Antenatal Vitamin A Decreases Ventilation-induced Lung Injury in the Lamb Model of Congenital Diaphragmatic Hernia

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OBJECTIVE: Infants with congenital diaphragmatic hernia (CDH) are susceptible to ventilation-induced lung injury. Vitamin A may protect the lung from injury during ventilation. The authors investigated the effects of antenatal vitamin A on ventilation-induced lung injury in CDH lambs using lung myeloperoxidase (MPO) activity as an indicator of lung injury.

METHODS: Left-sided diaphragmatic defects were created in 10 lambs at 79–81 days’ gestation. Six CDH lambs had right jugular venous catheters inserted at 120 days’ gestation and were given vitamin A until 135 days’ gestation. Four CDH lambs were not treated. Twin littermates \((n=3)\) served as controls. All lambs were delivered at 136–139 days of gestation and ventilated for 2 hours. Lambs were sacrificed following ventilation and samples of left lung were snap frozen. MPO was extracted from lung tissue and MPO activity was assayed.

RESULTS: CDH lambs treated with antenatal vitamin A demonstrated significantly lower MPO activity than untreated CDH lambs \((0.0477 \pm 0.0150 \text{ vs. } 0.1106 \pm 0.0230 \text{ units/mg protein}, p < 0.05)\).

CONCLUSION: This is the first study to look at the effect of vitamin A on lung injury in CDH. In the lamb model of CDH, antenatal vitamin A decreases ventilation-induced lung injury. [Asian J Surg 2006; 29(3):193–7]

Key Words: CDH, congenital diaphragmatic hernia, lung injury, myeloperoxidase activity, ventilation-induced lung injury, vitamin A

Introduction

Barotrauma, volutrauma and oxygen toxicity occur to varying degrees during ventilation. Barotrauma and volutrauma lead to diffuse alveolar injury, disruption of the alveolar capillary barrier, oedema and protein leakage.\(^1\) Moreover, during hyperoxia, highly reactive oxygen metabolites are produced: superoxide radical \((\text{O}_2^-)\), hydrogen peroxide \((\text{H}_2\text{O}_2)\), hydroxyl free radical \((\text{OH}^-)\) and singlet oxygen \((^{1}\text{O}_2)\).\(^2\) When these radicals are produced in excess, they react with cell proteins, lipids and nucleic acid, leading to damage and death of capillary endothelial cells and type I pneumocytes and interstitial oedema.

Infants with congenital diaphragmatic hernia (CDH) are susceptible to ventilation-induced lung injury. Post-mortem examinations of the lungs of ventilated CDH infants show diffuse alveolar damage, hyaline membrane formation, pulmonary haemorrhage and interstitial fibrosis.
in over 90% of cases. In addition, bronchopulmonary dysplasia is seen in approximately 40% of CDH survivors.  

Pulmonary hypoplasia and decreased lung compliance contribute to the increased susceptibility to ventilation-induced lung injury seen in CDH. Pulmonary hypoplasia and decreased lung compliance lead to the tidal volume being distributed to a few opened alveoli. This enhances barotrauma and volutrauma in CDH lungs.  

Immaturity of the pulmonary antioxidant enzyme (AOE) system also potentially contributes to lung injury in CDH. The AOE system is one of the primary means of defence against the active oxygen metabolites produced during hyperoxia. The lung possesses a battery of antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase scavenges the initial reactive product of oxygen (O2); catalase catalyzes the conversion of H2O2 to H2O and O2; glutathione peroxidase detoxifies OH radicals and lipid peroxides. In CDH, a low to normal baseline AOE exists at the time of delivery. More importantly, CDH lungs fail to mount a protective increase of AOE activity during ventilation with high FiO2. The CDH lung is therefore also susceptible to lung injury secondary to hyperoxia.  

Vitamin A may play a role in protecting the lung from ventilation-induced lung injury. Vitamin A is necessary for normal lung growth and vitamin A deficiency is associated with histopathological changes in the lung that are similar to bronchopulmonary dysplasia. Furthermore, clinical studies have shown that vitamin A supplementation reduces the incidence of bronchopulmonary dysplasia or chronic lung disease in the extremely low birth weight premature infant.  

