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Influence of 100% and 40% oxygen on penumbral blood flow, oxygen level and T₂-weighted MRI in a rat stroke model.*

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Abstract

Accurate imaging of the ischaemic penumbra is a prerequisite for acute clinical stroke research. T₂* MRI combined with an oxygen challenge (OC) is being developed to detect penumbra based on

changes in blood deoxyhaemoglobin. However, inducing OC with 100%O₂ induces sinus artefacts on human scans and influences CBF which can affect T₂^{*} signal. Therefore, we investigated replacing 100%O₂ OC with 40%O₂ OC (5mins 40%O₂ versus 100%O₂) and investigating effects on blood pressure (BP), CBF, tissue pO₂ and T₂^{*} signal change in presumed penumbra in a rat stroke model. Probes implanted into penumbra and contralateral cortex simultaneously recorded pO₂ and CBF during 40%O₂ (n=6) or 100%O₂ (n=8) OC. In study 2, T₂^{*} signal change to 40%O₂ (n=6) and 100%O₂ (n=5) OC was compared. OC (40% and 100%O₂) increased BP by 8.2% and 18.1%, penumbra CBF by 5% and 15%, and pO₂ levels by 80% and 144%, respectively. T₂^{*} signal increased by 4% and 8.7% in penumbra compared to 2.78% and 2.8% in contralateral cortex and 2.2% and -0.34% in ischaemic core, respectively. For diagnostic imaging, 40%O₂ OC induced sufficient T₂^{*} signal change to detect penumbra with limited influence in BP and CBF.

Keywords: CBF, hyperoxia, penumbra, pO₂, T₂^{*}, oxygen challenge

Introduction

Stroke is the second most common cause of death worldwide (Donnan et al. 2008) and a major cause of severe disability (Bonita et al. 1990). Potentially salvageable ischaemic penumbra, hypoperfused brain tissue with preserved oxygen metabolism and an increased oxygen extraction fraction (Astrup et al. 1981), is of key clinical importance since early restoration of blood flow can lead to penumbral salvage and improved neurologic outcome in acute stroke patients (Christou et al. 2000). However, penumbral tissue has a limited lifespan and without timely intervention becomes incorporated into the irreversibly damaged ischaemic core.

The amount and rate at which penumbra is lost varies widely amongst stroke patients. Consequently, dynamic brain imaging to accurately detect penumbra is crucial in providing key information regarding a patient's suitability for intervention therapies such as thrombolysis. Current imaging techniques used to identify penumbra include positron emission tomography (PET) which characterises penumbral tissue as metabolically active, yet hypoperfused (Baron, 1999) and magnetic resonance imaging (MRI), where the mismatch between the perfusion deficit (from perfusion-weighted imaging, PWI) and the irreversibly damaged ischaemic core (from diffusion-weighted imaging, DWI) serves as a surrogate marker of penumbra (Donnan and Davis, 2002). However, routine clinical use of these two imaging modalities is hampered by recognised economic (PET) and diagnostic (MRI) limitations. (Heiss, 2010)

Our group are developing a novel MRI technique which employs a period of normobaric hyperoxia (oxygen challenge, OC) to detect penumbra (Dani et al. 2010; Santosh et al. 2008). T_2^* -weighted MRI combined with 100%O₂ inhalation (oxygen challenge, OC) detects penumbra based on its increased oxygen extraction fraction and consequent higher blood deoxyhaemoglobin levels.

T_2^* OC technique is being translated for use on clinical scanners and has shown potential clinical utility in stroke patients (Dani et al. 2010). However, some limitations of using 100% O_2 for the OC have been identified: 100% O_2 produces artefacts around the air sinuses on human brain T_2^* maps which precludes analysis of brain regions anterior to the lateral ventricles. Given the heterogeneity of human stroke and varied vessel occlusion sites, it is important that the T_2^* OC technique can detect penumbra throughout the brain. The technique employs blood oxygen level-dependent (BOLD) T_2^* -weighted imaging, which is influenced by a number of physiological factors. In addition to blood deoxyhaemoglobin:oxyhaemoglobin ratio, tissue pO_2 , cerebral blood volume and most notably, CBF have all been shown to affect T_2^* signal (Corfield et al. 2001; Ramsay et al. 1993). Therefore, to improve the utility and accuracy of the T_2^* OC technique, it is important to identify and minimise the influence of factors, other than tissue metabolism, on T_2^* signal change. Consequently, we have investigated the potential to employ lower levels of oxygen to induce OC in order to establish if ability to detect penumbra can be achieved whilst minimising imaging artefacts..

