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Partial Liquid Ventilation with Perfluorocarbon Improves Gas Exchange and Decreases Inflammatory Response in Oleic Acid-induced Lung Injury in Beagles

The aim of this study was to determine the effect of partial liquid ventilation (PLV) using a perfluorocarbon (PFC) on gas exchange and lung inflammatory response in a canine acute lung injury model. After inducing severe lung injury by oleic acid infusion, beagle dogs were randomized to receive either gas ventilation only (control group, n=6) or PLV (PLV group, n=7) by sequential instillation of 10 mL/kg of perfluorodecalin (PFC) at 30 min intervals till functional residual capacity was attained. Measurements were made every 30 min till 210 min. Then the lungs were removed and bronchoalveolar lavage (BAL) (35 mL/kg) was performed on the right lung and the left lung was submitted for histologic analysis. There was significant improvement in PaO_2 and $PaCO_2$ in the PLV group compared to the control group ($p<0.05$) which was associated with a significant decrease in shunt ($p<0.05$). There was no significant difference in parameters of lung mechanics and hemodynamics. There was a significant decrease in cell count and neutrophil percentage in BAL fluid and significantly less inflammation and exudate scores in histology in the PLV group ($p<0.05$). We conclude that PLV with perfluorodecalin improves gas exchange and decreases inflammatory response in the acutely-injured lung.

Key Words : *Respiratory distress syndrome, adult; Fluorocarbons; Respiration, artificial; Bronchoalveolar lavage; Histology*

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INTRODUCTION

Acute respiratory distress syndrome (ARDS) is still a lethal disease with mortality typically reported to be around 50% (1). Because there is no effective, specific therapy in ARDS, mechanical ventilation that maintains gas exchange has been a vital part of the management for this syndrome. Although medical opinions are gradually converging, there is still no firm consensus regarding the appropriate ventilatory management of ARDS patients. Therefore, the development of new therapeutic strategies are needed.

Partial liquid ventilation (PLV), also known as "perfluorocarbon (PFC)-associated gas exchange" (2) is a new ventilation technique which supports gas exchange in the injured lung, combining the ease and familiarity of conventional gas ventilation with the benefits of liquid ventilation using PFC. This technique has been studied in healthy animals as well as in experimental lung injury models (3-6) and some encouraging human data have emerged (7, 8). These studies seem to show the remarkable effects of PLV on gas exchange and lung mechanics in surfactant deficient models (3). However, the effect was less dramatic in models with increased permeability at the endothelial-epithelial barrier (4, 6) and in patients with ARDS (8).

Several mechanisms have been proposed for the improvements observed in PLV. First, high density and low surface tension of PFC opens collapsed alveoli units thereby recruiting them for ventilation (9). Also lungs filled with perfluorocarbon may redistribute pulmonary blood flow, favoring enhanced ventilation-perfusion matching (10, 11). Another possible mechanism is the anti-inflammatory effect of PLV using PFC (12-15).

Thus, this study was performed to investigate the effect of PLV using perfluorodecalin on gas exchange and

inflammatory response in an acute lung injury model with increased permeability.

MATERIALS AND METHODS

Animal preparation

This study was reviewed and approved by the animal use and care committee of Samsung Life Science Research Institute. Thirteen beagle dogs of either sex (Choong-ang Laboratory Animals, Seoul, Korea) and more than 10 months of age [7.8-11.5 kg (median 9.6 kg)] were anesthetized with thiopenthal sodium (35 mg/kg), restrained in the supine position, intubated with internal diameter 6.0 mm cuffed endotracheal tubes. They were then ventilated with Servo 900C ventilator (Siemens-Elma, Solna, Sweden) at a fixed rate of 20 breaths/min, positive end expiratory pressure (PEEP) of 4 cmH_2O, total inspiratory percentage of 43% (inspiration 33%, pause 10%), inspired O_2 fraction of 1.0, and tidal volume (V_T) (120-190 mL or 13.4-21.2 mL/kg) adjusted to keep $PaCO_2$ between 35 and 45 mmHg. These initial ventilator settings were kept until the end of the experiment for both groups. The animals were paralyzed by bolus injection of 1 mg and then hourly injection of 0.1 mg/kg of norcuronium and anesthetized by continuous infusion of sodium pentobarbital at a rate of 6 mg/kg/hr. Physiologic saline was infused at a rate of 4 mL/kg/hr as maintenance fluid. Right femoral artery was catheterized for blood gas sampling and monitoring of arterial pressure, and a cut down of right femoral vein was performed to insert a Swan-Ganz catheter (5 Fr, Baxter Healthcare Corp., Irvine, CA, U.S.A.). Heart rate, electrocardiogram, arterial pressure (AP), pulmonary artery pressure (PAP), and pulmonary capillary wedge pressure (PCWP) were monitored using Hewlett-Packard Monitoring System Model 78354C (Hewlett-Packard GMBH, Boeblingen, Germany). A separate catheter was inserted via external jugular vein to right atrium to infuse oleic acid. Core body temperature was monitored with the pulmonary artery catheter and maintained between 36 and 38℃ with the use of heat lamps and heating pad.

Induction of acute lung injury

Animals were hydrated to maintain PCWP $\geq$6 mmHg. After a period of stability, preinjury baseline data were collected. All measurements were performed every 30 min thereafter. To induce lung injury, oleic acid 0.10 mL/kg (0.89 g/mL, Sigma Chemical, St. Louis, MO, U.S.A.) was infused into the right atrium for 5 min. During induction of lung injury, Dextran-40 was administered to maintain PCWP $\geq$6 mmHg. If PaO_2 >200 mmHg after 60 min, an additional 0.3 mL/kg of oleic acid was injected over 1 min (two beagles in the PLV group). When PaO_2 <100 mmHg, sufficient severe lung injury was thought to have occurred and the experiment was begun (postinjury baseline, 0 min).

