Protective Strategies of High-Frequency Oscillatory Ventilation in a Rabbit Model

JONATHAN MEYER, PETER N. COX, COLIN MCKERLIE, AND DOROTHEE BIENZLE

Department of Pathobiology [J.M., D.B.], University of Guelph, Guelph, Ontario, N1G 2W1, Canada; Departments of Critical Care [P.N.C.] and Pediatric Laboratory Medicine [C.M.], The Hospital for Sick Children, University of Toronto, Toronto, Ontario, M5G 1X8, Canada

ABSTRACT: High-frequency oscillatory ventilation (HFOV) causes less severe lung injury than conventional mechanical ventilation (CMV) but the optimal frequency for HFOV has not been determined. We hypothesized that 15 Hz HFOV would be more protective than 5 Hz HFOV in a rabbit model of acute lung injury. Surfactant-depleted rabbits were ventilated at 15 Hz or 5 Hz HFOV for 4 h, or not ventilated, to characterize the extent of lung injury before HFOV. PaO₂ and PaCO₂ were measured throughout the experiment, and lung myeloperoxidase (MPO) activity, neutrophil infiltration, and histopathological changes were determined. There were no statistically significant differences in PaO₂ and PaCO₂ between groups ($p > 0.05$). Neutrophil counts ($p = 0.013$), airway injury scores ($p = 0.007$), airspace injury scores ($p = 0.029$), and total lung injury scores ($p = 0.014$) differed between non-HFO-ventilated and HFOV animals. Comparing the 2 HFOV regimens, 15 Hz ventilation yielded a lower tissue neutrophil score ($p = 0.005$). MPO activity, neutrophil count, airway injury score, airspace injury score, and total lung injury score parameters did not differ significantly between the HFOV groups ($p > 0.150$). We concluded that both frequencies of HFOV efficiently restored O₂ and CO₂ exchange in a rabbit model of severe lung injury, and that 5 Hz HFOV increased neutrophil infiltration relative to 15 Hz HFOV. (*Pediatr Res* 60: 401–406, 2006)

Intensive care physicians generally agree that conventional mechanical ventilation (CMV), with relatively large tidal volumes (V_T) or low positive end expiratory pressure (PEEP) levels, contributes to ventilator associated lung injury (VALI) in patients with acute respiratory distress syndrome (ARDS) (1). Convective bulk flow is believed to be the means by which gas is moved from the proximal airways to the alveoli in this form of ventilation with large volume excursions of the lung as a consequence. The mechanisms by which gas is delivered to the distal airspaces in high-frequency oscillatory ventilation (HFOV) differ from those of CMV, in that volumes smaller than dead space are moved within the lung during ventilation (2,3). The protective benefit gained through HFOV is believed to be due to a reduction in expansion and contraction of the small airways and airspaces. Although HFOV is

considered protective in nature, it remains undetermined whether all frequencies of HFOV are equally protective.

The relationship of V_T to frequency (f) is approximated by the formula: $\text{DCO}_2 \propto V_T^2 f$ (where DCO_2 represents CO₂ elimination) during HFOV (3,4). From this relationship it is clear that if CO₂ were to be maintained at constant normocapnic levels, then a lower frequency would result in a higher V_T . The high V_T potentially increases delta V and lung distension, and likely leads to VALI, possibly by a mechanism similar to that of convectional bulk airflow in CMV. In contrast, the higher the frequency, the lower the V_T and the bulk flow, and the less distension of the small airways and airspaces will occur. Until recently, due to technical ventilator limitations, particularly in older children and adults, oscillators did not have the power to generate sufficiently high V_T to maintain normocapnea (5). The clinical implications were that to maintain normocapnea the frequency had to be reduced, thereby leading to increased minute ventilation, presumably through increased V_T . HFOV in a large animal model showed that V_T was proportional to amplitude and inversely proportional to ventilation frequency (4). However, in a small animal model or neonate the relationship between amplitude and frequency of HFOV to lung injury has not been determined (6–8). While HFOV appears to be a more protective mode of ventilation relative to CMV, optimal frequencies for HFOV remain to be determined. Hence, this pilot investigation addressed the hypothesis that lower-frequency HFOV was likely to cause more lung damage than higher-frequency HFOV in the rabbit model of acute lung injury.

