Arteriovenous CO_2 Removal Improves Survival Compared to High Frequency Percussive and Low Tidal Volume Ventilation in a Smoke/Burn Sheep Acute Respiratory Distress Syndrome Model

Frank C. Schmalstieg, MD, PhD, Susan E. Keeney, MD,*† Helen E. Rudloff,* Kimberly H. Palkowetz, BS,* Manuel Cevallos, MD,† Xiaoquin Zhou, MD,† Robert A. Cox, PhD,‡ Hal K. Hawkins, MD,‡ Daniel L. Traber, PhD,§ and Joseph B. Zwischenberger, MD†¶‖*

Objectives and Summary Background: Low tidal volume ventilation (LTV) has improved survival with acute respiratory distress syndrome (ARDS) by reducing lung stretch associated with volutrauma and barotrauma. Additional strategies to reduce lung stretch include arteriovenous carbon dioxide removal ($AVCO_2R$), and high frequency percussive ventilation (HFPV). We performed a prospective, randomized study comparing these techniques in our clinically relevant LD_{100} sheep model of ARDS to compare survival, pathology, and inflammation between the 3 ventilator methods.

Methods: Adult sheep (n = 61) received smoke inhalation (48 breaths) and a 40% third-degree burn. After ARDS developed (Pa_{O_2}/FiO_2 <200), animals were randomized. In experiment 1, animals were killed at 48 hours after randomization. Hemodynamics, pulmonary function, injury scores, myeloperoxidase (MPO) in lung tissues and neutrophils, IL-8 in lung tissues, and apoptosis were evaluated. In experiment 2, the end point was survival to 72 hours after onset of ARDS or end-of-life criteria with extension of the same studies performed in experiment 1.

Results: There were no differences in hemodynamics, but minute ventilation was lower in the $AVCO_2R$ group and Pa_{CO_2} for the HFPV and $AVCO_2R$ animals remained lower than LTV. Airway obstruction and injury scores were not different among the 3 ventilation strategies. In experiment 1, lung tissue MPO and IL-8 were not different among the ventilation strategies. However, in experiment 2, lung tissue MPO was significantly lower for $AVCO_2R$-treated animals ($AVCO_2R$ < HFPV < LTV). TUNEL staining showed little DNA breakage in neutrophils from experiment 1, but significantly increased breakage in all 3 ventilator strategies in experiment 2. In contrast, $AVCO_2R$ tissue neutrophils showed significant apoptosis at 72 hours post-ARDS criteria as measured by nuclear condensation ($P < 0.001$). Survival 72 hours post-ARDS criteria was highest for $AVCO_2R$ (71%) compared with HFPV (55%) and LTV (33%) ($AVCO_2R$ vs. LTV, $P = 0.05$).

Conclusions: Significantly more animals survived $AVCO_2R$ than LTV. In experiment 2, Lung MPO was significantly lower for $AVCO_2R$, compared with LTV ($P < 0.05$). This finding taken together with the TUNEL and neutrophil apoptosis results, suggested that disposition of neutrophils 72 hours post-ARDS criteria was different among the ventilatory strategies with neutrophils from $AVCO_2R$-treated animals removed chiefly through apoptosis, but in the cases of HFPV and LTV, dying by necrosis in lung tissue.

(*Ann Surg* 2007;246: 512–523)

Reduction of volutrauma and barotrauma in patients with acute respiratory distress syndrome (ARDS) result in significant reductions in mortality.[1] Low tidal volumes (LTV) and pressure limitation decrease mortality from ARDS as observed in multiple trials.[2–8] Controversy still exists as to which gentle ventilation mode provides the greatest benefit.[3,9,10] One gentle ventilation technique uses high-frequency percussive ventilation (HFPV) in which rapid sequence, subtidal volume breaths are delivered with a shortened expiratory time. Successive breaths are permitted to stack to a cumulative tidal volume.[11–13] Studies of HFPV in the smoke inhalation model have demonstrated improved oxygenation at low peak airway pressures.[11,14–19] An advantage of HFPV, which may uniquely suit it to the inhalation model, is the effect of its percussive action on optimization of pulmonary toilet.[13]

Another extraventilatory strategy for managing respiratory failure is the use of extracorporeal gas exchange. Arteriovenous CO_2 removal ($AVCO_2R$) uses a commercially available, low-resistance gas exchanger in a simple percutaneous arteriovenous shunt to achieve near-total extracorporeal removal of CO_2 production with only 800 to 1200 mL/min flow.[20–25] $AVCO_2R$ allows CO_2 normalization at low tidal volumes and respiratory rate. We have previously

From the Departments of *Pediatrics, †Surgery, ‡Pathology, §Anesthesiology, ¶Internal Medicine, and ‖Radiology, University of Texas Medical Branch, Galveston, TX.

Supported by Shriners Hospitals for Children (8530 and 8850) and National Institutes of Health (P01 GM066312).

Reprints: Frank C. Schmalstieg, MD, PhD, Department of Pediatrics, University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555. E-mail: fschmals@utmb.edu.

ISSN: 0003-4932/07/24603-0512
DOI: 10.1097/SLA.0b013e318148c6e6

demonstrated in an LD_{50} model that $AVCO_2R$ results in significantly higher survival compared with standard ventilation at moderately high tidal volume.[24] Because LTV has now resulted in improved survival, we chose to study an LD_{100} model previously shown to have 100% mortality by 120 hours postinjury.[26] Our present study compares 3 gentle ventilation techniques (LTV, HFPV, and $AVCO_2R$) utilizing our clinically relevant, LD_{100} ovine model of smoke/burn-induced ARDS.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Texas Medical Branch, Galveston, Texas, in compliance with the "Guide for the Care and Use of Laboratory Animals." All sheep received 24 hours/d cage-side care and physiologic monitoring with oversight by a veterinary anesthetist. An independent veterinarian supervised the experiments for compliance with IACUC guidelines.

Induction of ARDS

Adult free-range ewes (n = 61, 30–45 kg) were instrumented with femoral and pulmonary arterial catheters to obtain baseline variables (HR, CO, MAP, PAP, CVP, PAWP, and ABG). After endotracheal intubation, 1% to 2.5% isoflurane was administered via an anesthesia ventilator (Ohmeda 7000; BOC Health Care, Liberty Corner, NJ). To induce ARDS, a third-degree cutaneous flame burn to 40% total body surface area (20% on each flank) with 48 breaths of cotton smoke insufflation was performed as previously described.[27] Combined with moderately high tidal volume (15 mL/kg) ventilation (Servo 900C; Siemens-Elema, Sweden), this "3-hit" model of ARDS produces 100% mortality in animals that develop P/F ratios <200.[26] Fluid resuscitation used lactated Ringer's solution at 160 mL/kg/24 hours according to standard burn protocols.[28] Hemodynamic variables, ventilator parameters, and ABGs were recorded every 6 hours. Minute ventilation and FiO_2 were adjusted to maintain arterial pH 7.35 to 7.45, $Pao_2 > 60$ mm Hg and $Paco_2 < 40$ mm Hg. Antibiotics (Nafcillin, 1 g every 6 hours; Gentamicin sulfate, 80 mg every 8 hours) were administered throughout the experiments. Mild sedation and airway suctioning were done every 4 hours or as needed.

After ARDS criteria were met, the sheep were randomly assigned to LTV, $AVCO_2R$, or HFPV. After randomization, all animals received systemic anticoagulation (300 IU/kg bovine lung heparin; Upjohn, Kalamazoo, MI). Activated clotting time (ACT, Hemochron 400; International Technidyne, Edison, NJ) was maintained between 180 and 300 seconds by continuous heparin infusion.

Two separate experiments were performed. In experiment 1 the animals were killed at 48 hours after randomization to provide a midcourse window to compare pathophysiologic parameters. Twenty-four animals were randomized in experiment 1 [LTV (n = 8), $AVCO_2R$ (n = 7), and HFPV (n = 9)]. Three animals in the LTV group died before the 48-hour end point and were deleted from the analysis, leaving a total of 21 animals. In experiment 2, 30 animals were randomized to LTV (n = 10), $AVCO_2R$ (n = 10), and HFPV (n = 10) to assess survival to 96 hours post smoke/burn injury or killed if end-of-life criteria (defined as systolic blood pressure <60 mm Hg or heart rate <40 bpm, $Paco_2 > 100$ mm Hg or $Pao_2 < 45$ mm Hg for 1 hour) were met. Autopsy was performed and lung tissue was obtained after death or killing.