Protocol

The animals were randomized to receive two kinds of ventilation. In the control group (n=6), gas ventilation using the initial ventilator setting was continued throughout the experiment. In the PLV group (n=7), along with gas ventilation using the initial ventilator setting, sequential instillation of 10 mL/kg of perfluorodecalin (perfluorodecahydronapthalin, Fluka Chemie AG, Buchs, Switzerland) was done at 0, 30, 60 min and if needed to FRC at 90 min (total dose of PFC=33.3±1.8 mL/kg). Instillation was done slowly over a 10 min period through the side port of closed suction catheter (Trach Care, Ballard Medical Products, Draper, Utah, U.S.A.) during inspiration while ventilation was continued; 1/3 in the supine, right lateral decubitus and left lateral decubitus positions, respectively. Evaporated losses of perfluorodecalin were not replaced. After 210 min of ventilation, dogs were sacrificed with bolus injection of potassium chloride. The chest was immediately opened and heart and lungs were removed en bloc. After carefully aspirating secretions from the trachea, a 16F foley catheter was advanced to lower trachea and balloon inflated. After clamping the left main bronchus, bronchoalveolar lavage (BAL) was done in the right lung by instilling 35 mL/kg of sterile normal saline and then gently aspirating the fluid. From the left lung, two or three pieces of tissue block of 1 cm×1 cm×1 cm were removed from both dependent (posterobasal posterior segment of left caudal lobe) and non-dependent (tip of caudal part of left caudate lobe) lungs.

Data collection

Arterial and venous blood gas tensions, oxygen saturation and arterial hemoglobin concentration were measured with blood drawn from the femoral artery and pulmonary artery using a blood gas analyzer (288 Blood Gas Analyzer, CIBA-Corning, Medfield, MA, U.S.A.). Oxygen content (C_xO_2) was calculated as $1.35\times\%Sat\times Hb+PaO_2\times 0.003$ where %Sat is percent oxygen saturation of blood and Hb is hemoglobin. Shunt fraction was calculated as $(C_cO_2\text{-}C_aO_2)/(C_cO_2\text{-}C_vO_2)\times 100$ where subscripts c, a, and v denote pulmonary capillary, arterial and mixed venous blood, respectively.

Exhaled V_T and mean and peak airway pressures were recorded from the ventilator display. Effective tidal vol-

ume (V_{Teff}) was calculated by subtracting the compressible volume of the ventilator circuit from V_T. Plateau pressure (P_{plat}) was measured by occluding the expiratory valve for 3 seconds while observing pressure display to confirm stable pressure. Static compliance of the respiratory system was calculated by dividing V_{Teff} by (P_{plat}-PEEP) and then normalized for body weight.

Cardiac output (CO) was measured by CO monitor (COM-2 Cardiac Output Computer, Baxter Healthcare Corp., Irvine, CA, U.S.A.), using at least three 5 mL injections of cold normal saline at end-expiration. Cardiac index (CI) was calculated by dividing measured CO by body surface area (BSA) where BSA=0.12×(body weight)$^{2/3}$. Systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI) was calculated by the following formulas and expressed as dyne×sec×cm^{-5}/m^2 of BSA [SVRI=80×(mean AP/CI), PVRI=80×(mean PAP-PCWP)/CI].

BAL fluid and histologic analysis

After gentle mixing, aliquots of unfiltered BAL fluid was placed in a hemocytometer and total cell count was undertaken in duplicate by a blinded observer. Cytospin preparations were made and stained with Diff-Quik staining. A total of 300 cells were counted for differential cell counts. A part of BAL fluid was centrifuged at 1,500 rpm for 10 min and its supernatent stored at -70℃ for later protein quantification. Protein quantification was done using a modification of Lowry micro method which used a protein quantificaiton kit (Sigma, St. Louis, MO, U.S.A.) at 700 nm.

The removed lung tissues were fixed in 10% formalin and embedded in paraffin and stained with hematoxylin and eosin for microscopic examination. Lung tissues from the dependent and nondependent regions were assessed semi-quantitatively using five point, four variable scoring system. The specimen were scored separately for hemorrhage, edema, inflammation, and exudate on a 0-4 scale (0: no change, 1=minimal, 2=mild, 3=moderate, 4=severe changes), a modification of the method used by Kaisers et al. (16) (Table 1) which was performed by one of the authors (JHH) who was blinded to the treatment protocol.

Statistical analysis

Results are expressed as mean±SEM. Baseline characteristics, parameters at preinjury baseline and postinjury baseline (0 min), total and differential cell counts, and histologic changes were compared by Wilcoxon rank sum test. Intergroup and intragroup comparisons over time were made using repeated measures analysis of variance (ANOVA). Specific time points of intergroup differences were assessed by using post-hoc Bonferroni's test for selected pairs and intragroup differences were analyzed using post-hoc Dunnette's test using time 0 min as the baseline. Statistical analysis was done using SAS (SAS Institute Inc., Cary, NC, U.S.A.) and Prism (GraphPad Software Inc., San Diego, CA, U.S.A.) softwares.

RESULTS

The animals in the two groups had similar body weights (9.1±0.5 kg for the control group, 9.5±0.5 kg for the PLV group) and tidal volumes (15.5±0.8 mL/kg for the control group, 16.9±1.0 mL/kg for the PLV group). There was also no significant difference in gas exchange, lung mechanics and hemodynamic parameters at preinjury baseline and postinjury baseline (0 min) for the two groups (Table 2) ($p>0.05$). All animals in the PLV group completed the trial but two animals in the control group did not survive the trial, thus data were analyzed to 150 min due to the small number of surviving animals in the control group.

Gas exchange

In the control group, PaO_2 decreased to 68.7±3.5 mmHg at 0 min and it showed gradual deterioration to

Table 1. Criteria used in scoring histologic changes

Score	Hemorrhage	Edema	Inflammation	Exudate
0	None	None	None	None
1	Mild congestion with focal microscopic hemorrhage	Minimal focal edema	Scattered focal microscopic infiltration	Minute focal
2	Focal patchy hemorrhage, area <10%	Perivascular edema, area <10%	Diffuse scattered, but not patchy	Multifoci with inflammation, area <10%
3	Multifocal hemorrhage, area 10-30%	Perivascular edema, area 10-50%	Foci of patchy infiltration intraalveoar space involvement	Area ≥10% or mucin pool formation
4	Diffuse hemorrhage	Diffuse edema	Multifoci of microabscess formation	Mucin lake

Table 2. Respiratory and hemodynamic parameters of the two groups before lung injury and at postinjury baseline

