Effects of Vaporized Perfluorocarbon on Pulmonary Blood Flow and Ventilation/Perfusion Distribution in a Model of Acute Respiratory Distress Syndrome

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Background: Perfluorocarbon (PFC) liquids are known to improve gas exchange and pulmonary function in various models of acute respiratory failure. Vaporization has been recently reported as a new method of delivering PFC to the lung. Our aim was to study the effect of PFC vapor on the ventilation/perfusion ($\dot{V}_A/\dot{Q}$) matching and relative pulmonary blood flow ($\dot{Q}_{rel}$) distribution.

Methods: In nine sheep, lung injury was induced using oleic acid. Four sheep were treated with vaporized perfluorohexane (PFX) for 30 min, whereas the remaining sheep served as control animals. Vaporization was achieved using a modified isoflurane vaporizer. The animals were studied for 90 min after vaporization. $\dot{V}_A/\dot{Q}$ distributions were estimated using the multiple inert gas elimination technique. Change in $\dot{Q}_{rel}$ distribution was assessed using fluorescent-labeled microspheres.

Results: Treatment with PFX vapor improved oxygenation significantly and led to significantly lower shunt values ($P <$ 0.05, repeated-measures analysis of covariance). Analysis of the multiple inert gas elimination technique data showed that animals treated with PFX vapor demonstrated a higher $\dot{V}_A/\dot{Q}$ heterogeneity than the control animals ($P <$ 0.05, repeated-measures analysis of covariance). Microsphere data showed a redistribution of $\dot{Q}_{rel}$ attributable to oleic acid injury. $\dot{Q}_{rel}$ shifted from areas that were initially high-flow to areas that were initially low-flow, with no difference in redistribution between the groups. After established injury, $\dot{Q}_{rel}$ was redistributed to the nondependent lung areas in control animals, whereas $\dot{Q}_{rel}$ distribution did not change in treatment animals.

Conclusion: In oleic acid lung injury, treatment with PFX vapor improves gas exchange by increasing $\dot{V}_A/\dot{Q}$ heterogeneity in the whole lung without a significant change in gravitational gradient.

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A NUMBER of studies have demonstrated the ability of total liquid ventilation and partial liquid ventilation (PLV) with perfluorocarbon (PFC) liquids to improve gas exchange and pulmonary function in various animal models of acute respiratory failure.[1-4] These findings were confirmed in preliminary clinical trials.[5-7] Several, not mutually exclusive mechanisms of action of PFC are likely to be responsible for the observed improvements[8-11]: the high oxygen carrying capacity of PFC liquids, effects on surface tension at the air–liquid and liquid–tissue interfaces, antiinflammatory effects, and effects on pulmonary ventilation ($\dot{V}_A$) and perfusion ($\dot{Q}$) distributions.

Vaporization is a new delivery technique for PFC, recently reported by Bleyl *et al.*[12] The authors found distinct positive effects on the arterial oxygen partial pressure (Pa_{O_2}), lung compliance, and physiological shunt in an ovine model of adult respiratory distress syndrome (ARDS). Vaporization was achieved using perfluorohexane (PFX) in an anesthesia machine with modified bypass vaporizers. One advantage of this application technique compared with PLV is that the concentration of PFC delivered can be easily adjusted during administration without disconnecting the ventilator.

The aims of this study were twofold. First, we hoped to replicate the positive results obtained by Bleyl *et al.*[12] by vaporizing PFX in a sheep model of oleic acid (OA)–induced lung injury. Second, we hoped to shed light on the proposed mechanisms of the effects of vaporized PFC. Specifically, we hypothesized that vaporized PFX redistributes pulmonary blood flow to better ventilated lung regions, thus improving the matching of ventilation to perfusion.

Materials and Methods

The study was approved by the University of Washington Animal Care Committee (Seattle, Washington), and National Institutes of Health (Bethesda, Maryland) guidelines for animal use and care were followed throughout.

Animal Preparation and Experimental Protocol

The experiments were performed on nine adult sheep, ranging in weight from 18.2 to 33.6 kg. The animals were premedicated with 0.6 mg/kg xylazine (Phoenix Pharmaceutical, Inc., St. Joseph, MO), anesthetized with 20 mg/kg thiopental sodium (Abbott Laboratories, North Chicago, IL), intubated, and ventilated with a Servo 900