METHODS

Experimental procedures. The study was conducted in accordance with National Institutes of Health guidelines for the use of experimental animals, and with the approval of the Institutional Animal Care and Use Committee of The Hospital for Sick Children, Toronto, Canada. The warmed saline broncho-alveolar lavage model of acute lung injury has been previously described (6–9). Eighteen adult, specific pathogen free (SPF), male New Zealand White

Received November 18, 2005; accepted May 30, 2006.

Correspondence Dr. Dorothee Bienzle, D.V.M., Ph.D., Department of Pathobiology, University of Guelph, Guelph, ON, N1G 2W1, Canada; e-mail: dbienzle@uoguelph.ca

Supported in part by infrastructure funding from the Canadian Foundation for Innovation (CFI) and by research funds from the Department of Critical Care at the University of Toronto.

DOI: 10.1203/01.pdr.0000238248.42925.f4

Abbreviations: ARDS, acute respiratory distress syndrome; CMV, conventional mechanical ventilation; HFOV, high-frequency oscillatory ventilation; PaO₂, arterial oxygen saturation; PEEP, positive end expiratory pressure; PIP, peak inspiratory pressure; VALI, ventilator associated lung injury volume; V_T , tidal volume

rabbits (*Oryctolagus cuniculus*) weighing approximately 3 kg were used in the study.

Animal preparation. All animals were assessed as being healthy by clinical examination before anesthesia. After premedication with acepromazine (0.5 mg/kg intramuscularly), anesthesia was induced with a bolus of IV sodium pentobarbital (10–20 mg/kg) and the animals were placed in dorsal recumbency on a heated pad. An auricular artery catheter allowed for blood collection (blood gas determination) and blood pressure monitoring (Model 1280, Hewlett-Packard, Colorado Springs, CO) during the experiment. Anesthesia was maintained by constant infusion of pentobarbital (6 mg/kg/h), mixed with 0.9% saline and dextrose, through a catheter placed in the left auricular vein. After skin and s.c. dissection, the trachea was transected and a 3.5- to 4-mm non-cuffed endotracheal tube was secured without air leaks. Rabbits were connected to an infant CMV ventilator (Humming V, Senko Medical Instruments, Tokyo, Japan) and paralyzed by constant infusion of pancuronium bromide (0.2 mg/kg/h). V_T was measured with a pneumotachometer (BEAR NVM-1, BEAR Medical Systems, Riverside, CA) connected to the endotracheal tube, and ECG's were recorded during CMV.

Induction of lung injury. The lung injury was created by repeated saline lavages in aliquots of 25 mL/kg. Briefly, the rabbits were disconnected from the ventilator and a funnel attached to the endotracheal tube. The saline was slowly infused through the funnel while the chest was gently massaged and compressed in a caudo-cranial direction. After the massage maneuver the fluid was allowed to drain by gravity and excessive fluid was removed by negative pressure suction through the proximal portion of the endotracheal tube. The rabbits were reconnected to the conventional ventilator, and lavages were repeated until a defined end point was reached: Oxygen saturation (SpO_2) stabilized at 80% and arteriolar blood gases (ABG) reached and maintained an oxygen partial pressure (PaO_2) of less than 60 mm Hg for 10 min. Once the rabbits were stabilized, time was recorded as $T = 0$. The rabbits were placed into equal groups in a random manner: HFOV at 5 Hz ($n = 6$), HFOV at 15 Hz ($n = 6$), and a non-HFO ventilated group ($n = 6$). Animals from the latter group were euthanized immediately; other animals were ventilated for 4 h with a HFO ventilator designed for use in pediatric patients (High-frequency Oscillatory Ventilator 3100, Sensor Medics Corporation, Yorba Linda, CA).

