High Frequency Oscillatory Ventilation Versus Conventional Ventilation in a Newborn Piglet Model with Acute Lung Injury

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BACKGROUND: High frequency oscillatory ventilation (HFOV) is considered a protective strategy for human lungs. This study was designed to define microscopic structural features of lung injury following HFOV with a high lung volume strategy in newborn piglets with acute lung injury. METHODS: After acute lung injury with saline lavage, newborn piglets were randomly assigned to 5 study groups (6 in each group): control (no mechanical ventilation), conventional mechanical ventilation for 24 hours, conventional ventilation for 48 hours, HFOV for 24 hours, and HFOV for 48 hours. The right upper lung tissue was divided into the gravitation-dependent and gravitation-nondependent regions after the completion of mechanical ventilation. Under light microscopy, the numbers of polymorphonuclear leukocytes (PMNLs), alveolar macrophages, red blood cells, and hyaline membrane/alveolar edema were assessed in all lung tissues. Oxygenation index was continuously monitored. RESULTS: Our results showed that the degree of histopathologic lung damage in the gravitation-dependent region was greater than that in the gravitation-nondependent region. Compared with the control group, PMNLs, red blood cells and hyaline membrane/alveolar edemas were significantly increased and alveolar macrophages were significantly decreased in lung tissues of conventional ventilation and HFOV piglets. In HFOV with high lung volume strategy piglets, lung tissues had significantly fewer PMNLs, red blood cells, and hyaline membrane/alveolar edemas, and oxygenation was improved significantly, compared to those of the conventional ventilation piglets. CONCLUSIONS: Histopathologic lung damage in newborn piglets with lung injury was more severe in the gravitation-dependent region than in the gravitation-nondependent region. HFOV with high lung volume strategy reduced pulmonary PMNL infiltration, hemorrhage, alveolar edema, and hyaline membrane formation with improved oxygenation. *Key words: acute lung injury; ventilator-induced lung injury; conventional ventilation; high frequency oscillatory ventilation; newborn piglet.* [Respir Care 2013;58(5):824–830. © 2013 Daedalus Enterprises]

Introduction

Mechanical ventilation is an important therapeutic measure to reduce mortality and improve prognosis for acute lung injury (ALI) and ARDS. However, mechanical ventilation may aggravate pre-existing lung injury or cause injury in healthy lungs,[1,2] a phenomenon that is frequently referred to as ventilator-induced lung injury (VILI). In high frequency oscillatory ventilation (HFOV), small tidal volumes, which are usually equal to 20–80% of the anatomic dead space, are oscillated around a relatively high, fixed mean airway pressure, using a high frequency piston pump or oscillatory diaphragm movements. HFOV has been regarded as a protective ventilation strategy to relieve and attenuate VILI.

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Previous studies have shown that HFOV improves gas exchange in newborns with ALI or ARDS.[3,4] However, little is known about HFOV regarding the histopathologic features of lung injury in newborns with ALI or ARDS. Therefore, we used a newborn piglet model with VILI to study the effects of conventional mechanical ventilation and HFOV with a high lung volume strategy on lung histopathology. The objective of this study was to define microscopic structural features of lung injury following HFOV with a high lung volume strategy in a newborn piglet model with ALI.

QUICK LOOK

Current knowledge

High-frequency oscillatory ventilation (HFOV) uses a high mean airway pressure to recruit the lung, and small pressure oscillations around the mean, which might be a lung protective strategy. In infants, HFOV improves gas exchange and lowers the peak airway pressure, but there have been mixed results on HFOV's effects on important outcomes.

What this paper contributes to our knowledge

In a piglet model of acute lung injury the lung damage from mechanical ventilation was more severe in the gravitationally dependent lung regions. Lung injury occurred with both conventional ventilation and HFOV, but HFOV improved oxygenation and reduced lung damage, as evidenced by reduced pulmonary polymorphonuclear leukocyte infiltration, alveolar edema, and hyaline membrane formation.

Methods

Animals and Surgery

Thirty newborn piglets (≤ 3 days old, weighing 1.0–1.97 kg) were used in this study. All animal experiments were performed with the approval of the Guangdong Second Provincial People's Hospital Animal Care and Use Committee (2009-XEK-028).

