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Hyperoxia During One Lung Ventilation: Inflammatory and Oxidative Responses

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Summary

Background—It is common practice during one lung ventilation (OLV) to use 100% oxygen, although this may cause hyperoxia- and oxidative stress-related lung injury. We hypothesized that lower oxygen (FiO₂) during OLV will result in less inflammatory and oxidative lung injury and improved lung function.

Methods—Twenty pigs (8.88 ± 0.84 kg; 38 ± 4.6 days) were assigned to either the hyperoxia group (n = 10; FiO₂ = 100%) or the normoxia group (n = 10; FiO₂ < 50%). Both groups were subjected to 3 hr of OLV. Blood samples were tested for pro-inflammatory cytokines and lung tissue was tested for these cytokines and oxidative biomarkers.

Results—There were no differences between groups for partial pressure of CO₂, tidal volume, end-tidal CO₂, plasma cytokines, or respiratory compliance. Total respiratory resistance was greater in the hyperoxia group ($P = 0.02$). There were higher levels of TNF- α , IL-1 β , and IL-6 in the lung homogenates of the hyperoxia group than in the normoxia group ($P = 0.01$, 0.001 , and 0.001 , respectively). Myeloperoxidase and protein carbonyls (PC) were higher ($P = 0.03$ and $P = 0.01$, respectively) and superoxide dismutase (SOD) was lower in the lung homogenates of the hyperoxia group ($P = 0.001$).

Conclusion—Higher myeloperoxidase, PC, and cytokine levels, and lower SOD availability indicate a greater degree of injury in the lungs of the hyperoxia animals, possibly from using 100% oxygen. In this translational study using a pig model, FiO₂ 50% during OLV reduced hyperoxic injury and improved function in the lungs.

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Keywords

single-lung ventilation; thoracic surgery; acute lung injury

INTRODUCTION

Temporary, intentional collapse of a lung, commonly referred to as one lung ventilation (OLV), is an anesthetic procedure performed to facilitate thoracic surgeries. To prevent systemic hypoxemia and increase the margin of safety during OLV, it is common to increase the fraction of inspired oxygen (FiO₂) to 100% for the duration of OLV.

While this procedure is medically necessary, there is ample evidence to suggest that OLV procedures can lead to lung injury through various mechanisms. These include hypoxia, ischemia/reperfusion,¹ mechanical injury in the collapsed lung,² hyperperfusion of the ventilated lung³ (due in part to its gravitationally dependent positioning during surgery and in part to pulmonary shunting and overdistension of alveoli),⁴ and oxidative injury from high FiO₂.⁵ Hyperoxia-induced acute lung injury is characterized by increased pulmonary permeability, increased inflammatory-cell counts, and injury to endothelial and epithelial cells, which may result in necrosis.⁶ This acute response is partially mediated by cytokine signaling, and the cytokines interleukin (IL)-6 and IL-8, two well-studied cytokines related to lung injury, are known to be involved in this signaling cascade.^{7,8} In addition to the physiological effects that result from the inflammatory cascade, hyperoxia results in the formation of reactive oxygen species, which can damage and disrupt various proteins, leading ultimately to the death of oxygen-sensitive cells.⁹

We have previously reported on the lung injury associated with OLV in an animal model and found the use of both protective ventilation¹⁰ and the anti-inflammatory drug methylprednisolone¹¹ to reduce the levels of pro-inflammatory cytokines. In this study, we focus on characterizing inflammatory and oxidative injury to the lungs during OLV, and we hypothesize that a lower FiO₂ during OLV will result in less inflammatory and oxidative injury in the lung and in improved lung mechanics.

MATERIALS AND METHODS

The study was designed as a single-blinded, two-group, preclinical study using an established animal model of OLV.¹² Juvenile pigs were chosen because of their comparable size to pediatric patients and because of their previous application in pediatric pulmonary research.^{13,14} The methodology was designed to closely mimic the clinical scenario typical for OLV procedures such that the study would have more clinical relevance. This study was approved by the Institutional Animal Care and Use Committee, Department of Biomedical Research, Nemours, in accordance with the National Institutes of Health guidelines.