	Preinjury baseline		Postinjury baseline (0 min)	
	Control group	PLV group	Control group	PLV group
pH	7.34±0.01	7.32±0.02	7.03±0.01	7.08±0.04
PaO_2 (mmHg)	597.9±8.1	573.5±15.7	68.7±3.5	78.3±4.7
$PaCO_2$ (mmHg)	38.6±1.7	39.9±2.2	76.7±5.1	67.7±5.6
Shunt (%)	9.0±1.3	12.0±2.1	64.5±2.7	60.7±2.7
P_{plat} (cmH_2O)	12.8±1.0	12.6±0.8	25.4±0.6	26.4±0.8
Cst/kg ($mL/cmH_2O/kg$)	1.94±0.21	1.79±0.31	0.64±0.06	0.59±0.02
CI ($L/min/m^2$ of BSA)	4.7±0.5	5.1±0.4	4.8±0.4	5.1±0.5
MAP (mmHg)	143.5±7.0	147.1±4.5	129.2±4.8	113.4±7.3
SVRI (dyne sec cm^{-5}/m^2)	2,646±361	2,422±235	2,234±213	1,866±197
MPAP (mmHg)	15.7±1.0	17.4±0.9	21.7±1.5	20.1±1.1
PVRI (dyne sec cm^{-5}/m^2)	155.2±24.4	171.8±20.1	237.0±19.6	206.1±22.2
PCWP (mmHg)	6.7±0.5	6.9±0.6	6.5±0.2	7.7±1.0

PLV, partial liquid ventilation; P_{plat}, plateau airway pressure; Cst/kg, static compliance of the respiratory system normalized to body weight; CI, cardiac index; MAP, mean arterial pressure; SVRI, systemic vascular resistance index; MPAP, mean pulmonary artery pressure; PVRI, pulmonary vascular resistance index; PCWP, pulmonary capillary wedge pressure
Data shown as mean±SEM.

50.6±6.9 and 46.7±7.1 mmHg at 120 and 150 min, respectively (p<0.05 vs. 0 min) (Fig. 1A). In the PLV group, PaO_2 was 78.3±4.7 mmHg at 0 min and showed dose-dependent increase in response to sequential instillation of PFC to 100.8±8.1 mmHg at 90 min which was significantly improved compared to that of the control group (p<0.05). This difference was maintained till 150 min (Fig. 1A). At 0 min, $PaCO_2$ was similar for the two groups (p>0.05) (Table 2, Fig. 1B). $PaCO_2$ increased with time in both groups but to a lesser degree in the PLV group to show a significant difference at 90 min (104.3±11.4 mmHg in the control group, 73.2±8.2 mmHg in the PLV group), and the difference was maintained till 150 min (Fig. 1B). Arterial pH showed similar trend with PLV group showing significant differences at 120 and 150 min compared with those of the control group (p<0.05, Fig. 2A).

In the control group, shunt markedly increased after lung injury to 64.5±2.7%, which increased to 77.9±5.6 and 75.3±5.0% at 120 and 150 min (p<0.05 vs. 0 min) (Fig. 2B). In the PLV group, shunt decreased from 60.7±2.7% at 0 min to 52.2±4.2% at 90 min (p<0.05 vs. 0 min, p<0.05 vs. control group) and then showed a slight increase to the level of 0 min and remained

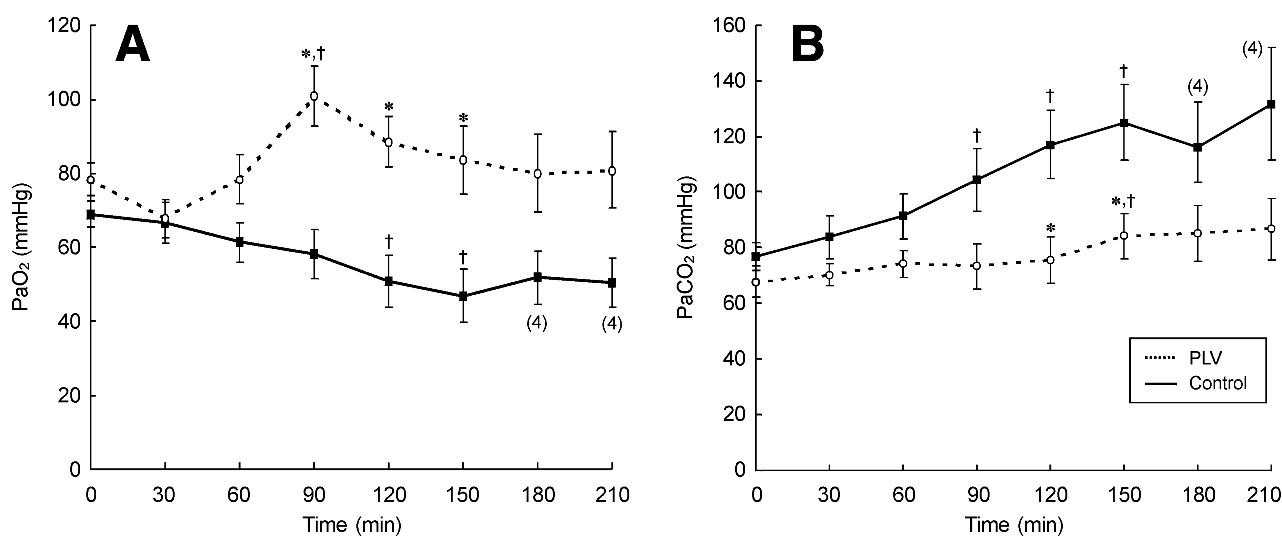

Fig. 1. Sequential changes in PaO_2 (A) and $PaCO_2$ (B) in the control group (Control, closed circles, solid line, n=6) and the partial liquid ventilation group (PLV, open circles, dotted line, n=7). Numbers in the parentheses denote numbers of animals surviving. *p<0.05 vs. Control, †p<0.05 vs. 0 min within groups.