LTV Ventilation

LTV followed the low tidal volume arm of the ARDS Network trial.[2] Sheep were ventilated in assist control mode and tidal volume was decreased from 15 to 6 mL/kg stepwise over 4 to 6 hours after meeting ARDS criteria. Respiratory rates (RR) were adjusted between 2 and 35 per min with a goal of $Paco_2 < 55$ (RR of 2–30 per min). FiO_2 and PEEP were titrated based on the FiO_2/PEEP grid used in the ARDS Network trial[2] with a goal of $Pao_2 > 60$ mm Hg.

$AVCO_2R$

After randomization, animals in the $AVCO_2R$ group were reanesthetized and then underwent cannulation of the left common carotid artery (percutaneous 12F TF018LH; Research Medical, Midvale, UT) and the left jugular vein (percutaneous 14F TF022L; Research Medical). A commercially available, low-resistance membrane gas exchanger (Affinity; Avecor Cardiovascular, Plymouth, MN) was primed with 0.9% NaCl solution (270 mL) and connected to the vascular cannulas forming an arteriovenous loop.[21,23,24] After the initiation of $AVCO_2R$, ventilator management was identical to the LTV arm according to the ARDS Network trial.[2] $AVCO_2R$ blood flow was monitored by an ultrasonic flow probe (model H6X; Transonic Systems, Ithaca, NY) on the arterial cannula interfaced with a real-time flowmeter (model HT 109; Transonic Systems). Sweep gas flow (100% oxygen) was maintained at 1.5 to 2.0 times blood flow. CO_2 removal was calculated as the product of gas flow and its exhaust CO_2 concentration measured by an in-line capnometer (SaraTrans, Lenexa, KS).

HFPV Ventilation

The HFPV group used the VDR ventilator (VDR, Percussionaire Corp., Sandpoint, ID), a time-cycled, pressure-limited ventilator with a piston driven by high-frequency delivery of gas superimposed on conventional pressure-controlled ventilation.[13,17] Rapid subtidal breath stacking creates each tidal breath. Initial ventilator settings included: a pressure limit set at 30 cm H_2O, a pulse frequency of 400 to 600 cycles/min superimposed on a RR of 5 to 20 breaths/min, an oscillatory PEEP of 5 cm H_2O, and a 1:1.5 to 2 inspiratory-to-expiratory ratio. When $Paco_2 \leq 35$ mm Hg, stepwise adjustments to HFPV were made as follows: (1) increase pulse frequency; (2) decrease phase rate by prolonging inspiratory and expiratory time; and (3) decrease pressure limit. When $Paco_2 \geq 50$ mm Hg, adjustments were made in the opposite direction Pao_2 was maintained between 60 and 100 mm Hg by adjusting FiO_2. To prevent cast formation, humidification during HFPV was delivered via a high-volume dual nebulizer into the ventilator.

Pulmonary Measurements

Bronchial and bronchiolar obstruction in 1 cm lung slices was determined by our published methods.[29] Bronchi (8–27) and bronchioles (89–220) per slide were evaluated for percentage of total airway lumen obstructed by casts. Lung wet to dry ratios were measured according to Pearce et al[30] Lung pathologic scores were assigned by a pathologist blinded to study group for the variables of congestion, edema, hemorrhage, PMNs, hyaline membranes, septal thickness, and fibrosis. MPO of lung homogenates was assayed enzymatically.[31] Protein was measured using a bicinchonic acid protein assay kit (Pierce). Detection of IL-8 in homogenized lung tissue was by ELISA[32] with K2.2 (a human monoclonal antibody that cross-reacts with sheep IL-8[33]) as the capture antibody and goat polyclonal antihuman IL-8 as the detector (R&D Systems, Minneapolis, MN).

Neutrophil Apoptosis

Immunohistochemical staining was performed on frozen sections (15–20 μm) fixed with 4% paraformaldehyde and permeabilized with Triton X-100. Dual staining was performed with polyclonal antibovine type IV collagen (Biodesign International) (1:40) and human antihuman MPO cross reacting with ovine MPO (US Biologicals, M9760). Detecting antibodies were absorbed goat antihuman IgG conjugated to Alexa Fluor 594 (1:1000) (Molecular Probes, A1104) and goat antirabbit IgG (Sigma, F1262) (1:40). Nuclei were stained with Hoechst 33258 (bis-benzamide H, Sigma, B2883) and visualized with a Zeiss LSM 510 laser scanning confocal microscope. *Volocity* software (Improvison, Lexington, MA) was used to construct 3-dimensional models to allow an accurate volume of the tissue examined. Improvision software allowed automation of detection of both MPO and nuclear staining to determine the number of cells with MPO, MPO volume, and the extent of nuclear condensation as measured by the increase in intensity of the nuclear stain Hoechst 33258, a measure of the extent of apoptosis of the neutrophils and other cells in the tissue.[33] The MPO volume determinations were limited to objects $\geq$50 μm^3. The intensities of Hoechst staining were collected and assigned to previously determined ranges (binning). Nuclear condensation agrees well with measurement of nondiploid DNA in neutrophils by propidium iodide staining.[34]

TUNEL

DNA fragmentation in neutrophils and other lung cells was determined using an ApoAlert DNA Fragmentation kit (BD Clontech) according to the manufacturer's instructions. Cells positive for MPO and DNA fragmentation were identified using confocal microscopy and Improvision software as described earlier.

Analysis of Data

Physiologic parameters were analyzed using analysis of variance (ANOVA) for a balanced, 2-factor experiment with repeated measures in time. A post hoc Tukey correction procedure was employed for multiple comparisons. Analyses of the apoptosis distributions were compared with a 1-way simple random design ANOVA with appropriate post hoc corrections. These data fulfilled the requirements of equal variances and normality. Error sources were identified and appropriately considered to allow meaningful comparison of the effects of the 3 ventilation strategies. All tests assumed the 0.05 level of significance. Survival statistics (relative risk and *P* values) were obtained using Kaplan-Meier methods (SPSS, Chicago, IL).

RESULTS

Fifty-four of 61 animals successfully completed smoke/burn injury, resuscitation, and initial ventilator management) to meet ARDS entry criteria for the study. ARDS entry criteria was reached at 24.36 $\pm$ 1.14 hours and did not differ between the 3 groups.

Cardiopulmonary Responses and Survival (Experiment 2)

There were 5 cases of mortality due to iatrogenic causes in experiment 2 (outcomes). We deleted these animals from the analysis. All remaining animals included in the survival analysis died of respiratory or multiorgan failure. The number of animals in the survival analysis was n = 7 for $AVCO_2R$, n = 9 for HFPV, and n = 9 for LTV. Survival at 96 hours postinjury was highest for $AVCO_2R$ (71%) as compared with HFPV (55%) and LTV (33%) (Fig. 1). Survival of $AVCO_2R$ and HFPV-treated animals were compared with the LTV animals representing the present standard of care. The relative risk of death (LTV vs. $AVCO_2R$) was 4.64 $\pm$ 3.76, (P = 0.05) for the $AVCO_2R$ group and (LTV vs. HFPV) 2.68 $\pm$ 1.69 (P = 0.12) for the HFPV group. The time of death tended to be earlier for the LTV group (55.56 $\pm$ 10 hours) compared with the $AVCO_2R$ (84.71 $\pm$ 6.13 hours) and HFPV (75.78 $\pm$ 8.30 hours) groups, but this difference was not significant (P = 0.07).

There were no significant differences in hemodynamics (Table 1) before randomization. In all 3 groups there was an

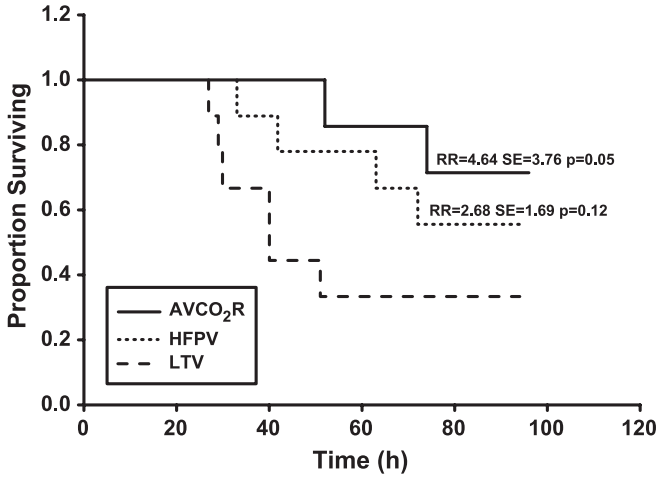

FIGURE 1. Kaplan-Meier curve showing survival over time in the LTV, HFPV, and $AVCO_2R$ groups. The number of animals were LTV (n = 9), HFPV (n = 9), and $AVCO_2R$ (n = 7). Data are expressed as mean $\pm$ SEM; *$P \leq 0.05$ for LTV versus $AVCO_2R$.