In the present study, we have characterised the influence of 40% O_2 on the following parameters in rats exposed to MCAO: mean arterial blood pressure (MABP), CBF, pO_2 , MRI T_2^* signal change in presumed penumbra, and contralateral cortex and compared this with the responses generated by 100% O_2 . The development of imaging techniques such as T_2^* OC that more precisely identify penumbra on the basis of ongoing metabolism would greatly aid in improving patient selection for thrombolysis and patient recruitment into clinical trials of new therapeutic agents.

Material and Methods

All experiments were performed under a UK Home Office license and were subject to the Animals (Scientific Procedures) Act, 1986. Male Sprague-Dawley rats (300-350g Harlan, UK) had free access to food and water and were maintained on a 12 hour light-dark cycle.

Model of middle cerebral artery occlusion (MCAO)

Isoflurane in N₂O:O₂ (70%:30%) was used for induction (5%), surgery (2.5-3%) and maintenance (2-2.5%) anaesthesia. N₂O:O₂ was replaced with medical air supplemented with ~5% O₂ (26% O₂) following surgery. Animals were artificially ventilated and body temperature maintained at 37°C. Femoral arteries were cannulated for mean arterial blood pressure (MABP) measurement and blood gas analysis. MABP and heart rate were continuously recorded (AcqKnowledge, Biopac Systems, CA, USA). MCAO was induced with an intraluminal filament (Longa et al. 1989). A 3.0 uncoated nylon filament was advanced along the left internal carotid artery for approximately 20-21mm from the bifurcation of the external carotid artery. Successful MCAO was confirmed from CBF data or histology post-mortem. Sham rats had carotid arteries exposed but no filament introduced.

Stereotaxic surgery

MRI perfusion- and diffusion-weighted (PWI/DWI) mismatch scans from a separate cohort provided spatial information on penumbra location (Figure 1A) to define stereotaxic co-ordinates. Rats were secured in a stereotaxic frame (Kopf Instruments, CA, USA), the skull exposed, and Oxylite/Oxyflo optodes (Oxford Optronics, Oxford, UK) implanted into presumed penumbra and contralateral cortex at 0.5mm anterior and 2mm lateral to bregma, depth 2.5 mm (retracted 0.5mm to reduce pressure around the probe tip).

Measurement of tissue pO₂ and relative cerebral blood flow

Oxylite/Oxyflo optodes simultaneously measures brain tissue pO₂ and relative CBF (rCBF) from the same brain region as previously described (Kuo et al. 2003). Pre-calibrated, stereotaxically

implanted optodes detected the effect of OC (40%O₂ or 100%O₂) on tissue oxygenation and rCBF in presumed penumbra and contralateral cortex following MCAO. Optodes were left to stabilise for 30mins following implantation before making measurements. After each experiment, optodes were placed in blood samples to ensure pO₂ readings matched those from a blood gas analyser (RapidLab 248 system, Siemens).

MRI scanning

The T₂^{*} sequence during OC was a single shot, gradient echo (EPI) sequence (TE: 20ms, TR: 10s, matrix 96x96, FOV 25x25 mm, 8 contiguous slices, 1.5mm thick, 2 averages, temporal resolution 20s, 75 repetitions). Two coronal slices within middle cerebral artery territory were selected for analysis. The paradigm for T₂^{*}-weighted OC was 5 minutes air ventilation, 5 minutes 40%O₂ (n=6) or 100%O₂ (n=5), and then back to air ventilation.

