A Temporal MRI Assessment of Neuropathology following Transient MCAO in the rat: Correlations with Behaviour.

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Introduction

\textit{In-vivo} MRI has become a highly recognised research tool to investigate the early diagnosis and ensuing effects of focal cerebral ischaemia. Numerous studies have documented quantitative changes in MCAO parameters at 'acute' time points following Middle Cerebral Artery Occlusion (MCAO) in the rat; however few studies have attempted to delineate the dynamic changes across a 'chronic' time course\textsuperscript{1,2}. In addition, functional, as well as neuropathological, outcome is a necessary requirement for elucidating the consequences of stroke. Although there is an increasing body of literature examining 'acute' functional outcome, few studies have extended behavioural assessment to determine the 'chronic' consequences of MCAO in the rat. The purpose of this study was to: 1) evaluate the temporal and spatial changes of pathological water from a sub-acute to a chronic time point; 2) employ a rigorous functional assessment concurrently with MRI; 3) attempt to correlate appropriate MR regions of interest (ROI) with specific behavioural profiles, following transient MCAO in the rat.

Methods

Twelve male Sprague-Dawley rats (300-350g) were initially trained on the following behavioural tasks, prior to surgery: Bilateral Sticky Label test (measure of tactile extinction/contralateral neglect - latency to contact /remove labels, in secs); Beam Walking (measure of hindlimb co-ordination - distance travelled across beam, in cm); Staircase test (measure of skilled forelimb paw-reaching total contralateral & ipsilateral food pellet retrieval). After successful training animals were randomly assigned to receive either transient right-sided MCAO (90 mins, n = 6), via the intraluminal thread technique, or sham surgery (introduction of thread to ICA, followed by rapid removal, n=6). All surgical procedures were performed under halothane (2% v/v in O\textsubscript{2}) anaesthesia.

For MRI procedures, anaesthesia was induced and maintained with halothane (1% v/v O\textsubscript{2}). MRI was performed on a 4.7T Biospec. Proton density, T\textsubscript{2} and T\textsubscript{2}-diffusion weighted MR images were acquired, interleaved with each phase encode step (NEX=2), using TE (ms)/TR (ms) / b (s/mm\textsuperscript{2}) values of 36/5000/0 and 68/5000/590, respectively. 32 contiguous slices starting at the level of the eyes, were acquired, using a 128x128 acquisition matrix, in an imaging time of 68 mins. The slice thickness was 0.6mm and the in-plane resolution was 0.3 x 0.3 mm. Scanning was employed at 1, 7, 14 and 28 days post occlusion (p.o.). Images were then smoothed and both T\textsubscript{2} relaxation and ADC maps were produced. ROI (3 x 3 pixels) for MCAO and sham groups, both ipsilateral and contralateral to the occlusion site, were then interrogated. ROI for T\textsubscript{2} maps were at: \textbullet~bregma -0.8mm, Forelimb Cortex (FCL), Dorsolateral Caudate-Putamen (CPU), Lower Parietal Cortex (LPC); and at ~ bregma -1.8mm, Hindlimb Cortex (HCL), according to the rat stereotactic atlas. Behavioural tests were instigated daily from 1 to 28 days, apart from the Staircase test (appetitively-motivated task) which resumed at 7 days p.o.

Results

MRI assessment

Lesion size visualised by T\textsubscript{2} WI hyperintensity in the MCAO group reduced significantly across the time course [Repeated Measures ANOVA: F(3,27) = 44.96, P<0.001]. The lesion was less conspicuous around the ipsilateral CPU at later time points (% reduction from 1 day p.o.: 7 days = 30%; 14 days = 44%; 28 days = 48%). T\textsubscript{2} map assessment demonstrated increased relaxation times for ipsilateral CPU, LPX and FLC at 1, 14 and 28 days p.o., which significantly differed from the contralateral side and sham group [F's (1,14) > 10, P<0.001]. Elevated T\textsubscript{2} values were also apparent for the HLC at 1 day (Post-hoc Newman Keul, P<0.01) with a subsequent decline to contralateral and sham group levels from 7 to 28 days p.o. (P<0.05, NS). However, reduced T\textsubscript{2} values were obtained at 7 days p.o. for each of the above ROI; only the ipsilateral CPU, LPC and FLC remained significantly different from the contralateral side and sham group (P<0.05).

Discussion

This chronic study has delineated subtle changes in the temporal profile of T\textsubscript{2} MRI measures, following transient MCAO in the rat. Lesion size, evaluated by T\textsubscript{2} WI, reduced over time, particularly within the traditional 'core' region (CPU) of the MCA territory. However, absolute T\textsubscript{2} values within the CPU were significantly elevated across the time course, suggesting evidence for edema and cell necrosis. Other ROI, the LPC and FLC, were shown to progressively increase over time, potentially indicating the transition from a 'penumbra' to irreversible damage. The HLC, on the other hand, demonstrated high T\textsubscript{2} relaxation times on day 1, but returned to control levels thereafter, suggesting that this region was 'salvaged' following extended reperfusion. Objective behavioural assessment corroborates this argument, in that stable, long-lasting deficits correlate with CPU, LPC and FLC markers; while restitution of hindlimb function is mirrored by a return of the HLC to T\textsubscript{2} control levels. Current work is directed towards assessing changes in ADC, PD, and T\textsubscript{1} values across the time course, in order to establish a consensus concerning the temporal alterations of ischaemic tissue.

It is also clear from this study that functional outcome provides an informative index to neuropathological assessment, that should serve as an 'adjunct' for evaluating neuroprotective compounds relevant for the clinic.

References