Ventilation parameters. During the animal preparation stage and the tracheotomy procedure the rabbits were ventilated conventionally at the following settings: peak inspiratory pressure (PIP): 10 cm H_2O , PEEP: 0 cm H_2O , respiratory rate (RR) 30/min, gas flow rate 10L/min, inspiration:expiration ratio (I:E) 0.65 and fraction of inspired oxygen (FiO_2) 1. The goal of ventilation was to maintain V_T at approximately 10 mL/kg. During the induction of lung injury the settings were adjusted in an attempt to maintain the V_T at this level. This was achieved by increasing the PIP to approximately 20 cm H_2O , PEEP to 5 cm H_2O , and I:E to 0.95. Once the end point of lung injury was reached ($T = 0$), animals from treatment groups A and B were transferred from CMV to HFOV. Immediately after connection to the HFO ventilator a recruitment maneuver (sustained inflation for 30 s at 30 cm H_2O) was performed on each animal, followed by ventilation at the predetermined frequency. During the HFOV phase of the experiment, the mean airway pressure (MAP) was fixed at 15 cm H_2O in an attempt to attain similar lung distension/recruitment between groups. Normocapnea ($PaCO_2$ 35–45 mm Hg) was targeted by adjusting the power/pressure amplitude (ΔP) to attempt similar ventilation among groups.

Monitoring. To determine the lung injury endpoint, arterial blood gases were analyzed several times during the induction period. Once the lung injury was established and the animals were placed on HFOV, blood gas analysis was performed and recorded hourly. Heart rate, ABP, rectal temperature, SpO_2 and ECG (ECG) tracings were monitored throughout the study and recorded hourly. During the HFOV period, ventilator settings (ΔP) were adjusted to maintain normocapnea and recorded hourly.

Tissue analysis. On completion of the experiment the rabbits were humanely euthanized, while still under anesthesia, with an IV overdose of pentobarbital, and samples were immediately collected. The lungs were removed *en bloc* by means of a ventral midline sternotomy. Excess hemorrhage on the visceral pleura was gently rinsed with saline and dried with sterile swabs. A small lung tissue sample (approximately 0.8 g) was taken from the right caudal lobe from each rabbit, placed in a sealed cryovial, and immediately frozen in liquid nitrogen. These samples were stored at $-70^\circ C$ for batch MPO activity determination. Thirty mL of 10% neutral buffered formalin was slowly instilled directly into the trachea by gravity. The lungs were immersed in formalin and allowed to fix for at least 48 h before further processing. A single slice of tissue, taken perpendicular to the long axis of the lung, from the cranial, middle and caudal lobes of the left lung was embedded in paraffin. Five-micron sections of the samples were cut and stained for histopathological evaluation.

Two pathologists blinded to the experimental groups independently examined each section. The sections examined were scored as previously described

(9–11) with the following modifications: An airway score (bronchiolar epithelial cells) and an airspace score (alveoli) were generated for semi-quantitative evaluation of the lung lesions. The severity of changes observed in each section was graded on a scale from 0 to 4 (0 = no change, 1 = minimal change, 2 = mild change, 3 = moderate change, 4 = marked change). The two criteria used to evaluate changes in the airways were epithelial cell sloughing and epithelial cell flattening. Changes in the airspace evaluated were: neutrophil accumulation, macrophage accumulation, septal thickening, emphysema, and the presence of proteinaceous exudates. The total lung score was calculated by adding the airway score and the airspace score for each animal. All areas of each section were evaluated in a systematic manner at both low and high magnification.

Neutrophil counts. Images of 5 random nonoverlapping high power fields (HPF, 630 $\times$ magnification) from each of the three lung lobes of the left lung were captured. Fields composed only of alveoli were accepted for analysis, while fields with large vascular structures or major airways were rejected. Neutrophils were identified by cytological criteria and manually enumerated. The average number of neutrophils/HPF was calculated by dividing the total number of neutrophils by the number of fields reviewed for each lobe.