TABLE 1. Hemodynamic Variables

	Time (h)			Time (h)		
Variables*	**Baseline 1**	**24**	**Baseline 2**	**24**	**48**	**60**
MAP (mm Hg)						
$AVCO_2R$	96.0 ± 2.3	96.7 ± 2.2	112.3 ± 8.2	91.4 ± 6.9	109.8 ± 2.4	105.8 ± 2.5
HFPV	102.7 ± 3.1	95.5 ± 7.8	92.6 ± 6.5	98.0 ± 5.2	89.8 ± 12.2	108.4 ± 7.0
LTV	112.1 ± 4.4	103.0 ± 1.5	102.9 ± 4.2	86.7 ± 13.1	106.7 ± 3.5	103.3 ± 10.5
PAP (mm Hg)						
$AVCO_2R$	17 ± 1.2	24 ± 2.6	24 ± 2.0	24 ± 2.6	30 ± 4.2†	26 ± 5.5
HFPV	17 ± 1.7	27 ± 1.4	25 ± 2·	31 ± 0.8†	29 ± 3.0	32 ± 5.6
LTV	15 ± 2.3	28 ± 2.5	28 ± 1.4	26 ± 3.4	22 ± 1.9	31 ± 10.5†
PAWP (mm Hg)						
$AVCO_2R$	10 ± 0.9	14 ± 1.0	11 ± 1.4	12 ± 1.5	13 ± 2.0	11 ± 1.2
HFPV	11 ± 1.6	13 ± 3.6	15 ± 2.3	19 ± 1.2	16 ± 2.7	22 ± 6.6
LTV	11 ± .1.7	16 ± 0.7	13 ± 0.6	14 ± 2.2	12 ± 1.0	9 ± 0
CVP (mm Hg)						
$AVCO_2R$	2 ± .0.6	7 ± 2.6	7 ± 1.6	8 ± 1.5	11 ± 2.4†	8 ± 2.7
HFPV	5 ± 1.1	7 ± 1.7	5 ± 0.9	8 ± 1.4	16 ± 6.4	11 ± 4.5
LTV	3 ± 0.9	6 ± 0.9	7 ± 1.0	8 ± 3.0	3 ± 0.9	4 ± 2.1
CO (L/min)						
$AVCO_2R$	4.6 ± 0.3	4.1 ± 0.1	4.8 ± 0.4	5.0 ± 0.5	8.3 ± 0.8†	9.4 ± 0.7†
HFPV	5.4 ± 0.8	4.3 ± 0.9	5.0 ± 0.4	5.5 ± 0.2	5.6 ± 0.8	5.5 ± 0.2
LTV	6.4 ± 0.8	3.9 ± 1.1	4.7 ± 1.4	4.3 ± 0.8	7.5 ± 0.9	5.3 ± 0.5
Temp. (°C)						
$AVCO_2R$	38.1 ± 0.2	38.5 ± 0.4	37.8 ± 0.2*	38.6 ± 0.2	39.1 ± 0.2†	39.3 ± 0.4†
HFPV	38.6 ± 0.2	39.4 ± 0.3	38.9 ± 0.2	39.2 ± 0.2*	38.7 ± 0.5	39.2 ± 0.3
LTV	38.1 ± 0.2	39.1 ± 0.1	39.1 ± 0.1	38.2 ± 0.4	39.2 ± 0.8	39.2 ± 0.4

Means ± SEM.
*Significant difference from LTV animals.
†Significant difference from baseline value, $P < 0.05$.
MAP indicates mean arterial pressure; PAP, pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; CVP, central venous pressure; CO, cardiac output.

increase in pulmonary artery pressure over the 60 hours after randomization, but there were no differences between the 3 groups at any time point. CVP did not differ between the 3 groups, nor did pulmonary artery wedge pressure. The cardiac output (CO) rose in the $AVCO_2R$ group at 48 and 60 hours post randomization. Because of the possibility of sepsis, we analyzed body temperature and found no differences between the groups at 48 or 60 hours. As to ventilator settings (Table 2), none of the groups differed before randomization. We were unable to accurately measure mean airway pressure, tidal volume, peak airway pressure, or minute ventilation in the HFPV group because of the unique characteristics of the ventilator. However, we compared LTV to $AVCO_2R$ and found that tidal volume dropped in both groups over time, but did not differ between groups, nor did mean airway pressure, peak airway pressure, or PEEP.

In the $AVCO_2R$ group, minute ventilation was significantly lower after randomization and at all later time points compared with the initial baseline. Minute ventilation was significantly lower (approximately half) in the $AVCO_2R$ compared with the LTV groups at the 24, 48, and 60-hour post randomization time points. Pa_{O_2} dropped in all groups over time (Table 3). However, Fi_{O_2} and Pa_{O_2} did not vary between the 3 groups at any of the time points. Pa_{CO_2} rose and pH fell in the LTV group at 24, 48, and 60 hours, but not in the $AVCO_2R$ group until 60 hours. Pa_{CO_2} was significantly lower in the $AVCO_2R$ and HFPV groups compared with LTV at 24 hours. Calculated blood bicarbonate rose over time in the LTV group and was higher at all time points compared with the $AVCO_2R$ and HFPV groups.

Bronchial and Bronchiolar Obstruction

Figure 2A shows the obstruction scores for experiment 1. There were no differences in the obstruction scores for either the bronchi or bronchioles in the 3 study groups. In experiment 2 (Fig. 2B), animals treated with HFPV had significantly less bronchial ($P < 0.05$) obstruction than animals treated with $AVCO_2R$. The HFPV animals also tended to have less bronchiolar obstruction, but the differences did not reach significance.

Lung Wet to Dry Ratios

In experiment 1, there were no statistically significant differences in lung wet/dry ratios between the values for the treatment groups (6.23 ± 1.23 for LTV, 7.3 ± 1.1 for $AVCO_2R$, and 6.71 ± 1.38 for HFPV). Similarly in experiment 2, differences in lung wet to dry ratios for LTV, $AVCO_2R$, and HFPV animals at killing (7.0 ± 1.76, 7.21 ±

TABLE 2. Ventilatory Variables

	Time (h)			Time (h)		
Variables*	**Baseline 1**	**24**	**Baseline 2**	**24**	**48**	**60**
Tidal volume						
$AVCO_2R$	530 ± 16.1	560 ± 27.3	337 ± 26.2	250 ± 11†	232 ± 11.9	218 ± 9.3†
HFPV	519 ± 21.3	543 ± 14.3	—	—	—	—
LTV	559 ± 34.0	521 ± 42.8	303 ± 44†	260 ± 36.4†	212 ± 23.2†	210 ± 22.9†
PAP (cm H_2O)						
$AVCO_2R$	19.0 ± 0.8	24.1 ± 1.7	27.6 ± 2.8	23.0 ± 4.4	23.7 ± 4.7	23.7 ± 3.9
HFPV	17.4 ± 1.2	25.6 ± 0.9	25.9 ± 3.0	26.2 ± 3.3	25.2 ± 3.8	21.8 ± 4.4
LTV	18.2 ± 0.9	20.6 ± 2.0	18.2 ± 3.0	24.5 ± 4.9	14.8 ± 3.1	24.4 ± 3.5
MAP (cm H_2O)						
$AVCO_2R$	9.3 ± 0.5	11.8 ± 1.8	13.4 ± 1.3†	12.5 ± 1.9	14.6 ± 3.1	13.4 ± 2.7
HFPV	8.8 ± 0.5	10.8 ± 1.0	—	—	—	—
LTV	8.6 ± 0.5	9.3 ± 0.4	8.5 ± 1.4	16.0 ± 3.8	12.8 ± 0.8	12.8+1.8
PEEP (cm H_2O)						
$AVCO_2R$	5.0 ± 0.0	6.0 ± 1.0	7.4 ± 1.4	9.8 ± 1.0†	11.3 ± 1.9	11.5 ± 3.0
HFPV	5.0 ± 0.0	5.0 ± 0.0	5.7 ± 0.6	11.5 ± 2.4	9.2 ± 2.4	6.8 ± 1.4
LTV	5.1 ± 0.1	5.0 ± 0.0	6.8 ± 0.8	13.2 ± 2.9	11.3 ± 1.3	10.0 ± 1.2
Min. Vent.						
$AVCO_2R$	19.4 ± 0.6	11.4 ± 0.0	9.6 ± 0.8†	7.3 ± 0.7†*	8.4 ± 0.5†*	6.2 ± 0.5†*
HFPV	16.4 ± 1.6	14.9 ± 1.2	—	—	—	—
LTV	17.0 ± 1.3	14.4 ± 1.9	12.9 ± 1.5	12.5 ± 1.6	12.5 ± 1.1	12.6 ± 1.6

Means ± SEM.
*Significant difference from LTV animals.
†Significant difference from baseline value, $P < 0.05$.
PAP indicates peak airway pressure; MAP, mean airway pressure.