Piglets were given 10% chloral hydrate (1 mL/kg) by mouth and placed in the supine position under an infant radiant warmer. The body temperature of the animals was kept within a range of 38.0–39.5°C. Catheters were inserted in the axillary vein and the femoral artery for medications and fluids, and for blood gas analysis and arterial blood pressure monitoring. The maintenance fluids were provided by the continuous infusion of 0.9% saline solution containing 5% dextrose (120 mL/kg/d). A dopamine infusion (5 μg/kg/min) was continuously administered. The piglets were anesthetized using diazepam (0.5 mg/kg, intramuscularly) followed by ethyl carbamate (0.6 g/kg, intravenously). The piglets were intubated with endotracheal tubes (4.0 mm internal diameter) and ventilated using conventional ventilation (Servo-i, Maquet, Sölna, Sweden) or HFOV. After the initiation of mechanical ventilation, each piglet was intravenously infused with a bolus of cefotiam hydrochloride (100 mg/kg).

Using the pressure control mode, the initial ventilator settings were set to a PEEP of 2 cm H_2O, a peak inspiratory pressure of 10 cm H_2O, an inspiratory to expiratory ratio of 1:2, and an F_{IO_2} of 0.30. The breathing frequency was set at a level of 25–30 breaths/min and adjusted to maintain P_{aCO_2} in the normal range (35–45 mm Hg).

Acute Lung Injury and Treatment Regimens

After baseline measurements of arterial blood gases, arterial blood pressure, and heart rate, ALI was induced by lavaging the whole lung with normal saline. During lavage, all piglets were ventilated using a conventional mechanical ventilator, using the pressure control mode. Briefly, warmed (37°C) normal saline (35 mL/kg) was instilled into the lung via the endotracheal tube. Saline was allowed to remain in the lung for 10 s before removal. Lung lavage was repeated at 5-min intervals until P_{aO_2} was below 100 mm Hg for 60 min at the following ventilator settings: peak inspiratory pressure 24 cm H_2O, PEEP 6 cm H_2O, inspiratory to expiratory ratio 1:2, F_{IO_2} 1.0, and breathing frequency 35 breaths/min.

After lung injury was established, the piglets were randomly assigned to one of the 5 study groups (6 in each group): control (with no ventilation), conventional ventilation for 24 hours, conventional ventilation for 48 hours, HFOV for 24 hours, and HFOV for 48 hours. After the establishment of lung injury, the control piglets were sacrificed using an overdose of 10% potassium chloride under deep anesthesia. In the conventional ventilation group, the piglets were ventilated using a conventional mechanical ventilator (Servo-i) in the pressure control mode at the following ventilator settings: peak inspiratory pressure 20 cm H_2O, PEEP 4 cm H_2O, inspiratory to expiratory ratio 1:2, and F_{IO_2} 1.0. The breathing frequency was set at a level of 25–30 breaths/min and adjusted to maintain P_{aCO_2} in the normal range (35–45 mm Hg). In the HFOV group the piglets were placed on HFOV (SLE-5000, Tokibo, Tokyo, Japan), with an oscillatory frequency of 10 Hz, a fractional inspiratory time of 33%, and an F_{IO_2} of 1.0. The mean airway pressure was set to 2 cm H_2O higher than that during conventional ventilation, which meant a high lung volume strategy in HFOV. The amplitude was set at a level of 20–25 cm H_2O and adjusted to maintain P_{aCO_2} in the normal range (35–45 mm Hg). During ventilation, F_{IO_2} was decreased by 10% every 6 h until

it reached 40%. At the end of ventilation, the piglets were sacrificed using an overdose of 10% potassium chloride under deep anesthesia. The lungs were immediately removed and fixed in 10% buffered formalin.