C ventilator (Siemens, Solna, Sweden). Volume-controlled intermittent positive pressure ventilation was initiated with a tidal volume of 12 ml/kg, a respiratory frequency of 26 breaths/min, a positive end-expiratory pressure (PEEP) of 5 cm H_2O, an inspiratory oxygen concentration (F_{IO_2}) of 1.0, and an inspiratory/expiratory ratio of 1:1. Anesthesia was maintained using a continuous infusion of thiopental sodium (mean infusion rate between 24.9 ± 3.1 and 59.9 ± 8.1 mg $\cdot$ kg$^{-1} \cdot$ h^{-1}). A cuffed endotracheal tube (Rüsch; Waiblingen, Germany) was inserted *via* a tracheotomy. A catheter was placed in a femoral artery to monitor systemic arterial pressure and to draw blood gas and inert gas samples. A No. 7 French Swan-Ganz thermodilution catheter (Baxter, Irvine, CA) was advanced into the pulmonary artery *via* the right external jugular vein to measure pulmonary arterial pressure, pulmonary capillary wedge pressure, and temperature and for blood sampling. A femoral venous catheter was inserted for infusion of anesthetic drugs and maintenance fluids. A central venous catheter was inserted through the left external jugular vein and used as the injection port for OA and microspheres. After the end of instrumentation, the animals were turned to prone position. The animals were paralyzed thereafter with pancuronium (0.1 mg/kg) (Ohmeda PPD Inc., Liberty Corner, NJ), which was administered in regular intervals throughout the experiment. Airway pressures, arterial pressure, and pulmonary arterial pressure were measured continuously with amplifiers (Validyne, Northridge, CA) and recorded on a Mark12 data management system (Model DMS 1000; Western Graphtec, Irvine, CA). The end-tidal carbon dioxide partial pressure (PET_{CO_2}) was measured with a mass spectrometer (medical gas analyzer Model MGA-1100; Perkin-Elmer, Pomona, CA). Cardiac outputs (thermodilution technique) and blood temperatures were measured with a cardiac output computer (Model Sat-2; Baxter Edwards Irvine, CA). Arterial and venous pH, oxygen partial pressures, and carbon dioxide partial pressures were measured with a blood gas analyzer (Radiometer Model ABL 330; Acid Base Laboratory, Copenhagen, Denmark) and corrected for temperature. The partial pressure of alveolar oxygen (PA_{O_2}) was derived from

$$PA_{O_2} = P_B \cdot F_{IO_2} - 47 - PET_{CO_2},$$

where P_B is the barometric pressure. Physiologic oxygen-shunt fraction ($\dot{Q}_{sp}/\dot{Q}_t$) was calculated using the Berggren shunt equation,

$$\dot{Q}_{sp}/\dot{Q}_t = (Cc'_{O_2} - CA_{O_2})/(Cc'_{O_2} - C\bar{v}_{O_2}),$$

where $\dot{Q}_{sp}$ is the physiologic shunt, $\dot{Q}_t$ is the total cardiac output, Cc'_{O_2} is the capillary oxygen content (assuming equilibration with PA_{O_2}), CA_{O_2} is the arterial oxygen content, and $C\bar{v}_{O_2}$ is the mixed venous oxygen content. The oxygen contents were estimated from the subrou-

tines of Olszowka and Farhi.[13] Static lung-chest compliance (C_{RS}) was calculated using

$$C_{RS} = V_t/(P_{plat} - PEEP) \cdot kg^{-1},$$

where V_t is the tidal volume and P_{plat} is the end-inspiratory plateau pressure during pressure hold.

After an initial stabilization period during which the respiratory minute volume was adjusted to maintain Pa_{CO_2} between 36 and 46 mmHg, baseline measurements (t_{base}) were recorded. Lung injury was induced by injecting 0.1 ml/kg OA ($C_{17}H_{33}COOH$; Sigma Chemical Company, St. Louis, MO). During the injection of OA, 500 ml hydroxyethyl starch, 6%, was given to maintain blood pressure. Severe lung injury was considered established (t_{inj}) when the clinical criteria for the definition from the American/European consensus conference on ARDS were fulfilled (ratio Pa_{O_2} to $F_{IO_2} < 200$, pulmonary capillary wedge pressure < 19 mmHg).[14] If Pa_{O_2} stabilized before reaching the criteria for ARDS, an additional 0.02 ml/kg OA was given. The mean amount of injected OA was 0.12 ± 0.02 (SD) ml/kg per animal with no differences between the groups ($P = 0.6$). The lungs of animals in the treatment group (n = 4) were then ventilated for 30 min with approximately 20 vol% vaporized PFX (ABCR, Karlsruhe, Germany), whereas the ventilation protocol of the control animals (n = 5) was not changed. All animals were studied for 120 min after the injury was established.

Vaporized Perfluorocarbon

All experiments were performed using PFX with a purity of 95%. Its chemical and physical properties and similarity to volatile anesthetics are described elsewhere.[12] PFX was vaporized using a modified vaporizer for Servo ventilators (Isoflurane Vaporizer Model 952; Siemens-Elema, Solna, Sweden). The concentration of the vaporized PFX in volume percent was monitored continuously using an Amis Model 2000 mass spectrometer system (Innovision A/S, Odense, Denmark) with a sampling time and frequency of 8.33 ms and 40 kHz, respectively. The Amis Model 2000 system was calibrated for an atomic mass peak of 69.13 and 25 vol% using the vapor in the head space of a sealed PFX bottle at room temperature (22.5°C). This PFX vapor concentration was calculated with the Clausius-Clopeyron equation $P = A \cdot e^{-B \cdot T}$, where P is vapor pressure in millimeters of mercury, T is temperature in Kelvin, and A and B are molecule-specific constants. A and B were derived using the vapor pressure (177 mmHg at 20°C) and boiling point (57°C) of PFX.