1.74, and 6.59 ± 1.66, respectively) did not reach statistical significance.

Lung Pathology

Table 4 summarizes the scores for the 5 pathologic variables in the 2 experiments. There were no significant differences in the scores between the groups in either experiment.

MPO in Lung Tissue and Neutrophils

Figure 3A depicts the values for lung MPO in experiment 1. Lung MPO was relatively low and did not differ between the groups. In experiment 2, the values for lung MPO were higher than in experiment 1 in all 3 groups (Fig. 3B). Lung MPO was greater in both the LTV ($P = 0.012$) and HFPV ($P = 0.046$) groups compared with $AVCO_2R$. The lung MPO values at death paralleled mortality in the groups.

The volume of MPO for cells in lung tissue for the 3 ventilator modalities did not differ between the ventilator strategies in experiment 1 (Fig. 4A) or experiment 2 (Fig. 4B).

IL-8 in Lung Homogenates

The amount of IL-8 was significantly larger in animals in experiment 1 (Fig. 5A) compared with experiment 2 (Fig. 5B). This is consistent with the apparent influx of neutrophils between 48 and 72 hours after injury. IL-8 did not differ between the groups in either experiment.

Apoptosis of Lung Tissue Neutrophils

Differences in the degree of nuclear condensation (measure of apoptosis) were apparent among the 3 modes of ventilation in both experiment 1 and 2. At 48 hours postcriteria, lung tissue neutrophils showed significantly increased nuclear condensation in LTV-treated animals compared with $AVCO_2R$ ($P < 0.001$) (Fig. 6A) with about the same number of neutrophils in the 3 arms, whereas in experiment 2, neutrophils in the $AVCO_2R$ group were the most apoptotic. The apparently fewer neutrophils in the $AVCO_2R$ group are accounted for by 2 less animals in this arm (Fig. 6B).

DNA Fragmentation of Lung Tissue Neutrophils

DNA fragmentation in lung tissue neutrophils was estimated by TUNEL staining. Little fragmentation was evident at 48 hours postcriteria (Fig. 7). However, DNA fragmentation was found in the majority of lung tissue neutrophils at 72 hours postcriteria in $AVCO_2R$, HFPV, and LTV (Fig. 7). There was no significant difference among these groups within each time period ($P > 0.05$), but a comparison of the 3 ventilation strategies at the 2 time points was significant ($P < 0.01$).

DISCUSSION

We found that $AVCO_2R$ resulted in a significant improvement in survival as compared with standard low tidal volume ventilation (LTV). High-frequency percussive venti-

TABLE 3. Blood Metabolic Variables

	Time (h)			Time (h)		
Variables*	**Baseline 1**	**24**	**Baseline 2**	**24**	**48**	**60**
FiO_2						
$AVCO_2R$	1.00 ± 0.0	0.57 ± 0.22	0.90 ± 0.07	0.82 ± 0.06	0.81 ± 0.11	0.70 ± 0.18
HFPV	1.00 ± 0.0	0.28 ± 0.04†	1.00 ± 0.0	0.79 ± 0.11	0.60 ± 0.11	0.41 ± 0.10†
LTV	0.995 ± 0.5	0.33 ± 0.07†	0.80 ± 0.08	0.74 ± 0.08	0.63 ± 0.09	0.60 ± 0.10
PaO_2 (mm Hg)						
$AVCO_2R$	517.1 ± 23.6	59.3 ± 4.8†	97.6 ± 27.5†	61.4 ± 4.9†	89.0 ± 8.9	74.2 ± 8.4
HFPV	485.4 ± 46.7	79.8 ± 13.2†	126.6 ± 25.5†·	85.6 ± 9.7†	84.7 ± 8.0†	86.8 ± 3.8†
LTV	522.4 ± 20.5	72.0 ± 8.5†	85.0 ± 7.1	75.5 ± 10.7†	67.0 ± 7.4†	73.7 ± 4.7
P/F ratio						
$AVCO_2R$	515.0 ± 24.2	140.7 ± 46.9†	107.0 ± 26.3†	79.7 ± 13.0†	131.9 ± 32.6†	155.2 ± 46.3†
HFPV	482.4 ± 46.3	261.8 ± 78.7†	120.4 ± 23.6†	138.4 ± 30.0†	170.7 ± 33.6†	243.8 ± 35.2†
LTV	524.1 ± 19.1	225.5 ± 26.3†	119.1 ± 40.0†	113.7 ± 17.8†	105.7 ± 13.4†	134.3 ± 34.6†
$PaCO_2$ (mm Hg)						
$AVCO_2R$	27.6 ± 2.2	25.5 ± 2.0	36.7 ± 7.3	33.5 ± 2.2*	46.0 ± 2.2	53.6 ± 4.8†
HFPV	32.1 ± 2.2	25.6 ± 3.8	38.5 ± 3.0	48.0 ± 2.4*	53.9 ± 10.6	36.5 ± 3.3
LTV	33.1 ± 3.3	33.7 ± 3.6	49.0 ± 3.2	66.6 ± 12.2†	65.5 ± 7.9†	65.5 ± 8.4†
pH ($-\log[H+]$)						
$AVCO_2R$	7.56 ± 0.03	7.58 ± 0.06	7.44 ± 0.07†	7.52 ± 0.04*	7.41 ± 0.03	7.42 ± 0.03
HFPV	7.50 ± 0.03	7.54 ± 0.01	7.43 ± 0.03	7.44 ± 0.02	7.36 ± 0.07	7.51 ± 0.02*
LTV	7.54 ± 0.02	7.49 ± 0.02	7.36 ± 0.03†	7.32 ± 0.02†	7.36 ± 0.06†	7.35 ± 0.06†
$[HCO_{3-}]$ (mM)						
$AVCO_2R$	24.1 ± 0.8	24.0 ± 1.4	23.0 ± 1.2	25.7 ± 1.4	29.8 ± 1.4*	33.2 ± 1.9†
HFPV	24.6 ± 1.1	21.5 ± 2.8	25.6 ± 1.6	28.9 ± 1.5	27.9 ± 1.0*	28.9 ± 1.4
LTV	24.9 ± 1.1	25.4 ± 1.5	27.1 ± 1.1†	31.9 ± 2.6†	35.9 ± 2.5†	34.7 ± 4.3†

Means ± SEM.
*Significant difference from LTV animals.
†Significant difference from baseline value, $P < 0.05$.

lation (HFPV) resulted in a trend toward improved survival compared with LTV but the difference was not statistically significant. The cause of differences in mortality among the 3 ventilation strategies remains to be proven. Three possibilities might explain the improvement in survival seen with $AVCO_2R$. First, minute ventilation was significantly decreased (approaching 2-fold) in the $AVCO_2R$ group, despite similar PIP and tidal volume between the LTV and $AVCO_2R$ groups. An improvement in survival due to decreased minute ventilation (in our experiments, less frequency) would be disputed by previous research showing that the magnitude of stretch correlates more closely with lung injury.[35] However, recent work by Gattinoni et al[36] indicates that the volume of recruitable lung is a positive indicator of mortality in ARDS. These observations coupled with recent meta analyses of tidal volumes in ARDS patients that show an increase in mortality even at peak airway pressures that ordinarily would be assumed to be safe, suggest the possibility that mortality in ARDS is related to opening and closing injured alveoli. $AVCO_2R$, therefore, may allow a significant decrease in this deleterious activity. A second difference between $AVCO_2R$ and LTV was the $Paco_2$, which was maintained at low to

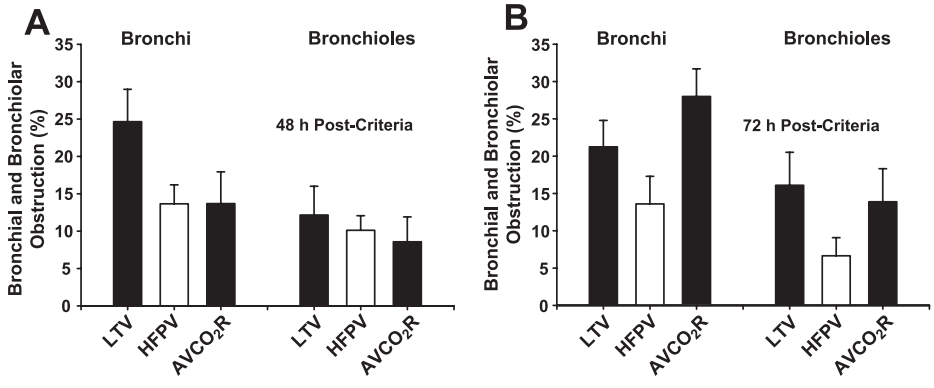

FIGURE 2. Obstruction scores for bronchi and bronchioles expressed as % of total airway lumen obstructed by casts. Measurements were made 48 hours after criteria were met for ARDS (A) and at killing (B) for the 3 treatment groups. Data are expressed as mean ± SEM. There were no differences in obstruction scores for groups in experiment 1 (A). In experiment 2 (B), bronchial obstruction scores were significantly lower in the HFPV group compared with $AVCO_2R$.