Arterial blood gases were obtained regularly. Arterial blood pressure and heart rate were continuously monitored using a multifunctional electrocardiogram monitor (IntelliVue MP20, Philips Healthcare, Best, The Netherlands). Arterial blood gases, arterial blood pressure, and heart rate were recorded at baseline, after induction of ALI (ALI 0 h), and at 1, 6, 12, 24, and 48 h after initiation of either conventional ventilation or HFOV. To compare gas exchange between groups, oxygenation index (OI) was calculated as:

$$OI = \text{mean airway pressure (cm } H_2O) \times F_{IO_2} \times 100/P_{aO_2} \text{ (mm Hg)}$$

Mean arterial blood pressure was calculated as:

$$\text{Mean arterial pressure} = \text{diastolic blood pressure (mmHg)} + [\text{systolic blood pressure (mm Hg)} - \text{diastolic blood pressure}]/3.$$

Light Microscopy and Morphometry

Histologic and morphometric analyses were performed using right upper lung tissue from the piglets. Fixed lungs were divided into gravitation-dependent and gravitation-nondependent regions. Each lung sample was cut into 2 serial sections: slices I and II. Slice I was deparaffinized using xylene, dehydrated in graded alcohol, and stained with hematoxylin and eosin. Slice II was stained with modified Mallory's phosphotungstic acid hematoxylin.

A total of 5–10 random fields of view were evaluated in each section under a light microscope at a magnification of 400. In slice I the numbers of polymorphonuclear leukocytes (PMNLs), alveolar macrophages (AMs), and red blood cells (RBCs) were counted. In slice II, the number of hyaline membrane/alveolar edemas (HM/AEs) were counted. All of the values were expressed as the numbers per 100 alveoli of the gravitation-dependent or gravitation-nondependent regions.

Statistical Analysis

Statistical analysis was performed using statistics software (SPSS 13.0, SPSS, Chicago, Illinois). The data are expressed as mean ± SD. Data were assessed by multiple linear regression and by analysis of variance for the intergroup comparison. The post hoc analysis was performed using the least significant difference test (when the homogeneity of variances was equal) or the Dunnett T3 test (when homogeneity of variances was unequal). Statistical comparisons between gravitation-dependent and gravitation-nondependent regions of the same group were performed using the nondependent-samples t test. Oxygenation and hemodynamic parameters (mean arterial pressure and heart rate) over time were compared between groups using a 2-way analysis of variance for repeated measures. Statistical significance was defined as $P \leq .05$.

Table. Oxygenation and Hemodynamics Before and After Lung Injury in Piglets

	Oxygenation Index	Mean Arterial Blood Pressure	Heart Rate
Control group			
Baseline	1.16 ± 0.07	47.78 ± 2.34	159.33 ± 11.59
0 h	17.90 ± 1.20	45.44 ± 6.16	181.33 ± 11.59
Conventional ventilation group			
Baseline	1.14 ± 0.12	47.11 ± 4.56	162.83 ± 15.25
0 h	17.43 ± 1.10*	47.17 ± 4.63	166.33 ± 9.07
1 h	11.89 ± 2.17*†	43.72 ± 8.84	166.50 ± 23.11
6 h	7.81 ± 0.77*†	47.17 ± 8.63	173.67 ± 17.41
12 h	6.11 ± 0.79*†	46.72 ± 7.28	165.33 ± 20.65
24 h	4.39 ± 0.28*†	44.45 ± 7.77	164.83 ± 12.75
48 h	3.81 ± 0.42*†	45.11 ± 10.22	165.33 ± 23.25
HFOV group			
Baseline	1.16 ± 0.04	50.00 ± 4.37	171.33 ± 24.25
0 h	17.51 ± 1.43*	46.00 ± 2.12	176.33 ± 14.35
1 h	11.39 ± 2.84*†	49.56 ± 11.48	172.33 ± 26.00
6 h	5.62 ± 0.51*†‡	47.83 ± 3.02	166.50 ± 15.00
12 h	4.05 ± 0.54*†‡	50.45 ± 10.78	176.17 ± 19.03
24 h	3.06 ± 0.39*†‡	48.33 ± 3.79	186.00 ± 22.34
48 h	2.74 ± 0.25*†‡	48.67 ± 5.84	172.00 ± 30.20