Inert Gas Measurements

The multiple inert gas elimination technique was used to assess gas exchange[15,16] at five time points during the experiments: t_{base}, t_{inj}, and 30, 60, and 120 min after injury (t_{30}, t_{60}, and t_{120}, respectively). A dilute solution of

six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) dissolved in dextrose, 5%, was infused and allowed to equilibrate in the animal for at least 30 min before baseline samples were drawn. Inert gas partial pressures were measured in arterial (Pa) and mixed venous (P$\bar{v}$) blood and in mixed expired gas (P$\bar{E}$). The mixed expired gas samples were collected from a heated Plexiglas mixing chamber that was fitted to the expiratory limb of the ventilator. All samples were collected simultaneously and in duplicate. Exhaled gas specimens were maintained at more than 40°C before analysis to avoid condensation and loss of highly soluble gases. The concentrations of inert gases in the gas samples were measured using a gas chromatograph (Varian, Walnut Creek, CA) equipped with a flame ionization detector and an electron capture detector. Because the high PFX concentration in the gas samples at t_{30} influenced the measured concentrations of the inert gases, P$\bar{E}$ at this time point was calculated from Pa, P$\bar{v}$, $\dot{Q}_t$, and $\dot{V}_E$, where $\dot{V}_E$ is the minute ventilation. The double extraction method of Wagner *et al.*[17] was used to determine the concentration of the inert gases in the blood samples.

Gas exchange was assessed by changes in $\dot{V}_A$ and $\dot{Q}$ distributions predicted by the 50-compartment model and by dispersion indices and arterial-alveolar difference ([a − A]D) areas derived from retention (R) and excretion (E) data. Inert gas shunt ($\dot{Q}_S/\dot{Q}_T$), inert gas dead space ($\dot{V}_D/\dot{V}_T$), and percentage of $\dot{V}_A$ and $\dot{Q}$ to regions with a different $\dot{V}_A/\dot{Q}$ ratio were calculated from the 50-compartment model.[15,16] Because OA lung injury primarily results in increases in shunt, and low $\dot{V}_A/\dot{Q}$ units are infrequently observed, the percentage of $\dot{Q}$ to shunt and low $\dot{V}_A/\dot{Q}$ units was grouped together to indicate $\dot{Q}$ to injured lung.[18] The 50-compartment distributions of $\dot{V}_A$ and $\dot{Q}$ were divided into five regions for further analysis: (1) inert gas shunt ($\dot{V}_A/\dot{Q} < 0.01$), (2) low $\dot{V}_A/\dot{Q}$ regions ($\dot{V}_A/\dot{Q} = 0.01–1$), (3) midrange $\dot{V}_A/\dot{Q}$ regions ($\dot{V}_A/\dot{Q} = 1–10$), (4) high $\dot{V}_A/\dot{Q}$ regions ($\dot{V}_A/\dot{Q} = 10–100$), and (5) inert gas dead space ($\dot{V}_A/\dot{Q} > 100$). The R and E components of the inert gas ([a − A]D) area[19] and the parameters of dispersion (DISP) adapted from Gale *et al.*[20] (DISP$_{R* − E*}$, DISP$_{R*}$, and DISP$_{E*}$) were calculated from direct analysis of R and E data.[21] The DISP parameters, corrected for shunt and dead space, and [a − A]D areas are derived from the difference between heterogeneous and homogeneous R and E curves. DISP$_{R*}$ and R[a − A]D are parameters of $\dot{Q}$ distribution, and DISP$_{E*}$ and E[a-A]D are parameters of $\dot{V}_A$ distribution. Increases in any of the above parameters are indicative of increased $\dot{V}_A/\dot{Q}$ heterogeneity.

Determination of Pulmonary Blood Flow

Details of the methods are described elsewhere.[22] Five fluorescent polystyrene microspheres (red, orange, blue green, yellow green, and crimson) of 15-μm diameter (Molecular Probes, Eugene, OR) were used in a randomized order to measure regional pulmonary blood flow. The time points of injection were t_{base}, t_{inj}, t_{30}, t_{60}, and t_{120}. The microspheres (1.5×10^6) were vortexed, then sonicated for 90 s and injected over a period of 30 s, followed by a saline flush.

After completion of the study, the animals were given 1,000 U/kg heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) and 3 mg/kg papaverine hydrochloride (American Regent Laboratories, Inc., Shirley, NY), exsanguinated, and sternotomized. The lungs were flushed *via* the main pulmonary artery with 50 ml/kg dextran, 2% (Sigma), removed, inflated to 30 cm H$_2$O, punctured, and dried with warm air for 7 days. When dry, the lungs were coated with a one-component polyurethane foam (DAP Inc., Dayton, OH), suspended vertically in a square box, and embedded in rapidly setting urethane foam (2 pounds, Polyol and Isocyanate; International Sales, Seattle, WA). The foam block was cut into slices approximately 1.2 cm thick. With the use of a 12-mm-diameter core, the slices were sampled. Cores were obtained in a rigid X-Y grid system, with 2 cm between the centers of adjacent cores. The height of every core was measured using a caliper, and the volume was calculated. Two hundred one ± 47 (SD) samples were obtained from each sheep lung after discarding samples with airways occupying more than 25% of the core's volume. The average volume of the samples was 1.35 ± 0.23 cm^3 (SD). The samples were soaked for 7 days in 3 ml 2-ethoxyethyl acetate (Aldrich Chemical Co., Milwaukee, WI). The fluorescence was read in a luminescence spectrophotometer (Model LS-50B; Perkin-Elmer, Beaconsfield, Buckinghamshire, UK) fitted with a flow cell and a red-sensitive photomultiplier tube. The volume-normalized relative blood flow ($\dot{Q}_{rel,i}$) of the piece i at every time point was calculated:

$$\dot{Q}_{rel,i} = \frac{x_i}{(\Sigma x_i)/n}$$

where x_i is the obtained fluorescence divided by the volume of the piece (in cubic centimeters), and n is the number of pieces of the lung. Therefore, the mean normalized relative flow was 1.0.

