Monitoring tissue response to hyperbaric oxygen intervention using PISTOL

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Introduction: The ability to quantitatively measure tissue oxygen tension (pO_2) non-invasively could have a significant impact on understanding the mechanisms of tissue function and in clinical prognosis of cancer and peripheral vascular disease [1]. To improve therapeutic efficacy, efforts have concentrated on eliminating the hypoxic state of tumors by the use of normobaric oxygen (NBO) or hyperbaric oxygen (HBO) breathing. Previous research showed PISTOL (Proton Imaging of Siloxanes to map Tissue Oxygenation Levels) [2] as an oximetry technique to quantitatively measure tissue oxygen tension using hexamethyldisiloxane (HMDSO). We now present data demonstrating the use of HMDSO based nanoemulsions for dynamic mapping of tissue oxygenation using PISTOL technique, in response to hyperbaric oxygen challenge following intra muscular injection. Furthermore we determined a kinetic parameter (k) related to oxygen consumption using pO_2 data post oxygen challenge (NBO or HBO) as described elsewhere [3] and compared the two cases.

Materials and Methods: Nanoemulsions were prepared by ultrasonic emulsification of HMDSO, de-ionized water and the surfactant: Polyethylene glycol hydroxystearate (HS-15) in the ratio 40:55:5 (%v/v) respectively. An R_1 vs pO_2 calibration curve of the nanoemulsion was determined using a spin-echo based pulse sequence by spectroscopy in a Varian 4.7 T MR scanner at 37° C. A home built MR compatible hyperbaric chamber equipped with MR volume coil, pressure and temperature sensors, was used to monitor tissue oxygen dynamics *in vivo*. After an intra muscular injection of 100µl of nanoemulsion, slice containing the emulsion was imaged using PISTOL technique. T_1 datasets (subsequently pO_2 maps) were obtained using this sequence at every 5 min intervals. In order to modulate tissue oxygenation, the rats were subjected to respiratory challenge in the sequence: air (20 min) – normobaric oxygen (60 min) – air (30 min) – hyperbaric oxygen (60 min) – air (30 min). k_{NBO} and k_{HBO} (kinetic parameters related to oxygen consumption) were calculated by fitting the data, obtained after switching back to air breathing post oxygen challenge, to the equation $pO_2(t) = pO_2^{air} + A\, e^{-kt}$ for each animal (where pO_2^{air} = baseline muscle pO_2 while breathing air, A = maximal pO_2 increase over baseline and t is time post switching to air breathing).

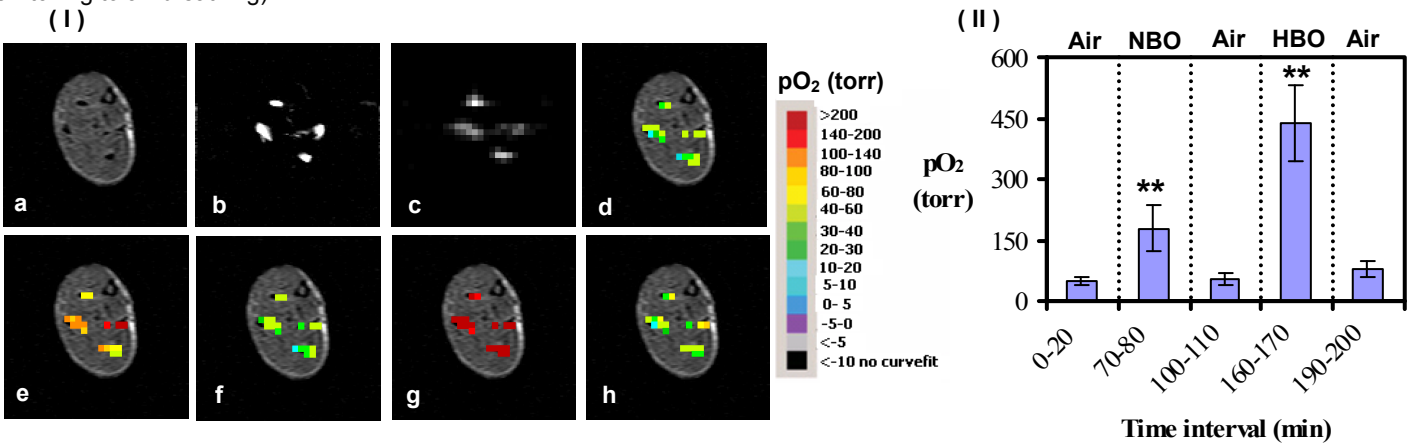

Figure I: *Tissue oximetry in rat thigh muscle using PISTOL. a) Scout image b) shift selective spin-echo and c) PISTOL image of the HMDSO distribution. PISTOL pO_2 maps while the rat breathes d) air (baseline), e) normobaric O_2 (1 atm), f) return to air, g) hyperbaric O_2 (2 atm) and h) return to air. a) and b) were acquired at 128X128, c)-h) at 32X32.* **II:** *Mean tissue pO_2 (n=6) during air (baseline) breathing and the last 10 minutes of each gas intervention. ** represent p<<0.05 with respect to baseline air and error bars represent standard deviation.*

Results and Discussion: During *in vivo* imaging studies, 100 µl of the nanoemulsion was administered into healthy thigh muscles of Athymic nude rats (n=6) and dynamic changes in mean thigh tissue oxygenation (pO_2) were measured in response to NBO and HBO challenge, using PISTOL technique. Changing the inhaled gas from air to NBO increased the mean thigh muscle pO_2 from 49.7 ± 10 torr to 178.5 ± 55 torr which then returned back to baseline pO_2 value of 55.8 ± 14 torr on air breathing. During HBO challenge the mean thigh muscle pO_2 increased to 437± 97 torr. 1-way ANOVA followed by Dunnett's multiple comparison test found significant increase in pO_2 values during NBO and HBO interventions when compared to initial baseline pO_2 and no significant differences while animal breathed air in between interventions. Applying a first order exponential fit to the pO_2 data after switching back to air breathing following NBO intervention yielded a kinetic rate constant k_{NBO} = 0.149 ± 0.03 min^{-1} (n=6). This is not significantly different statistically (p>>0.05, paired t-Test) to the value determined from the return kinetics after HBO intervention (k_{HBO} = 0.122 ± 0.03 min^{-1}). Given that the parameter 'k' also has contributions from flow, apart from consumption and diffusion, the lack of difference in k_{NBO} and k_{HBO} would indicate that flow contribution to 'k' is small. This study shows the feasibility for use of HMDSO emulsions as pO_2 nanoprobes for monitoring tissue/tumor pO_2 response to gas breathing interventions.

Acknowledgements: This work was supported by the Norman Hackerman ARP grant #010019-0056-2007, NIH NCI 1R21CA132096-01A1, Small Animal Imaging Resource (U24 CA126608) and BTRP P41 RR02584.

References:1) Zhao *et al., Methods Enzymol* 2004; 386:378-418. 2) Kodibagkar *et al., NMR Biomed* 2008; 21: 899–907. 3) Diepart *et al., NMR Biomed*; 2010; Epub ahead of print.