Characterising The Role Of Haptoglobin In Experimental Subarachnoid Haemorrhage

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Abstract

Subarachnoid haemorrhage (SAH) is a devastating event associated with significant mortality and morbidity. A large proportion of SAH patients either die or suffer permanent disability due to a delayed multifactorial injury processes involving blood vessels, diverse inflammatory processes and secondary injury mechanisms. Extracellular haemoglobin (Hb), released from lysed red blood cells after SAH, is thought to be one of the prime culprits that incite these pathological processes. The role of haptoglobin (Hp), a systemic acute phase protein and the primary Hb-scavenging molecule, has recently been postulated to play a role in the pathogenesis of cerebral arterial vasospasm and delayed neurological deterioration in patients suffering SAH. The aim of this project was to demonstrate the relationship between free Hb and Hp within the cerebrospinal fluid following SAH, using the previously validated rat filament model. The results show that whilst free Hb levels peaked at 24hr post-SAH, there was marked free Hb within the CSF as early as the 1hr post-SAH. In addition, there was an increase in CSF Hp and soluble CD163 macrophage haemoglobin scavenger receptor from baseline, concomitant with the Hb peak at the 24hr mark, with a steady and rapid taper off, in keeping with clearance of free Hb by 72hrs post-SAH. Additionally, histological assessment to examine macrophage receptor for uptake and subsequent degradation of the Hb/Hp complex showed sporadic parenchymal staining within the basal brain surface of rats that sustained SAH, but was absent in sham and control animals. This study adds further to the understanding of the way haemoglobin is handled in the central nervous system following SAH.

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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Author Contributions

The following people have contributed to authorship of the manuscripts enclosed in this thesis (in alphabetical order): Thomas C Morris, Renée J Turner, Robert Vink.

The individual contributions of each author can be summarised as:

Conceptualisation and documentation of the work: TM, RJT, RV.

Realisation of the work: TCM.

I give my consent for any manuscript(s) in which I am a co-author to be included in this thesis:

Thomas C Morris

Renée J Turner

Robert Vink

Publications

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

Papers submitted for publication:

Morris T MBBS, Vink R PhD DSc, Turner R PhD. The Temporal Profile of Free Haemoglobin and Haptoglobin in a Rat Model of Subarachnoid Haemorrhage. Submitted for publication in *Neuroscience* November 2014

Abstracts presented:

Morris T MBBS, Vink R PhD DSc, Turner R PhD. The Role of Haptoglobin in Subarachnoid Haemorrhage. Presented at Neurosurgical Society of Australasia Annual Scientific Meeting, Perth, October 3rd 2014

Abbreviations

ABP – Arterial Blood Pressure

C – Centigrade

C1 – First Cervical Vertebra

CD163 – Cluster of Differentiation 163

CNS – Central Nervous System

CT – Computer Tomography

CSF – Cerebrospinal Fluid

HMG-CoA - 3-hydroxy-3-methyl-glutaryl-CoA reductase

DAB - 3,30 diaminobenzidine

DCI – Delayed Cerebral Ischaemia

DIND – Delayed Ischaemic Neurological Deficit

DND – Delayed Neurological Deterioration

ELISA – Enzyme Linked Immunosorbent Assay

Fe – Iron

Hb – Haemoglobin

Hb-Hp – Haemoglobin/Haptoglobin Complex

HO – Haem Oxygenase

Hp – Haptoglobin

ICA – Internal Carotid Artery

ICP – Intracranial Pressure

IgG – Immunoglobulin G

mm – Millimetres

mmHg – Millimetres of Mercury

NO – Nitrous Oxide

NHS – Normal Horse Serum

nm - Nanometre

PBS – Phosphate buffered saline

rpm – Revolutions Per Minute

SAH – Subarachnoid Haemorrhage

sCD163 – Soluble Cluster of Differentiation 163

SEM – Standard Error of the Mean

μm – Micrometre

μl - Microlitre

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Fig 14. ELISA quantification of CSF sCD163

Fig 15. CD163 immunohistochemistry

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