries, Indianapolis, IN) were used for all experiments.

Following induction of anesthesia and analgesia with intramuscular ketamine HCl (50 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine HCl (10 mg/kg, Vedco, St. Joseph, MO), a tracheotomy was performed. Animals were mechanically ventilated with room air using a respiratory rate of 60 breaths/min, a tidal volume of 8 ml/kg, and 2 cm H₂O positive end-expiratory pressure (Harvard Apparatus, Model 643, South Natick, MA). The thoracic cavity was entered by first making a transverse abdominal incision at the costal margin. The diaphragm was incised along its ventral insertion and the sternum divided in a caudal-to-cranial fashion. Fifteen units of heparin then were injected into the left ventricle and allowed to circulate for 5 min. After ligating the caudal vena cava, an infusion cannula was placed by incising the wall of the right ventricle and advancing the catheter tip across the pulmonic valve into the main pulmonary artery, taking care to not entrain air into the pulmonary vasculature. A left atrial drainage cannula was placed in a similar fashion through the lateral wall of the left ventricle and retrograde across the mitral valve. Both cannulae were secured with 0–0 silk ties and umbilical tape, using the heart tissue only as a scaffold for securing the infusion and drainage lines.

Upon cannulation, the ventilator gas was switched to a custom mixture approximating alveolar gas (FiO₂ 0.21, FiCO₂ 0.05, FiN₂ 0.74). The trachea, heart, and lungs were then removed en bloc and suspended by the endotracheal tube from a strain gauge (TSD125C, range 0–50 g, Biopac Systems, Santa Barbara, CA) and placed in a humidified chamber (Fig. 1).

2.2. Organ perfusion

Lungs were perfused via an open, recirculating, constant-flow apparatus filled with Earle's balanced salt solution supplemented 4% bovine serum albumin and buffered to a pH of 7.40 with NaHCO₃ (Fig. 1). Manometers (Transpac IV, Abbott Critical Care, North Chicago, IL) were placed in both the infusion and drainage lines and zeroed to the level of the left atrium, allowing continuous monitoring of pulmonary arterial and left atrial pressure. The left atrial cannula drained into a warm water-jacketed reservoir the height of which could be adjusted to control left atrial pressure. Baseline conditions included a flow rate of 6 ml/min (approximately 2 ml/min per kg, 5–10% normal cardiac output [10]) and a left atrial pressure of 1.5–2.0 cm H₂O (hereafter referred to as low P_{LA}). Manometer and strain gauge signals were digitized and recorded using a commercially available system (MP100, Biopac Systems, Santa Barbara, CA).

2.3. Liquid ventilation protocol

Perfused organs were allowed to equilibrate at low P_{LA} for 10 min. (Likewise, following each part of the experimental protocol lungs were allowed to equilibrate for 10 min according to previously published protocols and preliminary observations that hemodynamic parameters uniformly stabilized during this period.) Next, pulmonary arterial and venous pressure measurements were recorded, as was pulmonary capillary pressure (see below). The left atrial reservoir was then raised approximately 10 cm (hereafter referred to as high P_{LA}) and the lungs were allowed to equilibrate. Next, a second set of pressure readings were recorded. These pre-treatment measures were performed to ensure baseline similarities between treatment groups prior to initiation of PLV, and indeed no statistically significant differences between groups were found (data not shown).

The atrial reservoir was then returned to low P_{LA} . Simultaneously, the lungs were either maintained with gas ventilation or received 5 or 10 ml/kg intratracheal perflubron. Room-temperature perflubron was instilled by briefly removing the endotracheal tube from the ventilator circuit and allowing the perflubron to run from the end of a syringe down the side of the endotracheal tube, avoiding the formation of fluid plugs along the large airways. Following instillation of the liquid ventilation agent, lungs were ventilated for 10 min. Measurements were again recorded at low P_{LA} and, 10 min after raising the left atrial reservoir, high P_{LA} . Lungs from any one animal were subjected to only one (i.e. gas or PLV, 5 or 10 ml/kg) ventilation strategy.

3. Measurements

3.1. Determination of capillary pressure and vascular resistances

In this organ perfusion system, occluding the infusion and drainage cannulae ceases flow and causes the pulmonary arterial and left atrial pressures to rapidly equilibrate to capillary pressure [9,11]. With pulmonary arterial (P_{PA}), left atrial (P_{LA}), and pulmonary capillary (P_{CAP}) pressures measured, and perfusate flow (Q , ml/min) fixed, total, pre-, and post-capillary vascular resistances (cm H_2O /ml per min) were calculated as follows:

$$\begin{aligned} R_{TOTAL} &= \frac{(P_{PA} - P_{LA})}{Q} \\ R_{PRECAP} &= \frac{(P_{PA} - P_{CAP})}{Q} \\ R_{POSTCAP} &= \frac{(P_{CAP} - P_{LA})}{Q} \end{aligned}$$

3.2. Determination of capillary filtration coefficient ($K_{f,c}$)

The term $K_{f,c}$ quantifies the rate of fluid flux across a vascular bed for a given transvascular hydrostatic pressure gradient. To measure this flux, continuous measurement of lung weight was made during the high P_{LA} phase of the experiment and $K_{f,c}$ (in mg/min per cm H_2O) calculated as follows:

$$K_{f,c} = \frac{\Delta \text{Weight}}{\Delta \text{Time}} \times \left(\frac{1}{P_{\text{cap, High } P_{LA}} - P_{\text{cap, Low } P_{LA}}} \right),$$

where ΔWeight is the change, in grams, in lung weight in the final 2 min of the high P_{LA} phase, ΔTime is 2 min, and P_{cap} is the measured capillary pressure (in cm H_2O) during high and low P_{LA} conditions.