Statistics

The values are reported as means ± SD. We used the unpaired *t* test to compare physiological parameters between the control and treatment groups at t_{base} (preinjury) and at t_{inj} before treatment. We used repeated measures analyses of covariance (RANCOVA) to determine whether values of a given variable differed between the treatment and control groups. The measurements taken after the treatment had begun (and after

Table 1. Respiratory Data

Variable	Group	Baseline*	Injury*	t_{30}	t_{60}	t_{90}	t_{120}	Overall Mean† P	Time Pattern‡ P
				Postinjury Time Points				RANCOVA	
Pao_2 (mmHg)	CTL	620 ± 17	102 ± 42	71 ± 21	65 ± 18	59 ± 15	57 ± 18		
	PFX	619 ± 18	129 ± 53	158 ± 101	291 ± 176	305 ± 183	338 ± 187	0.03	< 0.001
$Paco_2$ (mmHg)	CTL	37 ± 3	50 ± 7	55 ± 13	64 ± 16	66 ± 24	72 ± 28		
	PFX	39 ± 6	49 ± 12	51 ± 15	68 ± 35	71 ± 36	70 ± 30	0.8	0.7
PIP (cm H_2O)	CTL	20 ± 2	39 ± 2	44 ± 6	43 ± 6	44 ± 6	46 ± 6		
	PFX	19 ± 3	32 ± 3	36 ± 9	39 ± 12	36 ± 11	36 ± 10	0.8	1.0
C_{RS}	CTL	1.04 ± 0.19	0.56 ± 0.08	0.46 ± 0.07	0.43 ± 0.10	0.42 ± 0.09	0.41 ± 0.09		
($l \cdot cm\ H_2O^{-1} \cdot kg^{-1}$)	PFX	1.74 ± 1.03	0.70 ± 0.14	0.68 ± 0.19	0.62 ± 0.19	0.63 ± 0.19	0.61 ± 0.17	0.2	1.0
O_2 shunt (%)	CTL	3.7 ± 1.5	36.2 ± 7.1	51.8 ± 10.2	52.4 ± 12.5	58.7 ± 15.1	62.4 ± 22.1		
	PFX	4.1 ± 1.6	34.4 ± 13.0	36.0 ± 26.4	33.6 ± 30.8	30.7 ± 25.8	28.9 ± 25.5	0.02	1.0

Values are mean ± SD.

* Unpaired t test yielded significant differences for the PIP at t_{inj} ($P = 0.04$). All other parameters were not significantly different.   † Null hypothesis: the overall mean from the beginning of treatment to t_{120} (averaged across all time points) is the same in both groups.   ‡ Null hypothesis: the mean difference between both groups does not vary across time points (from the beginning of treatment to t_{120}).

RANCOVA = repeated-measures analysis of covariance; Pao_2 = arterial partial pressure of oxygen; $Paco_2$ = arterial partial pressure of carbon dioxide; PIP = peak inspiratory airway pressure; C_{RS} = static lung/chest compliance; CTL = control group; PFX = treatment group.

injury) were the repeated values of the dependent variable in the RANCOVA. For respiratory and cardiovascular variables, we used observations at 10, 20, and 30 min after t_{inj} and every 15 min from 45 to 120 min after t_{inj} as dependent variables in the RANCOVA analysis. For inert gas variables and pulmonary blood flow data, we used the observations at 30, 60, and 120 min after t_{inj} as the dependent variables. For economy of space, table 1 presents means only at selected times. For each response, the measurement taken after injury and before treatment had begun was used as a covariate (independent variable) in the RANCOVA. The use of the injury value as a covariate has the effect of comparing the groups on changes after injury, with injury considered as the starting point for each animal. Because the several animals start off after injury with varying values of the response variable, the treatment effect is best measured while controlling for this starting value. Without the covariate, treatment differences might simply be due to mean differences in the starting (injury) value of the response variable. The RANCOVA tests three null hypotheses of interest (H01, H02, H03) as follows. (1) The mean of the combined groups is constant after injury (H01). (2) The time patterns are parallel between groups (*i.e.*, the mean difference between the two groups is constant over all time points after injury) (H02). In the RANCOVA model this hypothesis is tested using an interaction term between time and treatment group. (3) The two groups have the same overall mean, where the mean is taken across all observation times after injury, once treatment has been initiated (H03). In the RANCOVA model, this is the main effect of the treatment term. Only hypotheses H02 and H03 involve treatment effects and are reported. Throughout the present study, $P < 0.05$ is used to designate statistical significance.

Results

The criteria for ARDS were attained after a mean time of 88 ± 41 (SD) min after the injection of OA with no differences between the groups ($P = 0.2$).

Respiratory and Hemodynamic Data

The analysis of the hemodynamic parameters heart rate, cardiac output, systemic arterial pressure, and pulmonary arterial pressure did not yield any statistically significant differences between the groups at the time points t_{base}, t_{inj}, and thereafter (data not shown). The respiratory data and the oxygen-shunt values are presented in table 1. The Pao_2 diverged significantly over time when comparing the two groups resulting in a significantly higher overall Pao_2 in the PFX group. The $C_{R/S}$ decreased in both groups over time (not significant). The time pattern in oxygen-shunt did not differ significantly between the groups, but the overall mean values were significantly lower in the PFX group when compared with control animals.

