Magnesium polyethylene glycol: a novel therapeutic agent for traumatic brain injury

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Medical Science
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Dedication

This thesis is dedicated to my mother Topers Sabiiti, who has always believed in me, encouraged and supported me in every way possible.
Publications, presentations and awards

The following articles have been published or accepted for publication or presentation during the period of my Masters candidature, and sections of these articles have been included in the present thesis.

Book Chapter


Presentation Abstracts


Award

Sir Grafton Elliot-Smith award for best student poster presentation, the Australian Neuroscience Society Conference, Adelaide, January 2014.
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To my husband Joseph thank you for always being there through thick and thin, standing by me, supporting me, encouraging me, helping with my poster presentation and formatting my thesis. You are my rock, best friend and biggest cheerleader.

Last but not least, to my beautiful daughter Tara. Coming home to your smile, cuddles and kisses after a long day meant the whole world to me.
# ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>APP</td>
<td>Amyloid Precursor Protein</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>AQP</td>
<td>Aquaporin</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>Calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>DAB</td>
<td>Diaminobenzidine tetrahydrochloride</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin-gene Related Peptide</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral Perfusion Pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse Axonal Injury</td>
</tr>
<tr>
<td>EB</td>
<td>Evans Blue</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
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ICP  Intracranial Pressure

IV  Intravenous

Mg^{2+}  Magnesium

NHS  Normal Horse Serum

NMDA  N-methyl-D-aspartate

NO  Nitric Oxide

PBS  Phosphate Buffered Solution

rpm  Revolutions per minute

SD  Standard Deviation

SEM  Standard Error of Measurement

SP  Substance P

SPC  Streptavidin Peroxidase Conjugate

TBI  Traumatic Brain Injury
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ABSTRACT

A number of experimental studies have shown that decline in intracellular free magnesium is a ubiquitous feature of traumatic brain injury (TBI), and that restoration of magnesium homeostasis improves both cognitive and motor outcome. However, a recent large, randomized clinical trial of magnesium in TBI failed, in part because of poor central penetration of the magnesium salt. Subsequent experimental studies in spinal cord injury have shown that magnesium penetration into the CNS can be facilitated if the magnesium salt is administered in a solution containing polyethylene glycol (PEG), a polymer that facilitates transport across the blood brain barrier and throughout the extracellular space. Accordingly, the current study characterised the therapeutic potential of high and low dose magnesium chloride, either alone or in combination with PEG, on oedema, blood brain barrier permeability, brain histology and functional outcome following moderate diffuse TBI in rats.

Adult male Sprague Dawley rats (350-380 g) exposed to moderate diffuse TBI induced using the impact acceleration injury model, were administered intravenous magnesium polyethylene glycol (Mg PEG) (254 µmoles/kg MgCl₂ in 1g/kg PEG), the same concentration (optimal dose) of MgCl₂ or PEG alone, or equal volume vehicle at 30 min postinjury. A separate group of surgically prepared animals were neither injured or treated and served as shams. All animals were subsequently assessed for oedema, blood barrier permeability, brain histology and functional outcome for up to 1 week after trauma. Administration of either Mg PEG or optimal dose MgCl₂ alone significantly improved all outcome parameters compared to vehicle treated or PEG treated controls with no significant difference between the magnesium treatment groups. Indeed,
magnesium treatment restored all parameters to sham levels. However, intravenous administration of one-tenth the magnesium concentration (25.4 µmoles/kg; low dose) had no beneficial effect on any of the outcome parameters whereas one-tenth the magnesium concentration in PEG (25.4 µmoles/kg MgCl₂ in 1g/kg PEG) had the same beneficial effects as optimal dose MgCl₂. We conclude that PEG facilitates movement of the magnesium salt across the blood brain barrier following TBI and that the combination of low dose magnesium in PEG significantly attenuates oedema, blood brain barrier permeability and improves motor and cognitive outcome following TBI.