* $P < .05$ compared with baseline in same group.
† $P < .05$ compared with same group at acute lung injury hour 0.
‡ $P < .05$ compared with same time in the conventional ventilation group
HFOV = high frequency oscillatory ventilation

Results

Oxygenation Index and Hemodynamic Measurements

There were no significant differences in OI, mean arterial pressure, and heart rate between the groups at baseline and at ALI 0 h, respectively. The Table shows the OI, mean arterial pressure, and heart rate in different groups before and after lung injury was induced as well as over the ventilation periods. With the extension of ventilation time, oxygenation was improved, and mean arterial pressure and heart rate were stable. From the 6th hour to the 48th hour, OI in HFOV was significantly lower than that in conventional ventilation.

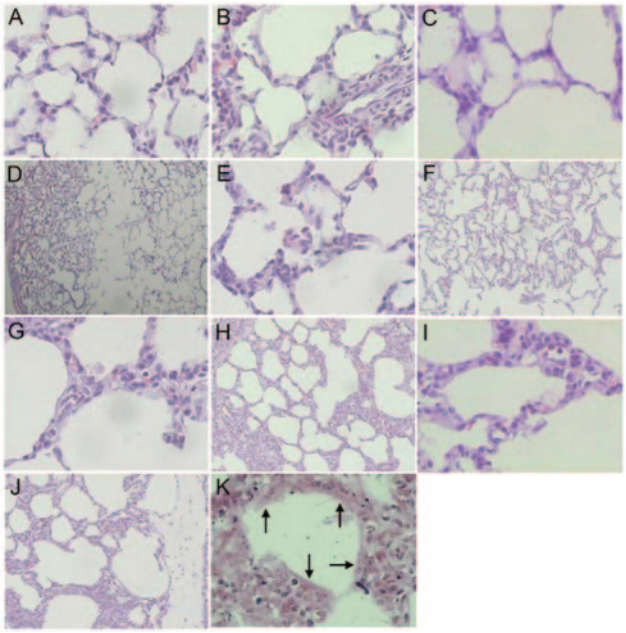

Fig. 1. Photomicrographs of the right upper lung tissue in all study groups were observed using light microscopy, including tissues from the gravitation-dependent region (A, C, E, G, I, and K) and tissues from the gravitation-nondependent region (B, D, F, H, and J). K: Hyaline membranes (arrowheads) in the gravitation-dependent region of the conventional ventilation 24 h group. The magnification of A, B, C, E, G, I, and K is 400. The magnification of D is 40. The magnification of F, H, and J is 100.

Gross and Microscopic Lung Histopathology

Morphologically, the lungs appeared smooth with no obvious swelling in the control group. In piglets with conventional ventilation or HFOV the lungs were swollen with punctiform and flaky bleeding, which were more pronounced in the gravitation-dependent region than in the gravitation-nondependent region. The lung damage was more severe in the conventional ventilation group than in the HFOV group. In the conventional ventilation or HFOV groups, the degree of lung damage was less intense after 24 h, compared to that after 48 h.

Under light microscopy, alveolar collapse and hyaline membrane formation were observed in the control group (Fig. 1A and 1B). After ventilation for 24 hours with conventional ventilation (see Figs. 1C and 1D), the lungs exhibited a large area of alveolar atelectasis. In addition, we observed alveoli rupturing; interstitial and septal pulmonary edema; intra-alveolar hemorrhaging; PMNL infiltration; hyaline membrane formation; microangium congestion, lymphangiectasia, and cilia dislodgement in the bronchioles; and cellulose exudation in the bronchiole spaces. Compared to the conventional ventilation 24 h group, piglets in the conventional ventilation 48 h group exhibited less alveolar atelectasis but increased alveoli rupturing and cilia dislodgement (see Figs. 1E and 1F). Less severe lung injury was observed in the HFOV 24 h (see Figs. 1G and 1H) and 48 h (see Figs. 1I and 1J) groups, compared with the conventional ventilation 24 h and 48 h groups, respectively.