Inert Gas Exchange

In one animal of the control group, no inert gas data could be obtained because of a malfunction of the gas chromatograph. The $\dot{V}_A$ and $\dot{Q}$ distributions to areas with a different $\dot{V}_A/\dot{Q}$ ratio are shown in table 2. Analysis of the $\dot{V}_A$ distribution showed that the $\dot{V}_A$ increased significantly in areas with a high $\dot{V}_A/\dot{Q}$ ratio in the PFX group when compared with the control group. Treatment with PFX vapor also shifted the $\dot{V}_A$ distribution toward areas with a low $\dot{V}_A/\dot{Q}$ ratio, although the comparison of the overall means of the two groups did not yield a statistically significant difference. The changes in inert gas shunt were comparable with the values obtained from the oxygen-shunt calculation.

Table 2. Inert Gas Exchange (I)

	$\dot{Q}$ or $\dot{V}_A$	Group	Baseline*	Injury*	Postinjury Time Points t_{30}	t_{60}	t_{120}	RANCOVA Overall Mean† P	Time Pattern‡ P
Shunt	$\dot{Q}$	CTL	0.7 ± 0.8	36.4 ± 8.9	46.5 ± 10.6	46.6 ± 3.1	53.2 ± 13.6		
($\dot{V}_A/\dot{Q} < 0.01$)		PFX	1.7 ± 1.8	35.3 ± 13.9	34.3 ± 20.4	29.1 ± 25.4	26.7 ± 26.7	0.01	0.3
Low $\dot{V}_A/\dot{Q}$	$\dot{Q}$	CTL	69.7 ± 15.6	18.3 ± 14.4	16.3 ± 10.4	15.6 ± 11.4	11.3 ± 4.9		
		PFX	81.9 ± 9.2	28.8 ± 17.4	34.2 ± 6.8	39.9 ± 24.1	43.1 ± 21.8	0.1	0.4
	$\dot{V}_A$	CTL	17.6 ± 2.3	5.2 ± 6.0	4.5 ± 5.7	2.8 ± 3.2	1.8 ± 1.5		
		PFX	11.2 ± 9.5	7.8 ± 5.6	17.3 ± 21.1	25.3 ± 22.4	25.8 ± 26.7	0.1	0.02
Midrange $\dot{V}_A/\dot{Q}$	$\dot{Q}$	CTL	28.6 ± 15.8	44.3 ± 13.6	36.9 ± 12.0	36.9 ± 11.9	32.1 ± 13.1		
		PFX	16.2 ± 8.9	35.4 ± 23.1	28.4 ± 30.5	28.3 ± 20.4	28.3 ± 15.1	0.9	0.9
	$\dot{V}_A$	CTL	14.8 ± 7.5	28.1 ± 10.4	26.8 ± 12.1	25.2 ± 12.8	21.7 ± 8.8		
		PFX	11.2 ± 9.5	22.8 ± 19.4	17.3 ± 21.1	25.2 ± 22.4	25.8 ± 26.6	0.1	0.2
High $\dot{V}_A/\dot{Q}$	$\dot{Q}$	CTL	1.4 ± 1.3	0.6 ± 1.0	0.3 ± 0.4	1.0 ± 1.1	3.3 ± 4.7		
		PFX	1.6 ± 1.3	0.5 ± 0.7	3.0 ± 2.6	2.7 ± 2.0	1.9 ± 2.1	0.5	0.3
	$\dot{V}_A$	CTL	17.0 ± 4.2	3.3 ± 4.3	4.0 ± 4.4	6.1 ± 4.3	10.8 ± 8.2		
		PFX	18.7 ± 8.0	11.7 ± 13.6	25.0 ± 13.6	20.7 ± 2.9	16.5 ± 7.4	0.02	0.02
Dead space	$\dot{V}_A$	CTL	49.3 ± 5.9	63.4 ± 7.5	64.7 ± 7.4	66.0 ± 9.2	65.8 ± 5.5		
($\dot{V}_A/\dot{Q} > 100$)		PFX	48.8 ± 7.1	57.7 ± 16.3	57.7 ± 12.3	48.6 ± 22.2	50.0 ± 14.8	0.2	1.0

Values are mean ± SD.

* Unpaired *t* test yielded no statistically significant differences between the two groups.   † Null hypothesis: the overall mean from the beginning of treatment to t_{120} (averaged across all time points) is the same in both groups.   ‡ Null hypothesis: the mean difference between both groups does not vary across time points (from the beginning of treatment to t_{120}).

$\dot{Q}$ = perfusion; $\dot{V}_A$ = ventilation; $\dot{V}_A/\dot{Q}$ = ventilation/perfusion ratio; RANCOVA = repeated measures analysis of covariance; CTL = control group; PFX = treatment group. $\dot{Q}$ or $\dot{V}_A$ given as percent of total $\dot{Q}$ or $\dot{V}_A$.

The dispersion indices and the [a − A]D areas derived from the R and E data are shown in table 3. Statistically, the values did not differ significantly between the groups at t_{base} and at t_{inj}, but were consistently somewhat higher in the PFX group than in the control group. All six parameters of heterogeneity continued to increase after t_{inj} as is typically seen in pulmonary edema. RANCOVA analysis of the dispersion indices and the [a − A]D areas (from 10 min after t_{inj} to t_{120}) yielded significant higher overall means in the PFX group. The overall increase in heterogeneity in the PFX group was probably due to both the increase in $\dot{V}_A$ heterogeneity and the increase in $\dot{Q}$ heterogeneity.