3.3. Confirmation of physiological vascular reactivity in the model

In separate experiments, isolated lungs were perfused with various concentrations of the prostaglandin H_2 /thromboxane A_2 analog U-46619 (Cayman Chemical, Ann Arbor, MI) to confirm smooth muscle function [12]. Lungs ($n = 8$) received escalating doses were compared to control lungs ($n = 3$) that did not receive the agent perfused for the same period of time. U-46619 at a concentration of 300 nM increased precapillary resistance 182% over control, and at a concentration of 3 μM increased precapillary resistance 458% over control values ($P < 0.05$ for each), indicating arterial smooth muscle responsiveness to a physiological agonist and serving as a positive control for the model system.

3.4. Confirmation of perfusate conditions between groups

As configured, the isolated lung apparatus did not permit the evaluation of gas exchange. (To do so would require the use of a deoxygenator in the perfusion circuit). While the effects of PLV on gas exchange have been considered extensively in previous *in vivo* studies, we felt it important nonetheless to examine perfusate gas tensions, pH, and ionized Ca^{2+} concentrations as a further means of confirming homogeneity between the two groups. At the conclusion of each experiment, perfusate sampled from the reservoir was evaluated using a clinical blood gas analyzer (ABL 606, Radiometer, Copenhagen, Denmark).

4. Data analysis

All values are reported as mean $\pm$ S.D. unless otherwise stated. Segmental pressures and resistances at low and high P_{LA} conditions were analyzed by linear regression against PLV dose using the following equation:

$$\text{Parameter} = b + m(\text{PLV Dose in ml/kg})$$

In this model, the y-intercept b represents the parameter of interest for a lung in which no perflubron is present. The mean and standard error of the mean for b and the coefficient m , as well as the R^2 and P values for the regression were reported. $K_{f,c}$ values for the three PLV doses were compared with analysis of variance, as were perfusate characteristics. All analyses were performed with SAS 8.2 (SAS, Cary, NC).

5. Results

PLV, in either the 5 or 10 ml/kg dose, produced very little effect on pulmonary vascular pressures or vascular resistances. Total pulmonary vascular resistance was inversely proportional to left atrial pressure, an effect dominated by decreases in pulmonary venous resistance in the face of elevated left atrial pressure (Figs. 2 and 3). Linear regression of total pulmonary vascular resistance, as well as segmental pulmonary vascular pressures and resistances revealed no statistically significant relationship to the administered PLV dose (Table 1). Furthermore, transcapillary flux as characterized by $K_{f,c}$ was not significantly altered by liquid ventilation (Table 2). Lastly, comparison of perfusate gas tensions, pH, and $[\text{Ca}^{2+}]$ revealed preservation of normal pH and gas tensions in all study groups (Table 3).

6. Discussion

In the current studies, we found that in doses as high as 10 ml/kg, PLV with perflubron did not adversely affect pulmonary segmental hemodynamics during simulated low cardiac output conditions. These *ex vivo* measurements are in agreement with our previous observation that despite profound hemodynamic compromise *in vivo*, PLV in doses as high as 10 ml/kg are well tolerated in rats [3]. From a pulmonary hemodynamic perspective, PLV with cold perfluorocarbon liquids should be possible during efforts to resuscitate individuals from cardiac arrest.

The isolated rat lung system is a useful means of characterizing a variety of hemodynamic parameters that are difficult if not currently impossible to measure *in vivo* in small or large animal species. As applied in the current study, some of the system's limitations deserve comment. While many of the hemodynamic determinants in isolated perfused rat lungs are independent of scale, some are likely not. In large animal or human applications of PLV, the hydrostatic forces exerted by the perflubron will be greater than those encountered in the rat lung. Radiological studies of humans undergoing liquid ventilation would indicate that following a perflubron dose comparable to the lung's functional residual capacity (corresponding to 10 ml/kg in rats), the hydrostatic pressure exerted by the material may in

some regions may exceed transmural pressure [13]. Given the relatively high density of this material, the compressive effects on dependent pulmonary vessels, particularly the thinner walled venous capacitance vessels, could be hemodynamically significant. The current studies, because of the much smaller size of the rat lung, would be unable to detect these differences.