HM/AE, RBC, PMNL, and AM Analyses

Different ventilation methods, different ventilation time, and different lung regions made a significant difference to the numbers of HM/AEs, RBCs and PMNLs in the 5 study groups (no mechanical ventilation, conventional ventilation for 24 h, conventional ventilation for 48 h, HFOV for 24 h, and HFOV for 48 h). To the numbers of AMs in inter-ventilation-groups, different ventilation methods and different ventilation time were made a significant difference.

As shown in Figure 2, the numbers of HM/AEs, RBCs, and PMNLs were significantly increased in lung tissues of all the conventional ventilation and HFOV groups, compared to those in the control group ($P < .01$). However, the HFOV 24 h or 48 h groups had significantly fewer HM/AEs or RBCs, compared to the conventional ventilation 24 h or 48 h groups, respectively ($P < .01$). In addition, the numbers of PMNLs were significantly decreased in the HFOV and conventional ventilation 48 h groups ($P < .01$) but not the HFOV and conventional ventilation 24 h groups. In the conventional ventilation or HFOV groups the numbers of HM/AEs and RBCs were significantly increased in lung tissues of the 48 h groups, compared to those in the 24 h groups of the same ventilation mode ($P < .01$). In addition, the numbers of HM/AEs and RBCs were significantly higher in the gravitation-dependent region than in the gravitation-nondependent region in the lung tissues of piglets with conventional ventilation or HFOV ($P < .05$).

As shown in Figure 3, the numbers of AMs were not significantly different between the gravitation-dependent and gravitation-nondependent regions in lung tissues of piglets with conventional ventilation (24 h group 102.79 ± 11.08 vs 84.47 ± 10.52, respectively; 48 h group 76.01 ± 14.96 vs 63.26 ± 11.10, respectively) or HFOV (24 h group 77.40 ± 8.48 vs 63.30 ± 8.45, respectively; 48 h group 69.58 ± 8.67 vs 70.27 ± 2.83, respectively). However, the numbers of AMs were significantly decreased in the lung tissues of all conventional ventilation and HFOV groups, except for the conventional ventilation 24 h group compared to those in the control group ($P = .40$). The HFOV 24 h group displayed significantly less AMs, compared to the conventional ventilation 24 h group ($P < .001$). In addition, the numbers of AMs was significantly decreased in the lung tissues of the conventional ventilation 48 h group, compared to the conventional ventilation 24 h group ($P < .001$).