We studied 20 juvenile Landrace-Yorkshire pigs that were approximately 4–5 weeks old. We chose this sample size to achieve sufficient statistical power while minimizing the number of animals sacrificed and to remain consistent with our previous studies on this model. The pigs were randomly assigned to two groups: the hyperoxic ventilation group (n = 10) and the normoxic ventilation group (n = 10). An anesthetic mixture (ketamine: 23 mg/ml, azepromazine: 0.1 mg/ml, and xylazine: 0.05 mg/ml) was administered via two injections (1 ml/kg) at a 10-min interval. The animals were subsequently placed on a radiant warmer bed (Resuscitaire, Hill-Rom Air-Shields, Hatboro, PA) to maintain a rectal temperature of 36–39°C. Following initial anesthesia, access was secured to the internal jugular vein and carotid artery using 8-Fr umbilical catheters, and the trachea was intubated with a 6.0-mm, uncuffed endotracheal tube. The leak around the endotracheal tube was

maintained <20–25 cm by inserting tonsil packs made of gauze around the tube. Both groups were mechanically ventilated with an 8–10 ml/kg tidal volume with no positive-end expiratory pressure applied (Ohmeda, Modulus II Plus™; GE Healthcare, Waukesha, WI). The normoxia group was ventilated with FiO₂ at or below 50% for the entire experiment, and the hyperoxia group was ventilated with 100% FiO₂ during the entire experiment. In both groups, the oxygen saturation (SaO₂) was maintained 96% and the end-tidal carbon dioxide (ETCO₂) was kept between 45 and 55 mmHg by adjusting, in some cases, the ventilatory rate, and inspiratory/expiratory ratio.

In both groups, following intubation and mechanical ventilation initiation, a 30-min stabilization period commenced during which the ventilatory parameters and anesthetic mixture were titrated to maintain the parameters mentioned above. Following the stabilization period, the left primary bronchus was blocked using a fiberoptic bronchoscope and a 5-Fr Arndt endobronchial blocker (Cook Medical, Bloomington, IN). The piglets were turned to the right-lateral position to simulate surgical positioning during OLV. Following local anesthesia with 0.5 ml of 1% lidocaine, a 5-mm trocar was placed through the left thoracic wall between the seventh and eighth ribs to simulate thoracoscopic instrumentation. The investigator observed the collapsed lung through the trocar using a Stryker endoscopy system (San Jose, CA). Breath sounds and the position of the bronchial blocker were checked half-hourly throughout the entire OLV period.

Throughout the duration of the experiment, vital signs were monitored using a standard pediatric anesthesia monitoring system (Model M1175A; Hewlett Packard, Palo Alto, CA), and arterial blood gas was measured half-hourly (Stat Profile pHox arterial blood gas/critical care analyzer; Nova Biomedical, Waltham, MA). Pulmonary mechanics were measured with a noninvasive cardiac output monitor (NICO; Respironics Novamatrix LLC, Wallingford, CT). Based on lung function evaluations in humans and animals, lung compliance, and lung volume are dependent upon weight,^{15,16} so total respiratory compliance was normalized to weight for analysis.

Anesthesia was maintained with intravenous sufentanil infusion at 0.2–0.3 µg/kg/hr and 1% inhaled isoflurane. Intravenous pancuronium (0.2 mg/kg) was administered half-hourly to maintain muscle relaxation. The depth of anesthesia was monitored using changes in vital signs as the primary criteria.

Blood samples were drawn half-hourly in sodium citrate (10% by blood volume), and after centrifugation, plasma was obtained for subsequent analysis of inflammatory mediators. These samples were drawn after a 30-min stabilization period (baseline), half-hourly during the OLV period (OLV), and again 30 min after re-expansion of the collapsed lung (endpoint).

