

## Imaging of tissue pH response to oxygen therapy in a rodent model of acute stroke

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**Introduction** Ischemic tissue suffers a cascade of molecular, metabolic and structural damages after acute stroke<sup>1</sup>. Recently, it has been shown that normobaric oxygen (NBO, high flow rate O<sub>2</sub>) remains promising to improve blood oxygenation saturation in ischemic tissue, and remains promising as a novel therapy to extend the thrombolysis window<sup>2</sup>. However, there is no readily available imaging markers with adequate spatiotemporal resolution to characterize tissue metabolic response to NBO. Recently, quantitative pH-sensitive amide proton (APT) MRI, a variant of chemical exchange saturation transfer (CEST) imaging, has been developed for mapping brain pH changes during acute stroke<sup>3-6</sup>. We postulated that brain tissue response to NBO treatment can be characterized with multi-parametric MRI, particularly, the emerging pH imaging, which may help guide and elucidate NBO therapy.

**Materials and Methods** Animal model: Middle cerebral artery occlusion (MCAO) was induced in adult male Wistar rats (n=3), following the guidelines approved by the institutional ethics committee. MRI: All experiments were conducted at a 4.7T small-bore MRI scanner after acute MCAO. We acquired perfusion (TR/TS/TE=6500/3250/14.8ms, NSA=32), pH-weighted APT (NA1/NA2=8/32, TR/TE=6500/14.8ms), diffusion (TR/TE=3250/54ms, b=250 and 1000 s/mm<sup>2</sup>, NSA=16), T<sub>1</sub> (inversion recovery, TI from 250 to 3000 ms, NSA=4) and T<sub>2</sub> (SE MRI, TR/TE1/TE2=3250/30/100 ms, NSA=16) MRI. In addition, cerebral tissue pH was calculated as  $pH=6.4+\log_{10}(R_{1w}/f_s*(\alpha*(1-\sigma)/(MTR_{asym}-\Delta MTR'_{asym})-1)/5.57)$  from pH-weighted MRI, where  $\alpha$  is the labeling coefficient,  $\sigma$  is the RF spillover factor,  $MTR_{asym}$  is pH-weighted APT asymmetry and  $\Delta MTR'_{asym}$  is the native asymmetry shift (i.e. -7.4%)<sup>6</sup>. Images were repeated before, during and immediately after NBO treatment, the duration of which was approximately 30 min.

**Results and Discussion** Fig. 1 shows a representative acute MCAO rat undergoing NBO treatment. MCAO induces severe hypoperfusion in the ipsilateral ischemic side, which also displayed significant pH and diffusion deficits. It is necessary to note that pH MRI captures heterogeneous pH deficit: severely acidic in the region displaying DWI deficit with mildly acidic lesion in the PWI/DWI mismatch. This suggests that DWI lesion suffers more severe pH deficit than the PWI/DWI mismatch. We manually outlined the contralateral normal (black), ischemic core (red) and pH penumbra (blue) region of interest (ROI) using pH MRI. Notably, core ADC recovered slightly from  $0.48\pm 0.03$  to  $0.51\pm 0.05$   $\mu\text{m}^2/\text{ms}$  upon NBO, which decreased to its baseline after the end of NBO treatment ( $0.48\pm 0.03$   $\mu\text{m}^2/\text{ms}$ ). The contralateral ADC showed very little change, as expected. In comparison, there is very little CBF change in the core and penumbra with respect to NBO. Interestingly, CBF of the contralateral tissue showed significant decrease upon NBO, from  $0.72 \pm 0.2$  to  $0.48\pm 0.20$  ml/g.min, which recovered ( $0.70\pm 0.21$  ml/g.min) after the termination of NBO. Importantly, complex pH changes were observed. Ischemic core showed very little pH response, likely due to little change in tissue oxygenation because of severe hypoperfusion. Similarly, pH of the contralateral normal tissue showed negligible change, as expected due to its unaltered aerobic metabolism and sufficient oxygen and nutrient delivery. Most interestingly, whereas the penumbral tissue showed minor pH recovery during NBO, its pH collapsed after the termination of NBO. We hypothesize that this is caused by secondary metabolic disruption. NBO treatment transiently reversed anaerobic glycolysis. However, without reperfusion, due to the use of permanent MCAO model, tissue reverted to anaerobic glycolysis after termination of NBO therapy. Therefore, penumbral tissue suffered secondary metabolic challenge, resulting in severe tissue acidosis and poor outcome (data now shown).

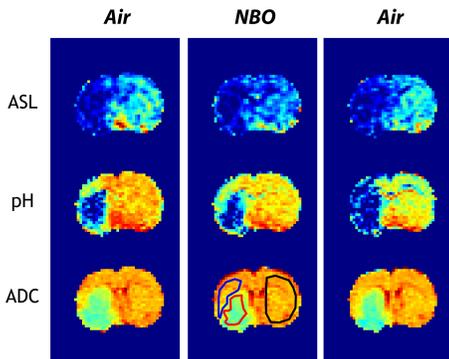


Fig. 1, Representative multi-parametric images (CBF, pH and ADC) of NBO therapy in an acute ischemic stroke.

In summary, our study showed that NBO therapy may transiently mitigate ischemic tissue injury, and reperfusion may be needed for long-term improvement. Future experiments will evaluate the optimal dosage and duration of NBO treatment, and the efficacy of NBO/thrombolytic combinational treatment. Nevertheless, our pilot study showed that pH imaging provides a unique imaging tool for monitoring tissue metabolic status following NBO therapy, complementing the commonly used perfusion and diffusion MRI.

**References** 1) Hossman. Ann Neurol 1994;36(4):557-65. 2) Singhal AB, Neurol Res. 2007;29(2):173-83. 3) Zhou et al., Nat. Med 2003; 9:1085-90. 4) Jokivarsi et al, MRM 2007; 57 (4):647-53. 5) Sun et al. JCBFM 2011; 65:1743-50. 6) Sun et al. Neuroimage 2012; 60(1):1-6.