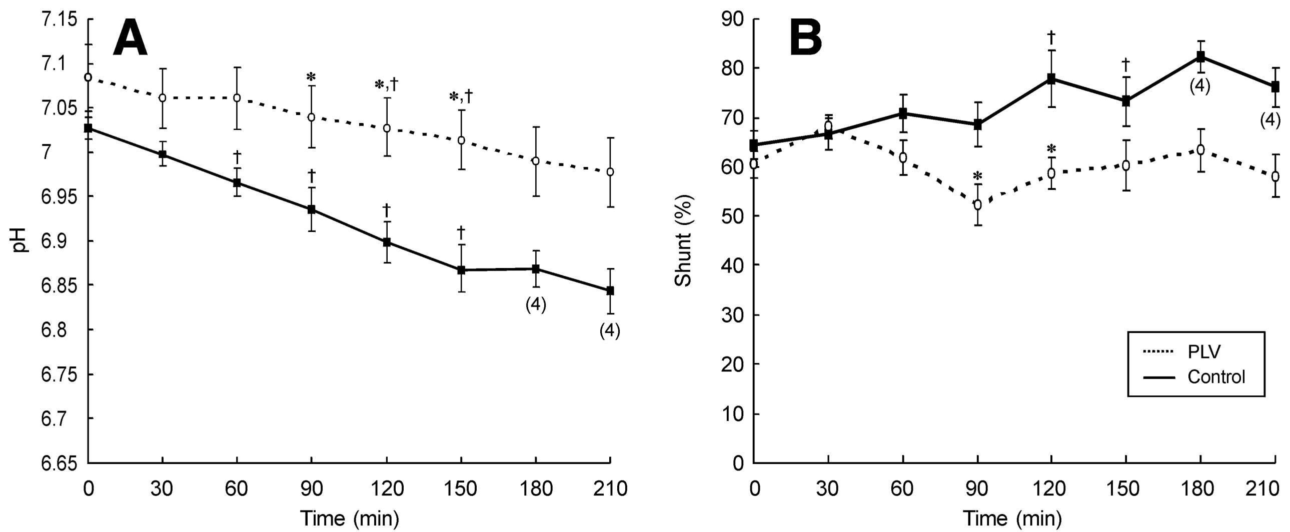

Fig. 2. Sequential changes in pH (A) and shunt (B) in the control group (Control, closed circles, solid line, n=6) and the partial liquid ventilation group (PLV, open circles, dotted line, n=7). Numbers in parentheses denote numbers of animals surviving. $^*p<0.05$ vs. Control, $^†p<0.05$ vs. 0 min within groups.

stable for the duration of the study (Fig. 2B).

Lung mechanics

Static compliance in both groups at 0 min was similar (Table 2, Fig. 3A). In the PLV group, six of the seven animals showed increase in compliance (from 0.64±0.07 mL/cmH_2O/kg at 0 min to 0.70±0.02 mL/cmH_2O/kg at 30 min) but there was no significant difference in compliance between the two groups (Fig. 3A). P_{plat} was decreased in the PLV group at 30 min with 24.4±0.5 cmH_2O compared with that of postinjury baseline of 26.3±0.8 cmH_2O ($p<0.05$). However, there was no significant difference between the control and the PLV group (Fig. 3B).

Hemodynamic parameters

Hemodynamic data are shown in Table 3. There was no significant difference in all the parameters shown between the two groups. Both groups showed stable CI and PCWP during the course of study while mean AP, mean PAP, SVRI, and PVRI showed a similar trend for increment throughout the study duration.

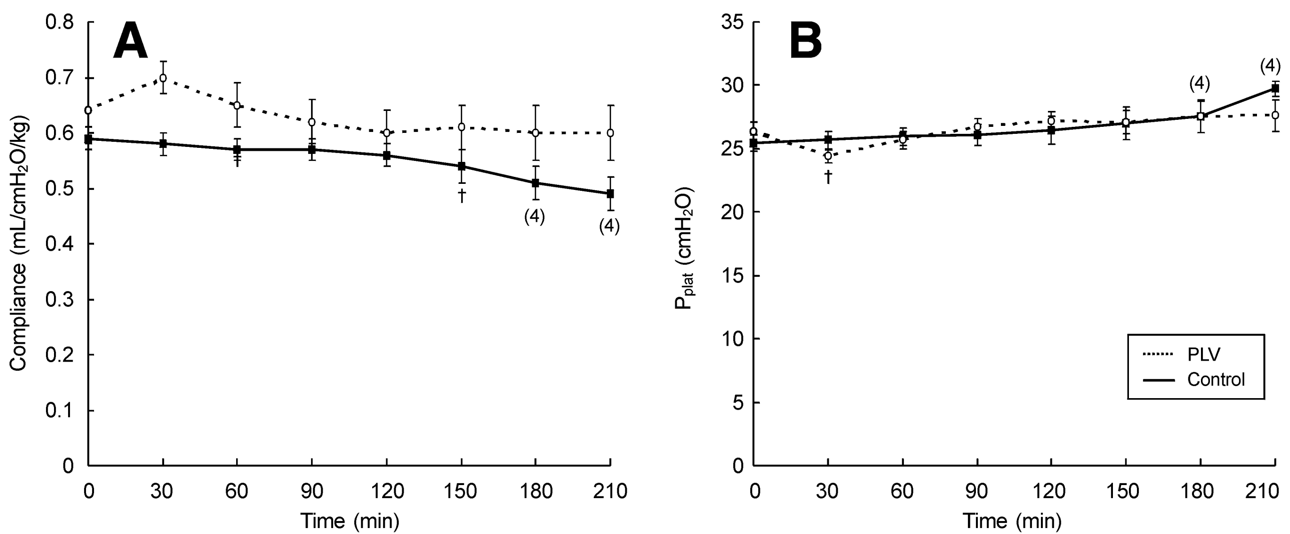

Fig. 3. Sequential changes in static compliance of the respiratory system (A) and plateau pressure (B) (P_{plat}) in the control group (Control, closed circles, solid line, n=6) and the partial liquid ventilation group (PLV, open circles, dotted line, n=7). Numbers in parentheses denote the number of animals surviving. $^*p<0.05$ vs. Control, $^†p<0.05$ vs. 0 min within groups.

Table 3. Sequential changes in hemodynamic parameters in the two groups

Time (min)	Group	CI (L/m^2)	MAP (mmHg)	SVRI (dyne sec cm^{-5}/m^2)	MPAP (mmHg)	PVRI (dyne sec cm^{-5}/m^2)	PCWP (mmHg)
0	Control	4.79±0.40	129.2±4.8	2,234±521	21.7±1.5	237.0±19.6	6.5±0.2
	PLV	5.07±0.45	113.4±7.3	1,866±197	20.1±1.1	206.1±22.2	7.7±1.0
30	Control	4.34±0.27	142.0±4.8	2,683±219	24.5±1.6	288.6±18.3	7.3±0.6
	PLV	5.09±0.47	114.0±9.0	1,869±185	21.7±1.5	218.3±32.3	8.7±0.8
60	Control	4.32±0.50	156.17±7.1	2,918±180	28.2±2.4†	342.8±32.7	7.7±1.1
	PLV	4.82±0.65	117.7±13.0	2,165±342	23.9±1.6	271.2±37.5	8.7±1.0
90	Control	4.50±0.50	169.2±8.82†	3,131±254	30.3±2.3†	380.5±41.4†	7.7±1.4
	PLV	4.48±1.26	128.0±15.9	2,451±440	26.1±1.7†	331.3±52.4†	8.6±0.6
120	Control	4.73±0.44	178.2±12.1†	3,109±250	34.0±1.9†	440.1±41.9†	7.8±1.7
	PLV	4.54±1.20	143.3±16.4†	2,734±512†	29.1±2.1†	372.7±58.8†	9.3±0.6
150	Control	4.66±0.45	162.8±14.7†	2,933±411	34.3±3.9†	450.0±70.6†	9.2±2.8
	PLV	4.81±1.30	150.7±15.3†	2,707±467†	32.4±2.7†	403.8±68.5†	9.7±0.6
180	Control	4.25±0.74	171.3±9.04	3,578±697	36.3±1.8	558.2±146.4	10.5±3.8
	PLV	4.72±1.24	157.0±12.5	2,916±532	34.6±3.0	449.4±85.8	10.0±0.8
210	Control	4.82±0.51	153.8±21.4	3,499±725	36.8±1.9	625.5±163.0	10.0±3.3
	PLV	4.62±1.13	164.3±14.2	3,102±583	35.3±2.9	468.7±80.8	10.0±0.8