Pulmonary Blood Flow

The injection of OA changed significantly the pattern of $\dot{Q}_{rel}$ at t_{inj} compared with t_{base}. $\dot{Q}_{rel}$ was redistributed from pieces that were high-flow at t_{base} to pieces that were low-flow at t_{base} (fig. 1). The mean slope of the change in $\dot{Q}_{rel}$ ($\dot{Q}_{rel}$ at t_{inj} minus $\dot{Q}_{rel}$ at t_{base} *vs.* $\dot{Q}_{rel}$ at t_{base}) was −0.50 ± 0.29 and significantly different from 0 ($P < 0.001$; one-sample, two-tailed *t* test). This slope

Table 3. Inert Gas Exchange (II)

Variable	Group	Baseline*	Injury*	Postinjury Time Points t_{30}	t_{60}	t_{120}	RANCOVA Overall Mean† P	Time Pattern‡ P
[a-A]D area	CTL	0.618 ± 0.21	0.236 ± 0.16	0.404 ± 0.15	0.489 ± 0.29	0.469 ± 0.21		
	PFX	0.720 ± 0.21	0.513 ± 0.46	1.112 ± 0.32	1.092 ± 0.36	1.034 ± 0.12	0.008	0.8
E[a-A]D area	CTL	0.422 ± 0.16	0.126 ± 0.10	0.206 ± 0.11	0.254 ± 0.16	0.234 ± 0.13		
	PFX	0.465 ± 0.17	0.318 ± 0.30	0.543 ± 0.22	0.519 ± 0.18	0.502 ± 0.13	0.08	0.7
R[a-A]D area	CTL	0.196 ± 0.06	0.094 ± 0.05	0.198 ± 0.10	0.235 ± 0.14	0.238 ± 0.11		
	PFX	0.254 ± 0.13	0.195 ± 0.17	0.569 ± 0.17	0.572 ± 0.20	0.531 ± 0.17	0.001	0.9
DISP$_{R^*-E^*}$	CTL	15.95 ± 4.9	6.71 ± 4.3	10.39 ± 3.6	12.28 ± 6.8	13.24 ± 6.0		
	PFX	19.57 ± 8.5	12.8 ± 10.9	27.39 ± 11.6	27.52 ± 11.6	25.65 ± 4.5	0.02	0.7
DISP$_{E^*}$	CTL	11.52 ± 3.7	3.83 ± 2.7	5.86 ± 2.4	7.10 ± 4.0	6.86 ± 4.1		
	PFX	13.60 ± 5.5	8.63 ± 7.8	15.35 ± 6.3	15.33 ± 6.1	14.53 ± 3.1	0.06	1.0
DISP$_{R^*}$	CTL	6.11 ± 1.9	3.50 ± 2.0	5.68 ± 2.6	6.66 ± 3.9	7.75 ± 3.4		
	PFX	8.25 ± 4.5	5.73 ± 4.5	15.71 ± 6.0	16.25 ± 7.6	14.75 ± 5.1	0.006	0.06

Values are mean ± SD.

* Unpaired *t* test yielded no statistically significant differences between the two groups.   † Null hypothesis: the overall mean from the beginning of treatment to t_{120} (averaged across all time points) is the same in both groups.   ‡ Null hypothesis: the mean difference between both groups does not vary across time points (from the beginning of treatment to t_{120}).

RANCOVA = repeated measures analysis of covariance; [a-A]D area = inert gas arterial-alveolar difference area; E[a-A]D = excretion component of [a-A]D; R[a-A]D = retention component of [a-A]D; DISP$_{R^*-E^*}$ = retention minus excretion index of dispersion, corrected for shunt and dead space; DISP$_{E^*}$ = excretion index of dispersion, corrected for dead space; DISP$_{R^*}$ = retention index of dispersion, corrected for shunt; CTL = control group; PFX = treatment group.
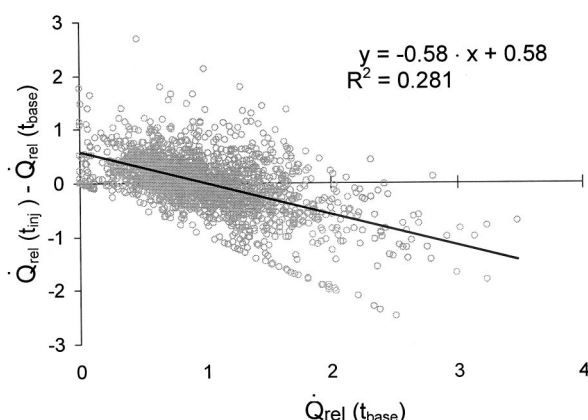
Fig. 1. Oleic acid–induced change in $\dot{Q}_{rel}$ distribution. Plot shows pooled lung pieces of all animals. $\dot{Q}_{rel}$ decreased in pieces with initial high flows and increased in pieces with initial low flows, suggesting that high-flow pieces were more injured. $\dot{Q}_{rel}$ (t_{base}) = relative pulmonary blood flow at baseline; $\dot{Q}_{rel}$ (t_{inj}) = relative pulmonary blood flow at injury.

was approximately the same in both groups ($P = 0.2$). These changes in $\dot{Q}_{rel}$ are also substantially larger than random variation observed over time.[23]