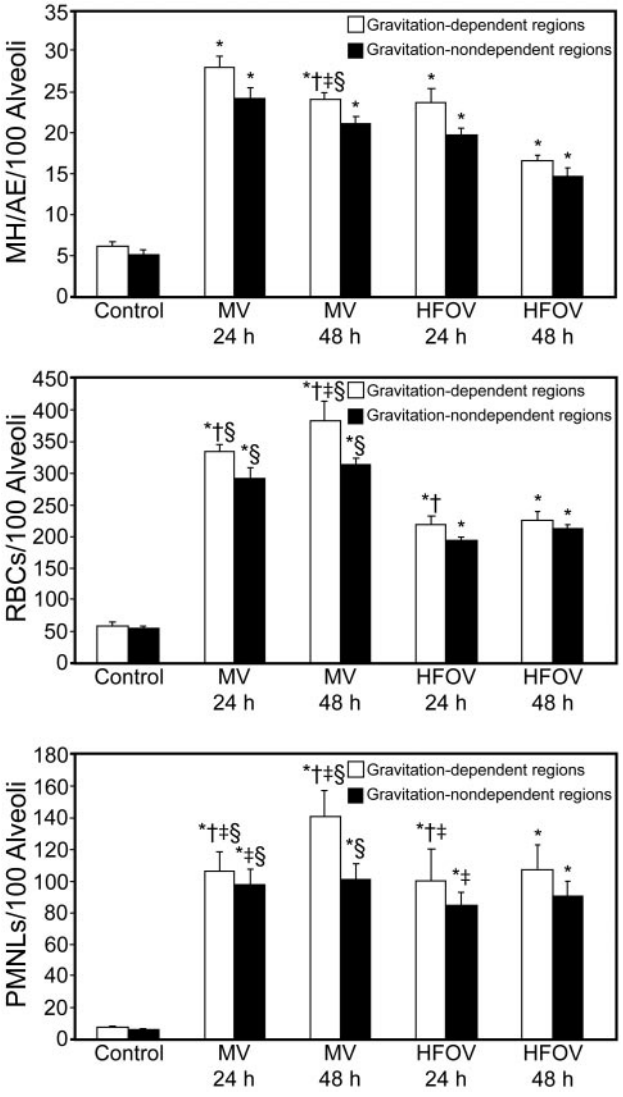

Fig. 2. Hyaline membrane/alveolar edema (HM/AE), red blood cells (RBCs), and polymorphonuclear leukocytes (PMNLs) in the gravitation-dependent region and gravitation-nondependent region of all study groups. * Significant difference compared to the same region of the control group. † Significant difference compared to the gravitation-nondependent region of same group. ‡ Significant difference compared to the same region and same mode of the other groups. § Significant difference compared to the same region and same time of the other groups. MV = mechanical ventilation. HFOV = high frequency oscillatory ventilation.

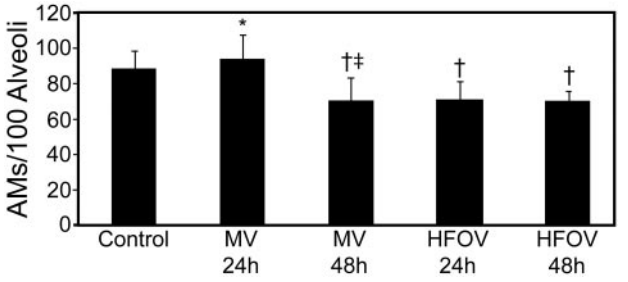

Fig. 3. Alveolar macrophages (AMs) per 100 alveoli. * Significant difference compared to the same time in the other groups. MV = mechanical ventilation. † Significant difference compared to the control group. ‡ Significant difference compared to the same mode in other groups. HFOV = high frequency oscillatory ventilation.

Discussion

Due to the special growth and developmental characteristics of newborn infants, VILI is thought to play an important role in the pathogenesis of neonatal chronic lung disease, which has an adverse effect on the survival rate and the quality of life for newborns with ALI/ARDS. To study newborn ALI/ARDS, newborn animals, such as piglets,[5,6] rats,[7] and rabbits,[8] have been used as animal models. Because the organ development, lung volume, and birth weight of newborn piglets resemble those of newborn infants, the current study used induced ALI/ARDS in newborn piglets.

One of the aims in this study was to compare the histopathological features of the lung damage in gravitation-dependent and gravitation-nondependent regions. Compared to the gravitation-nondependent region, the gravitation-dependent region displayed increased lung swelling and hemorrhaging, and damage to the alveolar integrity and organization. We observed increased inflammatory cell infiltration, edema formation, and hemorrhages, using a quantitative analysis of the numbers of PMNLs, AMs, RBCs, and HM/AEs. Consistent with the result from Viana et al,[9] we demonstrated that histopathologic lung damage was more severe in the gravitation-dependent region than in the gravitation-nondependent region during ventilation. In ALI/ARDS, lung pathological changes are diffuse, and extraordinary disparities in lung compliances among different regions are observed. When ventilated in the supine position, the dorsal region (gravitation-dependent region) displays limited lung ectasis, compared to the gastric region (gravitation-nondependent region), due to the existence of the spinal support and increased pulmonary capillary blood flow volumes, which result in a decreased ventilation/blood flow ratio and the disturbance of gas diffusion. Furthermore, the shear force that is induced by damaged alveoli damages the structures and functions of type II alveolar epithelial cells, resulting in inactivation and decreased pulmonary surfactant secretion. After lung damage, pro-inflammatory cytokines and mediators are activated and released to further aggravate lung injury and eventually increase histopathologic lung damage in the gravitation-dependent region.