Control, control group; PLV, PLV group; CI, cardiac index; MAP, mean arterial pressure; SVRI, systemic vascular resistance index; MPAP, mean pulmonary artery pressure; PVRI, pulmonary vascular resistance index; PCWP, pulmonary capillary wedge pressure
Data shown as mean±SEM. *p<0.05 vs. control group; $^†p$<0.05 vs. time 0 within groups

BAL fluid analysis and histologic findings

The number of nucleated cells in BAL fluid was significantly decreased in the PLV group compared with that of the control group ($p<0.05$) (Table 4). The percentages of neutrophils and lymphocytes were significantly different between the two groups ($p<0.05$) (Table 4). The protein content in BAL fluid showed no significant difference between the two groups (Table 4).

There were no significant differences in any of the histological injury scores between dependent and non-dependent regions in both groups; although there was a tendency for more severe injury in the dependent lung regions (data not shown). There was also no significant difference in scores for hemorrhage or edema. However, inflammation (3.3±0.4 vs. 2.1±0.3) and exudate (2.2±0.4 vs. 0.7±0.2) scores were significantly lower in the PLV group compared with those in the control group ($p<0.05$) (Fig. 4, 5).

Table 4. Cell count, differential count and protein concentration in bronchoalveolar lavage (BAL) fluid in the two groups

	Control	PLV
Cell count (×10^6/mL of BAL fluid)	1.01±0.30	0.33±0.13*
Differential count		
Macrophage (%)	4.5±1.4	19.3±8.2
Neutrophil (%)	91.3±2.1	65.0±10.1*
Lymphocyte (%)	3.3±0.7	15.0±3.5*
Protein (mg/dL of BAL fluid)	403.9±63.3	314.9±44.0

Control, control group; PLV, partial liquid ventilation group
Data shown as mean±SEM. *p<0.05 vs. Control

DISCUSSION

The major findings of this study are that PLV using perfluorodecalin improved gas exchange and decreased lung inflammatory responses in an acute lung injury model with increased permeability. PLV significantly improved PaO_2, $PaCO_2$, and pH compared with the control group, which was accompanied by a decrease in shunt. There were significantly less inflammatory cells in BAL fluid and less inflammatory responses in histology in animals ventilated with PLV.

Since its first description (2), PLV has been demonstrated to be effective in various animal models of ARDS including surfactant-depletion by saline lavage (3, 16, 17), acid instillation (18), combination of saline lavage and oleic acid infusion (19) and oleic acid infusion only (4, 20, 21). These studies showed remarkable dose-dependent improvement in gas exchange and sustained improvement of lung mechanics in surfactant deficient models (3, 17), but somewhat less of an effect in models in which oleic acid was used (4, 19-21). We chose oleic acid-induced lung injury model because it exhibits a disruption of the epithelial-endothelial barrier, which is the major pathophysiologic mechanism for lung injury seen in ARDS patients.

The extent of injury induced by oleic acid is dependent on the dose, duration of injection period, location of injection and also fluid management (22). The method

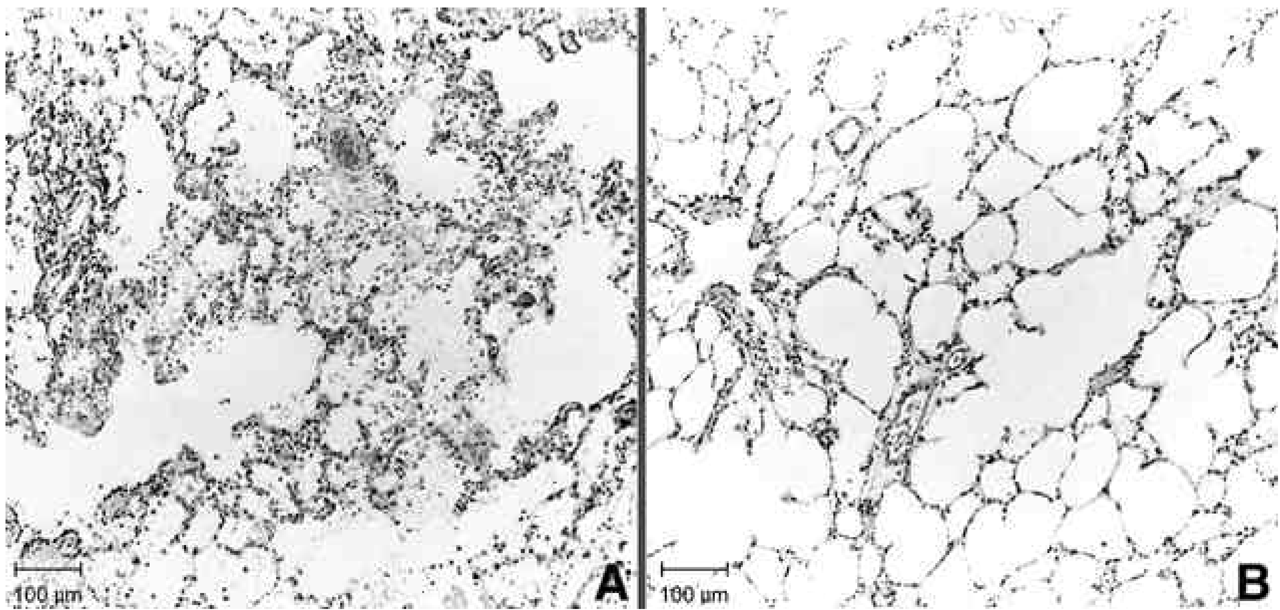

Fig. 4. Photomicrograph of representative section of dependent lung (H&E, ×100). A: Contol group specimen shows diffuse interstitial and intraalveolar inflammatory cell infiltraion with edema along with intraalveolar exudate. B: Partial liquid ventilation group specimen shows markedly less inflammatory cell infiltration and exudate compared with the control group.