Immediately before the piglets were killed, their anesthesia was deepened with boluses of sufentanil and ketamine, and isoflurane was increased to 4%. A modified Millonig's buffer solution (0.11 M NaOH, 0.12 M NaH₂PO₄·H₂O, 0.01 M glucose, 100 U/L heparin, pH 7.45) was introduced to the pulmonary artery to flush blood from the vasculature of the lungs before they were harvested. Base nondependent lung-tissue samples (approximately 5 cm³) were immediately collected from both groups and snap-frozen in liquid nitrogen. The samples were stored at –70°C until assaying for cytokines and oxidative injury markers. Lung-tissue homogenates were prepared as described below, and total protein concentrations of lung-tissue homogenates were determined using a bicinchoninic acid protein assay (BCA Protein Assay Kit; Thermo Scientific, Rockford, IL).

Myeloperoxidase Assay

Myeloperoxidase (MPO) in lung tissue was assayed, as modified from the procedure developed by Goldblum et al.¹⁷ Snap-frozen lung tissue was homogenized on ice in 300 μ l of 50 mM potassium phosphate, pH 6.0 (phosphate buffer). The total volume was adjusted to 1 ml. Following centrifugation at 10,000 rpm for 15 min, the pellet was resuspended in 300 μ l hexadecyltrimethylammonium bromide buffer (50 mM potassium phosphate, pH 6.0; 50 mM hexadecyltrimethylammonium bromide) and re-homogenized for 30 sec. Next, 700 μ l of phosphate buffer was added to the homogenate, and the mixture was homogenized for 20 sec using an ultrasonic homogenizer (BioLogics, Manassas, VA) before being snap-frozen in liquid nitrogen. The frozen samples were thawed at room temperature and homogenizing, snap freezing, and thawing were repeated twice more. After the third thaw, the samples were centrifuged at 10,000 rpm for 10 min to obtain a supernatant for measurement of MPO activity.

Myeloperoxidase in supernatants was measured using a standard spectrophotometric assay.¹⁷ One hundred microliter of the lung-tissue homogenate supernatant was mixed with 2.9 ml of phosphate buffer containing 0.167 mg/ml *O*-dianisidine dihydrochloride (Sigma–Aldrich, St. Louis, MO) and 0.0005% hydrogen peroxide (Sigma–Aldrich). The optical density at 460 nm was read at 15-sec intervals for 3 min using a kinetic program on a commercial spectrophotometer (SmartSpec Plus; BioRad Laboratories, Hercules, CA). The change in $A_{460} \times \text{minute}^{-1} \times \text{g total protein}^{-1}$ was analyzed.

Superoxide Dismutase Assay

Frozen base nondependent lung tissue was homogenized on ice in 5 ml of cold lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 0.5% Triton-100) per gram of tissue. The homogenate was centrifuged at 12,000 rpm for 10 min and the supernatant was collected for measurement of superoxide dismutase (SOD). The SOD activity was measured using a commercially available assay kit (Oxiselect™ Superoxide Dismutase Activity Assay; Cell BioLabs, San Diego, CA) according to manufacturer's instructions.

Protein Carbonyl Assay

Protein carbonyls (PC) were detected and quantitated in lung homogenates using an enzyme linked immunoassay (ELISA) kit (Oxiselect™ Protein Carbonyl ELISA Kit; Cell BioLabs). Linear standard curves were optimized by diluting reduced or oxidized bovine serum albumin with sensitivities ranging from 0.00 to 7.5 nmol/mg; inter-assay and intra-assay coefficients of variance were <10% and <6%, respectively. The bovine serum albumin standards and protein samples (10 μ g/ml) were derivatized with dinitrophenyl hydrazone and probed with an anti-dinitrophenyl antibody followed by a horseradish peroxidase-conjugated secondary antibody. The plate was read at 450 nm in an automated plate reader. All standards and samples were run in duplicate, and data are expressed as nmol/mg.

Cytokine Assays

Snap-frozen lung-tissue samples were homogenized on ice in 300 μ l phosphate-buffered saline (PBS), pH 7.4, then centrifuged for 1 min at 13,000 rpm. The supernatant was then collected in a clean tube. The pellet was rinsed in another 300 μ l PBS and centrifuged as before. The supernatant was combined with the first, and the volume was adjusted to 1 ml with PBS.

