A photothrombosis-induced ischaemic infarct model for study of hind brain stroke

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The mortality rate following a cerebellar infarction is 23% greater than for an infarct in any other part of the brain (Macdonell *et al.*, 1987). Moreover, vertebrobasilar stroke has a mortality rate of 85% (Kaye, 2011). Despite the high mortality and morbidity of hind brain strokes, there are limited animal models to aid investigations into novel therapeutic options. Currently, small animal models of stroke focus on middle cerebral artery occlusion or global ischaemia. Neither of these models supports an investigation of hind brain infarction. We therefore investigated whether photothrombosis may provide the necessary spatio-temporal control of infarct volume; further, we resolved to settle the controversy over whether this technique provides a thrombotic infarct, or whether, as recently suggested (Frederix *et al.*, 2007; Kleinschnitz *et al*., 2008), the brain injury is independent of the platelet aggregation-mediated occlusion of cerebrovascular microvessels. Here we show in the mouse cerebellum that photothrombosis can induce hind brain ischaemia, based on real-time imaging of thrombis development, magnetic resonance imaging (MRI) of the progression of these cerebellar infarcts, and histological analysis of the thrombis in the brain tissue. The infarcts were created following a protocol approved by the University of New South Wales Animal Care and Ethics Committee.

Photothrombosis in the cerebellum was achieved by intravenous administration of rose bengal followed immediately by coherent illumination at 561nm of the region of interest within the cerebellum of the isoflurane anaesthetized mouse (after Watson *et al.*, 1985). The progression of clot formation in the vermis region of the cerebellum was visualized in real-time (established in ~10 minutes) by intravital multi-photon imaging of the platelet aggregation (using Cy7-labelled anti-CD42). Infarcts were subsequently examined by high-field (9.4T) T2 weighted MRI at days 1, 4, and 7 post-ischaemia *in situ*, subsequent to deeply anaesthetizing the mouse and intracardiac perfusion with paraformaldehyde. Following MRI, the tissue was paraffin embedded, sectioned, and stained with haematoxylin and eosin to enable histological verification of the infarct and surrounding regions.

The MRI scans showed acute infarction of ~1mm^3 that corresponded to the primary vascular occlusion that was imaged using intravital microscopy. The anti-CD42 platelet marker showed the clot was restricted to the same area. This confirmed that the posterior circulation targeted using photo-induced thrombosis provided exclusive vascular support to the infarcted brain region. Further, the MRI and histology indicated that ventral to this site, there was altered diffusion (MRI) and histology, consistent with a penumbrum, which reflected propagation of collateral ischaemic injury. The key control study delivered the equivalent photonic flux in the absence of rose bengal infusion. This brain tissue lacked platelet aggregation and an infarct. These data indicated that endothelial activation by photo-sensitizing the cells with rose bengal was required for site-specific ischaemic brain injury. This model will enable study of neuroprotective signal pathways in the hind brain in the mouse model.

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AC is supported by a UNSW Vice Chancellor's post-doctoral Fellowship and UNSW Goldstar funding.