TABLE 4. Pathological Correlation

	Congestion	Edema	Hemorrhage	Hyaline Membranes	Septal Thickness
Experiment 1					
LTV	1.25 ± 0.25	1.50 ± 0.29	0.25 ± 0.25	0.0	1.25 ± 0.25
$AVCO_2R$	1.88 ± 0.23	2.13 ± 0.23	1.13 ± 0.23	0.0	0.88 ± 0.23
HFPV	1.78 ± 0.15	1.38 ± 0.02	0.67 ± 0.29	0.56 ± 0.38	0.67 ± 0.29
Experiment 2					
LTV	2.29 ± 0.29	1.86 ± 0.26	0.57 ± 0.20	0.29 ± 0.18	0.57 ± 0.30
AVCO2R	1.57 ± 0.37	1.86 ± 0.40	0.86 ± 0.40	0.43 ± 0.30	0.57 ± 0.20
HFPV	1.50 ± 0.29	1.0 ± 0.41	0.33 ± 0.33	1.25 ± 0.75	1.0 ± 0.58

normal levels in the $AVCO_2R$ group until 60 hours, whereas it was high in the LTV group from 24 hours after randomization. The $Paco_2$ was also low in the HFPV group, which showed a trend toward improved survival compared with LTV. However, the actual flux of CO_2 through the lung tissue was presumably even less in the $AVCO_2R$ group because of CO_2 removal directly from the blood via the $AVCO_2R$ circuit. It is possible that hypercapnia contributes to lung injury and that carbon dioxide diffusion in damaged lung parenchyma may exacerbate or sustain inflammatory damage.[37,38] Finally, the $AVCO_2R$ gas exchange device is known to transiently trap polymorphonuclear leukocytes which could result in removal of activated cells from the circulation. This mechanism is less likely because $AVCO_2R$ without sweep gas was not beneficial (n = 4, all died, early unpublished data) in prolonging survival of these injured animals.

PMNs are considered to be of prime importance in causing lung damage associated with ARDS.[39–43] Previous studies have shown increased numbers and activation of neutrophils in patients with ARDS and that the mode of ventilation may impact neutrophil activation.[16,44–47] In support of those previous reports, the significantly lower lung MPO in the $AVCO_2R$ group at 72 hours postcriteria compared with HFPV and LTV, indicates that neutrophil disposition might be causally associated with the improved survival. Although lung MPO for all groups was significantly lower at 48 hours than at 72 hours, the numbers of neutrophils in the tissue determined by confocal microscopy at these time points did not differ. This suggests that lung MPO includes both MPO in neutrophils and MPO remaining in tissue after degranulation or disintegration of the cells. Therefore, although MPO is often used as a surrogate marker for neutrophil numbers, this assumption may be unjustified in certain cases. To interpret these data, the flux of neutrophils into the lung over time and the fate of these cells must be considered. After release from the bone marrow, neutrophils spend only

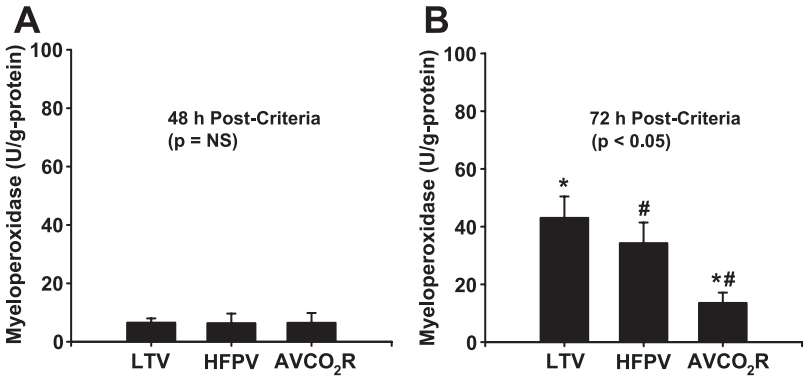

FIGURE 3. Myeloperoxidase activity in lung homogenates expressed as units/g protein. Measurements were made 48 hours after criteria were met for ARDS (A) and at killing (B) for the 3 treatment groups. Data are expressed as mean ± SEM; $*P < 0.05$ for LTV versus $AVCO_2R$.

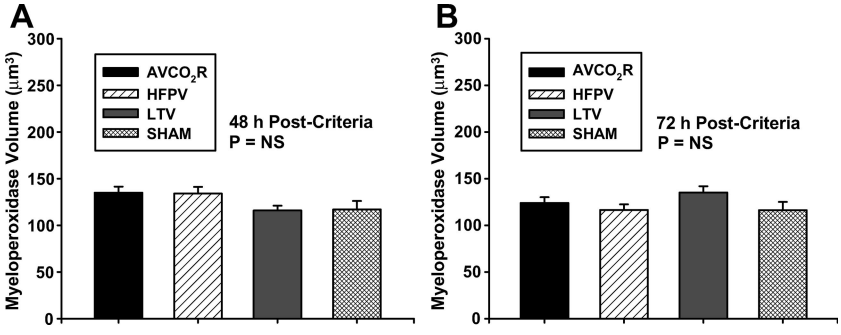

FIGURE 4. Myeloperoxidase volume in lung tissue neutrophils. Laser scanning confocal microscopy was used to measure the average volume of MPO in lung tissue neutrophils at 48 hours (A) and 72 hours (B) after randomization. Data is expressed as histograms for each mode of ventilation. There is no significant difference among the 3 data distributions ($P > 0.05$).

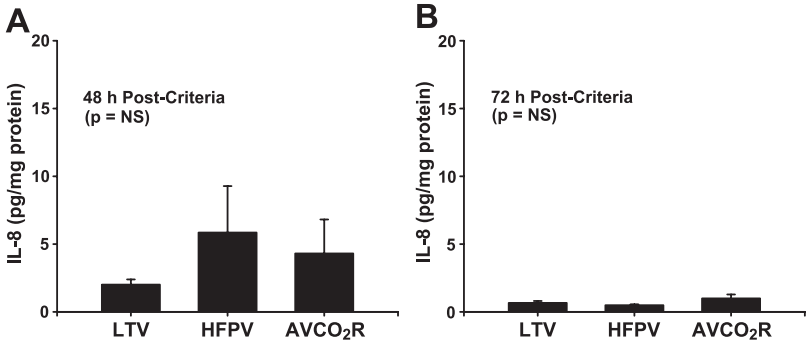

FIGURE 5. IL-8 concentration in lung homogenates expressed as picograms/mg protein. Measurements were made at 48 hours after criteria were met for ARDS (A) and at killing (B) for the 3 treatment groups. In all 3 groups, IL-8 was higher at 48 hours than at killing, but there were no differences between treatment groups in IL-8 concentrations at either time point.

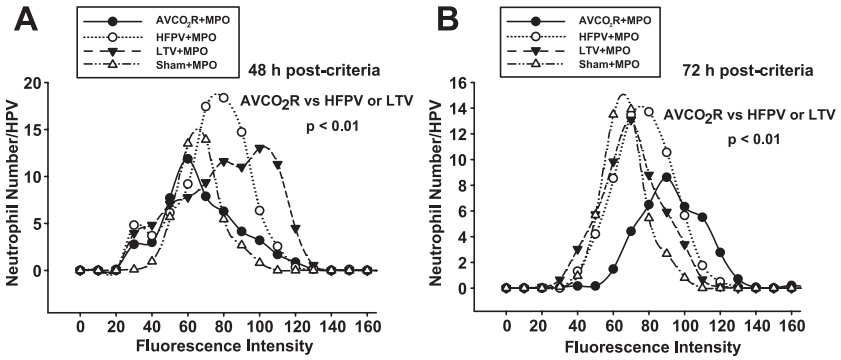

FIGURE 6. Apoptosis as detected by fluorescent intensity of Hoechst staining in confocal micrographs of lung tissue. The confocal software counted the number of cells with each Hoechst staining intensity. Data are presented for neutrophils with positive MPO staining in experiment 1 (A) and in experiment 2 (B). By analyzing the morphometry of the cells, we determined that a small number of the MPO-negative cells are neutrophils that have lost their MPO, but that the majority are other lung cells. Analysis of variance techniques indicated significant differences in Hoechst intensity between LTV (higher) and $AVCO_2R$ (lower) for experiment 1. In experiment 2, the Hoechst intensity is higher for the $AVCO_2R$ group in MPO containing cells compared with LTV.