Myeloperoxidase assays. MPO activity assays of frozen tissue samples used in experimental models as an index of neutrophil sequestration are well described (12–14). MPO activity was assessed using a spectrophotometric technique with tetramethylbenzidine as substrate (15,16). Thawed lung samples weighing approximately 50 mg were homogenized in buffer, and centrifuged. One mL of potassium phosphate buffer containing hexadecyltrimethylammonium bromide was added to the pellet and sonicated. The suspension was freeze-thawed once in N_2 , re-sonicated for 25 s on ice and centrifuged. The supernatant was used for analysis. Each cuvette contained 25 μL of the undiluted sample, 25 μL of 16 mM tetramethylbenzidine dissolved in dimethylformamide, and 175 μL of 220 mM potassium phosphate buffer containing 110 mM sodium chloride at pH 5.4. The reaction was initiated with 25 μL of 3 mM hydrogen peroxide. One unit of activity was defined as one unit change in absorbance per minute at 37 $^\circ C$. Protein content of lung tissue samples was determined by a BCA assay (Pierce, Rockford, IL), and a standard curve was prepared with dilutions of BSA.

Statistical analysis. Data were analyzed with SigmaStat software (Systat software, Point Richard, CA). Analysis of variance of parameters from the different lung lobes (cranial, middle and caudal) in each group was assessed using either 2-way ANOVA or Kruskal-Wallis tests, depending on the distribution and the homogeneity of variation within the groups. Tukey's pair-wise comparisons were performed to assess whether there were differences between the three lung lobes in each group. The lung was then assessed as a whole, using mean scores of the three lung lobes, and the three treatment groups were compared using ANOVA or Kruskal-Wallis tests. Subsequently, the two HFOV treatment groups were compared with 2-way *t*-tests, or Mann-Whitney tests when the non-ventilated group was omitted from the analysis. The parameters analyzed were airway score, airspace score, total lung score, neutrophil count and MPO activity.

The individual parameters comprising the airway (epithelial sloughing and flattening), airspace (accumulation of neutrophils and macrophages, septal thickening, emphysema and protein exudates) and total lung scores for each group were also compared. Again, analysis was initially done on all 3 groups using either ANOVA or Kruskal-Wallis tests (with Tukey's pair-wise comparisons), followed by either 2-way *t*-tests or Mann-Whitney tests on the HFOV groups.

Correlation between MPO activity and neutrophil count was assessed with Spearman's rank test. For all comparisons and the correlation, differences were considered significant when $p < 0.05$. The scores generated independently by the two pathologists were evaluated with a kappa (κ) test of agreement. Agreement was considered "poor" if $\kappa < 0.20$, "fair" if $0.21 \geq \kappa < 0.40$, "moderate" if $0.41 \geq \kappa < 0.60$, "substantial" if $0.61 \geq \kappa < 0.80$, and "good" if $\kappa \geq 0.81$ (17).

RESULTS

Physiologic data. The time from start of induction of lung injury to HFOV or euthanasia ranged from 10–15 min with no significant differences between groups. Results for $PaCO_2$ and PaO_2 did not significantly differ between the 5 Hz and 15 Hz HFOV groups during the 4-h ventilation period or during the lung injury induction period with CMV between all three groups ($p = 0.624$ and $p = 0.447$ for $PaCO_2$ and PaO_2 , respectively) (Fig. 1). The recorded pressure amplitude (ΔP) required to maintain normocapnea during HFOV at 5 Hz

Figure 1. PaCO₂ (A) and PaO₂ (B) measured during lung injury induction and high-frequency oscillatory ventilation. Ventilator pressure amplitude was adjusted to maintain normocapnea (CO₂ 35–45 mm Hg) during HFOV, the end point of lung injury induction was at T = 0 min. Significant treatment or time differences were not present. (C) The amplitude necessary to maintain normocapnea during HFOV at 5 Hz was significantly lower than at 15 Hz ($p = 0.000$). Six animals per group, ● = 5 Hz, ○ = 15 Hz, ▼ = non-HFO ventilated. Bars = Standard error.

Figure 2. Lung sections from non-HFO ventilated animals lack neutrophil accumulation in the alveoli (A), while animals ventilated at 5 Hz (B) and 15 Hz (C) have neutrophil accumulations in the alveoli (arrows). H&E, bar = 20 μ m.