The levels of tumor necrosis factor-alpha (TNF- α), IL-1 β , IL-6, and IL-8 in lung homogenates and in plasma samples collected during the experiment were measured with quantitative ELISA using porcine-specific Quantikine ELISA kits (R&D Systems,

Minneapolis, MN). Lung homogenates and plasma samples were appropriately diluted to fall within the detection range of each assay, and all standards and samples were assayed in duplicate. The test sensitivities for respective immunoassays were as follows: TNF- α , 3.7 pg/ml; IL-1 β , 10 pg/ml; IL-6, 10 pg/ml; and IL-8, 0.039 pg/ml. Inter-assay and intra-assay coefficients of variance were <10%.

Preparation and Analysis of Histological Samples

Formalin-fixed lung tissue was processed and paraffin-embedded, and 5- μ M sections were cut for slide preparation. Tissue was stained with hematoxylin and eosin according to CLIA-approved protocols and visualized at 10 $\times$ magnification using a Nikon Eclipse 80i light microscope (Nikon, Tokyo, Japan) equipped with a digital camera (Digital Sight DS-SM; Nikon). Random fields from each slide were digitally imaged for qualitative analysis (ACT-2U; Nikon).

Statistical Analysis

Confounding variables were analyzed for equality of groups by *t* test for age and weight. Plasma cytokines and physiological and hemodynamic parameters were analyzed by repeated measures ANOVA. Two-factor ANOVA was used to analyze biomarkers in the lung-tissue homogenates following log-transformation of the data. The software used for statistical analysis was SPSS 17.0 for Windows (SPSS, Chicago, IL). Probability values <0.05 were considered significant.

RESULTS

On average, the pigs in the hyperoxia group weighed more than those in the normoxia group (9.5 ± 0.6 kg vs. 8.3 ± 0.6 kg, respectively; $P = 0.001$); therefore, weight was used as a covariate in all statistical analyses. Both groups were similar for temperature, mean arterial pressure, heart rate, peak inflating pressure, mean airway pressure, arterial partial pressure of carbon dioxide, and respiratory compliance, but there was a significant group $\times$ time difference for total respiratory resistance ($P = 0.02$; Table 1). Arterial partial pressure of oxygen in the hyperoxia group was higher as expected ($P = 0.02$; Fig. 1). There were no significant differences in the pro-inflammatory cytokines measured in the plasma. The lung homogenates in the hyperoxia group contained higher levels of TNF- α , IL-1 β , and IL-6 compared to the normoxia group ($P = 0.01$, 0.001, and 0.001, respectively), although these markers did not show a group $\times$ lung difference when analyzed by two-way ANOVA (Fig. 2). In addition to pro-inflammatory cytokines, MPO was higher in the hyperoxia group ($P = 0.03$). Oxidative injury markers were also significantly different (Fig. 3) between groups; SOD was lower in the lung-tissue homogenates of the hyperoxia group ($P = 0.001$). Protein carbonyl concentration in lung homogenate was greater ($P = 0.01$) in the hyperoxia group than the normoxia group, regardless of whether the lung was collapsed or ventilated. Qualitative histological analysis of the lung tissue revealed greater alveolar wall thickening and polymorphonuclear infiltrates in the collapsed lungs of both groups as well as increased numbers of red blood cells in the ventilated lungs of the hyperoxia group (Fig. 4).

DISCUSSION

One lung ventilation is increasingly used in pediatrics as both pediatric surgeons and pediatric anesthesiologists are gaining more experience in the involved techniques.¹⁸ Hyperoxic ventilation is a common anesthetic practice during OLV procedures, which makes sense in a clinical practice of increasing the safety margin when a child is being ventilated with 40–60% (left vs. right) of his or her lung volume. There have been several studies examining lung injury resulting from the interaction of high FiO₂ and mechanical

ventilation,^{19–23} and some of the studies focused on OLV specifically.^{24–27} These studies have been performed in animals or adult patients. The lack of studies in children led us to examine this injury in the context of pediatric OLV.

