Experimental Neurology 277 (2016) 252–260

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Systemic inflammation affects reperfusion following transient cerebral ischaemia

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ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 13 January 2016
Accepted 15 January 2016
Available online 18 January 2016

Keywords:
Stroke
Co-morbidity
Systemic inflammation
Middle cerebral artery occlusion
Spectroscopy
Optical imaging
Reperfusion
Interleukin-1
Interleukin-1β

ABSTRACT

Reperfusion after stroke is critical for improved patient survival and recovery and can be achieved clinically through pharmacological (recombinant tissue plasminogen activator) or physical (endovascular intervention) means. Yet these approaches remain confined to a small percentage of stroke patients, often with incomplete reperfusion, and therefore there is an urgent need to learn more about the mechanisms underlying the no-reflow phenomenon that prevents restoration of adequate microvascular perfusion. Recent evidence suggests systemic inflammation as an important contributor to no-reflow and to further investigate this here we inject interleukin 1 (IL-1) i.p. 30 min prior to an ischaemic challenge using a remote filament to occlude the middle cerebral artery (MCA) in mice. Before, during and after the injection of IL-1 and occlusion we use two-dimensional optical imaging spectroscopy to record the spatial and temporal dynamics of oxyhaemoglobin concentration in the cortical areas supplied by the MCA. Our results reveal that systemic inflammation significantly reduces oxyhaemoglobin reperfusion as early as 3 h after filament removal compared to vehicle injected animals. CD41 immunohistochemistry shows a significant increase of hyper-coagulated platelets within the microvessels in the stroked cortex of the IL-1 group compared to vehicle. We also observed an increase of pathophysiological biomarkers of ischaemic damage including elevated microglial activation co-localized with interleukin 1α (IL-1α), increased blood brain barrier breakdown as shown by IgG infiltration and increased pyknotic morphological changes of cresyl violet stained neurons. These data confirm systemic inflammation as an underlying cause of no-reflow in the post-ischaemic brain and that appropriate anti-inflammatory approaches could be beneficial in treating ischaemic stroke.

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1. Introduction

Stroke is a leading cause of global morbidity and mortality with treatment options limited to reperfusion through thrombolysis or thrombectomy. Ischaemic stroke accounts for 85% of all human strokes and is mainly caused by occlusion of the middle cerebral artery (MCA), a major arterial branch supplying blood to the brain (Doyle et al., 2008, Lloyd, 2010; Rosamond, et al., 2008). The resulting occlusion results in hypoperfusion of brain tissue creating an energy depleted state within the infarct core, triggering acute pathophysiological processes which result in neuronal injury (Ankolekar et al., 2012, Cramer et al., 2003, Semenza, 2009). Hypoperfusion starves tissue of glucose resulting in neuronal dysfunction and release of pro-inflammatory cytokines (Mori et al., 1992, Vila et al., 2000). Future successful treatment of ischaemic stroke requires a comprehensive understanding of the pathophysiological changes that occur within the acute phase of cerebral ischaemia.

Despite promising results in experimental studies of stroke in animal models there has been a lack of translation to clinical success (Ankolekar et al., 2012, Denes et al., 2010a, 2010b). Pre-clinical studies of ischaemic stroke to date have largely failed to take into account relevant co-morbidities for stroke, which experimentally we have shown can dramatically exacerbate ischaemic injury (Denes et al., 2010a, 2010b, McColl et al., 2007a, 2007b, McColl, 2008). A key hallmark of stroke co-morbidities is systemic inflammation and it is now well accepted that inflammatory processes are a major contributor to cerebral ischaemia (Denes et al., 2010a, 2010b, McColl et al., 2007a, 2007b, McColl, 2009; McColl, 2008; McColl et al., 2010; Maysami et al., 2015). The pro-inflammatory cytokine interleukin-1 (IL-1) in particular has been identified as a key mediator of neuronal injury. Experimental models of stroke have found that IL-1 signalling on endothelial cells, microglia, astrocytes and neutrophils stabilizes mRNA for pro-inflammatory mediator expression (Brandolini et al., 1997, Ericsson et al., 1995). Furthermore IL-1 acts on cerebral microvasculature endothelial cells, upregulating adhesion molecule expression and subsequent neutrophil transmigration (Brandolini et al., 1997). IL-1 has also been shown as the main driver in lipopolysaccharide (LPS) mediated worsening of damage (Denes et al., 2010a, 2010b, McColl et al., 2007a, 2007b). Recent findings demonstrate