Spatial analysis of the $\dot{Q}_{rel}$ distributions after t_{inj} showed differences between the groups: In the control group, the mean slope of $\dot{Q}_{rel}$ *versus* the dorsal-to-ventral axis became more negative, showing that $\dot{Q}_{rel}$ was redistributed from dependent, ventral areas to nondependent, dorsal areas (mean slope at t_{inj}: -0.031 ± 0.046; mean slope at t_{30}: -0.06 ± 0.03; mean slope at t_{60}: -0.081 ± 0.066; and mean slope at t_{120}: -0.10 ± 0.057). In contrast, the mean slope of $\dot{Q}_{rel}$ *versus* the dorsal-to-ventral axis remained almost unchanged in the PFX group (mean slope at t_{inj}: -0.024 ± 0.079; mean slope at t_{30}: -0.019 ± 0.068; mean slope at t_{60}: -0.033 ± 0.071; and mean slope at t_{120}: -0.012 ± 0.073). Statistical analysis yielded no differences in the overall means but a significantly different time pattern when comparing the two groups ($P = 0.048$). Figure 2 shows plots of $\dot{Q}_{rel}$ *versus* the dorsal-to-ventral axis in one representative animal from each group.

Discussion

The important findings of our study are that after OA injury, treatment with PFX vapor leads to a higher overall $\dot{V}_A/\dot{Q}$ heterogeneity in comparison with control animals. The injury caused by OA redistributed $\dot{Q}_{rel}$ from high-flow to low-flow pieces when compared with baseline values. The pattern of this redistribution, as measured with the microsphere method, was similar for both treatment and control groups. Short treatment with PFX vapor modified the further redistribution of $\dot{Q}_{rel}$.

Our microsphere data showed that $\dot{Q}_{rel}$ shifted from areas that were initially high-flow to areas that were initially low-flow after inducing the injury with OA. It

may be reasonable to suppose that more OA was delivered to lung regions with a high initial $\dot{Q}_{rel}$. However, whether this correlates with a greater degree of injury to these lung areas is not known. The spatial analysis of the $\dot{Q}_{rel}$ distribution after t_{inj} showed that, in the control group, $\dot{Q}_{rel}$ was redistributed over time from dependent to nondependent lung areas, whereas the spatial $\dot{Q}_{rel}$ distribution remained unchanged in the PFX group. $\dot{Q}_{rel}$ was not redistributed from dependent to nondependent lung areas as described for PLV. The inspiratory PFX fraction was 0.2, which yields according to Dalton's law to a partial pressure of PFX of approximately 142 mmHg inside the alveoli. Solving the Clausius-Clapeyron equation ($P = A \cdot e^{-B \cdot T}$) for the animals' body temperature yields a vapor pressure of 390 mmHg. Because of the difference in these pressures, we think that a thin film of PFX covers the bronchial tree but that a net accumulation of PFX with bulk deposition of condensed PFX does not occur. PLV redistributes $\dot{V}_A$ from dependent to nondependent lung areas in OA-induced lung injury.[10] PLV in noninjured lungs changes the pulmonary blood flow distribution in the same direction.[24] Therefore, the better matching of $\dot{V}_A$ and $\dot{Q}$ in the nondependent lung areas might be one reason, beside others, for the improved gas exchange during PLV. In our experiments, we did not measure the spatial $\dot{V}_A$ distribution but found an increase in $\dot{V}_A$ and $\dot{Q}$ heterogeneity. However, at the same time, we observed improved oxygenation and a decrease in shunt. The later observation suggests that the overall matching of $\dot{V}_A$ and $\dot{Q}$ improved after treatment with PFX vapor. These seemingly contradictory statements can be explained if, despite an increase in $\dot{V}_A$ and $\dot{Q}$ heterogeneity, the regional $\dot{V}_A$ and $\dot{Q}$ was better matched at each $\dot{V}_A/\dot{Q}$ ratio. There may be a much wider range of $\dot{V}_A/\dot{Q}$ ratios in the lung (increased heterogeneity), but the $\dot{V}_A$ and $\dot{Q}$ plots might be almost identical (increased matching). Decreased alveolar $\dot{V}_A$ (attributable either to atelectasis or decreased ventilation) leads to alveolar hypoxia and subsequent hypoxic pulmonary vasoconstriction. Probably, the PFX vapor prevents a further collapse of alveoli and $\dot{V}_A$ does not decrease further. This is consistent with the proposed effect of PFX vapor on the surface tension at the air–liquid interface in the alveoli as one mechanism of action.[12] Alveolar expansion is facilitated and, consecutively, the hypoxic pulmonary vasoconstriction is either attenuated or even released, which leads to a better gas exchange. According to our data, this effect appears to be independent from gravitational factors.

In the first report about the use of PFX vapor, vaporization was achieved using an anesthesia machine with modified bypass vaporizers.[12] In our experiments, we used a Servo 900 C ventilator equipped with a modified Isoflurane vaporizer Model 952 and measured the PFX