PWI-DWI mismatch maps of penumbra were prepared for comparison using the following sequences: DWI (Spin-echo planar (EPI) TE: 43ms, TR: 4000.3ms, in plane resolution 260um, 3 directions: x, y, z, B values: 0, 1000 s/mm², 8x1.5mm thick slices). PWI using a form of pseudo-continuous ASL (Moffat et al. 2005): (Spin-echo planar (EPI) imaging module, TE 20ms, TR 7000ms, matrix 96x96, FOV 25x25mm, slice thickness 1.5mm, 16 averages, 4 shots) preceded by 50 hyperbolic secant inversion pulses in a 3s train.

Image Analysis

T₂^{*}OC percentage signal change maps were generated using Image J software (<http://rsb.info.nih.gov/ij/>). Penumbra tissue was defined by thresholding T₂^{*} signal change maps to 2 Standard Deviations (SD) above the mean contralateral signal. Time course and size of the T₂^{*} signal change was analysed on regions of interest within penumbra, ischaemic core (from DWI) and equivalent contralateral regions. T₂^{*}OC percentage signal change was taken as the peak signal during OC and expressed as a percentage of the mean signal during baseline.

Experimental protocol

Animals were randomly assigned to one of four hyperoxic groups for bench experiments; 40%O₂ sham (n=6), 40%O₂ MCAO (n=6), 100%O₂ sham (n=8) or 100%O₂ MCAO (n=8) and two hyperoxia groups for MRI studies 40%O₂ MCAO (n=6), 100%O₂ MCAO (n=6). Approximately 90mins after MCAO or sham procedure animals were exposed to OC. Arterial blood samples were collected for blood gas analysis immediately prior to the 5mins baseline and toward the end of the hyperoxic challenge (approximately 4½min).

Histology

Animals used in bench experiments were culled by anaesthetic overdose, the brain removed and placed in isopentane (-35°C) for 15mins. Coronal cryostat sections (30µm) were collected within MCA territory and stained with haematoxylin and eosin to confirm correct optode placement. Anatomical location of optodes was identified using light microscopy at x20 magnification.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (Version 4.03, CA, USA). Data are presented as mean ± SD. Comparison of physiological variables before and during OC, rCBF and pO₂ between ipsilateral and contralateral cortex and T₂^{*} signal change intensities between penumbra, contralateral cortex and ischaemic core were assessed using a 2-tailed paired Student's t-test. Data from different groups were assessed using a 2-tailed unpaired Student's t-test. P<0.05 was considered statistically significant.

Results

Study 1 – changes in brain tissue oxygen and CBF during oxygen challenge

Mortality and exclusions

In the 100%O₂ sham group, one animal died under procedure. Post-mortem analysis revealed a mucus plug in the tracheal tube as the likely cause of death. Exclusion criteria covered incorrect placement or evidence of haemorrhage around the optode tip, determined from histological sections. Two animals in the 100%O₂ MCAO group were excluded. In one animal pO₂ recordings were uncharacteristically low and a large cortical haemorrhage was found around the ipsilateral optode which would have severely impacted on recording accuracy. In the other, the ipsilateral optode was incorrectly positioned (striatum).

Baseline brain pO₂ and rCBF

Under air ventilation, basal brain pO₂ was 47% lower ($p < 0.0005$) and rCBF 28% lower ($p < 0.05$) in ipsilateral cortex (presumed penumbra) compared to contralateral cortex in MCAO rats ($n=14$, Figure 1B&C). There was no hemispheric difference in baseline brain pO₂ or rCBF in shams ($n=14$).

OC-induced changes in Physiological Parameters

Pre-OC, blood gases, pH and MABP did not differ significantly between groups (Table 1). OC, started 90mins post-MCAO, significantly increased arterial paO₂ in sham and MCAO rats: 40%O₂ by 83±44% and 74±18%; and 100%O₂ by 363±48% and 337±57%, respectively. No significant changes in pH or paCO₂ were detected in any group or hyperoxic regime.

OC transiently increased MABP in sham and MCAO rats: 40%O₂ by 8±5% and 8±6%, and 100%O₂ by 19±8% and 19±15%, respectively (Table 1, Figure 2A).