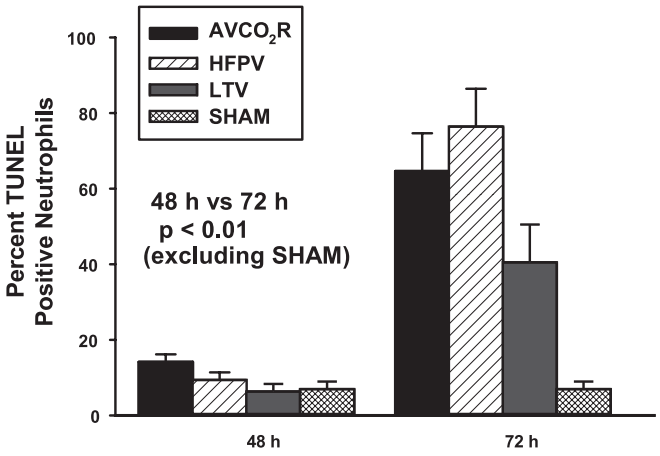

FIGURE 7. DNA fragmentation in lung tissue neutrophils. MPO-containing cells were analyzed for TUNEL staining at 48 hours postcriteria and 72 hours postcriteria for DNA fragmentation ($P > 0.05$, for LTV, HFPV, and $AVCO_2R$ for differences within groups at the 2 times). Little fragmentation is present at 48 hours postcriteria, but a substantial increase in fragmentation is noted at 72 hours postcriteria ($P < 0.05$, for comparison between times).

a few hours in the peripheral circulation before migration into tissues. The half-life of neutrophils in lung tissue is uncertain and has been estimated to be as short as 2.6 hours in mice[48] and 6 days in rabbits.[49] Normally, the fate of lung neutrophils is likely controlled largely by resident macrophages and is then dependent on the rate of phagocytosis by these cells. Normally, only a few neutrophils die by necrosis. Presumably, the apoptotic neutrophils are phagocytized, and the MPO is degraded via highly specific proteolytic pathways (via ubiquitination) and transported to the proteasome where it is metabolized.[50] In that case, the MPO would not be measured by an enzymatic assay. Alternatively, those cells that either degranulate or die by necrosis might deposit enzymatically detectable MPO in the tissues. The present data may be interpreted as a large influx of neutrophils beginning at the 48-hour time point after randomization in all 3 study arms. In support of this, the lung IL-8 concentrations were consistent with increased neutrophil migration into lung tissue at 48 hours postcriteria (high IL-8 concentration) and lesser migration at the 72 hours time point (lower concentration of IL-8).

The significantly lower MPO found at 72 hours postcriteria in the $AVCO_2R$-treated sheep compared with the other ventilatory modalities may be due to one or more of the

following processes: (1) partial degranulation of neutrophils in the circulation before migration into the tissues; (2) migration of fewer neutrophils into the lung; (3) more efficient phagocytosis of neutrophils by resident macrophages in the lung. Little support for possible differential degranulation in the circulation was found because the numbers of neutrophils and the volume of MPO differed little among the 3 groups at 48 or 72 hours postcriteria. However, it is still possible there were differences in the rate of migration of neutrophils among the 3 arms in the period between 48 hours after randomization and killing time points. The uncertainty of neutrophil flux between the 2 time points is an unavoidable limitation inherent in the design of the study. In regard to the third possibility, there were significantly more apoptotic neutrophils at the time of killing in the $AVCO_2R$ group compared with LTV or HFPV. Increased apoptosis of neutrophils would presumably result in more efficient phagocytosis of neutrophils in the $AVCO_2R$ group. The TUNEL staining is consistent with this interpretation because it suggests that in the case of LTV and HFPV, more of the neutrophils were dying by necrosis because most of the neutrophils at the 72-hour postcriteria time point in all the ventilation groups showed DNA fragmentation. It will be important to answer this question in future studies because phagocytosis of apoptotic neutrophils by resident macrophages has an anti-inflammatory effect[51] on those cells due to elaboration of less pro-inflammatory cytokines.[51,52] If a similar process occurs in other organs, it might have a moderating effect on the evolution of multiple organ failure that often accompanies severe respiratory failure.[53]

In summary, we have shown in a burn/smoke model of lung injury that $AVCO_2R$ results in improved survival as compared with HFPV and LTV. Lower MPO in the $AVCO_2R$ group suggests a possible causal association between lung neutrophils and mortality in this model. The difference in MPO between the $AVCO_2R$ group and the LTV and HFPV groups was associated with differences in extent of apoptosis. Further study of neutrophil dynamics in the lung and other organs and of CO_2 flux in the lungs of animals treated with these ventilatory techniques are indicated to further illuminate the mechanism of the survival differences.

ACKNOWLEDGMENTS

The authors thank Dr. Elbert Whorton for his help with statistical analysis of the data, and the Optical Imaging Core at The University of Texas Medical Branch for their valuable assistance with technical aspects of laser scanning confocal microscopy.

REFERENCES

1. Jardin F, Fellahi JL, Beauchet A, et al. Improved prognosis of acute respiratory distress syndrome 15 years on. *Intensive Care Med*. 1999; 25:936–941.
2. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med.* 2000;342:1301–1308.
3. Amato MB, Barbas CS, Medeiros DM, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med*. 1998;338:347–354.
4. Brower RG, Lanken PN, MacIntyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. *N Engl J Med*. 2004;351:327–336.
5. Brower RG, Shanholtz CB, Fessler HE, et al. Prospective, randomized, controlled clinical trial comparing traditional versus reduced tidal volume ventilation in acute respiratory distress syndrome patients. *Crit Care Med*. 1999;27:1492–1498.
6. Hickling KG, Henderson SJ, Jackson R. Low mortality associated with low volume pressure limited ventilation with permissive hypercapnia in severe adult respiratory distress syndrome. *Intensive Care Med*. 1990; 16:372–377.
7. Kallet RH, Jasmer RM, Pittet JF, et al. Clinical implementation of the ARDS network protocol is associated with reduced hospital mortality compared with historical controls. *Crit Care Med*. 2005;33:925–929.
8. Slutsky AS. Mechanical ventilation. American College of Chest Physicians' Consensus Conference. *Chest*. 1993;104:1833–1859.
9. Brochard L, Roudot-Thoraval F, Roupie E, et al. Tidal volume reduction for prevention of ventilator-induced lung injury in acute respiratory distress syndrome. The Multicenter Trail Group on Tidal Volume reduction in ARDS. *Am J Respir Crit Care Med*. 1998;158: 1831–1838.
10. Stewart TE, Meade MO, Cook DJ, et al. Evaluation of a ventilation strategy to prevent barotrauma in patients at high risk for acute respiratory distress syndrome. Pressure- and Volume-Limited Ventilation Strategy Group. *N Engl J Med*. 1998;338:355–361.
11. Cioffi WG, Graves TA, McManus WF, et al. High-frequency percussive ventilation in patients with inhalation injury. *J Trauma*. 1989;29:350–354.
12. Cortiella J, Mlcak R, Herndon D. High frequency percussive ventilation in pediatric patients with inhalation injury. *J Burn Care Rehabil*. 1999; 20:232–235.
13. Salim A, Martin M. High-frequency percussive ventilation. *Crit Care Med*. 2005;33(3 suppl):S241–S245.
14. Carman B, Cahill T, Warden G, et al. A prospective, randomized comparison of the Volume Diffusive Respirator vs conventional ventilation for ventilation of burned children. 2001 ABA paper. *J Burn Care Rehabil*. 2002;23:444–448.
15. Cartotto R, Ellis S, Smith T. Use of high-frequency oscillatory ventilation in burn patients. *Crit Care Med*. 2005;33(3 suppl):S175–S181.
16. Cioffi WG, deLemos RA, Coalson JJ, et al. Decreased pulmonary damage in primates with inhalation injury treated with high-frequency ventilation. *Ann Surg*. 1993;218:328–337.
17. Mlcak R, Cortiella J, Desai M, et al. Lung compliance, airway resistance, and work of breathing in children after inhalation injury. *J Burn Care Rehabil*. 1997;18:531–534.
18. Reper P, Wibaux O, Van Laeke P, et al. High frequency percussive ventilation and conventional ventilation after smoke inhalation: a randomised study. *Burns*. 2002;28:503–508.
19. Rue LW III, Cioffi WG, Mason AD, et al. Improved survival of burned patients with inhalation injury. *Arch Surg*. 1993;128:772–780.
20. Alpard SK, Zwischenberger JB, Tao W, et al. Reduced ventilator pressure and improved P/F ratio during percutaneous arteriovenous carbon dioxide removal for severe respiratory failure. *Ann Surg*. 1999; 230:215–224.
21. Brunston RL Jr, Zwischenberger JB, Tao W, et al. Total arteriovenous CO_2 removal: simplifying extracorporeal support for respiratory failure. *Ann Thorac Surg*. 1997;64:1599–1604; discussion 1604–1595.
22. Conrad SA, Zwischenberger JB, Grier LR, et al. Total extracorporeal arteriovenous carbon dioxide removal in acute respiratory failure: a phase I clinical study. *Intensive Care Med*. 2001;27:1340–1351.
23. Zwischenberger JB, Alpard SK, Conrad SA, et al. Arteriovenous carbon dioxide removal: development and impact on ventilator management and survival during severe respiratory failure. *Perfusion*. 1999;14:299–310.
24. Zwischenberger JB, Alpard SK, Tao W, et al. Percutaneous extracorporeal arteriovenous carbon dioxide removal improves survival in respiratory distress syndrome: a prospective randomized outcomes study in adult sheep. *J Thorac Cardiovasc Surg*. 2001;121:542–551.
25. Zwischenberger JB, Conrad SA, Alpard SK, et al. Percutaneous extracorporeal arteriovenous CO_2 removal for severe respiratory failure. *Ann Thorac Surg*. 1999;68:181–187.

