Hyperpolarized 1-13C pyruvate metabolism as marker of inflammation and progression of lung injury

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Introduction: Acute lung injury (ALI) is defined as a diffuse injury of alveolar or capillary endothelial cells. Pulmonary interstitial, alveolar edema, and acute hypoxic respiratory failure are the main result of ALI. ALI can progress to acute respiratory distress syndrome (ARDS) and it causes acute hypoxemic respiratory failure (ALI: $PaO_2/FiO_2 \leq 300$ mmHg, ARDS $PaO_2/FiO_2 \leq 200$ mmHg). Mechanical ventilation is an essential component of the care of patients with ARDS to improve gas exchange and minimize any additional hypoxic damage. However mechanical ventilation itself can further injure damaged lungs; considerable effort has been directed toward identifying the best ventilation strategies to avoid additional damage while maintaining adequate gas exchange. Low tidal volume and high positive end-expiratory pressure is the suggested ventilation strategy and it is the only method of mechanical ventilation that has been shown to improve survival. The main goal of this work is to study the metabolic changes of ALI during the ventilation with a protective strategy which avoids additional damage as measured using systematic blood biomarkers and X-ray computed tomography (X-ray CT). For this purpose, hyperpolarized 1-13C pyruvate metabolism was applied in an acid-aspiration lung injury model. This model was chosen for its tendency to propagate in a manner similar to ARDS, although it duplicates a clinically relevant injury as well.

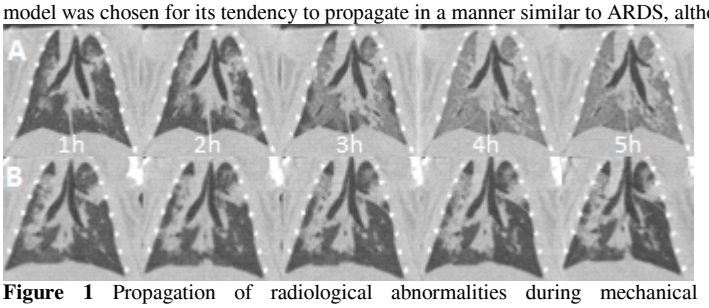
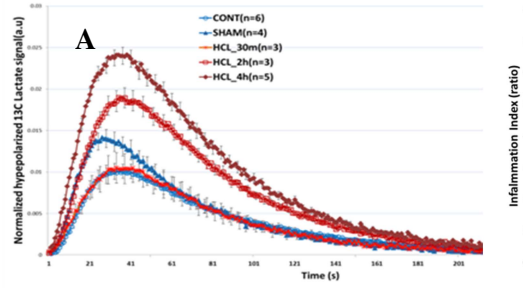
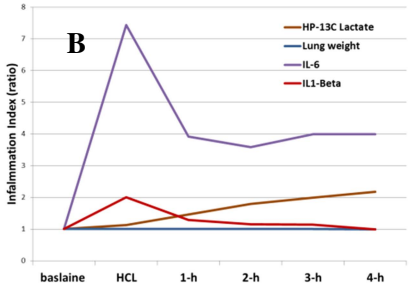


Figure 1 Propagation of radiological abnormalities during mechanical ventilation following HCl aspiration. Two patterns were recognizable: rapid dissemination of lesions (PEEP,3 cmH_2O/V_T, 12ml/Kg, FiO 1.0) (A) and limited propagation (PEEP,10 cmH_2O/V_T, 6ml/Kg, FiO 1.0) (B).

