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Model of minor stroke with mild peri-infarct ischemic injury

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HIGHLIGHTS

- Few animal models can produce a mild ischemia, minor stroke and its recurrence.
- A minor photothrombosis was developed to produce small cortical infarcts.
- This minor stroke was accompanied by a peri-infarct region of mild ischemic injury.
- A minor recurrent stroke was produced by repeating the mild photothrombosis.
- T_2 magnetic resonance imaging was key to identify regions of mild ischemic injury.

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ABSTRACT

Background: Transient ischemic attack, minor stroke and stroke recurrence need improved treatment but lack animal models for research. The aim was to modify photothrombosis methods thereby producing both a minor stroke (with adjacent mild damage) or a minor recurrent stroke.
New method: A minor stroke, as detected using magnetic resonance imaging and histology, was produced using a low intensity beam of white light with a bright centre, a low dose of Rose Bengal and a short 5 min illumination of thinned skull. A recurrent minor stroke was produced by repeating the procedure two days later except the cortical mask was positioned 1.5 mm posteriorly.
Results: The minor photothrombosis procedure produced a small superficial infarct surrounded by a region of scattered necrosis detected histologically. Marked hyperintensities in diffusion weighted and T_2 images identified the infarct. Peri-infarct regions with modest T_2 increases corresponded to regions of scattered cell death. A recurrent minor photothrombosis produced additional damage in regions with overlapping mild injury.
Comparison with existing methods: Previous photothrombosis methods usually produce large cortical infarcts with little penumbra. The current method produces small infarcts with diffuse mild peri-infarct ischemic injury that can be diagnosed using T_2 imaging.
Conclusions: The modified photothrombotic procedure will produce a minor stroke consisting of a small infarct in a region with marked diffusion and T_2 hyperintensities and a peri-infarct region of selective necrosis with modest T_2 changes. Minor recurrent stroke is readily produced but imaging is key for assessing size and location of each insult.

1. Introduction

Minor stroke or TIA is relatively common and often precedes major stroke (Easton et al., 2009; Kernan et al., 2014). Mild transient focal cerebral ischemia, as occurs with a TIA, can be produced experimentally by brief direct occlusion of the middle cerebral artery to produce selective cell necrosis (Ejaz et al., 2015; Qiao et al., 2009). Such mild episodes of cerebral ischemia are not necessarily associated with MR changes. In addition to potential mild ischemic changes, patients with TIA, despite functional recovery, often have small Dw lesions indicative of minor stroke (Brazzelli

Abbreviations: Dw, diffusion weighted; MRI, magnetic resonance imaging; Iba1, Ionized Calcium-Binding Adapter Molecule 1; T_{2w}, transverse relaxation time weighted; TIA, transient ischemic attack.

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et al., 2014). How frequently this is accompanied by peri-infarct tissue with milder ischemic injury is not clear but there are reports of atrophy following TIA and minor stroke (Li et al., 2015; Weiller et al., 1993) indicating that despite good functional recovery there may be diffuse ischemic damage difficult to detect with MR imaging. Furthermore, minor stroke and TIA are predictive of stroke recurrence; and, as an increasing proportion of our population ages the incidence of TIA, minor stroke and recurrent stroke is anticipated to increase. However, their diagnosis, pathophysiology and management remains suboptimal with effective treatment options limited to thrombolytic or endovascular therapy.

A factor curbing advances in this area of stroke research is that there are relatively few simple animal models of minor and recurrent stroke available. Photothrombosis is a relatively simple method technically for producing rather non-invasively a focal infarct in experimental animals (Watson et al., 1985). However, the majority of previous studies using photothrombosis have produced rather large focal infarcts throughout the entire cortex that often include subcortical white matter (Dietrich et al., 1987a; Lee et al., 1996; Pierpaoli et al., 1993; Watson et al., 1985). A few studies have produced smaller infarcts or single vessel infarcts using photothrombosis (e.g. (Harrison et al., 2013; Moon et al., 2009; Pevsner et al., 2001; Shih et al., 2013)) and spontaneous reperfusion of thrombosed vessels has been observed (Zhang et al., 2005). However, producing infarction rather than mild ischemic injury in the peri-infarct region has been the focus of such previous studies. The aim of the current study was to investigate the conditions required to produce diffuse mild ischemic injury surrounding a small photothrombotic lesion—an insult that that could then model clinical minor strokes with a penumbra and could also be used to potentially model recurrent minor strokes.

Modifying the intensity and the duration of light illuminating the cerebral cortex following Rose Bengal injection produced small cortical infarcts with peri-infarct regions of scattered necrosis. T_2 imaging facilitated identification of the precise size and location of the small ischemic lesion and its peri-infarct region of mild ischemic injury. The photothrombotic insult could be repeated to produce a recurrent stroke and overlapping regions of mild ischemic damage.