Effect of OC on Cortical pO₂

Brain pO₂ levels increased markedly and remained elevated during 100%O₂ OC, (Figure 2A). Responses to 40%O₂ were smaller in magnitude with a similar time course (data not shown).

100%O₂ produced significant increases in brain pO₂ of similar magnitude across hemispheres and groups with penumbral pO₂ increased above control baseline levels (Table 2). 40%O₂ , significantly increased pO₂ in shams and contralateral hemisphere of MCAO rats. Penumbral pO₂ was increased to a value equivalent to baseline (sham) values which was not statistically different from baseline contralateral values (Table 2). With 40%O₂ the greatest percentage increase in pO₂ was recorded in penumbra (MCAO ipsi, P<0.05, versus contralateral cortex).

Effect of OC on rCBF

OC induced small, transient changes in rCBF in all groups relative to pre-OC values (Figure 2A&C). With 100%O₂ this reflected an increased rCBF in 6/8 sham rats, no change in 1/8 shams, reduced rCBF in 1/8 shams and an increase in all (MCAO rats. Mean increases from pre-OC baseline were 9%±3.8 (P<0.0001) and 15%±12.9, (P<0.05) in contralateral and ipsilateral cortex of MCAO rats, respectively (Figure 2C) . With 40%O₂, OC induced smaller, non-significant increases in rCBF.

Study 2 - T₂^{*} percentage signal change to OC

OC (100%O₂ and 40%O₂) induced increases in T₂^{*} signal which were greatest in magnitude in dorsolateral cortex corresponding to penumbra as defined by PWI/DWI mismatch (Figure 3C) and in large veins. 100%O₂ induced a greater T₂^{*} signal change in penumbra (P<0.05) but the signal achieved with 40%O₂ was sufficient to discriminate penumbra from surrounding tissue on thresholded T₂^{*} maps (Figure 3C). For 100% and 40%O₂ oxygen challenge, the T₂^{*} signal change was greater in penumbra than in ischaemic core and contralateral cortex (p<0.001 and p<0.06 for 100% O₂ and p<0.05 for both regions with 40%O₂). The smaller increases in T₂^{*} signal in the contralateral hemisphere were of similar magnitude for 100%O₂ and 40%O₂ (Figure 3B).

Discussion

Imaging of the moderately hypoxic, hypoperfused yet metabolically active penumbra is of key importance when determining a patient's suitability for intervention therapies such as thrombolysis, which carry a risk of haemorrhage. Accurate assessment of penumbral tissue will also be required for future drug trials so that only patients who stand to benefit from treatment are recruited. Transient administration of an oxygen challenge combined with T_2^* -weighted imaging to detect oxidative metabolism represents a novel non-invasive method of imaging penumbra with potential clinical utility in acute stroke (Dani et al. 2010; Santosh et al. 2008). However, using 100%O₂ results in artefacts within the brain (frontal lobes) in human scans due to increased susceptibility effects from the paramagnetic oxygen within the nasal passages and the paranasal sinuses (Dani et al. 2010). In addition, inhalation of 100%O₂ can influence cerebral haemodynamics which can confound interpretation of tissue metabolism using T_2^* signal change (Corfield et al, 2001).. Consequently, we have investigated the possibility of using lower levels of oxygen to detect penumbra using OC T_2^* -weighted MRI. This may mitigate sinus artefacts and minimise any potentially confounding effects of OC-induced changes in cerebral perfusion on T_2^* signal change.

