The Activation of Mitogen-Activated Protein Kinases in the Optic Nerve Head in a Model of Ocular Hypertension

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A thesis submitted to the University of Adelaide in fulfilment of Masters of Philosophy (Ophthalmology)

In

The Discipline of Ophthalmology & Visual Sciences, School of Medicine

August 2017
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IMPROVED IMMUNOHISTOCHEMICAL DETECTION OF PHOSPHORYLATED MAP KINASES IN THE INJURED RAT ONH

ABSTRACT

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Availability of data and materials ...

Conflict of interests ...

Funding ...

Author contribution ...

Acknowledgments ...

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SUPPLEMENTAL FIGURES

CHAPTER 2 PRELUDE

Is that the end of MAPK in the ONH?

CHAPTER 2

Statement of Authorship

Chapter 2

Evaluation of damage profiles

Verification of antibody detection of MAPK species by Western immunoblot

Immunolabelling of MAPK subtypes
ABSTRACT

Glaucoma is a neurological blinding eye disease, which results from the death of retinal ganglion cells. Although the pathogenesis of glaucoma remains unknown, changes in the tissue microenvironment of the optic nerve head (ONH), where insults are believed to be initiated, will cause signalling alterations in local cells. One important type of signalling involved in the control of cellular functions is protein phosphorylation. This is controlled by the balance between protein kinases and protein phosphatases, which add and remove phosphate groups respectively. One particularly important group of protein kinases is the mitogen-activated protein kinase (MAPK) family, whose activity is known to be altered in neurological diseases.

The first aim of this thesis was to determine whether specific MAPK family members (P42/44 MAPK, SAPK/JNK MAPK and P38 MAPK) were altered in a laser-induced ocular hypertension model, used to simulate the pressure elevation often associated with glaucoma. Techniques used for analysis included immunohistochemistry to observe changes in histopathological activation and location, Western immunoblotting to quantify changes in protein level expression, and real time reverse-transcriptase polymerase chain reaction to establish whether there were any changes in MAPK gene expression.

Total P42/44 MAPK expression was unaffected after intraocular pressure elevation, but a significant increase in its activation was detected in astrocytes in the ONH after 6-24 hours. Active SAPK/JNK was present throughout treated and untreated RGC axons, but accumulated in the ONH at 6-24 hours after pressure elevation, signifying axon transport disruption. P38 MAPK was expressed by a population of microglial cells throughout the retina, ONH and optic nerve, which were significantly increased in number following elevated intraocular pressure. However, this enzyme was only significantly activated in microglia after more than 3 days and
then not in the retina, where it was solely activated in retinal ganglion cell perikarya. These data imply both upregulation and activation of MAPK in the ocular hypertension model, in several distinct locations.

Levels of particular phosphoproteins are readily affected by minor perturbations in cellular homeostasis, as will occur when an animal is killed for tissue procurement. Thus, the second aim of this thesis was to identify whether activated MAPKs could be stabilised in procured tissues by perfusing animals with saline containing phosphatase inhibitors before fixation. Immunohistochemical analysis was used to observe differences in specific staining of phosphorylated MAPKs. The addition of phosphatase inhibitors to the perfusate had no significant effect on control animals or animals where there was a robust demonstration of tissue damage, but this procedure significantly reduced variability and improved clarity of outcome in labelling for activated MAPKs in animals with less extensive tissue damage, likely by stabilising levels of these phosphoproteins. These data suggest that phosphatase inhibitors stabilised phosphorylated MAPK levels and enabled a clearer dissemination of the activation of these enzymes, particularly when associated tissue damage was not extensive.

Having determined that MAPKs isoenzymes were activated in the ONH after sustained ocular hypertension, future work will concentrate on determining whether manipulation of these enzymes could play a useful role in the management of diseases such as glaucoma.
DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 in my name, 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. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Teresa Mammone       November 2017
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Wed 9/8/2017 1:14 PM

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1 attachment
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To Graduate Centre

cc Teresa Mamnone

I have looked through the attached AHEGS abstract for Teresa Mamnone and I am happy with this.

Thank you and best wishes

John W

Abstract:
The aim of this thesis was to investigate the activation of mitogen activated protein kinases (MAPKs) in the optic nerve head in a laser-induced model of ocular hypertension (OHT). Activation of three distinct sub-groups of MAPKs: P42/44 MAPK, SAPK/JNK and P38 MAPK was proven, by immunohistochemistry, Western immunoblot and reverse-transcriptase polymerase chain reaction. In addition to this, a more sensitive method of detection for activated MAPKs was developed in order to identify whether the magnitude of the observed activations in the ONH were directly proportional to the degree of tissue damage in the model.

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Hi Dr Wood,

Attached is the AHEGS thesis abstract.

Please review and confirm, by reply of email, you are satisfied with the wording and submission of this abstract and thesis.

Regards
Teresa Mamnone

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ABBREVIATIONS

AD; Alzheimer's disease
APP; Amyloid precursor protein
CNS; Central nervous system
HD; High damage
IOP; Intraocular pressure
LD; Low damage
MAPK; Mitogen-activated protein kinase
MPIs; Minus phosphatase inhibitors
NBF; Neutral buffered formalin containing 4 % formaldehyde
OHT; Ocular hypertension
ONH; Optic nerve head
P38 MAPK ; P38 mitogen-activated protein kinase
P42/44 MAPK; Extracellular signal-regulated kinases (ERK)
PIs; Phosphatase inhibitors
POAG; Primary open-angle glaucoma
PPIs; Plus phosphatase inhibitors
RGC; Retinal ganglion cells
SAPK/JNK; Stress-activated protein kinase/c-Jun N-terminal kinases