APOPTOTIC MARKERS IN THE LUNGS OF MECHANICALLY AND NASAL CONTINUOUS POSITIVE AIRWAY PRESSURE VENTILATED ANIMALS


Background
Mechanical ventilation in preterm infants has been shown to stress the lung and cause respiratory damage. Nasal CPAP (continuous positive airway pressure, nCPAP) ventilation lessens damage and allows for more normal pulmonary development. An analysis of apoptosis in the lungs will further our understanding of alveolar simplification in animals ventilated by mechanical and nCPAP ventilation. The lung naturally simplifies itself through the process of apoptosis, leading to thin alveolar walls which are ideal for gas exchange. Ventilation of preterm infants may disrupt this normal apoptotic simplification while also causing undesirable cell death in other areas of the lung tissue such as terminal airway cells. The purpose, then, of this study was to examine the apoptotic effects of mechanical and nasal CPAP ventilation on lung tissue.

Methods
Lung tissue was taken from five different groups of lambs: Preterm, mechanically ventilated for 72 hours (n=4); Preterm, mechanically ventilated and given vitamin A (IM, 5000 u/kg) for 72 hours (n=4); Preterm, nCPAP for 72 hours (n=4); One day term lambs, unventilated gestational control (n=4); Three week term lambs, unventilated gestational control (n=4). Tissue samples were analyzed through immunohistochemical staining for certain apoptotic molecules: Fas (Santa Cruz, monoclonal, 1:1500 and 1:2000), Antigen retrieval and Tyramide Signal Amplification method (AR-TSA method); Fas-Ligand (Santa Cruz), rabbit, 1:800 and 1:1200, Antigen retrieval and avidin-biotin complex method (AR-ABC method), Bax (Signal rabbit, 1:800 and 1:1200, AR-ABC method); p53 (BioGenex), monoclonal, 1:50 and 1:100, AR-TSA method; Cleaved caspase-3 (Cell Signalling), rabbit, 1:75 and 1:100, AR-TSA method; Active-form caspase-3 (BD Pharmingen), rabbit, 1:75 and 1:1000, AR-ABC method; Caspase-8 (Santa Cruz, goat, 1:500 and 1:800, AR-ABC method. A TUNEL (Roche) stain was also performed, using the following mixture: 10% TdT buffer, 10%CoCl2, 1.2% DIG-11-dUTP, 0.6% TdT, 72.8% water.

Results
The abundance of staining by these apoptotic markers was analyzed qualitatively in the terminal airway tissue of the study groups. The mechanically ventilated lamb groups showed more staining in the terminal airways, while staining in the nCPAP groups was markedly less abundant. The term lamb groups exhibited a minimal amount of apoptotic staining in the terminal airways.

Conclusion/Speculation
The low degree of apoptosis in the terminal airway cells of the term lambs shows that postnatal programmed cell death does not occur. The nCPAP ventilation group had apoptotic levels similar to the levels found in the term control animals. The mechanically-ventilated groups showed a higher incidence of cell death, denoting an increase in lung damage. Therefore, nCPAP ventilation, with its decreased incidence of apoptosis in the terminal airway cells, is gentler on the developing preterm lung. Future plans include a quantitative analysis to determine if statistical differences are found.