Another aim of our study was to compare the histopathologic lung damage between HFOV with high lung volume strategy and conventional ventilation. Cyclic stretch of alveoli is one of the characteristics of mechanical ventilation, and repeated opening and closing of atelectatic

lung segments at low lung volume (atelectrauma) is postulated to be partly responsible for VILI and inflammation in VILI. Hence we used a high lung volume strategy in HFOV. In this study the treatment of ALI in newborn piglets with mechanical ventilation, using the HFOV mode or the conventional ventilation mode for 24–48 hours, increased lung inflammatory infiltration. ALI/ARDS and VILI perturb the balance between pro-inflammatory and anti-inflammatory responses. The inflammatory response to this damage is primarily dependent on PMNL. Greater quantities of PMNLs are recruited and sequestrated in lung tissues after lung damage, and PMNLs play a critical role in the development and progression of lung injury.[10-12]

When piglets were ventilated with the conventional ventilation mode for 48 hours, PMNLs were increased and AMs were decreased, which may suggest that the activation of PMNLs and the inflammatory response are closely associated with the interactions between PMNLs and AMs. The phenotype of activated AMs is altered because induced PMNLs delay apoptosis in the early stage of ALI.[13] Activated AMs secrete pro-inflammatory cytokines that induce the PMNL-mediated delays in apoptosis[14] and increase the release of inflammatory factors via autocrine or paracrine effects of inflammatory cytokines and inflammatory effector cells. Therefore, great quantities of PMNLs are recruited and sequestered, due to the interactions between these inflammatory factors. However, abnormalities in AM apoptosis are present in ALI. The AM apoptosis ratio is significantly increased over time.[15,16] PMNLs that have undergone apoptosis are not removed due to decreased AMs. In this study we showed that in piglets that were ventilated with the HFOV mode for 48 hours, PMNLs were not significantly increased (see Fig. 2C) and AMs were not significantly decreased (see Fig. 3). PMNLs in the HFOV group were lower than the conventional ventilation group, especially in the HFOV 48 h group (see Fig. 2C). Our findings suggest that inflammatory infiltration in the HFOV group is decreased, compared to that in the conventional ventilation group, and are consistent with the findings of Capoluongo et al[17] and von der Hardt et al,[18] which showed that HFOV decreases the expression of pro-inflammatory cytokines.

With increased inflammatory infiltration we observed increased pulmonary hemorrhages, edema formation, and rupturing of the alveolar integrity and organization in piglets with VILI, suggesting a positive correlation between the inflammatory infiltration in the lung tissue and the severity of lung injury. Compared with the conventional ventilation group, the HFOV with a high lung volume strategy group displayed less inflammatory infiltration and damage to alveolar integrity and organization (see Fig. 1), implying that lung injury was less intense in the HFOV with high lung volume strategy group than in the conventional ventilation group.

Pulmonary inflammatory infiltration, hemorrhages, and edema formation have a close relationship with the degree of lung injury. First, these harmful factors injure type II alveolar epithelial cells and decrease the synthesis and secretion of pulmonary surfactant. Second, these harmful factors may directly inactivate pulmonary surfactant, resulting in impaired lung immunological functions and hypoxemia, which is difficult to treat and further decreases pulmonary surfactant. Therefore, reducing pulmonary inflammatory infiltration, hemorrhages, edema, and hyaline membrane formation is critical to treat ALI/ARDS and to relieve and attenuate VILI.

In this study a surfactant deficient piglet model of ALI was used without surfactant replacement. Our data indicated that, compared to conventional ventilation, HFOV with a high lung volume strategy improved oxygenation fast and significantly. Even though we found hemodynamics stable during the whole ventilation process with either conventional ventilation or HFOV, a further study has to be performed to observe returned blood volume, hemodynamics such as cardiac output and cardiac index, air leak, and neurologic outcome.

Conclusions

In conclusion, histopathologic lung damage in newborn piglets with ALI was more severe in the gravitation-dependent region than in the gravitation-nondependent region. Different ventilation methods resulted in different damaged effect on injured lungs. Despite the improvement in oxygenation, HFOV with a high lung volume strategy attenuated lung injury by reducing pulmonary PMNLs infiltration, hemorrhages, alveolar edema, and hyaline membrane formation.