(mean 21.5, range 19.9–22.7) was significantly lower than that at 15 Hz (mean 26.5, range 25.7–27.5) with $p = 0.000$ (Fig. 1C).

Histopathology. The non-ventilated group had relatively normal tissue architecture within the airspaces (Fig. 2A), whereas sections from the 15 Hz and 5 Hz HFOV groups showed increasing architectural changes and inflammation within the alveoli (Fig. 2B,C, respectively). Most severe lesions consisted of clusters of neutrophils within alveoli (Fig. 3A,B), intra-alveolar hemorrhage, rupture of alveolar septa and regional emphysema (Fig. 3C), fibrillar eosinophilic material within alveoli, and increases in the thickness of alveolar septa (Fig. 3D). However, rupture of alveolar septa and regional emphysema were uncommon lesions in animals from all three groups.

Examination of the airways from all groups showed variable morphologic changes and sloughing of epithelial cells (Fig. 4). Not all airways in the same histologic section were equally affected, in some areas of the section the bronchioles were largely denuded of epithelial cells; however, in other areas the bronchiolar epithelium was spared or only mildly affected. Focal areas of congestion within the lung parenchyma were present in all three groups. These areas of congestion were variable in size and not restricted to specific lung lobes or anatomical locations. Congestion was identified in both dorsal (dependent) and ventral (non-dependent) areas of the lung.

Statistical analysis. There was fair agreement between pathologists in the semi-quantitative scoring system (left cranial $\kappa = 0.210$, left middle $\kappa = 0.251$, left caudal $\kappa = 0.275$ lung lobes); therefore, the average scores were used for sta-

tistical analysis. There was a significant correlation between neutrophil count/HPF and MPO activity across all lung sections ($p = 0.005$, Fig. 5). Spearman's correlation coefficient (r) was 0.635 with a 95% confidence interval of 0.239–0.845. Within groups, there were no differences in the cranial, middle and caudal lung lobes with regard to airway score, airspace score, total lung score and neutrophil count ($p > 0.100$). Due to the lack of differences between lobes for all parameters, the lung was assessed as a whole, and averages of the three lobes were used in analysis.

Overall, the parameter most significantly different between the non-HFOV ventilated group and the two HFOV groups was the neutrophil count ($p = 0.013$, Fig. 6). Other significant differences between the non-HFO ventilated group and the 5 Hz group were in the airspace injury score ($p = 0.029$), airway injury score ($p = 0.007$) and total lung injury score ($p = 0.014$). Significant differences in these parameters were not identified between 5 Hz and 15 Hz groups.

Of the individual parameters comprising the scores generated, significant differences between the three groups were present for neutrophils ($p = 0.002$) and septal thickening ($p = 0.029$). In both cases the non-ventilated group had fewer lesions than both the 5 Hz and 15 Hz groups. None of the other parameters comprising the airspace score (macrophages, emphysema, protein exudate) and neither parameter comprising the airway score (epithelial flattening, epithelial sloughing) significantly differed among the three groups. The neutrophil score in airspaces significantly differed between all three groups (2.7 versus 2.3 versus 0.8; $p = 0.001$, Fig. 6), while differences in MPO activity approached significance (0.42 versus 0.36 versus 0.18; $p = 0.058$).

Figure 3. Lung sections illustrating neutrophil infiltration (A, bar = 10 μ m and B, bar = 20 μ m), emphysema and septal rupture (C, bar = 100 μ m) and proteinaceous exudates (D, bar = 20 μ m) in the airspaces of animals ventilated at 5 Hz. H&E.

Since the non-HFO ventilated group would be expected to differ significantly from the HFOV groups, the HFOV groups were compared independently. The neutrophil score differed significantly between the 2 HFO ventilated groups ($p = 0.005$). The other individual parameters comprising the airspace score (septal thickening [$p = 0.423$]; macrophages [$p = 0.132$]; emphysema [$p = 0.986$], protein exudates [$p = 0.101$]), and the two parameters comprising the airway score (epithelial flattening [$p = 0.224$] and epithelial sloughing [$p = 0.199$]) did not differ significantly (Fig. 6). MPO activity ($p = 0.579$), neutrophil count ($p = 0.471$), airspace score ($p = 0.460$), airway score ($p = 0.134$) and total lung score ($p = 0.300$) did not differ significantly between the 5 Hz and 15 Hz groups when the non-HFO ventilated group was omitted from the analysis.