Method: To create the acid-aspiration model, one dose of HCl (2.5 ml/kg, pH 1.25) was administered in the trachea with the animal in a 45° head up position. In one cohort of 15 animals, hourly computerized tomography (CT) was used to track the subsequent radiological propagation of injury in rat lungs. These animals were examined in two separate groups: seven were ventilated with high positive end expiratory pressure and low tidal volume (PEEP,10 cmH_2O/V_T, 6ml/kg, FiO2 1.0) and eight animals were ventilated with low PEEP (3 cmH_2O) and moderate V_T (12ml/kg). These ventilation conditions are hereafter referred to as high PEEP and low PEEP. Three-dimensional images of both lungs were obtained by a semi-automated, multi-landmark, registration-based scheme for lung segmentation. Images were then quantitatively analyzed to measure change of lung weight as an *in vivo* marker of edema. Blood gas and cytokines analysis were also utilized hourly during ventilation. In a second cohort of 21 animals, metabolic changes were evaluated in isolated perfused lung using hyperpolarized 1-13C pyruvate. Animals were induced and ventilated with high low V_T (6ml/Kg) with varying duration as enumerated in fig. 2A. All the lungs were then excised and placed in a 20-mm NMR tube (9.4T vertical bore magnet) while perfused with a modified Krebs-Henseleit buffer containing 3% (w/v) fatty acid free BSA at 10 ml/min. 28.5mg [1-^{13}C] pyruvate was polarized with a HyperSense DNP system (Oxford Instruments). 4 mL Tris-buffered saline with 100 mg/L EDTA was heated to 190°C at 10 bar, and was used to rapidly dissolve the frozen sample. This sample was added to the perfusate flow using a HPLC pump such that that the final HP-pyruvate concentration was 4mM. The health of the tissue was checked with ^{31}P spectroscopy before and after HP-pyruavte administration. Low flip-angle (α=15°) carbon spectra were acquired for the duration of the hyperpolarized signal. The spectra were fitted and analyzed using custom MATLAB routines.

Results: In hourly CT images, significant propagation was observed in low PEEP and moderate V_T ventilation strategy (fig 1.A) while the protective high PEEP cohort displayed no discernable propagation (Figure 1.B). Analysis of circulating inflammatory mediators (IL-6 and IL1-β) showed a significant increase in both ventilation strategies right after HCl-induction. The low PEEP cohort displayed continued increases of both mediators throughout the duration of the experiment, while the protective high PEEP group stabilized at a substantially reduced mediator concentration (fig. 1.B). Hyperpolarized lactate signal significantly increased (2.1-fold) four hours after intratracheal HCl administration (fig. 2.A) in high PEEP ventilated animals (the low PEEP cohort could not be studied because tissue perfusion was compromised by the widespread damage). Lactate production was unchanged 30 minutes after HCl administration; the first significant increases in lactate signal with respect to control animals were seen after 2h ventilation and these levels were further increased after 4h (fig. 2.B). Hyperpolarized alanine signals were not significantly different from those of the control group in any of the cohorts. Among the eight distinct 31P-containing species GPC/E and GP differ significantly between the sham and HCL-injured lung, and are elevated by a factor of 3 in inflammation on average (figure 2.C).





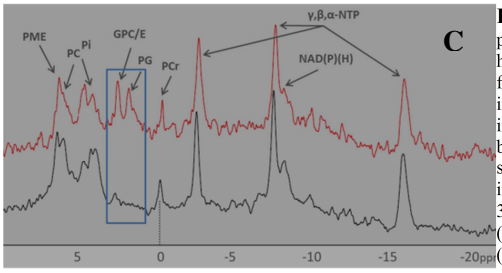


Figure 2: A: The full time-series of fit HP-Lactate peak area in HCl-induced (after 30 mins, two hrs or four hrs of protective ventilation), sham (saline-induced after four hrs of protective ventilation) and control (no-induction, no-ventilation). B: Comparison of time course in different inflammatory index ratio (fraction of baseline). To simplify figure the error bars did not shown. C: 31P NMR spectra of Sham (gray) and HCL-injured (red) lungs after 4h ventlation. Eight distinct 31P-containing species : three high energy phosphates (NTP), inorganic phosphate (Pi), phosphomonoesters (PME), phosphocreatine (PCr) phosphoglycans, phosphodiesters, (GPC/E)and phosphoglycans (PG)

Discussion and Conclusion: Among all of the inflammatory indexes (figure 2.B), only the lactate signal increases during protective ventilation. This indicates the qualitative differences among the indices, with HP 13C-labeled pyruvate showing the progress of neutrophil infiltration and/or activation (1) as distinct from the initial burst of inflammatory mediators or progress of edema. These data show that HP 13C-labeled pyruvate NMR is sensitive to lung injury and could be employed to evaluate the interaction between primary injury and mechanical ventilation during the course of inflammatory activation.

Reference: 1) Shaghaghi, H. et al. NMR in Biomed. 2014, 27: 939–947.