The initial indication of lung injury is an inflammatory response and lung injury is associated with neutrophil sequestration. Myeloperoxidase (MPO) is a major component of neutrophil cytoplasmic granules and lung MPO activity increases with lung injury. The purpose of this study is to investigate the effects of antenatal vitamin A on ventilation-induced lung injury in CDH lambs, using lung MPO activity as an indicator of lung injury.

Materials and methods

Experimental design  
Diaphragmatic defects were created at 79–81 days of gestation (term, 140–145 days). Lambs were divided into three groups. Group 1 lambs (CDH, n = 4) were not treated. In group 2 lambs (CDH + vitamin A, n = 6), an indwelling right jugular venous catheter was placed at 118–120 days, and intravenous vitamin A was administered. Group 3 lambs (control group, n = 3) consisted of twin littermates. All animals were delivered at 136–139 days and ventilated for 2 hours according to a set protocol.  

Fetal surgical procedures  
This study was approved by the Animal Care Committee of the State University of New York at Buffalo, and conforms to the National Institute of Health guidelines. The lamb model of CDH was created. Hysterotomy was performed using a GIA stapling device (US Surgical, Norwalk, CT, USA). The head and upper torso of the lamb were delivered into the wound and left posterolateral thoracotomy was performed. The head and upper torso of the lamb were delivered into the wound and left posterolateral thoracotomy was performed. The diaphragm was incised over the stomach, and the stomach and small bowel were gently manipulated into the chest. The thoracotomy was closed and the fetus returned to the uterus. Amniotic fluid lost during the procedure was replaced with warm sterile normal saline. All pregnant ewes were housed in the animal facility during the experimental period.  

In group 2 animals (CDH + vitamin A), long-term closed fetal venous access was gained at 118–120 days’ gestation. Open hysterotomy was performed, and the fetal head and neck were delivered into the wound. The tip of the venous catheter (Bard Access Systems, Salt Lake City, UT, USA) was advanced via venotomy into the right jugular vein. The catheter was secured above and below the venotomy site. The fetus was returned to the uterus and the hysterotomy was closed. The venous catheter was tunnelled from its point of exit from the uterus, through the subcutaneous tissue of the anterior abdominal wall to the right flank of the ewe. The body of the implanted port was connected to the venous catheter and secured in a subcutaneous pouch.  

Vitamin A administration  
Vitamin A was given as retinyl palmitate (Aquasol A parenteral, Astra USA Inc., Westborough, MA, USA), 7,500 IU on alternate days from 121 to 135 days in n = 3 animals (in keeping with the recommended dose of vitamin A supplementation for very low birth weight infants on parenteral nutrition) and 7,500 IU daily in n = 3 animals.  

Resuscitation and monitoring  
Lambs were delivered via caesarean section at 136–139 days of gestation. All lambs were instrumented while on
placental circulation. The fetal head and neck were delivered and an endotracheal tube (internal diameter, 3.5 or 4.0 mm) was inserted and secured via tracheostomy. Polyvinyl catheters were inserted in the carotid artery and jugular vein. The umbilicus was divided between clamps. The fetus was weighed, wrapped in plastic and placed in a warming blanket under a Servo radiant warmer (Vickens Air-Shields, Hatboro, PA, USA). Lambs were ventilated for 2 hours based on a set protocol (Servo ventilator 900C, Siemens, Elema, Sweden): respiratory rate of 60 breaths per minute; peak inspiratory pressure (PIP) of 28 cmH₂O; peak end expiratory pressure (PEEP) of 4 cmH₂O; inspired oxygen concentration of 100%. Control lambs were initially ventilated at these settings. Thereafter, settings were adjusted to maintain PaCO₂ within normal limits.

**Myeloperoxidase assay**

MPO was extracted from homogenized lung tissue sample by suspending the material in 0.5 hexadecyltrimethylammonium bromide (Sigma Chemical Co., St Louis, MO, USA) in 50 mmol/L potassium phosphate buffer, pH 6.0, before sonication in an ice bath for 30 seconds. The samples underwent three freeze-thaw cycles, after which sonication was repeated. Suspensions were then centrifuged at 40,000 g for 30 minutes and the resulting supernatant further assayed.