26. Alpard SK, Zwischenberger JB, Tao W, et al. New clinically relevant sheep model of severe respiratory failure secondary to combined smoke inhalation/cutaneous flame burn injury. *Crit Care Med*. 2000;28:1469–1476.
27. Soejima K, Schmalstieg FC, Sakurai H, et al. Pathophysiological analysis of combined burn and smoke inhalation injuries in sheep. *Am J Physiol Lung Cell Mol Physiol*. 2001;280:L1233–L1241.
28. Chandra A, Katahira J, Schmalstieg FC, et al. P-selectin blockade fails to improve acute lung injury in sheep. *Clin Sci (Lond)*. 2003;104:313–321.
29. Cox RA, Burke AS, Soejima K, et al. Airway obstruction in sheep with burn and smoke inhalation injuries. *Am J Respir Cell Mol Biol*. 2003;29(3 Pt 1):295–302.
30. Pearce ML, Yamashita J, Beazell J. Measurement of pulmonary edema. *Circ Res*. 1965;16:482–488.
31. Keeney SE, Mathews MJ, Haque AK, et al. Oxygen-induced lung injury in the guinea pig proceeds through CD18-independent mechanisms. *Am J Respir Crit Care Med*. 1994;149(2 Pt 1):311–319.
32. Schmalstieg FC, Chow J, Savage C, et al. Interleukin-8, aquaporin-1, and inducible nitric oxide synthase in smoke and burn injured sheep treated with percutaneous carbon dioxide removal. *ASAIO J*. 2001;47:365–371.
33. Nusbaum P, Laine C, Bouaouina M, et al. Distinct signaling pathways are involved in leukosialin (CD43) down-regulation, membrane blebbing, and phospholipid scrambling during neutrophil apoptosis. *J Biol Chem*. 2005;280:5843–5853.
34. Gardai SJ, Whitlock BB, Xiao YQ, et al. Oxidants inhibit ERK/MAPK and prevent its ability to delay neutrophil apoptosis downstream of mitochondrial changes and at the level of XIAP. *J Biol Chem*. 2004;279:44695–44703.
35. Quinn D, Tager A, Joseph PM, et al. Stretch-induced mitogen-activated protein kinase activation and interleukin-8 production in type II alveolar cells. *Chest*. 1999;116(1 suppl):89S–90S.
36. Gattinoni L, Caironi P, Cressoni M, et al. Lung recruitment in patients with the acute respiratory distress syndrome. *N Engl J Med*. 2006;354:1775–1786.
37. Doerr CH, Gajic O, Berrios JC, et al. Hypercapnic acidosis impairs plasma membrane wound resealing in ventilator-injured lungs. *Am J Respir Crit Care Med*. 2005;171:1371–1377.
38. Lang JD, Figueroa M, Sanders KD, et al. Hypercapnia via reduced rate and tidal volume contributes to lipopolysaccharide-induced lung injury. *Am J Respir Crit Care Med*. 2005;171:147–157.
39. Basadre JO, Sugi K, Traber DL, et al. The effect of leukocyte depletion on smoke inhalation injury in sheep. *Surgery*. 1988;104:208–215.
40. Belperio JA, Keane MP, Burdick MD, et al. Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. *J Clin Invest*. 2002;110:1703–1716.
41. Imai Y, Kawano T, Miyasaka K, et al. Inflammatory chemical mediators during conventional ventilation and during high frequency oscillatory ventilation. *Am J Respir Crit Care Med*. 1994;150(6 Pt 1):1550–1554.
42. Kawano T, Mori S, Cybulsky M, et al. Effect of granulocyte depletion in a ventilated surfactant-depleted lung. *J Appl Physiol*. 1987;62:27–33.
43. Wilson MR, Choudhury S, Goddard ME, et al. High tidal volume upregulates intrapulmonary cytokines in an in vivo mouse model of ventilator-induced lung injury. *J Appl Physiol*. 2003;95:1385–1393.
44. Matsuoka T, Kawano T, Miyasaka K. Role of high-frequency ventilation in surfactant-depleted lung injury as measured by granulocytes. *J Appl Physiol*. 1994;76:539–544.
45. Rivkind AI, Siegel JH, Littleton M, et al. Neutrophil oxidative burst activation and the pattern of respiratory physiologic abnormalities in the fulminant post-traumatic adult respiratory distress syndrome. *Circ Shock*. 1991;33:48–62.
46. Sugiura M, McCulloch PR, Wren S, et al. Ventilator pattern influences neutrophil influx and activation in atelectasis-prone rabbit lung. *J Appl Physiol*. 1994;77:1355–1365.
47. Zimmerman GA, Renzetti AD, Hill HR. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. Evidence for activated neutrophils in the pulmonary circulation. *Am Rev Respir Dis*. 1983;127:290–300.
48. Cheretakis C, Leung R, Sun CX, et al. Timing of neutrophil tissue repopulation predicts restoration of innate immune protection in a murine bone marrow transplantation model. *Blood*. 2006;108:2821–2826.
49. Cohen AB, Rossi M, Geczy D, et al. Neutrophil turnover in normal rabbit lungs. *J Clin Invest*. 1982;69:794–798.
50. DeLeo FR, Goedken M, McCormick SJ, et al. A novel form of hereditary myeloperoxidase deficiency linked to endoplasmic reticulum/proteasome degradation. *J Clin Invest*. 1998;101:2900–2909.
51. Knapp S, Leemans JC, Florquin S, et al. Alveolar macrophages have a protective antiinflammatory role during murine pneumococcal pneumonia. *Am J Respir Crit Care Med*. 2003;167:171–179.
52. Fadok VA, Bratton DL, Konowal A, et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest*. 1998;101:890–898.
53. Borelli M, Kolobow T, Spatola R, et al. Severe acute respiratory failure managed with continuous positive airway pressure and partial extracorporeal carbon dioxide removal by an artificial membrane lung. A controlled, randomized animal study. *Am Rev Respir Dis*. 1988;138:1480–1487.

Discussions

DR. ROBERT H. BARTLETT (ANN ARBOR, MICHIGAN): This study is very important in at least 3 areas of the pathophysiology and treatment of acute lung injury.

First of all, this model of ARDS is to my knowledge unprecedented. It is the only large animal clinical relevant model that can be studied for days, and in itself that is a major contribution.

Secondly, the use of selective CO_2 removal as opposed to oxygenation by a mechanical device allows the investigators to separate the effects of altered pulmonary mechanics, that is to say breathing and ventilation, and it offers the only real way to separate those 2 components of breathing, as Dr. Zwischenberger pointed out.

And thirdly, this technique allows the study of the basic mechanisms by which lung stretch or respiratory acidosis or other variables might impact the path of genesis and recovery from acute lung injury. We have journals full of studies on alveolar cells and lung capillary cells, all done in Petri dishes, and it is hard to understand the relevance of those studies. This model allows the molecular biology of the events occurring in ARDS to be studied in an appropriate model.