Another potential limitation of studies in ex vivo perfused lungs is being unable to comment on any contributions of the thoracic wall. Although distensible to a degree, the thoracic cavity can be over pressurized by hyperinflation of the lung. Adding a large volume of perfluorocarbon to the lung while continuing positive pressure mechanical ventilation, as would theoretically occur during cardiopulmonary resuscitation, risks compromising venous return and reducing cardiac output. Such effects would not be detectable in hemodynamic measurements made ex vivo but are a real concern for the use of this technology during cardiac arrest. PLV delivered during shock or cardiac arrest would likely require pressure, rather than volume, controlled ventilation or at least continuous monitoring of end-expiratory pressure.

In the current studies an FiO_2 of 0.21 was used, which could differ from clinical application, where higher fractions of inspired oxygen might be employed. Regional oxygen tension may have significant effects on local pulmonary blood flow through the hypoxic pulmonary vasoconstriction process. While the effect that PLV has upon regional oxygen partial pressure is not fully understood, other animal studies have indicated that compressive effects are probably more important [6].

Lastly, this work determined $K_{f,c}$ only in normal lungs undergoing liquid ventilation. Previous work by our group suggests that in acutely injured lungs, lung water (as measured by [^{17}O] positron emission tomography), is reduced during PLV [14]. In the current studies, $K_{f,c}$ was normal in gas and liquid-ventilated lungs. It is possible that PLV might alter $K_{f,c}$ in acutely injured organs. Although acute lung injury or ARDS are not present in the majority of cardiac arrests (with the exception perhaps of arrests occurring at the end of severe illness in an intensive care unit), our studies do not rule out the possibility that PLV might have clinically useful effects on capillary permeability in some settings, and the issue merits further study.

Our results indicate clinically negligible hemodynamic effects of PLV in the setting of low blood flow in the rat lung. These findings serve as further proof-of-concept that PLV during shock or cardiac arrest is feasible and warrants further investigation.

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Fig. 1. Isolated lung apparatus. *Upper panel:* Isolated, mechanically ventilated rat lungs were suspended from a strain gauge and instrumented for measurement of pulmonary arterial and venous pressure. Venous drainage from the left atrium flowed into a reservoir the height of which could be adjusted to vary left atrial pressure (P_{LA}) in a controlled fashion. See text for additional technical details. *Lower Panel:* Rat lungs immediately prior to study. Cannulae were secured across the pulmonic and mitral valves into the pulmonary artery and left atrium, respectively

Fig. 2.

Effect of partial liquid ventilation (PLV) on segmental pulmonary vascular pressures. Intratracheal perflubron doses as high as 10 ml/kg did not significantly change pulmonary vascular pressures during perfusion at low or elevated left atrial pressures ($n = 5$ for each perfluorocarbon dose, see text and Table 1 for details of analysis).

Fig. 3.

Effect of partial liquid ventilation (PLV) on total and segmental pulmonary vascular resistance. Intratracheal perflubron doses as high as 10 ml/kg did not significantly change segmental pulmonary vascular resistances during perfusion at low or elevated left atrial pressures ($n = 5$ for each perfluorocarbon dose, see text and Table 1 for details of analysis).

Table 1

Regression analysis of segmental hemodynamics during PLV

	Low left atrial pressure			High left atrial pressure		
	y-intercept	PFC dose coefficient	R ²	y-intercept	P value	P value
<i>Pressure (cm H₂O)</i>						
Pulmonary artery	10.8±0.7	-0.2 ± 0.1	0.13	20.7±0.9	0.18	0.47
Pulmonary capillary	6.0±0.4	-0.1±0.1	0.06	16.3±0.7	0.39	0.20
<i>Resistance (cm H₂O/ml per min)</i>						
Total	1.5±0.2	-0.0 ± 0.0	0.14	0.9±0.1	0.18	0.81
Precapillary	0.8±0.1	-0.0 ± 0.0	0.05	0.8±0.1	0.44	0.75
Postcapillary	0.7±0.1	-0.1±0.0	0.18	0.2±0.1	0.12	0.43

Table 2

Capillary filtration coefficient during PLV

	<u>Perflubron dose</u>			<i>P</i> value
	0 ml/kg	5 ml/kg	10 ml/kg	
$K_{f,x}$ (mg/min per cm H ₂ O)	8.2±5.4	6.9±4.5	6.1±4.8	0.82

Table 3

Perfusate characteristics at the conclusion of the experiment

Perfusate parameter	Perflubron dose			<i>P</i> value
	0 ml/kg	5 ml/kg	10 ml/kg	
pH	7.34±0.05	7.36±0.08	7.36±0.03	0.73
pO ₂ (mmHg)	163±5	166±5	163±2	0.75
pCO ₂ (mmHg)	36±3	35±4	34±3	0.24
[HCO ₃ ⁻] (mEq/l)	19.1±1.3	19.1±1.3	18.6±1.0	0.49
iCa ²⁺ (mEq/l)	1.75±0.08	1.78±0.06	1.70±0.06	0.38

Results reported as mean±S.D. *N*>5 in each group. *P* value reflects results of general linear modeling.