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http://dx.doi.org/10.1016/j.expneurol.2016.01.013
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that systemic IL-1 prevents microvascular reperfusion post-stroke through endothelin-dependent mechanisms (Murray et al., 2013). Furthermore we have previously shown that preceding pneumonia infection worsens stroke outcome via IL-1 and platelet dependent mechanisms (Denes et al., 2014). Patients with underlying inflammation-related co-morbidities for stroke, including obesity, diabetes, arthritis and smoking often have worse outcomes following a cerebral ischaemic event (Bottcher and Falk, 1999; Herz et al., 2015, Pinto et al., 2004). In human trials IL-1 receptor antagonist (IL-1RA) limits the action of IL-1, improving patient outcome by reducing the pathophysiological activity including reduction in neutrophil numbers (Emsley, et al., 2005; Emsley et al., 2008, Smith et al., 2004). Therefore we have identified IL-1 as a very relevant cytokine to co-administer with cerebral ischaemia. Despite these data supporting a key role of IL-1 in ischaemic brain injury it remains to be fully defined how IL-1 affects reperfusion post-stroke. To investigate this further we combine systemic administration of IL-1 with a remote filament model of middle cerebral artery occlusion (MCAo) (Burrows et al., 2015), using two-dimensional optical imaging spectroscopy (2D-OIS), to confirm the effects of IL-1 on cortical oxyhaemoglobin dynamics during reperfusion after stroke in mice.

2. Methods

2.1. Animals

All animal procedures were performed under an appropriate Home Office Licence and adhered to regulations as specified in the Animals (Scientific Procedures) Act (1986). Studies were performed in accordance with ARRIVE guidelines, with appropriate randomisation and blinding procedures in place, and ethical approval by the local ethics committee of the University of Manchester. Mice were kept at 21 °C and 65% humidity with a regulated 12-h light–dark cycle and free access to food and water. Twenty-nine male C57/BL6 mice (Harlan Laboratories, UK) weighing between 30 and 36 g (age 18 to 25 weeks) were used in this study. Ten C57/BL6 male mice underwent i.p. injection of 20 ng/kg mouse recombinant IL-1β (R&D systems, UK) in sterile PBS and 0.5% low endotoxin BSA, 30 min prior to a 30 min MCAo with 6 h reperfusion; the remaining ten mice underwent i.p. injection of sterile PBS with 0.5% low endotoxin BSA alone (vehicle), 30 min prior to a 30 min ischaemic insult with 6 h reperfusion. In addition four C57/BL6 mice underwent the IL-1 protocol and five C57/BL6 mice underwent the vehicle protocol but were sacrificed 10 min after removal of the MCAo filament (i.e. 70 min after injection) for further immunohistochemical analysis. Though IL-1β was injected in the interest of brevity the rest of the manuscript will simply refer to IL-1.

2.2. Remote filaments

Filament preparation was performed as described in (Burrows et al., 2015) producing 5 cm remote filaments with a Xantopren M Mucosa and Activator NF Optosil (Heraeus, GER) mix for tip coating, first described by (Engel et al., 2011). A 2 cm length of clear i.v. catheter tubing (Portex, Kent UK) was used to aid stability during insertion of the filament.