2. Methods

2.1. Animals

The care and handling of all animals were carried out in accordance with the guidelines of the Canadian Council on Animal Care for care and use of experimental animals. Experiments were approved by the University of Calgary Health Sciences Animal Care Committee (Protocol M11017). Male Wistar rats (Charles River, Montreal, Canada) were acclimatized to a 12 h light/dark cycle with free access to food and water. Animals weighed 226–395 g (median 263) at the time of surgery. Following stroke, rats were housed in separate cages with free access to soft and hard food, water and environmental enrichment. All surgical procedures were performed using aseptic techniques.

2.2. Minor photothrombosis model

The photothrombotic stroke method uses a photo-activatable dye, Rose Bengal, to generate coagulation within vessels using experimental conditions that have generally resulted in a relatively large ischemic infarct within the entire depth of the cortex and often also white matter and subcortical structures (Dietrich et al., 1987a; Lee et al., 1996; Pierpaoli et al., 1993). Injection and illumination conditions were chosen to potentially produce minor strokes by selecting conditions that could enhance early platelet disaggregation and thrombus clearance as has been reported to be visualized directly using confocal microscopy (Zhang et al., 2005). Thus the concentration of Rose Bengal (Sigma–Aldrich, St Louis, MO) administered IV (prepared as 10 mg/ml in sterile distilled water and filtered through an 80 μm filter) was low (10 mg/kg, 1 ml/kg) compared to many previous studies using 15–50 mg/kg (Jolkkonen et al., 2007; Pierpaoli et al., 1993; Van Bruggen et al., 1992; Verlooy et al., 1993). The pharmacokinetics of Rose Bengal was used to help select the duration of illumination (Fig. 1A). Rose Bengal levels were measured spectrophotometrically in plasma from timed samples of arterial blood. Concentrations were determined from means of triplicate measures using a standard curve of known Rose Bengal concentrations and absorptions measured at 532 nm. Rose Bengal concentration peaked within the first minute following IV administration, clearing over 10 min (Fig. 1A), similar to observations in the mouse (Boquillon et al., 1992; Zhang and Murphy 2007). Thus, for the duration of illumination, a rather short period of five minutes was selected compared to the 15–30 min periods of illumination used in previous studies (e.g. Jolkkonen et al., 2007; Moon et al., 2009; Pevsner et al., 2001; Pierpaoli et al., 1993; Schroeter et al., 2001; Van Bruggen et al., 1992; Watson et al., 1985).

The animal was prepared to optimize illumination of the cortex under normally maintained physiological conditions. Rats were anesthetized with isoflurane (1.5–2.5% in 30% oxygen remainder nitrogen) to allow restraint of the head in a stereotaxic frame and surgical exposure of the skull. A femoral vein catheter was inserted surgically to allow effective intravenous injection of Rose Bengal. Normothermia (rectal temperature 36.5–37.5 °C) was maintained using a heating pad. Anesthesia was adjusted to maintain normal spontaneous respiration (55–75 breaths/min, mean 65 ± 9) resulting in good oxygenation (>98% saturation) confirmed with pulse oximetry in a subgroup of animals. To limit potential variations in light transmission into cortex, the skull was thinned to translucence over an area somewhat larger than the illumination region using a saline cooled dental drill. Bleeding was controlled using bone wax to provide a clean field of illumination. In some animals, regional cerebral blood flow (rCBF) within the illuminated region was measured prior to and post illumination using laser-Doppler flowmetry (Periflux PF5010, Perimed) with a 1.0 mm probe (403, Perimed) placed perpendicular to the skull. Rectal temperature was monitored and maintained throughout the experiment and for 1 h after surgery confirming that thermoregulation and body temperature was normal. After illumination, surgical sites were closed with 3-0 nylon sutures and buprenorphine (0.03 mg/kg) was administered to provide analgesia.

For photothrombosis, an opaque foil mask with an opening size of 3 mm × 3 mm was fabricated in order to illuminate a region encompassing several 1 mm thick MR slices. This was placed directly on the skull, with the centre of the mask at stereotaxic coordinates of 1.5 mm anteroposterior from bregma and 2.5 mm mediolateral from the midline (Paxinos and Watson 1998). The illumination apparatus used light from a 150 W halogen bulb (NCL 150 illuminator, Volpi) transmitted perpendicularly from the source through an infrared filter (#46-386, Edmund Optics, Barrington, NJ) and a 30 cm length of 13 mm diameter fibre optic cable (Fig. 1B). In initial studies light intensity of illumination was varied from 200,000 to 400,000 lux as measured at the skull (Light Meter, Sper Scientific Ltd.) which is the equivalent of approx. 30–60 mW/cm^2 at 555 nm. Direct comparisons of intensities used between studies are difficult; intensities reported have ranged from 3 to 30 mW or 285 to 580 mW/cm2 for green laser/light (Dietrich et al., 1987b; Harrison et al., 2013; Kao et al., 2014; Zhang et al., 2005) and 100 to 580 mW/cm^2 for white light (Lee et al., 1996; Pevsner et al., 2001).