The Role of Cyclooxygenase 2 in Mechanical Ventilation Induced Lung Injury

Joshua A. Robertson, David Sauer, Jeffrey A. Gold, Stephanie A. Nonas

ONLINE DATA SUPPLEMENT

***In Vivo* Model of ALI/VILI**

All animal protocols were approved by the Oregon Health and Science University Institutional Animal Care and Use Committee and conform to NIH guidelines.

Adult male C57Bl/6J mice 24-30g (Jackson Laboratories, Bar Harbor, Maine) were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10mg/kg, i.p.) and redosed as needed. Animals were intratracheally intubated and ventilated with room air at either low (LTV, 7ml/kg) or high (HTV, 20ml/kg) tidal volume for four hours at a respiratory rate of 160 breaths/minute with 3cm H_2O positive end-expiratory pressure. Control animals were anesthetized and allowed to breathe spontaneously. Because respiratory rate is known to have a prominent effect on cytokine generation [1], we matched the rate in the LTV and HTV animals with that of control mice. In order to prevent hyperventilation in the HTV animals, external dead space was applied to the ventilator. All ventilated animals received an IV bolus of 0.5ml of sterile Ringer's lactate at the onset of mechanical ventilation to prevent hypotension. At the end of each experiment, a 0.5-1ml heparinized blood sample was obtained by cardiac puncture.

Blood Gas Analysis and Peripheral Blood Arterial Oxygen Saturation. Blood samples were immediately analyzed for blood gases using i-STAT point-of-care analyzer (iSTAT, Princeton, NJ) as previously described [2]. Arterial oxygen saturation (Sp_{O2}) was measured during ventilation using the MouseOx system (Starr Life Sciences Corp., Allison Park, PA), at 15-30 minute intervals throughout the experiment.

Myeloperoxidase (MPO) Activity.

MPO activity was used as a marker of inflammatory cell infiltration into the lung and measured using a modification of the technique previously described by Bradley et al[3]. Briefly, at the completion of the mechanical ventilation, the pulmonary vasculature was perfused clear as above and the right lung homogenized. Aliquots (0.1mL) of 10,000xg supernatants of these lung homogenates were added to 2.9mL of reaction mixture containing 0.167 mg/ml O-dianisidine dihydrochloride and 0.0005% hydrogen perioxide in 50mM potassium phosphate working buffer at pH 6.0. The absorbance of the supernatant was measured at 460nm at 15 second intervals for 2 minutes and the activity calculated as previously described. The activity is expressed as units/g lung tissue.

Western Blot –

After sample is quantified, a mix of 50ug of protein, water, and loading buffer (NP0007) is prepared. Samples are boiled for 10-15 minutes at 65 C, and load into SDS-page gel (NuPage 4-12% gradient Bis-Tris Gel 1.0mm/well NP0321BOX) in MOPS SDS Running Buffer (20X NP0001), then run at 150 volts for approximately 1.5 hrs. Gels are transferred onto .2um nitrocellulose (LC2000) in Invitrogen's Transfer Buffer (20X NP0006-1) at 15 volts for ~18hrs. Membrane is then blocked in 5% dried milk in PBS-T overnight. Membrane is washed 3X in PBS-T, new milk with preferred antibody is added and incubated for the amount of time and dilution as recommended by the manufacturer. Blot is washed again 3X with PBS-T. Secondary antibody is added for 1.5 hours, washed 3X again, and developed using Thermo Scientific's SuperSignal

West Pico Chemiluminescent Substrate (34080). Blots are exposed on Kodak's BioMax Light Film Scientific Imaging Film (868-9358).

E1. Rich, P.B., et al., *Effect of ventilatory rate on airway cytokine levels and lung injury.* J Surg Res, 2003. **113**(1): p. 139-45.
E2. Irikura, K., et al., *Moderate hypothermia reduces hypotensive, but not hypercapnic vasodilation of pial arterioles in rats.* J Cereb Blood Flow Metab, 1998. **18**(12): p. 1294-7.
E3. Bradley, P.P., et al., *Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker.* J Invest Dermatol, 1982. **78**(3): p. 206-9.