To our knowledge, this is the first study investigating the effect of oxygen on simultaneously measured CBF and tissue oxygenation in presumed penumbra. Inhalation of 100%O₂ increases blood pressure in anaesthetised rats (Lu et al. 2009), but not in the conscious human, and induces vasoconstriction in normal brain causing a 4-15% reduction in CBF in humans and rats (Lu et al, 2009; Bulte et al. 2007), due in part to decreased $paCO_2$ or a direct effect on the cerebral vasculature (Floyd et al. 2003). However, hyperoxia can have opposite effects on CBF in ischaemic tissue (Liu et al. 2006), increasing CBF in penumbra in humans (Singhal et al. 2005) and rodents (Shin et al, 2007). Our data are consistent with these reports, demonstrating a 5% and 15% increase in penumbral CBF during 40%O₂ and 100%O₂ OC, respectively. The increases in CBF were transient in nature, following a similar profile to OC-induced increases in blood pressure (8% and

19%, respectively, Figure 2A, Table 1). Modest increases in CBF were also detected in non-ischaemic tissue in sham and MCAO rats in animals breathing 40%O₂ (3-5%) and 100%O₂ (5-9%).

OC was associated with approximately 79% and 144% increases in penumbral pO₂ in animals breathing 40%O₂ and 100%O₂, respectively. Using the lower %O₂, penumbra sustained a greater % increase than the other regions of interest with tissue oxygen levels approaching control levels (Figure 2B, Table 2). During 100%O₂ inhalation, the % increase in penumbral pO₂ was similar to that in other brain regions examined (Figure 2B); however, penumbral tissue oxygen levels did exceed baseline values in the contralateral cortex of controls (Table 2).

Differences in baseline pO₂ measurements were detected in non-ischaemic hemispheres of all groups (pO₂ values ranging from 24-37mmHg). This could be due to small regional differences in optode placement, the oxygen-dependent fluorescence quenching abilities of the optode may be affected by tissue trauma after optode insertion and/or the region of tissue sampled (0.25-0.35m³) was comparatively smaller than other tissue oxygenation monitoring methods (O'Hara et al. 2005) which may lead to some inaccuracies in pO₂ measurements. Despite this, our baseline pO₂ values were not significantly different between groups and were similar to those published by others using the same method (O'Hara et al, 2005).

Hyperoxia is known to increase the BOLD MRI signal which is derived from blood oxygenation, CBF, CBV and cerebral metabolic rate of oxygen (Corfield et al, 2001). The T₂^{*} OC technique is based on the premise that hyperoxia will lead to an amplification in the BOLD T₂^{*} signal within the hypoxic penumbra where there is an increased OEF (Baron, 1999; Heiss, 2000) and so a greater deoxyhaemoglobin/oxyhaemoglobin ratio compared to healthy tissue. Subsequently, the conversion of deoxyhaemoglobin to oxyhaemoglobin during OC gives rise to the greater increase in T₂^{*} signal in this region. Thresholded maps of T₂^{*} signal change located penumbra to a region of dorsolateral cortex, corresponding approximately to penumbra as defined by PWI/DWI mismatch (Figure 3C).

Although the magnitude of the T_2^* signal change was smaller using 40%O₂, the signal was significantly greater in penumbra than in ischaemic core and control (contralateral) cortex and was sufficient to identify penumbra on thresholded T_2^* maps (Figure 3C).

Since the BOLD MRI signal is also sensitive to changes in CBF and CBV (Corfield et al, 2001) it is important to consider changes in these parameters during OC to confirm that they do not confound interpretation of T_2^* signal change. Oxylite/Oxyflo probes revealed small increases in penumbral CBF during both hyperoxic regimes (5% and 15% for 40%O₂ and 100%O₂ OC, respectively). Since hypercapnia-induced CBF increases of 25-40% have been shown to influence BOLD signal by ~ 2-3%,(Rostrup et al. 2000; Rostrup et al. 2005) it is unlikely that the small flow augmentations observed in our study had a significant influence on the BOLD T_2^* response. Similarly, changes in MR signal intensity when switching oxygenation status in ventilated rats, are shown to be predominantly due to the magnetic properties from blood oxygenation and not cerebral blood volume effects (Kennan et al. 1997) Furthermore, time course data illustrate a sustained T_2^* signal increase during OC which more closely matched tissue pO₂ time course than the rCBF time course. Whilst T_2^* and tissue pO₂ remained elevated during OC, the increase in CBF was transient, returning to pre-OC baseline values during OC (Figures 2A&3A). The ischaemic core reflects irreversibly damaged, non-metabolising tissue that has little or no blood flow. As expected, OC T_2^* MRI signal was reduced within ischaemic core.

