[14C]2-Deoxyglucose autoradiography confirms metabolism within ischaemic penumbra identified by two complementary, PFC-enhanced dynamic MR imaging techniques

Graeme A Deuchar[1], David Brennan[2], William M Holmes[1], Maria del Rosario Lopez Gonzalez[1], Martin Shaw[3], I Mhairi Macrae[1], and Celestine Santosh[2]
[1]*Institute of Neuroscience & Psychology, University of Glasgow, Glasgow, United Kingdom,* [2]*Institute of Neurological Sciences, Southern General Hospital, Glasgow, United Kingdom,* [3]*Clinical Physics, University of Glasgow, Glasgow, United Kingdom*

Background: Every 6 seconds, someone in the world dies or is permanently disabled as a result of stroke (http://www.worldstrokecampaign.org). Thrombolysis with Alteplase is the only approved therapy but, currently, only ~5% of patients are treated due to risk of haemorrhage. The introduction of an accurate technique to identify the target for thrombolysis, potentially salvageable ischaemic penumbra, is crucial for improving treatment decisions and treating more patients. The gold standard for penumbra detection is PET, and is based on metabolism. However this is expensive, has low resolution and involves injection of radioisotopes, making it impractical as a routine clinical tool. Current MRI-based techniques (diffusion/perfusion (DWI/PWI) mismatch) are indirect, lack accuracy and do not provide information on tissue metabolic status.

To provide a solution for this clinical need we have developed two independent but complementary MRI techniques (GOLD; Glasgow Oxygen Level Dependent), consisting of an oxygen challenge (OC) and an efficient carrier of oxygen (PFC) to identify the penumbra. Technique 1 utilises a T_2* signal based on the different magnetic properties of deoxy- and oxyhaemoglobin in blood (paramagnetic and diamagnetic, respectively), while technique 2 uses Lactate Change Imaging, which differentiates between anaerobic/aerobic metabolism [1,2] .

Our aim was to demonstrate an enhancement of these techniques working concurrently in a single scanning protocol, through the addition of an oxygen-carrying perfluorocarbon (PFC) emulsion (Oxycyte®; Oxygen Biotherapeutics). Furthermore we aim to provide validation of the combined techniques by confirming on-going tissue metabolism in hypoperfused regions of the brain identified as penumbra.

Methods: Following permanent middle cerebral artery occlusion (MCAO) in rats (n=9), serial scanning (Bruker 7T Biospec) produced maps of ischaemic injury (from apparent diffusion coefficient, ADC maps) and perfusion deficit (arterial spin labelling). The combined T_2* / Lactate Change penumbra imaging protocol was carried out following Oxycyte® injection (4.5ml/kg, i.v). At ~1.5hrs following MCAO an initial baseline Lactate scan was carried out on normoxia. This was followed by T_2* scanning with 3mins on normoxia and 5mins on 50%O_2 hyperoxia. Following an increase in the level of hyperoxia to 100%O_2, a further Lactate scan was carried out. The scanning protocol was repeated at ~2.5hrs following MCAO, followed immediately by a terminal [^{14}C]2-deoxyglucose (2-DG) autoradiography protocol to determine glucose metabolism in the region identified as penumbra.

Results: From region of interest (ROI) analysis on T_2* signal change maps it was found that in all cases and at both time points (1.5 and 2.5hrs post MCAO), the magnitude of T_2* signal change to 50%O_2 hyperoxia following Oxycyte® varied throughout the brain (Figure 1, Figure 2(i)). In the ipsilateral hemisphere, the region defined as penumbra displayed a T_2* signal increase significantly higher than the T_2* % signal change in adjacent ischaemic core ROI identified from thresholded ADC maps (Figure 2i &2ii) and contralateral ROI. The magnitude of response to 50%O_2 + Oxycyte® in the penumbra ROI was similar to that previously seen with 100%O_2 hyperoxia alone (which has limitations for use in man due to imaging artefacts and contraindications in some patients). The addition of Oxycyte® therefore provides an improvement on the T_2*OC method to detect penumbra using lower levels of inspired oxygen.

