Unilateral common carotid artery occlusion: a transient ischemia model well suited to MR studies

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Introduction

Stroke is one of the most prevalent and debilitating pathological conditions, causing direct and indirect costs of approximately US$ 56.8 billion in 2005 alone [1]. Many animal models have been developed to aid in the study of stroke's causes and effects, and many of these models have been employed in MRI studies of cerebral hypoxia and ischemia [2]. Middle cerebral artery occlusion is commonly employed to induce a reproducible focal ischemic lesion in rats. However, this model can present complications including inadequate vessel occlusion, vessel laceration, or hypothalamic damage resulting in hyperthermia [3,4,5]. The macrosphere occlusion model developed by Gerriets *et al* avoids these confounding factors but does not offer the possibility of studying reperfusion injury due to its irreversibility [6]. Most importantly, neither of these methods offer the ability to induce and reverse hypoxia during the course of an MRI experiment as both require manipulations that cannot be performed while the rat is positioned in the magnet.

We report the use of a transient focal cerebral hypoxia model well suited to MRI studies, consisting of ligation of one common carotid artery followed by exposure to a low-oxygen environment, based on the model developed by Levene *et al*. [7] and utilized in many studies as a simple focal cerebral hypoxia model [8]. This method holds special potential for MRI applications as it allows for the induction and reversal of hypoxia without requiring physical manipulation of the animal, as the hypoxic insult is initiated by addition of nitrogen gas into the anesthetic gas mixture. This enables one to acquire MR data prior to, during and after a hypoxic insult.

Methods

To demonstrate the feasibility of applying Levene's model to MRI experiments, three adult male Fisher 344 rats (250g) were anesthetized (isoflurane, 2.5%) and administered buprenorphine (0.1-0.2 mg/kg, i/p) prior to surgery. The right common carotid artery was excised by blunt dissection and occluded with two tightly-knotted sutures. Rats were allowed to recover for approximately two hours in a temperature and humidity controlled environment, then reanesthetized (isoflurane, 2 – 2.5% in air) for MRI studies. MR data were acquired using a 33cm 4.7T horizontal bore magnet interfaced to a Bruker console. Body temperature was maintained during imaging with a heating pad. Diffusion weighted images were acquired at *b*-values of 150 and 1500 s/mm^2, with an echo time of 36 ms and a repetition time of 2.3 s. Focal cerebral hypoxia was induced by incremental addition of nitrogen into the anesthesia gas mixture. Oxygen levels were reduced from 21% to 12%, 11%, 10%, 9%, and finally 8% as measured by a MSA MiniOx I oxygen meter. A 10 minute equilibration period was employed between oxygen level decrease and acquisition of diffusion-weighted MR data. After observation of a region of elevated signal in diffusion weighted images, anesthetic gases were exchanged for 100% nitrogen to cause global hypoxia, then MR data re-acquired.

Results

Figure 1A shows a diffusion-weighted image from a rat following ligation of the right carotid artery, but prior to induction of focal cerebral hypoxia. No regions of elevated signal intensity can be seen in the DWI data, indicating normoxia. Figure 1B shows DWI data from the same rat breathing 8% oxygen, balance nitrogen. Elevated signal intensity is visible in cortical and sub-cortical brain regions ipsilateral to artery ligation, demonstrating induction of a focal hypoxic lesion. Figure 1C shows DWI data acquired from the same rat *post mortem*. Elevated signal intensity is visible throughout the brain.

Discussion and Conclusions

Our data demonstrate successful induction of a focal hypoxic lesion in rats following carotid artery ligation and exposure to a hypoxic environment. The majority of studies employing the model developed by Levene *et al*. have involved exposure to a hypoxic environment in unanesthetised rats. As anesthesia is required for animal restraint purposes during MR imaging, we are unable to replicate the exact conditions of Levene's model. As isoflurane anesthesia results in respiratory suppression, we have observed a lower tolerance to hypoxic environments for anesthetized rats than for unanesthetised rats, resulting in failure of respiration. Future studies will employ ventilation during MR imaging to maintain respiration during and following hypoxic insult. We plan to employ this model for metabolic studies of cerebral hypoxia and the secondary injury cascade.

References and Acknowledgements

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Figure 1. Diffusion weighted images from rat brain under normoxic (A), 8% O_2 hypoxic (B) and *post mortem* (C) conditions