This technique of selective CO_2 removal in respiratory failure is now being applied clinically, primarily in Europe. And my questions make the jump to clinical use.

The ideal application of this technology would seem to be the disease with pure CO_2 retention, that is to say status asthmaticus. Do you have any experience in this area? Have you studied it? What do you think about that application?

For a group of surgeons it would seem the ideal application would be patients who have undergone thoracotomy, perhaps pulmonary resection, and are now faced with complications either in the pulmonary parenchyma or bronchopleural fistulas or blown stumps and so on. Has there been any application of this technique to that particular problem?

Finally, even though your studies have shown that oxygenation improves solely by the mechanical removal of

CO_2, there clearly are patients who reach a stage of ARDS where they cannot oxygenate at all. This technique would not improve that. What would you suggest for those types of patients? In summary, where do you see the clinical applications of this technique going?

Dr. Joseph B. Zwischenberger (Galveston, Texas): First of all, I want to express my admiration and gratitude to my mentor, Dr. Bartlett, for his pioneering efforts to develop, standardize and distribute the application of Extracorporeal Membrane Oxygenation (ECMO) to treat life-threatening cardiopulmonary failure. Certainly, ECMO has become the standard of care in neonates with respiratory failure and congenital cardiac repairs that are unresponsive to conventional therapy. Dr. Bartlett's contributions have been recognized by the American Surgical Association as he was awarded the Medallion for Scientific Achievement. Most importantly, Dr. Bartlett taught all of us that extrapulmonary gas exchange can assume many forms ranging from ECMO to the development of the artificial lung.

One thing I must call attention to is that in the conduct of a study like this we had to follow animal case management protocols exactly. We conducted low tidal volume exactly the way the ARDS NET Trial prescribed. We performed percussive ventilation exactly the way the manufacturer recommends for patients. And we performed $AVCO_2R$ exactly the way we wrote the protocols for our human studies. When treating an animal in an outcome study, there is always a tremendous temptation to exercise your knowledge and skills as a physician to try to intervene or change that protocol. So, one of the most important points is to follow the protocol.

Now, in terms of responding to your questions about clinical application, we have used this in patients with status asthmaticus. It is currently thought that virtually all patients with status asthmaticus can respond to different intravenous therapies and even general anesthesia. But we all know as clinicians that that is not true and hundreds of patients die of status asthmaticus each year across the country. At our institution, we have used this application in a child, who despite general anesthesia, had a PCO_2 of 160 and an $AVCO_2R$ was applied. That patient went home on the fifth day as soon as the asthma broke.

In terms of applying this technique to prolonged air leak, I have not had to do that in my practice. But I can tell you over in Europe there is a manuscript that was submitted for peer review publication that does report on 7 patients in which this technique was applied successfully.

Now, as to the problem of an inability to oxygenate, when teaching this technique the most difficult thing to communicate to clinical care physicians is the concept that when you uncouple CO_2 and oxygenation, physiology changes. If you ever watched somebody hold their breath for 2 minutes, they become desperate to breathe, but they never turn blue. The point is that once you initiate very slow volume control ventilation and remove CO_2 extracorporeally, oxygenation becomes much less of a physiologic challenge. However, for those few patients in whom we are unable to oxygenate, obviously more aggressive techniques such as venovenous or venoarterial ECMO are warranted.

Dr. Ronald V. Maier (Seattle, Washington): I want to focus on your proposed mechanism for the improvement in survival due to an increased apoptotic rate in neutrophils. You may have mentioned it and I missed it, but have you conducted the control of measuring the impact of extracorporeal circulation on the neutrophil apoptotic rate in this animal model? As you know, mere adherence of the neutrophils can initiate the caspase sequence and apoptosis. Did you assess low tidal volume ventilation and extracorporeal circulation without removing CO_2 in your circuit? I do not know the details of neutrophil adherence characteristics, but wonder whether exposure of the blood to the circuit without CO_2 removal would have any beneficial effect on increasing apoptosis and improving outcomes.

Dr. Joseph B. Zwischenberger (Galveston, Texas): We also asked that question and wondered whether we could separate the effects of the circuit from the gas exchange characteristics. If you put an animal that is this sick on an extracorporeal circuit and do not accomplish gas exchange, they all die. We performed a series of 5 animals fitted with $AVCO_2R$ but no sweep gas. I did not report these animals because they all died and the Institutional Animal Committee asked that we no longer perform that arm. So we have not obtained the data on that particular group.

One of the things I must mention is that we fully anticipated the conventional pathologic studies would yield some insight in the outcomes. But that was not the case. It was not until we looked at the physiology of the neutrophil that we were able to clearly see a difference in physiologic behavior between groups.

Dr. H. Gill Cryer (Los Angeles, California): I had a chance to visit this lab 1 time, and if you ever get a chance to do that, I would suggest you take it up because what they can do there is absolutely phenomenal.

I was really struck by the fact that the structural pathology in the different groups was the same. And I assume that the animals are dying of hypoxia. You did not really say, but I assume that is the case. I, like Dr. Maier, am having trouble making the jump between how it is that a decrease in neutrophils in the lung tissue is keeping the animals from becoming hypoxic with this technique.

As you know, attempts to use monoclonal antibodies for neutrophils and things like that in experimental animals and in humans have not led to a particularly profound effect. And I just ask you to explain why it is those neutrophils might be contributing to the problem.

DR. JOSEPH B. ZWISCHENBERGER (GALVESTON, TEXAS): Our interpretation is that every neutrophil that develops in the bone marrow is destined to be eliminated through apoptotic mechanisms. We interpret our data as indicating that apoptotic mechanisms are preserved by $AVCO_2R$ through CO_2 normalization that keeps the lung milieu from being subject to the degranulation of neutrophils and fueling of the inflammatory process.

For decades we thought that ARDS was a "fueling of fire" of inflammation in the lung that resulted in progressive respiratory failure. The finding that the apoptotic pathways and the apoptotic mechanisms are preserved by $AVCO_2R$ may be a clue to why this fueling of the fire no longer takes place when treated with $AVCO_2R$.

Although hypoxia was present to a degree, the cause of death likely involved multiple organ damage. Obviously, we did not do a study designed to test that. This was uncovered when we went back and did the pathologic studies and delved deeper into the different mechanisms by which this could occur. That is why we are specifically conducting those studies now in another prospective randomized animal study.

DR. BASIL A. PRUITT, JR. (SAN ANTONIO, TEXAS): Dr. Zwischenberger, your study addresses the most important comorbid factor in burn patients today, which is inhalation injury. I was a little disappointed that there was no difference in the pathology. Do you have some idea how long such treatment would have to be continued in the patient to allow the lung to heal and resume normal function? Is there some time limit to how long this can be done because of the membrane effects of the device? And does that membrane effect have any consequence for the formed elements of the blood? Are the neutrophils in the circuit rather than in the lung? And does the device induce coagulopathy as time passes? Lastly, if you intervened earlier, would use of the device have a prophylactic effect and minimize the pathologic changes?

DR. JOSEPH B. ZWISCHENBERGER (GALVESTON, TEXAS): We, too, were disappointed that the simple studies of pathology did not yield a difference. However, we were comforted by the fact that our model indeed represented quite a lung injury and would yield an LD 100 if carried to completion with conventional management techniques.

One of the problems of designing a study of this nature is, first of all, do you declare an animal injured from the get-go and then perform your study based on the assumption that they are injured or do you require that they meet criteria? We felt strongly that to mimic the clinical situation they had to meet criteria for ARDS.

Likewise, in terms of treatment period, we are able to go 96 hours post-injury. But at that point, you hit Friday evening, and staffing an intensive care unit with a sheep on an extracorporeal device with the restrictions and labor force required is onerous at best. We plan on longer studies, but, as you can well imagine, the labor force involved becomes outrageous.

Finally, what I can tell you is that there were not neutrophils in the circuit to excess of what we saw in the other animals. In other words, the number of neutrophils in the lung was the same. It was the physiologic behavior of the neutrophils that became so readily apparent to us in our pathologic studies.

Again, this was by retrospective study. We did not plan on finding the neutrophils to be the overall deciding factor. Therefore, I can only tell you what we found; I cannot say we designed the study to look at neutrophils. But the data showed a difference between the groups. I think the bottom line is that all 3 groups were severely injured and we did see a significant outcomes difference that we attempted to explain.

The idea of starting earlier or using the device more prophylactically, obviously, is very exciting. The difficult problem is convincing clinicians to use an invasive technique such as extracorporeal gas exchange, even when a patient is in the throes of death. I think that trying to move this technique to an earlier point in the treatment process will require considerably more experience.