2.3. Surgical preparation

Anaesthesia was induced with 4% isoflurane (Abbott, Berkshire, UK) in room air. Once the animals were unconscious with lack of pedal reflex, they were maintained under 2% isoflurane via a face mask for surgery and, once a tracheal cannula had been inserted, 1% to 1.5% isoflurane for the remainder of the experiment. MCAo was performed by advancing the custom made filament through the external carotid artery (ECA) up to the internal carotid artery to a point just before the MCA branch (Burrows et al., 2015). For imaging experiments, animals were fixed in a stereotactic frame (Narishige, Tokyo, Japan) with ear bars, mouth bar and a dorsal head post to prevent movement. Animals were artificially ventilated with 1% to 1.5% isoflurane in room air via a Zoovent Jetsys ventilator (Universal Lung Ventilators Ltd., Milton Keynes, UK). Body temperature was maintained at 37.5 °C via a heating blanket controlled with a rectal probe (Harvard Apparatus, Kent, UK), and the heart rate was monitored via ECG throughout the experiment. The scalp was dissected down the midline to expose the skull. The bone over the area of the somatosensory cortex supplied by the MCA in the stroked hemisphere was kept translucent with a saline filled paraffin well closed by a circular cover slip.

2.4. Two-dimensional optical imaging spectroscopy

Two-dimensional spectroscopic imaging data were collected through the imaging window over the intact skull using a high signal-to-noise charged coupled device (CCD) camera (Pantera 1M30, DALSA, Munich, Germany). The region of interest was illuminated sequentially by four different wavelengths of light (550 ± 10 nm, 560 ± 10 nm, 577 ± 10 nm, and 700 ± 10 nm) using a Lambda DG-4 high-speed filter changer (Sutter Instruments, Novato, CA, USA). Camera data collection was synchronized with the filter changer so that each image frame was recorded with one of the four different cortical illumination wavelengths in a sequential manner at a rate of 28 Hz. A ceramic attenuator (PI Instruments, Bedford, UK) was attached to a single whisker on the right whisker pad to enable computer-controlled mechanical stimulation of the barrel cortex throughout the experiment. A single imaging experiment consisted of a continuous recording of 30 trials. Each trial was 16 s long and contained a 4-s pre-stimulus period; 4 s of 8 Hz mechanical whisker stimulation and an 8-s recovery time. These 8-min experiments were recorded before injection, post injection, during, and after MCAo for both experimental groups as shown in Fig. 1.

2.5. Imaging protocol

A region of interest (ROI) for imaging was chosen to include the main branches of the MCA and the barrel cortex as identified by localized functional activity from mechanical whisker stimulation. Three sets of trials were recorded for baseline comparison after which the animals underwent i.p. injection with 20 ng/kg IL-1β or vehicle treatment (Fig. 1). Post injection of IL-1β or vehicle, a further three sets of trials were recorded during the 30 min prior to filament advancement. Then the remote filament was advanced ~2 to 4 mm to induce a 30 min occlusion of the MCA. During the MCAo three further trials were recorded. The remote filament was then retracted back 6 mm to allow full reperfusion of the MCA. Trials were then recorded every 30 min for 6 h after reperfusion. After 6 h reperfusion, the animal was transcardially perfused with 0.9% saline solution containing 0.5% sodium nitrate followed by 2% paraformaldehyde solution. After fixing, the brain was removed, stored in 2% paraformaldehyde for 24 h and submerged in a 30% sucrose solution for a further 24 h.

2.6. Tissue processing

Coronal brain sections (30 μm thick) were cut on a sledge microtome (Leica, Milton Keynes, UK) with freezing stage (Bright Instruments, Huntingdon, UK). Sections were stored in antifreeze solution (30% ethylene glycol and 20% glycerol (Sigma, Gillingham, UK) in phosphate-buffered saline (PBS)) at −20 °C, before histological staining.

2.7. Immunohistochemistry

Brain sections were pre-mounted on charged slides (Fisher Scientific, USA) before antigen-retrieval, where slides were submerged in citrate buffer (Invitrogen) diluted 1:100 in distilled water or 10%

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