The observed imaging artefacts on human T_2^* scans with 100%O₂ OC (Dani et al. 2010) limit its clinical utility as a contrast agent for metabolic imaging in acute stroke. From the published fMRI literature it is clear that 21% oxygen (air) does not result in any sinus artifact.

Here we show that 40%O₂ is similarly effective in detecting penumbra in a rat stroke model, with less effect on BP and CBF, and may be preferable, if it is shown on human scans to be free of air space imaging artefacts. However, further human studies, administering 40%O₂ in combination with T2*-MRI, are required to confirm its diagnostic capabilities..

In conclusion, hyperoxia is inexpensive, readily available and easily administered during MRI scanning. Combined with BOLD T₂*-weighted imaging it could deliver a novel mode of metabolic imaging in acute stroke. Our data demonstrate that an oxygen challenge with 40%O₂ or 100%O₂ - induces a stepwise increase in pO₂ and MRI T₂* signal allowing delineation of the ischaemic penumbra. CBF increases to OC were transient, small and unlikely to confound the ability of the BOLD T₂* signal to identify penumbra based on metabolic activity. Susceptibility artefacts limit the utility of 100%O₂ but 40%O₂ appears effective in distinguishing different metabolic compartments within hypoperfused tissue.

Disclosure/conflict of interest

The authors have no conflict of interest to declare concerning this research

Table and Figure legends

Figure 1: A. Representative composite MRI image revealing position of optodes in relation to penumbra (red). Ischaemic injury, (DWI, white) superimposed onto perfusion deficit (PWI, red) reveals PWI/DWI mismatch (presumed penumbra) ~ 90mins post-MCAO;

Baseline tissue pO₂ (B) and rCBF (C) in ipsilateral (Ipsi) and contralateral (Contra) cortices of sham (n=14) and MCAO (n=14) rats prior to OC. Data from all groups are pooled and presented as mean ± SD. *P<0.05, **P<0.0005 vs MCAO contra, Student's paired t-test, BPU – blood perfusion units.

Figure 2: A. Representative traces of MABP, cortex pO₂ and rCBF from a MCAO rat during 100% OC (blue-shaded box); B. Percentage increase in cortex pO₂; C. rCBF in the contralateral and

ipsilateral cortex of sham and MCAO rats breathing either 40% or 100% O₂. Data (n=6-8) are presented as mean ± SD. # P<0.05, paired Student's t-test ; * P<0.001, ** P≤ 0.0005, 100%O₂ vs 40%O₂ (unpaired Student's t-test). Contra=contralateral, Ipsi= ipsilateral.

Figure 3A. Time course of T2* signal change in penumbra, ischaemic core equivalent contralateral cortex and contralateral caudate nucleus. B. T2* % signal change to OC (40%O₂ or 100%O₂) in MCAO rats (n=5-6) . Data are expressed as mean±SD. * P<0.05 vs 40%O₂ inhalation (unpaired Student's t-test), # P<0.05, ##, P<0.001, versus equivalent ischaemic core, \$ versus equivalent contralateral cortex (paired Student's t test). Contra=contralateral. C. Representative thresholded T2* % signal change maps for 100%O₂ OC and 40%O₂ (top row) with the equivalent PWI/DWI mismatch maps (bottom row) where ischaemic injury, (DWI, white) superimposed onto perfusion deficit (PWI, red) reveals penumbra as PWI/DWI mismatch (P=OC-defined penumbra and IC=ADC-defined ischaemic core).

Table 1: Blood gas analysis prior to (baseline) and during oxygen challenge (OC). Data are presented as mean ± SD, * P<0.05, ** P<0.005, *** P<0.0005, Student's paired t-test.

Table 2: Effect of OC on cortex pO₂ (mmHg). Data are presented as means ± SD, * P<0.05, ** P<0.01, *** P<0.005, vs baseline, Student's paired t-test. OC=oxygen challenge.

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