DISCUSSION

It is well-established that mechanical ventilation predisposes the lung to injury through various mechanisms; however, mechanical ventilation remains the cornerstone of treatment in patients with acute lung injury or ARDS. The relative benefit of mechanical ventilation at lower V_T as a lung

Figure 5. Scatterplot of myeloperoxidase (MPO) activity versus the average number of neutrophils per high-power field (HPF). Spearman's correlation coefficient $r = 0.635$, 95% confidence interval 0.239–0.845.

protective strategy was recently confirmed in a large multi-center randomized clinical trial (1). In this trial, patients with acute lung injury or ARDS ventilated at 6 mL/kg had a significant reduction in mortality compared with those ventilated at the higher traditional levels. Although ventilation at lower V_T was beneficial, undesirable systemic effects of CO_2 retention have previously limited use in CMV. This, in part, has led to renewed interest in HFOV as an alternative ventilation strategy.

HFOV is a novel form of mechanical ventilation where patients are ventilated at V_T approximating or less than physiologic dead space and very high respiratory rates. Oxygen delivery to the tissue, and CO_2 removal from the lungs, is well maintained with HFOV, and may be superior to CMV. In contrast to CMV, methods other than convective bulk flow are believed to be operational in HFOV, but have yet to be fully characterized. HFOV can support gas exchange over a broad range of frequencies, from 3–30Hz (4,18). The higher the frequency, the lower the bulk flow and the less the lung distension; however, this benefit disappears around 15–20Hz (20). The converse may also hold true since at lower frequencies V_T and bulk flow increase. There are abundant data from animal models to show that ventilating at higher frequencies reduces VALI relative to CMV (7, 20–25), but there is a paucity of information regarding outcomes of lower-

Figure 4. Lung sections illustrating changes in the terminal bronchioles (airways) in non-HFO ventilated animals (A), and animals ventilated at 5 Hz (B) and 15 Hz (C). Epithelial cells are slightly flattened (C, arrow) in animals ventilated at 15 Hz compared with more marked flattening and sloughing of epithelium (B, arrow) in animals ventilated at 5 Hz. H&E, bar = 20 μ m.

a) Airspace

b) Airway

c) Lung

Figure 6. Lung lesion scores in animals ventilated at 5 Hz and 15 Hz, and non-HFO ventilated (NV) animals (6 animals per group). Medians with 25th and 75th quartiles of individual and cumulative scores are shown, bars are 5th and 95th percentiles. Significant ($p < 0.05$) differences were between NV and 5 Hz or 15 Hz ventilated animals (†), between NV and 5 Hz ventilated animals (*), and between all 3 groups (**).

frequency ventilation. Therefore, this study addressed the hypothesis that higher frequencies of HFOV cause less lung injury than lower frequencies.

The goal of this pilot investigation was to compare lung lesions resulting from two different HFOV regimes; direct comparison to CMV was not part of this study as it was shown previously that HFOV improves oxygenation and reduces inflammation relative to CMV (20–26). A 4-h period of ventilation was chosen since injury should manifest at this time. Physiologic measurements in this study confirmed that HFOV was an efficient method of ventilation with respect to tissue oxygenation and CO₂ elimination. HFOV rescued the animals when conventional means of ventilation were unable to maintain PaO₂ and PaCO₂ within normal limits. After induction of lung injury ($T = 0$), there was a dramatic drop in PaO₂ (Fig. 1), which was not improved by CMV even when PIP levels were increased and PEEP set at 10 cmH₂O. However, immediately after the animals were switched to HFOV and the lungs were recruited, oxygenation returned to normal and was maintained at these levels throughout the duration of

the experiments. Although the severity of lung lesions differed between 5 Hz and 15 Hz HFOV, there were no differences in the physiologic parameters. These findings imply that although a patient may appear to have adequate tissue oxygenation and CO₂ elimination when ventilated with HFO at both low and high frequencies, the lower frequency may be more likely to result in neutrophilic inflammation. The lower HFOV frequency resulted in higher scores for most indicators of lung damage, but reaching statistical significance was limited by individual variation in the relatively small number of animals in this pilot study.