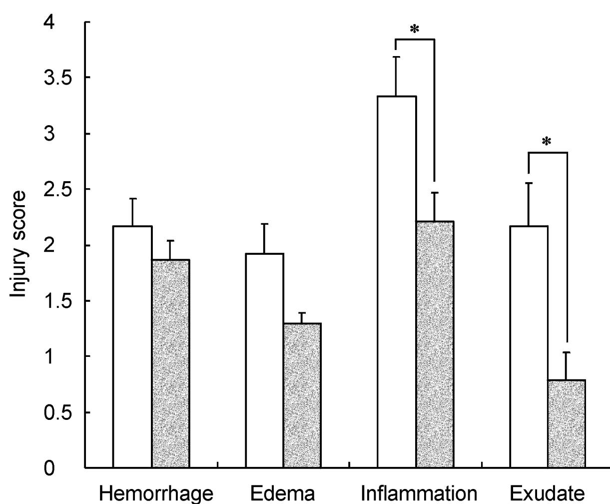

Fig. 5. Results of semiquantitative assessment of histopathologic changes in the control group (open bars) and the partial liquid ventilation (PLV) group (filled bars). Figures represent average scores for dependent and non-dependent regions (mean±SEM). See Table 1 for details. *$p<0.05$ vs. control.

used in this experiment had shown to produce consistent severe acute lung injury in our laboratory. We used perfluorodecalin (perfluoro-decanaphtalin) in our experiment because the availability of perflubron for experimental as well as for clinical purposes was restricted.

Our study reconfirmed the effectiveness of PLV using PFC in improving gas exchange in acute lung injury. By the third dose of perfluorodecalin (which was approximately FRC in our model), PaO_2 significantly improved compared to the control group and this improvement was maintained till 150 min. The decrease in oxygenation after the first dose of perfluorodecalin at 30 min seen in some dogs was noted by Parent et al. (23) using a similar model in sheeps. This might be due to insufficient recruitment of collapsed lung units by small dose of PFC. Since we did not compensate for perfluorodecalin lost through vaporization, the gradual decline seen in gas exchange during experiment could be explained in part by this phenomenon. The improvement in oxygenation was associated with a decrease in shunt. $PaCO_2$ and pH values showed similar improvements in the PLV group.

Although there was a tendency for dose-dependent increase in oxygenation, a significant difference was only seen after perfluorodecalin approximating FRC was used in our ventilator setting. These findings are different from models using surfactant depletion (3, 17) and consistent with the results of Curtis et al. (4) who also used oleic acid, suggesting that the mechanism of lung injury may be an important determinant of PLV efficacy. Another possible explanation for the smaller benefit than expected in gas exchange parameters might be differences in the PFC used. Most of the studies so far have examined the usefulness of perflubron which is the only medical grade PFC available. In this study perfluorodecalin was used. Although two PFCs are similar in surface tension (18 dynes/cm for perflubron and 15 dynes/cm for perfluorodecalin at 25℃) and solubility for oxygen (53 mL O_2/100 mL for perflubron, 49 mL O_2/100 mL for perfluorodecalin), perfluorodecalin has lower solubility

for CO_2 (140 vs. 210 CO_2/100 mL for perflubron), and more importantly, has higher viscosity than perflubron (2.90 vs. 1.10 centistokes at 25℃) (24). It is well known that viscosity is inversely correlated with the rate of diffusion and higher viscosity of perfluorodecalin as opposed to perflubron might have had an adverse effect on gas exchange (25).

Most of the studies on PLV have shown significant improvement in lung compliance. In surfactant-depleted model of ARDS in rabbits, a remarkable increase in lung compliance after a small dose of PFC was observed, after which further increase in compliance was not seen (3). In oleic acid-induced lung injury in mongrel dogs, a low dose of perflubron increased compliance significantly but further instillation on the contrary reduced lung compliance down to the level of the control group (4).

In our study a transient decrease in airway pressure, thus increase in compliance were seen in all of the animals in the PLV group and at 30 min, P_{Plat} was significantly lower than the baseline (0 min) value. Further instillation of PFC returned compliance and pressure to the baseline values, overall showing no differences between the two groups. This is similar to the results of other models using oleic acid showing less improvement in compliance than surfactant-deficient models. The physicochemical property of perfluorodecalin as opposed to perflubron also might have influenced our result. Higher viscosity of perfluorodecalin necessitates more pressure to move the fluid during ventilation (25) and this might be one of the reasons that we did not see a significant difference in compliance between the two groups. Equation for calculating SVRI usually includes right atrial pressure, but since its contribution to SVRI is minimal we have omitted right atrial pressure as some of the other investigators have (26, 27).

Most of the animals in our model had liquid FRC around 30 mL/kg (median 31.4 mL/kg) which is considerably less than liquid FRC reported elsewhere in dogs by Curtis et al. (4). These differences probably reflect differences in strains of dogs used. Curtis et al. used mongrels with average weight of around 18 kg while our model used beagles of around 9 kg in weight. Another cause of this difference might be the method of lung injury induction used. In our model, volume of fluid infused was considerably more than the model used by Curtis et al. as we tried to maintain adequate intravascular volume throughout the study.

Although some authors reported poor histologic improvement by PLV (4, 16), many studies reported improved histology in animal models with PLV (18, 21, 28-31). PLV decreased diffuse alveolar damage score while reducing capillary diameter and septal wall thickness and increased alveolar diameter in the lungs of oleic-acid injured sheeps that underwent PLV (29). PLV has also been reported to decrease inflammation, hemorrhage and edema (28) in surfactant-depletion model of neonatal piglets. Furthermore, PLV has shown to decrease lung neutrophil infiltration in the acutely-injured lung (32, 33) and in hemorrhagic shock (34) models. These findings are consistent with our findings of significantly less inflammatory cells in BAL fluid and decreased inflammation and exudate in histologic sections in animals ventilated with PLV.

