Hyperglycaemia in Experimental Glaucoma

Andreas Johannes Anton Ebneter, MD

School of Medicine

Discipline of Ophthalmology & Visual Sciences

A thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

May 2011

Papers produced during the candidature (included in this thesis)

Microglial Activation in the Visual Pathway in Experimental Glaucoma: Spatiotemporal Characterization and Correlation with Axonal Injury
Ebneter A, Casson RJ, Wood JP, Chidlow G.
Investigative Ophthalmology & Visual Science. 2010 Dec;51(12):6448-60.
Impact factor*: 3.466

The optic nerve head is the site of axonal transport disruption, axonal cytoskeleton damage and putative axonal regeneration failure in a rat model of glaucoma
Chidlow G, Ebneter A, Wood JP, Casson RJ.
Acta Neuropathologica. 2011 Jun;121(6):737-51.
Impact factor*: 7.695

Protection of Retinal Ganglion Cells and the Optic Nerve During Short-term Hyperglycemia in Experimental Glaucoma
Ebneter A, Chidlow G, Wood JP, Casson RJ.
Archives of Ophthalmology. 2011 Oct; 129(10)
Impact factor*: 3.516

Comparison of fixed-pattern sampling with targeted sampling for estimation of axon counts in a rat model of glaucoma
Ebneter A, Casson RJ, Wood JP, Chidlow G.
Clinical & Experimental Ophthalmology (accepted)
Impact factor*: 1.755

Paper Presentations

Retinal Neuronal Protection Via the Crabtree Effect
RANZCO Annual General Meeting and Scientific Congress 2008 - Melbourne

Microglial Activation in the Optic Nerve and Tract in Experimental Glaucoma
RANZCO Annual General Meeting and Scientific Congress 2009 – Brisbane

Hyperglycemia is Neuroprotective in a Rat Model of Experimental Glaucoma
RANZCO Annual General Meeting and Scientific Congress 2010 – Adelaide

Posters

Microglial Activation in the Optic Nerve and Tract in a Rat Model of Experimental Glaucoma
ARVO Annual Meeting 2009 – Fort Lauderdale (FL)

Hyperglycemia is Neuroprotective in a Rat Model of Experimental Glaucoma
ARVO Annual Meeting 2010 – Fort Lauderdale (FL)

Evaluation of Axonal Regeneration in a Rat Model of Experimental Glaucoma
ARVO Annual Meeting 2011 – Fort Lauderdale (FL)

Lab Talks

Quantification of optic nerve injury
Center for Molecular Ophthalmology & Neuroscience (Jeffrey Goldberg's Lab) – May 2010
Bascom Palmer Eye Institute – Fort Lauderdale (FL)

---

* As per THOMSON REUTERS Journal Citation Reports®, Science Edition 2010

TABLE OF CONTENTS

ABSTRACT

Glaucoma refers to a family of optic neuropathies with multi-factorial aetiology. The pathogenesis of glaucoma remains unclear, but there is good evidence that the optic nerve head is involved early in the pathogenesis of the disease. Inadequate blood supply to the optic nerve head may play a role, at least in some types of glaucoma. Given that vasculopathy is a hallmark of diabetes, one would expect that diabetes might exacerbate glaucoma; however, in large epidemiological studies no clear association was found. The Ocular Hypertension Treatment Study even suggested that diabetes protected against the conversion of ocular hypertension to glaucoma. In this thesis, I attempted to investigate the effect of short-term hyperglycaemia on retinal ganglion cell death and optic nerve damage in an experimental rat model of chronic ocular hypertension, which consisted of laser photocoagulation of the trabecular meshwork.

The thesis is made up of four papers. The first paper characterises the rat model for our laboratory and validates the laser parameters used, which, in comparison to the original publication describing the model, have been slightly modified to minimise the ocular complications. A combination of histology, immunohistochemistry, Western blotting and real-time polymerase chain reaction was used to portray the spatial and temporal nature of retinal ganglion cell pathology. The data provides robust support for the hypothesis that the optic nerve head is the pivotal site of retinal ganglion cell injury, with resulting anterograde degeneration of axons and retrograde injury and death of perikarya. It was found that disruption of axonal transport occurs very soon after ocular hypertension, prior to structural damage, substantiating the hypothesis that axonal dysfunction may be an important cause of retinal ganglion cell degeneration. Moreover, as a novel finding, restricted axonal regeneration were observed at the optic nerve head.

The second and third papers address the issue of damage quantification in the optic nerve. Axon counting on semi-thin optic nerve cross-sections represents the gold standard to evaluate the extent of axonal injury. However, this method is very laborious and time consuming. In search for alternatives, I investigated the accuracy of different sampling methods to estimate optic nerve axon numbers on cross sections and the usefulness of immunohistochemical markers on longitudinal optic nerve sections. Random sampling of pictures for automated axon counting was sufficiently accurate and the microglial response proved very valuable and effective for quantification of optic nerve damage.