To maintain normocapnia, the amplitude (ΔP) setting on the ventilator in the 5 Hz group was significantly less than that of the 15 Hz group (Fig. 1). We have previously observed that V_T and ventilator frequency were closely related by measuring V_T in animals ventilated at 5 and 15 Hz (26). Therefore, 5 Hz HFOV may have generated higher V_T than 15 Hz HFOV, which, together with data from other experiments, suggests an inverse relationship between V_T and ventilation frequency, whereby the former increases with decreasing ventilator fre-

quency (4,27). The clinical implication of this relationship would be for intensivists to consider changing the amplitude (ΔP) before decreasing the frequency, to maintain normocapnea in patients ventilated with HFO.

Histopathological examination of tissue remains the foundation of lesion characterization and diagnosis in research and clinical settings. In the current study, changes in the lung created by repeated lavages were compared with those from two different regimes of subsequent HFOV. This was considered the most suitable approach for identifying whether a higher or lower frequency of HFOV was more protective, but did not allow comparison to equivalent CMV. Lesions within the airways and airspaces, and the number of neutrophils in the lung, were significantly higher in the animals ventilated with HFOV compared with those that were not. The non-HFOV ventilated group had less architectural damage and fewer inflammatory cells within the airways and airspaces. These findings suggest that establishment of lung injury by repeated lavages did not significantly contribute to the lesions observed in HFOV. Although this procedure undoubtedly sets up or predisposes the pulmonary tissue to damage by ventilation, there was little histologic evidence of severe airway or airspace damage in animals from the non-HFO ventilated group within the time frame assessed. The higher frequency regime of HFOV yielded consistently lower scores of pathologic lung change than the lower frequency; however, only the neutrophil score statistically distinguished the two regimes. The neutrophil score was based on review of a relatively large area of lung at low magnification, which may account for differences to counts in smaller fields and in MPO activity since the latter was determined only for caudal lung lobe sections. Overall, findings from this study are consistent with other studies indicating that HFOV affords excellent gas exchange in an animal model of acute lung injury and suggest that differences between 5 and 15 Hz HFOV are relatively minor. Further studies involving a greater number of animals are needed to precisely identify the optimal combination of ventilatory frequency and V_T .

Acknowledgments. We thank W. Sears for assistance with statistical analysis, and H. Frndova for technical support.