CTL   **PFX**

Fig. 2. Relative pulmonary blood flow ($\dot{Q}_{rel}$) distribution *versus* the dorsal-to-ventral axis in one representative animal from each group. The numbers of pieces per lung were 236 for the control animal and 233 for the treatment animal, respectively. The slopes remained unchanged in treatment animals, whereas the slopes became more negative over time in control animals showing that $\dot{Q}_{rel}$ was redistributed from dependent (ventral) to nondependent (dorsal) lung areas. CTL = control group; PFX = treatment group; t_{inj} = established injury; t_{30} = 30 min after established injury; t_{60} = 60 min after established injury; t_{120} = 120 min after established injury.
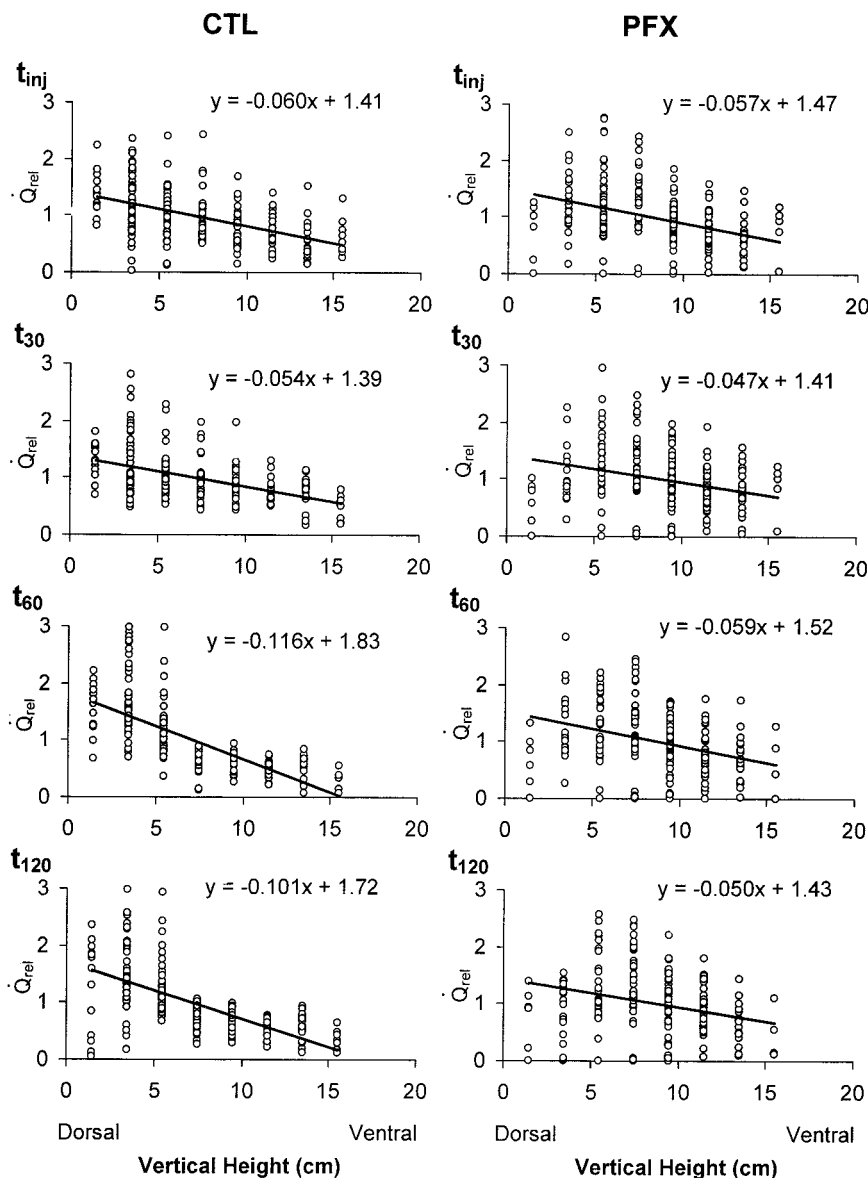
vapor concentration with a mass spectrometer. The aimed inspiratory PFX concentration was reached within 2 min after opening the vaporizer. When the vaporizers were closed at the end of the treatment period, the mass spectrometer was only able to detect PFX for approximately 3 min. One advantage of this type of setup (compared with an anesthesia machine) is that it is easy to use in an intensive care unit. However, it is known that PFC vapor may affect pneumotachometers and that the measured tidal volumes are falsely high.[25] Therefore, it is mandatory that the ventilator is adjusted appropriately to deliver the correct tidal volume to the patient or the animal, and to get the correct numbers for the calculation of the compliance. In our experimental setting, we had to increase the preset tidal volume of approximately 20% during vaporization to correct for this error.

Our study clarifies some issues of vaporized PFX, but the mechanism of action still remains poorly under-

stood. It is unlikely that the oxygen solubility in PFX is a major mechanism of action because molecules in the gaseous state of matter diffuse freely. It was shown that PLV attenuates lung injury when compared with conventional mechanical ventilation in endotoxin,[11] as well as in OA-induced models of ARDS.[10] Previous studies have suggested that PFC may have antiinflammatory properties[26,27] and that alveolar macrophage function may be impaired after exposure to PFC.[28] In a recent *in vitro* study it was shown that PFX reduces the expression and the release of proinflammatory and procoagulant mediators,[29] which is further evidence of direct antiinflammatory properties of PFCs on the cellular level. Therefore, it is possible that PFX vapor might have an effect in a less simple mechanistic manner than it appears.

In summary, we have shown in an animal model of ARDS that short-term treatment with PFX vapor increased $\dot{V}_A/\dot{Q}$ heterogeneity. This increase was indepen-

dent from changes in shunt and in dead space as shown by the dispersion indices. The relative blood flow distribution as measured by the microsphere method did not yield a gravitational effect of the vapor. Our results are consistent with an effect of PFX vapor on the surface tension, thus preventing further alveolar collapse and improving gas exchange over time.

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