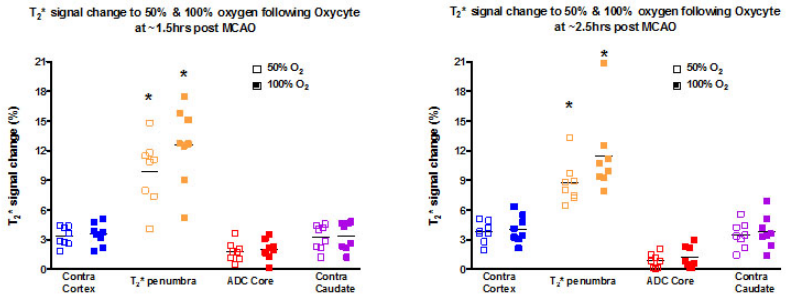

Figure.1 Graphs showing T_2* signal changes for selected ROI to 50 & 100% oxygen challenges carried out at approximately 1.5hours (left) and 2.5hours (right) following MCAO. Oxycyte was administered prior to the initial oxygen challenge. Bars indicate means. * indicates significantly greater signal change in T_2* defined penumbra compared to all other ROI; P<0.05 2-tailed paired Student's t-test. Contra cortex, contralateral cortex; T_2* penumbra, T_2*-defined penumbra; ADC core, ADC-defined ischaemic core; Contra caudate, contralateral caudate nucleus.

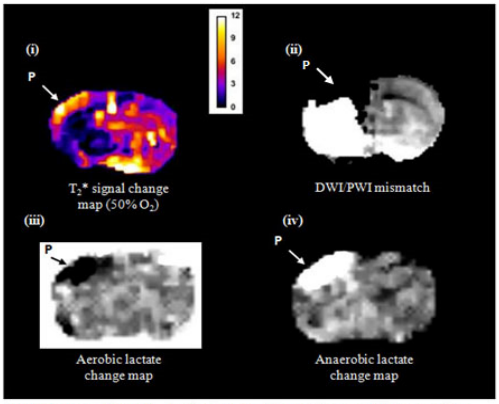

Figure 2 Images illustrating both T_2* OC and lactate change techniques working concurrently in a representative animal following administration of Oxycyte (4.5ml/kg). (i) shows T_2* signal change map to 50% OC at ~2.5hrs post MCAO. (ii) corresponding time matched DWI/PWI mismatch. (iii) Aerobic lactate change map in response to hyperoxia at ~3hrs post MCAO (black region indicates decrease in lactate) and (iv) anaerobic lactate change map in response to returning animal to normoxic ventilation at ~3hrs post MCAO (white area indicates increase in lactate). P; indicates penumbra as detected by GOLD techniques.

In all experiments increased lactate levels detected within the ischaemic hemisphere decreased in response to hyperoxia + Oxycyte® as indicated in the aerobic Lactate Change map (Figure 2iii). On returning to normoxia, lactate increased as indicated in the anaerobic Lactate Change map (Figure 2iv).

The addition of Oxycyte® represents an improvement in the sensitivity to detect lactate change to OC, when compared to hyperoxia (100%O_2) alone[2].

With both T_2^* and Lactate Change OC techniques, the region identified as penumbra displayed maintained glucose metabolism (25.5±2.6µmol/100g/min), comparable to values in contralateral cortex (23.2±2.5), while adjacent ischaemic core displayed low glucose use (12.4±7). Regions identified as penumbra by thresholded T_2* and Lactate Change were not identical to the region defined by DWI/PWI mismatch.

Conclusion: Penumbra detection with combined dynamic T_2* and Lactate Change OC imaging is improved with the addition of PFC Oxycyte®. In the clinical management of stroke it is important to accurately identify patients with potentially salvageable penumbra tissue and GOLD MR imaging has the potential to offer an improvement on current techniques by being primarily based on metabolism.

1. Santosh C, Brennan D, McCabe C et al. Potential use of oxygen as a metabolic biosensor in combination with T2*-weighted MRI to define the ischemic penumbra. *J Cereb Blood Flow Metab* 2008;28:1742-53.
2. Holmes WM, Lopez-Gonzalez MR, Gallagher L et al. Novel MRI detection of the ischemic penumbra: direct assessment of metabolic integrity. *NMR Biomed* 25:295-304.

Oxycyte® was provided by Oxygen Biotherapeutics Inc. (Morrisville, NC, USA).