Performing BAL in the PFC-filled lung is not standardized. Since PFC is much heavier than water and is immiscible in nearly all aqueous solutions, it can displace large volumes of secretion and inflammatory exudate into the larger airways (8). The inflammatory exudate floating on top of PFC in the large airways does not represent on-going alveolar inflammation and if this fluid is included in the BAL, this will increase cell counts and protein contents in the BAL fluid disproportionately. Conversely, large amount of PFC which is distributed preferentially to dependent regions (35) might fill the alveoli, blocking effective sampling of the alveoli in the dependent regions where inflammation should be the greatest. Thus to minimize the effect of PFC on BAL fluid analysis, we carefully aspirated PFC from the trachea before performing BAL and used relatively large volumes of saline (35 mL/kg).

Also to validate our method of BAL, we performed a separate set of experiments in uninjured dogs which were ventilated in the same manner as our experiment. BAL was done according to our original methods except that both right and left lungs were lavaged separately. Dogs ventilated with gas ventilation (n=2, 4 separate lungs) and PLV (n=2, 4 separate lungs) had similar protein levels (20.0±5.7 vs. 32.1±6.7, p=0.20 by Mann-Whitney U Test), cell counts ($0.31 \pm 0.06 \times 10^6$/mL vs. $0.30 \pm 0.08 \times 10^6$/mL, p=1.0), and differential counts for macrophages (76.0±2.5% vs. 75.3±6.6%, p=1.0), lymphocytes (14.5±2.6% vs. 17.8±7.1%, p=1.0) and neutrophils (6.3±2.0% vs. 7.0±0.7%, p=0.49). These data suggest that sampling of the alveoli could be done to the same extent even in the presence of PFC by our method of BAL. Similar BAL fluid protein concentration seen in this study (Table 4) also suggests BAL was effectively done in the PLV group. Even with these data, we cannot completely rule out the possibility that the inherent limitations in the BAL method might have influenced our results.

The mechanisms of this anti-inflammatory effect may be diverse. First, PFC itself has anti-inflammatory effects which may attenuate lung inflammation. PFC decreased in vitro production of reactive oxygen species (12) and nitrogen intermediates (36) by alveolar macrophages,

neutrophils exposed to PFC showed less H_2O_2 production and chemotaxis (13), and perflubron decreased inflammatory cytokine production in LPS-stimulated human alveolar macrophages (14). Moreover, PFC may affect receptor-ligand binding in neutrophils (37). Second, PLV may attenuate lung inflammation by reducing ventilator-induced lung injury. Injurious ventilatory strategies can cause inflammation and ARDS-like changes through overdistension and cyclic opening and closing of alveoli (38). PLV may prevent cyclic opening and closing of alveoli in the dependent lung through its "liquid PEEP" effect and its surface tension reducing properties recruiting them for ventilation. Third, PLV with PFC may decrease inflammation and exudate by clearing the airways of tissue debris and inflammatory cells with its lavage effect. Heavy density of PFC allows inflammatory exudate to float to the proximal airways, thus clearing inflammatory exudate from the site of lung damage (39) which would benefit the host by reducing inflammation. Whether the reduced inflammation that we observed reflects direct anti-inflammatory effect of PFC, decreased ventilator-associated lung injury, or lung lavage of inflammatory exudate is not clear from this study. It would be possible all three mechanisms contributed to the anti-inflammatory effect of PLV. Regardless of its mechanism, the effect of reduction in inflammation would be beneficial to patients with severe acute lung injury preventing further lung injury and possibly systemic organ failure (40).

In conclusion, PLV can improve gas exchange in the acutely-injured lung, which is accompanied by decrease in shunt and inflammatory response. Exact mechanism of this anti-inflammatory effect of PLV needs further clarification.

REFERENCES

1. Krafft P, Fridrich P, Pernerstorfer T, Fitzgerald RD, Koc D, Schneider B, Hammerle AF, Steltzer H. *The acute respiratory distress syndrome: definitions, severity and clinical outcome. An analysis of 101 clinical investigations. Intensive Care Med 1996; 22: 519-29.*
2. Fuhrman BP, Paczan PR, DeFrancisis M. *Perfluorocarbon-associated gas exchange. Crit Care Med 1991; 19: 712-22.*
3. Tutuncu AS, Faithfull NS, Lachmann B. *Intratracheal perfluorocarbon administration combined with mechanical ventilation in experimental respiratory distress syndrome: dose-dependent improvement of gas exchange. Crit Care Med 1993; 21: 962-9.*
4. Curtis SE, Peek JT, Kelly DR. *Partial liquid breathing with perflubron improves arterial oxygenation in acute canine lung injury. J Appl Physiol 1993; 75: 2696-702.*
5. Hirschl RB, Parent A, Tooley R, McCracken M, Johnson K, Shaffer TH, Wolfson MR, Bartlett RH. *Liquid ventilation improves pulmonary function, gas exchange, and lung injury in a model of respiratory failure. Ann Surg 1995; 221: 79-88.*
6. Hernan LJ, Fuhrman BP, Kaiser RE, Penfil S, Foley C, Papo MC, Leach CL. *Perfluorocarbon-associated gas exchange in normal and acid-injured large sheep. Crit Care Med 1996; 24: 475-81.*
7. Hirschl RB, Pranikoff T, Gauger P, Schreiner RJ, Dechert R, Bartlett RH. *Liquid ventilation in adults, children, and full-term neonates. Lancet 1995; 346: 1201-2.*
8. Hirschl RB, Pranikoff T, Wise C, Overbeck MC, Gauger P, Schreiner RJ, Dechert R, Bartlett RH. *Initial experience with partial liquid ventilation in adult patients with the acute respiratory distress syndrome. JAMA 1996; 275: 383-9.*
9. Kelley KP. *Partial liquid ventilation-turning back a PAGE on evolution. Br J Anesth 1997; 78: 1-2.*
10. Doctor A, Ibla JC, Grenier BM, Zurakowski D, Ferretti ML, Thompson JE, Lillehei CW, Arnold JH. *Pulmonary blood flow distribution during partial liquid ventilation. J Appl Physiol 1998; 84: 1540-50.*
11. Gauger PG, Overbeck MC, Koeppe RA, Shulkin BL, Hrycko JN, Weber ED, Hirschl RB. *Distribution of pulmonary blood flow and total lung water during partial liquid ventilation in acute lung injury. Surgery 1997; 122: 313-23.*
12. Smith TM, Steinhorn DM, Thusu K, Fuhrman BP, Dandona P. *A liquid perfluorochemical decreases the in vitro production of reactive oxygen species by alveolar macrophages. Crit Care Med 1995; 23: 1533-9.*
13. Rossman JE, Caty MG, Rich GA, Karamanoukian HL, Azizkhan RG. *Neutrophil activation and chemotaxis after in vitro treatment with perfluorocarbon. J Pediatr Surg 1996; 31: 1147-51.*
14. Thomassen MJ, Buhrow BS, Wiedemann HP. *Perflubron decreases inflammatory cytokine production by human alveolar macrophages. Crit Care Med 1997; 25: 2045-7.*
15. Kawamae K, Prinstine G, Chiumello D, Slutsky AS. *Partial liquid ventilation decreases circulating TNF-alpha in acid aspiration model. Am J Respir Crit Care Med 1997; 155: A87.*
16. Kaisers U, Max M, Walter J, Kuhlen R, Pappert D, Falke K, Rossaint R. *Partial liquid ventilation with small volumes of FC 3280 increases survival time in experimental ARDS. Eur Respir J 1997; 10: 1955-61.*
17. Tutuncu AS, Akpir K, Mulder P, Erdmann W, Lachmann B. *Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. Anesthesiology 1993; 79: 1083-93.*
18. Nesti FD, Fuhrman BP, Steinhorn DM, Papo MC, Hernan LJ, Duffy LC, Fisher JE, Leach CL, Paczan PR, Burak BA. *Perfluorocarbon-associated gas exchange in gastric aspiration. Crit Care Med 1994; 22: 1445-52.*
19. Hirschl RB, Tooley R, Parent A, Johnson K, Bartlett RH. *Evaluation of gas exchange, pulmonary compliance, and lung injury during total and partial liquid ventilation in the acute respiratory distress syndrome. Crit Care Med 1996; 24: 1001-*

