Assessment of the neuroprotective efficacy of poly-arginine-18 (R18) peptides in a pre-clinical model of perinatal hypoxic-ischaemic encephalopathy (HIE)

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Assessment of the neuroprotective efficacy of poly-arginine-18 (R18) peptides in a pre-clinical model of perinatal hypoxic-ischaemic encephalopathy (HIE)

by

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ABSTRACT

Hypoxic-ischaemic encephalopathy (HIE) is one of the leading causes of mortality and morbidity in infants, globally. This disorder eventuates following a reduction in oxygenated cerebral blood flow to the foetus in utero, leading to excitotoxic-mediated brain cell (e.g. neuron, glia and glial progenitor cell) death. Currently, there is no clinically appropriate neuroprotective treatment to reduce acute brain injury following HIE. Recent studies have demonstrated that poly-arginine and cationic arginine-rich peptides (CARPs; e.g. R18: R = arginine residues) exhibit potent neuroprotective properties in both in vitro and adult animal models of ischaemia, and therefore have the potential to be developed into a neuroprotective treatment to reduce brain injury following HIE. Therefore, the aim of this thesis was to assess the neuroprotective efficacy of CARPs in a model of perinatal HIE in the rat.

To elucidate the neuroprotective efficacy of CARPs, a novel surgical modification to the original in vivo Rice-Vannucci model of perinatal HIE was developed. Using 7-day old Sprague-Dawley rats, brain injury was induced following the permanent ligation of the common and external carotid arteries, followed by a period of transient hypoxia (8% O₂/92% N₂). Results from this experiment demonstrated that the occlusion of common and external carotid arteries reduced cerebral communicational and/or anastomotic blood flow, reducing variability and improving the reliability in the presence of a cerebral infarct. The demonstration and termination of cerebral communicational and/or anastomotic blood flow improved the pre-clinical assessment of neuroprotective therapies to treat HIE.
The CARPs, R18, R18D (D-enantiomer) and JNKI-1-TATD, were assessed in the modified Rice-Vannucci model of HIE when administered intraperitoneally, immediately after the cessation of hypoxia-ischaemia (HI; 8% O₂/92% N₂ for 2.5 h). Treatment with R18 and R18D significantly reduced infarct volume and improved behavioural assessments in this model. Surprisingly, the well-characterised neuroprotective peptide JNKI-1-TATD, used as a positive control and benchmark, did not exhibit any significant neuroprotection. Succeeding positive results obtained following R18D administration immediately after HI, its therapeutic window was further assessed. R18D significantly decreased infarct volume and improved behavioural assessments when administered intraperitoneally up to 1 hour after the cessation of HI; correlating to 3.5 hours since HI onset. To confirm the neuroprotective mechanism of action of CARPs in HIE, an established *in vitro* primary cortical neuronal excitotoxic injury model was used. Results from this experiment demonstrate that CARPs reduce excitotoxic intracellular calcium influx in a dose-dependent fashion, providing evidence for a role in the reduction of several calcium-dependent pro-cell death cascades. The demonstration of significant neuroprotection following R18 peptide administration provides evidence for a novel therapeutic, which has the potential to reduce brain injury in infants who suffer HIE.

In summary, this thesis has identified a novel surgical modification to improve the reliability and reproducibility of the original Rice-Vannucci model of HIE. In addition, the administration of the R18 and R18D peptides following perinatal HIE, significantly reduces brain injury and improves behavioural assessments when administered up to 3.5 hours after the onset of HI. These findings demonstrate that CARPs provide an exciting and novel approach to reduce brain injury following HIE.
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DECLARATION

I hereby declare that:

- This thesis is submitted as part of the requirement for a Doctor of Philosophy degree as a result of my own work and research. All other sources have been indicated and acknowledged.

- Permission has been granted by co-authors for any work that has been co-published to be included in this thesis.

- This thesis has been substantially completed during the course of enrolment and its content has not previously been submitted or accepted for any other degree in this or any other institution.

- I understand that this work may be electronically scanned for detection of plagiarism.

Signed

Adam B. Edwards

Signed

Coordinating Supervisor: Bruno Meloni

Approval of final thesis
PUBLICATIONS ARISING FROM THIS THESIS


CONFERENCE PROCEEDINGS ARISING FROM THIS THESIS


AWARDS ARISING FROM THIS THESIS

Fee Remission Postgraduate Scholarship

The University of Notre Dame Australia;

University Postgraduate Award

The University of Notre Dame Australia;

Young Investigator Travel Programme Award

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Fremantle, Australia (2018).

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TAT-mGluR1

c-Jun N-terminal kinase (JNK) inhibitors

TAT-BH4

Osteopontin (OPN) and TAT-fused OPN peptide (TAT-OPN)

P5-TAT

D-TAT-GESV

TAT-NR2B9c/NA-1

Poly-arginine-18 (R18 and R18D)

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COG133

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Rice-Vannucci and modified HIE surgical models

Infarct volume assessment

Functional testing

Magnetic resonance imaging

Processing of FAIR-EPI data

Statistical analysis

Results

Time-of-flight magnetic resonance angiography (TOF-MRA)

Phase-contrast velocity encoding

Pulsed arterial spin labelling (PASL)

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Functional outcomes after HIE

Discussion

Conclusion
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Author contributions

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Introduction

Materials and methods

Animal ethics and study design

Peptides used in the study

Surgical procedure for modified Rice-Vannucci model

Peptide administration

Animals used and sample size

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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BSS</td>
<td>Balanced salt solution</td>
</tr>
<tr>
<td>CARP</td>
<td>Cationic arginine-rich peptide</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CCAO</td>
<td>Common carotid artery occlusion</td>
</tr>
<tr>
<td>CPP</td>
<td>Cell penetrating peptide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECA</td>
<td>External carotid artery</td>
</tr>
<tr>
<td>ECAO</td>
<td>External carotid artery occlusion</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>FAIR</td>
<td>Fluid attenuation inversion recovery</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>HI</td>
<td>Hypoxic-ischaemic or hypoxia-ischaemia</td>
</tr>
<tr>
<td>HIE</td>
<td>Hypoxic-ischaemic encephalopathy</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ICV</td>
<td>Intracerebroventricular</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MCAO</td>
<td>Middle cerebral artery occlusion</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimum essential medium</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Magnesium sulfate</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>MPTP</td>
<td>Mitochondrial permeability transition pore</td>
</tr>
<tr>
<td>MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTS</td>
<td>3-(4, 5, dimethyliazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H–tetrazolium salt</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
</tr>
<tr>
<td>P7</td>
<td>7-day-old</td>
</tr>
<tr>
<td>PASL</td>
<td>Pulsed arterial spin labelling</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague-Dawley</td>
</tr>
<tr>
<td>SE-EPI</td>
<td>Spin-echo echo-planar imaging</td>
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</tbody>
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**TOF**  Time of flight

**TTC**  Triphenyl tetrazolium chloride
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