The thesis culminates in the fourth paper, which presents a limited reproduction of the Ocular Hypertension Treatment Study in a laboratory environment. Unilateral ocular hypertension was induced in two groups (n=26 per group) of Sprague-Dawley rats. One group remained normoglycaemic; the other was rendered hyperglycaemic by intraperitoneal injection of streptozotocin. After two weeks of elevated intraocular pressure, axonal and retinal damage were compared using the quantification methods introduced in the previous papers. There was convincing evidence for delayed axonal degeneration and retinal ganglion cell death in the hyperglycaemic rats. Axonal loss was reduced by about 50%. Survival of retinal ganglion cell somata was increased to a similar extent in hyperglycaemic rats. Hence, energy substrate availability may play a role in glaucomatous optic neuropathy. Targeted manipulation of neuronal energy metabolism may delay optic nerve degeneration and may represent a novel neuroprotective strategy for neurodegenerative diseases of the visual system such as glaucoma.

DECLARATION OF ORIGINALITY AND COPYRIGHT

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.

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.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holders 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 catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restricted access for a period of time.

Andreas Ebneter
Adelaide, October 2011

The optic nerve head is the site of axonal transport disruption, axonal cytoskeleton damage and putative axonal regeneration failure in a rat model of glaucoma
Acta Neuropathologica. 2011 Jun;121(6):737-51.
Copyright holder Springer-Verlag GmbH
Rights and Permissions
Tiergartenstr. 17
D - 69121 Heidelberg, Germany
Fax: +49 6221 487 8223

Microglial Activation in the Visual Pathway in Experimental Glaucoma: Spatiotemporal Characterization and Correlation with Axonal Injury
Investigative Ophthalmology & Visual Science. 2010 Dec;51(12):6448-60.
Copyright holder The Association for Research in Vision and Ophthalmology (ARVO)
1801 Rockville Pike, Suite 400
Rockville, MD 20852-5622
Phone: +1 240 221 2900 Fax +1 240 221 0370

Protection of Retinal Ganglion Cells and the Optic Nerve During Short-term Hyperglycemia in Experimental Glaucoma
Archives of Ophthalmology. 2011 Oct
Copyright holder American Medical Association
515 North State St
Chicago, IL 60654
Phone: +1 312 464 2513 Fax: +1 312 464 5834

Comparison of fixed-pattern sampling with targeted sampling for estimation of axon counts in a rat model of glaucoma
Clinical & Experimental Ophthalmology
Copyright holder John Wiley & Sons, Ltd.
Permissions Department
The Atrium, Southern Gate
Chichester, West Sussex, PO19 8SQ
Phone: +44 1243 843356 Fax: +44 1243 770620

ACKNOWLEDGMENTS

This study was partially supported by a grant from the foundation OPOS, Switzerland. This research was also supported by funds from ORIA and NHMRC.

First and foremost I would sincerely like to thank Prof Robert Casson and Prof Dinesh Selva for allowing me to undertake my PhD in the Ophthalmic Research Laboratories of the South Australian Institute of Ophthalmology. I am deeply grateful to Prof Robert Casson, an outstanding mentor in clinical and experimental ophthalmology, for his constant support as my principal supervisor throughout the last 3 years.

To Dr. Glyn Chidlow I say thank you for his support, his honest, thorough and constructive criticism, and for sharing his outstanding knowledge in the field of experimental ophthalmology. It has been a privilege to work with you. Thank you for your expert guidance and for finding answers to so many, at first glance, puzzling observations. Unfortunately we have not yet got a chance to test our skills on the downhill run on a snowfield. To Dr. John Wood I say thank you not just for expert supervision or teaching me many valuable technical and scientific skills but also friendship.

Mark Daymon contributed incredibly to this work through his creative input and exceptional expertise in theory and practice of histological techniques. Also, you were always prepared to give me a hand with immunostaining or cutting when I was short of time. Not to forget about his worldly wisdom and the after-work beers at the Elephant Pub.

Teresa Mammone brought the laboratory back to life after a transient quiet period. I thank you for continuing the work I started and wish you the best of luck in developing it further. I am certain that you have the necessary skills and attitude to succeed.

To the entire Neuropathology Laboratory including Kathy, Cai, Bernice, Sven, Serg, Yvonne and Sophie. You made my life easier with helpful advise and the occasional chat in the tearoom. In particular I would like to thank Kathy and Cai for introducing me to the resin processing.

Special thanks go to Jim Manavis for giving me access to his fantastic range of antibodies and for letting me use the laboratory facilities, which he manages so smoothly.

I would like to offer a big thank you to all the animal house staff for looking my rats during the experiments. In particular I would like to mention Briony, Brian and Brigit who taught me a lot about animal handling and gave me invaluable insight into the behaviour of laboratory rats.

Special thank you also to Dr. Tim Kuchel, who helped us to improve the experimental procedures and supported our research team with his knowledge when we encountered difficulties.

I am very grateful to my friend Martin for his forward-looking, anticipatory and creative thinking and his invaluable mental support during the long experiments.

Levon enriched the everyday laboratory routine with his philosophical and open-minded thoughts on life and science.

Lenny. We crossed our ways on a plane between Los Angeles and Miami in difficult times.

Last but certainly not least I wish to thank my parents and brothers for their support, appreciation and inspiring guidance during all these years on this long and eventful journey.

ABBREVIATIONS

ATP	adenosine triphosphate
GLUT	glucose transport protein
HIF	hypoxia inducible factor
IOP	intraocular pressure
NMDA	N-methyl-D-aspartate
NO	nitric oxide
pO_2	oxygen partial pressure
POAG	primary open-angle glaucoma
RGC	retinal ganglion cell
ROS	reactive oxygen species
STZ	streptozotocin