8.
20. Curtis SE, Tilden SJ, Bradley E, Cain SM. *Effects of continuous rotation on the efficacy of partial liquid (perflubron) breathing in canine acute lung injury. Adv Exp Med Biol 1994; 361: 449-56.*
21. Papo MC, Paczan PR, Fuhrman BP, Steinhorn DM, Hernan LJ, Leach CL, Holm BA, Fisher JE, Kahn BA. *Perfluorocarbon-associated gas exchange improves oxygenation, lung mechanics, and survival in a model of adult respiratory distress syndrome. Crit Care Med 1996; 24: 466-74.*
22. Schuster DP. *ARDS: clinical lessons from the oleic acid model of acute lung injury. Am J Respir Crit Care Med 1994; 149: 245-60.*
23. Parent AC, Overbeck MC, Hirschl RB. *Oxygen dynamics during partial liquid ventilation in a sheep model of severe respiratory failure. Surgery 1997; 121: 320-7.*
24. Cox CA, Wolfson MR, Shaffer TH. *Liquid ventilation: a comprehensive overview. Neonat Network 1996; 15: 31-43.*
25. Miller TF, Milestone B, Stern R, Shaffer TH, Wolfson MR. *Effect of perfluorochemical (PFC) properties on PFC distribution, elimination, and lung mechanics during partial liquid ventilation. Am J Respir Crit Care Med 1998; 157: A462.*
26. Hofman WF, Ehrhart IC, Granger WM, Miller DA. *Sequential cardiopulmonary changes after oleic-acid injury in dogs. Crit Care Med 1985; 13: 22-7.*
27. Schuster DP, Perez JE, Trulock EP, Williamson JR, Biello DR, Kenzora JL, Amundsen T, Lange LG. *Cardiac dysfunction during acute lung injury induced by oleic acid in dogs. Am Rev Respir Dis 1986; 133: 519-25.*
28. Smith KM, Mrozek JD, Simonton SC, Bing DR, Meyers PA, Connett JE, Mammel MC. *Prolonged partial liquid ventilation using conventional and high- frequency ventilatory techniques: gas exchange and lung pathology in an animal model of respiratory distress syndrome. Crit Care Med 1997; 25: 1888-97.*
29. Quintel M, Heine M, Hirschl RB, Tillmanns R, Wessendorf V. *Effects of partial liquid ventilation on lung injury in a model of acute respiratory failure: a histologic and morphometric analysis. Crit Care Med 1998; 26: 833-43.*
30. Hirschl RB, Tooley R, Parent AC, Johnson K, Bartlett RH. *Improvement of gas exchange, pulmonary function, and lung injury with partial liquid ventilation. A study model in a setting of severe respiratory failure. Chest 1995; 108: 500-8.*
31. Tutuncu AS, Faithfull NS, Lachmann B. *Comparison of ventilatory support with intratracheal perfluorocarbon administration and conventional mechanical ventilation in animals with acute respiratory failure. Am Rev Respir Dis 1993; 148: 785-92.*
32. Rotta AT, Steinhorn DM. *Partial liquid ventilation reduces pulmonary neutrophil accumulation in an experimental model of systemic endotoxemia and acute lung injury. Crit Care Med 1998; 26: 1707-15.*
33. Colton DM, Till GO, Johnson KJ, Dean SB, Bartlett RH, Hirschl RB. *Neutrophil accumulation is reduced during partial liquid ventilation. Crit Care Med 1998; 26: 1716-24.*
34. Younger JG, Taqi AS, Till GO, Hirschl RB. *Partial liquid ventilation protects lung during resuscitation from shock. J Appl Physiol 1997; 83: 1666-70.*
35. Quintel M, Hirschl RB, Roth H, Loose R, van Ackern K. *Computer tomographic assessment of perfluorocarbon and gas distribution during partial liquid ventilation for acute respiratory failure. Am J Respir Crit Care Med 1998; 158: 249-55.*
36. Kornbrust ES, Neslund G, Flaim S. *Perflubron (Liquivent) may attenuate nitric oxide-derived reactive intermediates in the setting of acute lung injury. Am J Respir Crit Care Med 1997; 155: A468.*
37. Gumnarsson B, Rich GA, Steinhorn DM. *Perfluorocarbon (PFC) affects receptor-ligand binding in neutrophils (PMNs) after in vivo exposure. Crit Care Med 1998; 26: A27.*
38. Dreyfuss D, Saumon G. *Ventilator-induced lung injury: lessons from experimental studies. Am J Respir Crit Care Med 1998; 157: 294-323.*
39. Richman PS, Wolfson MR, Shaffer TH. *Lung lavage with oxygenated perfluorochemical liquid in acute lung injury. Crit Care Med 1993; 21: 768-74.*
40. Dreyfuss D, Saumon G. *From ventilator-induced lung injury to multiple organ dysfunction ? Intensive Care Med 1998; 24: 102-4.*