Misthos et al.²⁵ found that the level of malondialdehyde in plasma samples was positively correlated with the duration of OLV. They hypothesized that the oxidative stress was due to ischemia–reperfusion (IR) injury. Ischemia–reperfusion is well known to result in increased levels of reactive oxygen species.^{28–32} In our study, animals in both groups had measurable levels of inflammation and oxidative injury markers, but animals in the hyperoxia group had significantly higher levels of MPO and PC than those in the normoxia group. This suggests that while IR may play a role in generation of reactive oxygen species, there is a significant contribution from the use of 100% inspired oxygen during this procedure. Further, there were lower levels of SOD in both lungs of animals in the hyperoxia group, which is likely due to a greater depletion of SOD in reaction with the increased amount of reactive oxygen species in these animals.

There were increased levels of pro-inflammatory cytokines in the lungs of the hyperoxia group animals, while no differences between groups were observed for these same markers in plasma samples. We expected to and did find evidence of inflammation in the lung tissue itself before being able to measure these markers systemically.

Qualitative histological analysis showed some evidence of greater injury in the lungs of animals in the hyperoxia group, but the sample preparation was insufficient for a more definitive analysis. Future studies should include perfusion-fixation of the tissue to better preserve the pulmonary architecture and a trichromic staining method to more clearly identify inflammatory cells.

One limitation to the study was the short post-OLV recovery period before the end of the experiment. A longer measurement and sampling duration following OLV would allow us to see more time-dependent effects following reperfusion of the collapsed lung and might enable us to detect these pro-inflammatory cytokines systemically. In addition, the absence of differences in the inflammatory profile in the plasma may also be related to the ventilatory strategy. In this regard, if the ventilatory strategy produced relatively little mechanical disruption to the alveolar-capillary membrane, there would be minimal translocation of lung-originating cytokines into the systemic circulation. Further study of ventilatory strategies during OLV is warranted.

We conclude that animals exposed to 100% oxygen during 1 hr of bilateral ventilation (total) and 3 hr of OLV have greater inflammatory injury in their lungs compared with animals breathing an oxygen concentration of 50% or less. The results from our study suggest that lung injury is caused not only by OLV, IR, and mechanical ventilation, but specifically by 100% FiO₂. In piglets undergoing simulated OLV, lowered FiO₂ (50%) reduces oxidative injury to the lungs yet maintains adequate partial pressure of oxygen (PaO₂). Admittedly, clinical scenarios of OLV are more complex and fraught with variables such as compression of the mediastinum, blood loss, and others that are not controlled by the anesthesiologist. However, by monitoring arterial PaO₂ for OLV patients, a safe oxygenation range could be kept, and the sustained hyperoxia that leads to increased oxidative injury could be minimized. Oxidative stress and inflammation were also associated with increased total respiratory resistance in our hyperoxia animals, and studies have shown other clinical complications following oxidative injury as well.^{25,33–35}

Our study supports the hypothesis that a lower FiO₂ during OLV will result in less inflammatory and oxidative injury in the lung and in improved pulmonary mechanics in a

piglet model of OLV. Further clinical research is necessary to confirm similar results during OLV in children.

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Fig. 1.

Partial pressure of oxygen (PaO₂) over time. This figure shows the hyperoxia group with a higher mean PaO₂ than the normoxia group from the baseline, through one lung ventilation, to the endpoint measurement ($P = 0.02$ by repeated measures ANOVA). This difference is expected, since the FiO₂ for the hyperoxia group was held at 100% for the duration of the experiment, while the FiO₂ for the normoxia group was maintained at or below 50%. There is also greater variability in dispersion of data points for PaO₂ in the hyperoxia group for each time point. *Significance at the $P = 0.001$ level.

Fig. 2.