MPO activity was assayed spectrophotometrically using the method of Bradley et al; 0.1 mL of supernatant was mixed with 2.9 mL of 50 mmol/L phosphate buffer, pH 6.0, containing 0.167 g/mL θ-dianisidine dihydrochloride (Sigma Chemical Co.) and 0.0005 hydrogen peroxide (Sigma Chemical Co.). The change in absorbance at 460 nm was measured using a spectrophotometer (U-2000; Hitachi Instruments, Webster, NY, USA). MPO activity was then derived from the observed change in absorbance per minute. MPO activity was normalized further to the total protein content of the supernatant, as measured by the micro-Lowry technique. Activity was expressed as units of MPO activity per milligram of protein.

**Statistical analysis**

Data are presented as mean ± SEM. Statistical differences were determined by using an unpaired Student’s t test for equal and unequal variances. The value of p < 0.05 was considered significant.

**Results**

One of the vitamin A treated CDH lambs was excluded from the study following the development of Q fever in the pregnant ewe. Following 2 hours of mechanical ventilation under similar conditions, CDH lambs treated with antenatal vitamin A demonstrated significantly lower lung MPO activity than untreated CDH lambs (0.0477 ± 0.0152 vs. 0.1106 ± 0.0230, p < 0.05). Control lambs demonstrated significantly lower MPO activity than untreated CDH or CDH + vitamin A lambs. No significant difference was demonstrated between control lambs and CDH + vitamin A lambs (Figure). The birth weights of CDH, CDH + vitamin A, and control lambs were similar. Treated and untreated CDH lambs had significant pulmonary hypoplasia (p < 0.001) (Table).

**Discussion**

Antenatal vitamin A as administered in this study decreases ventilation-induced lung injury in the lamb model of...
CDH. This study also provides additional evidence that the CDH lung is susceptible to ventilation-induced lung injury.

A previous study by our group confirmed that lung MPO is a reliable indicator of lung injury in the lamb model of CDH. We previously demonstrated that CDH lambs and control lambs have similarly low levels of MPO activity at birth. In both CDH and control lambs, MPO activity significantly increases following 4 hours of ventilation; the increase seen in CDH lambs is, however, significantly greater than that seen in control lambs. Our current findings correlate with this previous study in that following 2 hours of ventilation, CDH lambs had significantly greater MPO activity than the control group.

Antenatal vitamin A as administered in this study decreases ventilation-induced lung injury in the lamb model of CDH. This is in keeping with the protective effects of supplemental vitamin A on premature lung in human neonates; extremely low birth weight premature infants have a decreased incidence of chronic lung disease when supplemental retinoic acid (active metabolite of vitamin A) is given in the newborn period.

The mechanisms through which vitamin A leads to decreased ventilation-induced lung injury remain unknown. The decrease in ventilation-induced lung injury may be secondary to the increase in lung compliance noted in CDH lambs following administration of antenatal vitamin A or secondary to the antioxidant effect of vitamin A. Increased lung compliance may have decreased airway opening injury mechanism and mechanical stretch-induced injury in the lung. Also, vitamin A is an antioxidant and it may have neutralized some of the reactive oxygen metabolites produced during hyperoxia and lung injury. In addition, vitamin A is an important modulator of lung development and has been previously shown to stimulate antioxidant defences. We hypothesize that vitamin A may have resulted in the maturation of the AOE system in vitamin A treated CDH lambs. AOE levels at delivery and following ventilation were not measured in this study.

Treatment protocols using inhaled nitric oxide, high frequency oscillatory ventilation and “gentle ventilation” with permissive hypercapnia have been associated with increased survival in patients with CDH. Interestingly, Bagolan et al noted a significant increase in survival (from 50% to 90%) when gentle ventilation was utilized in addition to inhaled nitric oxide and high frequency oscillatory ventilation. This suggests that significant mortality is associated with ventilation-induced lung injury and that there is significant benefit to reducing ventilation-induced lung injury in CDH.

Antenatal betamethasone and tracheal occlusion are newer modalities in limited use in the management of antenatally diagnosed CDH. The effect of antenatal betamethasone on lung injury in CDH has not been investigated and antenatal betamethasone has variable effects on AOE levels in experimental models of CDH. Tracheal occlusion on the other hand has not been shown to improve the AOE system in CDH.

Neonates with CDH have increased susceptibility to ventilation-induced lung injury. This is the first study to look at the effects of antenatal vitamin A on ventilation-induced lung injury in CDH. In the lamb model of CDH, vitamin A decreases ventilation-induced lung injury. This study has significant implications for CDH neonates. The mechanisms by which vitamin A decreases lung injury as well as the optimum timing and dosage need to be explored.

References