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REFERENCES

1. Gajic O, Dara SI, Mendez JL, Adesanya AO, Festic E, Caples SM, et al. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. Criti Care Med 2004; 32(9):1817-1824.
2. Wolthuis EK, Vlaar AP, Choi G, Roelofs JJ, Juffermans NP, Schultz MJ. Mechanical ventilation using non-injurious ventilation settings causes lung injury in the absence of pre-existing lung injury in healthy mice. Crit Care 2009;13(1):R1.
3. Ben Jaballah N, Mnif K, Khaldi A, Bouziri A, Belhadj S, Hamdi A. High-frequency oscillatory ventilation in term and near-term infants with acute respiratory failure: early rescue use. Am J Perinatol 2006; 23(7):403-411.

4. Wallace MJ, Probyn ME, Zahra VA, Crossley K, Cole TJ, Davis PG, et al. Early biomarkers and potential mediators of ventilation-induced lung injury in very preterm lambs. Respir Res 2009;10(10):19.
5. Chada M, Nögel S, Schmidt A-M, Rückel A, Bosselmann S, Walther J, et al. Anakinra (IL-1R antagonist) lowers pulmonary artery pressure in a neonatal surfactant depleted piglet model. Pediatr Pulmonol 2008;43(9):851-857.
6. Kandler MA, von der Hardt K, Gericke N, Chada M, Dötsch J, Rascher W. Dose response to aerosolized perflubron in a neonatal swine model of lung injury. Pediatr Res 2004;56(2):191-197.
7. Lu HY, Chang LW, Li WB, Jiang N, Peng QL, Cai C. Effect of hyperoxia on the expression of surfactant protein C in premature rats. Pediatr 2007;25(5):386-388.
8. Hua SD, Du J, Shi XD, Liu XX, Feng ZC. Experimental research of lung injury caused by different condition mechanical ventilation and oxygen inhalation in newborn rabbits. J Appl Clin Pediatr 2008; 23(16):1276-1279.
9. Viana ME, Sargentelli GA, Arruda ALM, Wiryawan B, Rotta AT. [The impact of mechanical ventilation strategies that minimize atelectrauma in an experimental model of acute lung injury]. J Pediatr (Rio J) 2004;80(3):189-196. *Article in Portuguese.*
10. Fan J. TLR Cross-talk mechanism of hemorrhagic shock-primed pulmonary neutrophil infiltration. Open Crit Care Med J 2010;2:1-8.
11. Fan J, Marshall JC, Jimenez M, Shek PN, Zagorski J, Rotstein OD. Hemorrhagic shock primes for increased expression of cytokine-induced neutrophil chemoattractant in the lung: role in pulmonary inflammation following lipopolysaccharide. J Immunol 1998;161(1): 440-447.
12. Rotstein OD. Modeling the two-hit hypothesis for evaluating strategies to prevent organ injury after shock/resuscitation. J Trauma 2003;54(5):203-206.
13. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. 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(4):890-898.
14. Matute BG, Martin TR. Science review: apoptosis in acute lung injury. Crit Care 2003;7(5):355-358.
15. Bingisser R, Stey C, Weller M, Groscurth P, Russi E, Frei K. Apoptosis in human alveolar macrophages is induced by endotoxin and is modulated by cytokines. Am J Respir Cell Mol Biol 1996;15(1): 64-70.
16. Lu MC, Liu TA, Lee MR, Lin L, Chang WC. Apoptosis contributes to the decrement in numbers of alveolar macrophages from rats with polymicrobial sepsis. J Microbiol Immunol Infect 2002;35(2):71-77.
17. Capoluongo E, Vento G, Santonocito C, Matassa PG, Vaccarella C, Giardina B, et al. Comparison of serum levels of seven cytokines in premature newborns undergoing different ventilatory procedures: high frequency oscillatory ventilation or synchronized intermittent mandatory ventilation. Eur Cytokine Netw 2005;16(3):199-205.
18. von der Hardt K, Kandler MA, Fink L, Schoof E, Dötsch J, Brandenstein O, et al. High frequency oscillatory ventilation suppresses inflammatory response in lung tissue and microdissected alveolar macrophages in surfactant depleted piglets. Pediatr Res 2004;55(2): 339-346.