Aspects of Retinal Energy Metabolism

Supervised by Professor Robert J. Casson & Dr. John Wood

Guoge Han

Discipline of Ophthalmology, University of Adelaide

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Mechanisms of neuroprotection by glucose in rat retinal cell cultures subjected to respiratory inhibition.
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1801 Rockville Pike, Suite 400
Rockville, MD 20852-5622
Phone +1.240.221.2900 Fax +1.240.221.0370

Glucose-induced temporary visual recovery in primary open-angle glaucoma: a double-blind, randomized study. Ophthalmology. 2014 Jun; 121 (6):1203-11.

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Mayo Clinic, East 4, Mayo Building
Rochester, Minnesota 55905 USA
Phone: 415-447-0261 Fax: 415-727-4600

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An explanation for the Warburg effect in the adult mammalian retina. Clin Experiment Ophthalmol. 2013 Jul; 41(5):517

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The Atrium Southern Gate Chichester UK Phone: 44 (0)1865 778315

Papers included in this thesis

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Han G, Wood JP, Chidlow G, Mammone T, Casson RJ.

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The Mitochondrial Complex I Inhibitor Rotenone Induces Endoplasmic Reticulum Stress and Activation of GSK3β in Cultured Rat Retinal Cells.

Han G, Casson RJ, Chidlow G, Wood JP

Invest Ophthalmol Vis Sci. 2014. (Accepted, in press)

Expression of Pyruvate Kinase (PKM) in the mice retina

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Glucose-induced temporary visual recovery in primary open-angle glaucoma: a double-blind, randomized study.

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Paper Presentations

Mechanisms of neuroprotection by glucose in rat retinal cell cultures subjected to respiratory inhibition.
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Posters

The Mitochondrial Complex I Inhibitor Rotenone Induces Endoplasmic Reticulum Stress and Activation of GSK3β in Cultured Rat Retinal Cells. ARVO 2014 Orlando USA

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Abstract

Energy failure is a possible pathogenic component of a number of common blinding disorders, including vascular retinopathies, glaucoma, and age-related macular degeneration. An overarching premise of the bioenergetics-based research from the research group in which I conducted my studies is that if energy failure constitutes a pathogenic component in ocular disease, then provision of energy or the means of the diseased tissue to create its own additional energy may well presented a valid and viable therapeutic solution. One such approach has been to provide an additional supply of glucose to a tissue since this monosaccharide is used by the majority of cells as the primary fuel source for the generation of cellular energy in the form of adenosine triphosphate (ATP).

Previous *in vivo* research from the group has demonstrated that elevated vitreal glucose levels afforded robust neuroprotection to the retina and optic nerve in experimental model of acute and sub-acute ischaemic retinal injury, and in a rat model of laser-induced glaucoma. The current research focussed on aspects of retinal energy metabolism, and in particular, the mechanisms by which glucose can act as a neuroprotectant under conditions of compromised energy production in the retina. Another aim was to translate this research to the clinic and assess the effect of elevated vitreal glucose levels on visual function in glaucoma patients.

The current thesis comprises four original papers and one perspectives paper. The first paper characterised an *in vitro* model of metabolic impairment to rat retinal cultures, using the mitochondrial complex I inhibitor, rotenone. Subsequently, the protective effect to retina cells of glucose was investigated in this model, and the effects compared with other known energy substrates (pyruvate and lactate). A variety of methods, including immunocytochemistry, Western blot and TUNEL staining were used to determine neuronal and glia cell viability. Cellular energy levels were determined by luminescent ATP assays and reduction and oxidation (REDOX) power was assessed by nicotinamide adenine dinucleotide phosphate (NADPH) assay. Metabolic pathways were modified with specific inhibitors. The findings from this series of experiments supported the hypothesis that the mechanism by which glucose protects the retina in the presence of mitochondrial impairment is

principally via glycolysis- generated ATP. Moreover, the glucose- stimulated anti-oxidant production via the pentose phosphate pathway also contributed to the neuroprotection.

In the second paper, we investigated neuronal and glial death and damage mechanism in response to rotenone treatment, with a particular focus on endoplasmic reticulum (ER) stress, and the signalling pathways involving calcium-activated neutral protease-μ (calpain-μ) and glycogen synthase kinase 3β (GSK3β). We found that retinal cultures were modulated via different mechanisms according to the cell-type and the degree of reduction of ATP. Overall, retinal neurons that were subjected to rapid ATP depletion in response to rotenone, underwent non-apoptotic death involving generation of reactive oxygen species (ROS) and activation of calpain-μ. In contrast, glial cells, which are relatively resistant to mitochondrial damage, were damaged via a combination of endoplasmic reticulum (ER) stress and deactivation of GSK3β.

The appendix paper provided a brief perspective on a possible explanation for the existence of aerobic glycolysis (the Warburg effect) in the mammalian retina. It was hypothesized that the rhodopsin turnover drives the Warburg effect in a similar manner to a proliferating tissue. Recently, a specific pyruvate kinase isoenzyme (PKM2) has been suggested to be a key mediator of the Warburg effect in cancer. The third paper (manuscript in preparation) characterizes the distribution of PK isoenzymes in the rodent retina and brain. PKM2 was distributed in the outer retina, particularly at the level of the photoreceptor inner segments. Minimal PKM2 was detected in the inner retina of rodents, mirroring the pattern in the brain; however, the outer retina labelling was remarkably similar to cancerous tissue.

The current body of work culminates in the fourth paper, which translated the laboratory finding to a clinical trial. In a preliminary study on patients with epiretinal membranes scheduled for routine vitrectomy, we demonstrated that concentrated 50% topical glucose treatment significantly increased the vitreous glucose concentration in pseudophakic patients. We then conducted a randomized, double blind, crossover trial on 29 eyes of 16 pseudophakic patients with severe primary open-angle glaucoma. We assessed the effect of intensive topical glucose on visual psychophysical parameters. Saline (0.9%) was used as a control in an initial study and a follow-up study used osmotically-matched (8%) saline as a control. Glucose significantly improved the mean contrast sensitivity at 12

cycles/degree compared to 0.9% saline by 0.26 log units (95% confidence interval [CI]: 0.13 – 0.38; P < 0.001); and in the follow–up study by 0.40 log units (95% CI: 0.17 – 0.60; P < 0.001). Neither the intraocular pressure (IOP), refraction, nor the central corneal thickness were affected by glucose; age was not a significant predictor of the response.

In conclusion, these studies add valuable information to the literature concerned with bioenergetic-based protection to ocular and, in particular, retinal cells. Furthermore, the clinical study presented in the final chapter actually demonstrates a confirmation of the "proof-of-principle" for bioenergetic neurprotection as a treatment strategy for retinal and optic nerve diseases. Similar strategies could now conceivably be applied to other retinal diseases. Finally, a better understanding of the unusual retinal energy metabolism, as discussed in the preceding chapters, is likely to shed light on disease pathogenesis and provide information that could potentially be translated to the clinic.

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