REFERENCES

- Slutsky AS, Ranieri VM 2000 Mechanical ventilation: lessons from the ARDSNet trial. *Respir Res* 1:73–77
- Venegas JG, Fredberg JJ 1994 Understanding the pressure cost of ventilation: why does high-frequency ventilation work? *Crit Care Med* 22:S49–S57
- Chang HK 1984 Mechanisms of gas transport during ventilation by high-frequency oscillation. *J Appl Physiol* 56:553–563
- Kolton M, McGhee I, Bryan AC 1987 Tidal volumes required to maintain isocapnia at frequencies from 3 to 30 Hz in the dog. *Anesth Analg* 66:523–528
- Froese AB, Kinsella JP 2005 High-frequency oscillatory ventilation: lessons from the neonatal/pediatric experience. *Crit Care Med* 33:S115–S121
- McCulloch PR, Forkert PG, Froese AB 1988 Lung volume maintenance prevents lung injury during high-frequency oscillatory ventilation in surfactant-deficient rabbits. *Am Rev Respir Dis* 137:1185–1192
- Lachmann B, Robertson B, Vogel J 1980 In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 24:231–236
- Rimensberger PC, Cox PN, Frndova H, Bryan AC 1999 The open lung during small tidal volume ventilation: concepts of recruitment and “optimal” positive end-expiratory pressure. *Crit Care Med* 27:1946–1952
- Rimensberger PC, Pache JC, McKlerie C, Frndova H, Cox PN 2000 Lung recruitment and lung volume maintenance: a strategy for improving oxygenation and preventing lung injury during both conventional mechanical ventilation and high-frequency oscillation. *Intensive Care Med* 26:745–755
- Ginsberg HS, Moldawer LL, Sehgal PB, Redington M, Kilian PL, Chanock RM, Prince GA 1991 A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia. *Proc Natl Acad Sci USA* 88:1651–1655
- Hamilton PP, Onayemi A, Smyth JA, Gillan JE, Cutz E, Froese AB, Bryan AC 1983 Comparison of conventional and high-frequency ventilation: oxygenation and lung pathology. *J Appl Physiol* 55:131–138
- Harkin DW, Romaschin A, Taylor SM, Romaschin A, Rubin BB, Lindsay TF 2004 Complement C5a receptor antagonist attenuates multiple organ injury in a model of ruptured abdominal aortic aneurysm. *J Vasc Surg* 39:196–206
- Boyd AJ, Rubin BB, Walker PM, Romaschin A, Issekutz TB, Lindsay TF 1999 A CD18 monoclonal antibody reduces multiple organ injury in a model of ruptured abdominal aortic aneurysm. *Am J Physiol* 277:172–182
- Cox PN, Frndova H, Karlsson O, Holowka S, Bryan CA 2003 Fluorocarbons facilitate lung recruitment. *Intensive Care Med* 29:2297–2302
- Suzuki K, Ota H, Sasagawa S, Sakatani T, Fujikura T 1983 Assay method for myeloperoxidase in human polymorphonuclear leukocytes. *Anal Biochem* 132:345–352
- Lindsay TF, Walker PM, Romaschin A 1995 Acute pulmonary injury in a model of ruptured abdominal aortic aneurysm. *J Vasc Surg* 22:1–8
- Landis JR, Koch GG 1977 The measurement of observer agreement for categorical data. *Biometrics* 33:159–174
- Bohn DJ, Miyasaka K, Marchak BE, Thompson WK, Froese AB, Bryan AC 1980 Ventilation by high-frequency oscillation. *J Appl Physiol* 48:710–716
- Sugiura M, McCulloch PR, Wren S, Dawson RH, Froese AB 1994 Ventilator pattern influences neutrophil influx and activation in atelectasis-prone rabbit lung. *J Appl Physiol* 77:1355–1365
- Baden HP, Mellema JD, Bratton SL, O'Rourke PP, Jackson JC 1997 High-frequency oscillatory ventilation with partial liquid ventilation in a model of acute respiratory failure. *Crit Care Med* 25:299–302
- Rotta AT, Gunnarsson B, Fuhrman BP, Hernan LJ, Steinhorn DM 2001 Comparison of lung protective ventilation strategies in a rabbit model of acute lung injury. *Crit Care Med* 29:2176–2184
- Gerstmann DR, Fouke JM, Winter DC, Taylor AF, deLemos RA 1990 Proximal, tracheal, and alveolar pressures during high-frequency oscillatory ventilation in a normal rabbit model. *Pediatr Res* 28:367–373
- Yoder BA, Siler-Khodr T, Winter VT, Coalson JJ 2000 High-frequency oscillatory ventilation: effects on lung function, mechanics, and airway cytokines in the immature baboon model for neonatal chronic lung disease. *Am J Respir Crit Care Med* 162:1867–1876
- Kerr CL, Veldhuizen RA, Lewis JF 2001 Effects of high-frequency oscillation on endogenous surfactant in an acute lung injury model. *Am J Respir Crit Care Med* 164:237–242
- Piva J, Chatkew P, Choong K, Frndova H, Cox P 2000 High-frequency oscillation ventilation compared to conventional mechanical ventilation plus exogenous surfactant replacement in rabbits. *J Pediatr (Rio J)* 76:349–356
- Hatcher D, Watanabe H, Ashbury T, Vincent S, Fisher J, Froese A 1998 Mechanical performance of clinically available, neonatal, high-frequency, oscillatory-type ventilators. *Crit Care Med* 26:1081–1088