Pro-inflammatory cytokines in lung tissue. Levels of pro-inflammatory cytokines in lung-tissue homogenates from the collapsed and ventilated lungs of animals in the hyperoxia group and the normoxia group. There was an overall group difference observed for tumor necrosis factor (TNF)- α (A), interleukin (IL)-1 β (B), and IL-6 (C) at $P < 0.01$, 0.001, and 0.001, respectively, independent of whether the lung was collapsed or ventilated. No difference was seen between groups for IL-8 (D). Levels of these markers were higher in the lungs of animals in the hyperoxia group, thus indicating a greater degree of inflammation from exposure to a FiO_2 of 1. Note that to improve clarity for the IL-1 β graph, the split Y-axis excludes the 20–1000 range.

Fig. 3.

Oxidative injury markers in lung tissue. Levels of oxidative injury markers in lung-tissue homogenates from the collapsed and ventilated lungs of animals in the hyperoxia group and the normoxia group. Overall group differences existed for myeloperoxidase (MPO) (A; $P = 0.03$), protein carbonyls (PC) (B; $P = 0.02$) and superoxide dismutase (SOD) (C; $P = 0.001$) by two-way ANOVA, independent of whether the lung was collapsed or ventilated. The hyperoxia group shows higher levels of MPO and PCs, suggesting a greater amount of reactive oxygen species in this group. The abundance of reactive oxygen species in the lungs of hyperoxia group animals may have resulted in a depletion of available SOD, explaining why the hyperoxia group had lower levels of SOD compared to the normoxia group.

Fig. 4. Representative histology. Hematoxylin- and eosin-stained base nondependent lung tissue harvested following endpoint measures of one lung ventilation (OLV) experiment and viewed at 10× magnification. In both groups, the collapsed lung shows greater alveolar wall thickening and polymorphonuclear infiltrates when compared with the ventilated lung. In the hyperoxia group, greater numbers of red blood cells are visible in the alveolar lumen compared to the normoxia group, which may suggest greater pulmonary permeability due to oxidative injury.

TABLE 1

Physiological and Hemodynamic Data

	Normoxia group (mean ± SD)			Hyperoxia group (mean ± SD)			P-value
	T = 0	T = 2.5 hr	End	T = 0	T = 2.5 hr	End	
Temp (°C)	36.7 (0.6)	36.4 (0.6)	36.6 (0.7)	36.4 (0.6)	36.8 (0.5)	36.8 (0.6)	0.71
MAP (mmHg)	71.1 (4.2)	71.9 (2.8)	67.9 (5.0)	79.2 (9.0)	76.3 (9.9)	72.1 (6.2)	0.89
HR (min ⁻¹)	99.1 (10.5)	94.9 (14.2)	100.4 (19.8)	99.1 (20.4)	110.3 (21.8)	115.0 (19.6)	0.115
PIP (cmH ₂ O)	15.7 (2.3)	23.2 (4.0)	21.4 (3.2)	16.2 (2.3)	24.3 (1.8)	21.0 (3.1)	0.56
MAWP (cmH ₂ O)	4.5 (0.7)	6.0 (0.9)	5.6 (1.0)	4.4 (5.3)	6.6 (0.7)	5.6 (0.7)	0.95
PaCO ₂ (mmHg)	40.4 (6.5)	47.1 (3.3)	49.7 (5.7)	47.1 (7.9)	54.5 (8.6)	54.3 (9.4)	0.70
Compliance (ml/cmH ₂ O/kg)	0.84 (0.2)	0.53 (0.1)	0.61 (0.1)	1.0 (0.3)	0.7 (0.2)	0.8 (0.2)	0.27
Resistance (cmH ₂ O/L/min)	15.0 (3.6)	30.8 (4.1)	23.0 (3.8)	16.5 (3.0)	31.5 (7.7)	24.9 (5.4)	0.02 [†]

Confounding variables measured over time at baseline before one lung ventilation (OLV) (T = 0), 2.5 hr of OLV (T = 2.5), and one half-hour after re-expansion of the collapsed lung (End). P-value obtained by analysis using repeated measures ANOVA. Temp, temperature; MAP, mean arterial pressure; HR, heart rate; PIP, peak inspiratory pressure; MAWP, mean airway pressure; PaCO₂, partial pressure of carbon dioxide.

[†] Group × time statistical difference.