THE EFFECT OF HYPERGLYCAEMIA ON EXPERIMENTAL SUBACUTE ISCHAEMIC OPTIC NEUROPATHY AND RETINOPATHY

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ABSTRACT

The overarching aim of the work described in this thesis was to address perceived deficiencies in knowledge of the differences between retinal and brain metabolism in order to gain a greater understanding of the mechanisms involved in ischaemic retinal and optic nerve injury. Specifically, the aim of the project was to test the hypothesis that elevated blood and vitreal glucose levels induced by short-term diabetes would attenuate prolonged ischaemic retinal degeneration in the rat.

Simultaneous retinal and cerebral hypoperfusion was achieved by 2-vessel occlusion (2VO; permanent ligation of both common carotid arteries). Prior to testing the stated hypothesis, it was necessary to fully characterize the 2VO model in order to establish the optimal endpoint for analysing neuroprotection. Thus, at various times after surgery, retinas and optic nerves were removed form RNA or Western blot analysis or to be processed for histology and immunohistochemistry. In the retina, 2VO induceda progressive loss of retinal ganglion cells and horizontal cells, thinning of the inner retina, together with macroglial and microglial cell activation. One week was selected as the optimal time point at which to analyse neuroprotection. In the optic nerve, 2VO caused axonal transport disruption, followed by the loss of axonal cytoskeleton proteins, glial cell activation, infiltration of macrophages, upregulation of stress proteins by astrocytes and oligodendrocytes, and finally extracellular matrix remodeling.

To address the major aim of the thesis, rats were divided into 4 groups: normoglycemic and hyperglycemic sham-operated rats; normoglycemic and hyperglycemic 2VO rats. Hyperglycemia was induced 3 days prior to 2VO by streptozotocin injection. Rats were killed one week after 2VO or sham surgery. The retina of one eye were collected for histology/immunohistochemistry, whilst the fellow retina was dissected for real-time RT-PCR. Retinas were analysed for neuronal and glial markers and the inducible stress protein heat shock protein-27. Brains were processed for histology and immunohistochemistry.

Retinas of normoglycemic 2VO animals showed a marked loss of retinal ganglion cells and horizontal cells, thinning of the inner retina, together with macroglial and microglial cell activation. Hyperglycemic 2VO rats displayed a remarkable protection of retinal structure and reduced glial cell activation compared to normoglycemic 2VO animals. There was a significantly greater number of heat shock protein-27-positive retinal ganglion cells in

normoglycemic animals compared to hyperglycemic animals, indicating that a greater proportion of surviving retinal ganglion cells were stressed in normoglycemic animals as compared to hyperglycemic rats. Brains of both normoglycemic and hyperglycemic 2VO animals displayed scattered ischemic infarcts and mild white matter injury.

In conclusion, short-term hyperglycemia afforded a robust protection against retinal hypoperfusion injury, but in the same animals brain injury was not ameliorated. The mechanism of this retinal hyperglycemia-induced neuroprotection requires further study.

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STATEMENT OF ORIGINALITY

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Matthew C. Holman

Chapter 1 - INTRODUCTION

Disruption in blood supply to the retina and/or optic nerve (ON) is a leading cause of blindness worldwide, manifesting in a variety of ischemic-like diseases, including arterial and venous occlusions, diabetic retinopathy, ischemic optic neuropathies, and putatively glaucomatous optic neuropathy.[1] Currently, there are very limited, suboptimal treatment options available for many of these conditions, hence ischemia-related consequences are a common cause of blindness.

Ischemia results in a decreased tissue availability of oxygen and nutrients such that cellular energy demands cannot be met. Initially, ischemia results in a lowered tissue homeostasis, but as the duration of ischemia increases, the magnitude of cellular stress also rises, culminating in irreversible neuronal injury and death.[1] It is logical to envisage that supplementation of glucose, the major energy source for cells, would lessen the impact of ischemia on central nervous system (CNS) neurons. In fact, within the brain, the opposite is true: hyperglycemia exacerbates stroke and cerebral ischemia. Diabetes mellitus is a well-documented independent risk factor for the incidence of stroke and worsens the prognosis. Greater frequencies of all types of cerebral infarction have been found in diabetic patients compared with non-diabetics, while large clinical trials have revealed that diabetes at the time of admission is a predictor of worse outcomes following both focal and global ischemia admission.[2-5] The detrimental influence of diabetes is understandable given the knowledge that prolonged hyperglycemia damages the micro- and macro-circulation.[6] More surprisingly, hyperglycemia without pre-existing diabetes worsens outcomes for stroke patients.[7] Similarly in animal studies it has been repeatedly demonstrated that hyperglycemia exacerbates brain injury following ischemic insults. The negative impact of hyperglycemia is evident in both focal and global models of injury, when the degree of hyperglycemia is only moderate, and when the animals are rendered hyperglycemic only shortly before the insult commences.[3,8] The mechanisms by which hyperglycemia exacerbates brain ischemia injury remain unresolved but have been hypothesized to involve increased acidosis, edema, excitotoxicity, blood-brain barrier breakdown, and oxygen free radical damage.

In the retina, prolonged hyperglycemia is toxic to the vasculature, manifesting as thickening of capillary basement membranes, loss of pericytes and endothelial cells, blood-retinal barrier breakdown and leakage, and eventually neovascularization.[9] These attributes

are characteristic of diabetic retinopathy. An increasing body of evidence also points to a direct neurotoxic effect of chronic hyperglycemia on retinal neurons, in particular retinal ganglion cells (RGCs).[10,11] The onset of hyperglycemic neurotoxicity on RGCs within the laboratory animal setting has been shown to vary between studies, but typically occurs gradually over a period of months. Intriguingly, however, in vitro and in vivo experiments have demonstrated that short-term elevation of the glucose concentration within the eye actually elicits a remarkable degree of protection against an acute ischemia retinal injury,[12] while hypoglycaemia has a deleterious effect.[13]

The rationale by which hyperglycemia exerts opposing effects in the retina and brain is unknown, but it can be hypothesised that it exemplifies a fundamental difference between energy metabolism in the retina and brain. Retinal metabolism features high glycolytic activity even during aerobic conditions.[14,15] Indeed, as much as 80% of glucose supplied by the choroid to the photoreceptors is metabolised to lactate.[16] This is akin to the situation in some neoplasms and is known as the Warburg effect. Glycolysis increases further during conditions of reduced oxygen availability. By upregulating glycolysis, the retina can continue to synthesize the vast majority of its ATP requirement in the absence of oxygen, as long as glucose is abundant[14,15,17] — a manifestation of the Pasteur effect.[18] In contrast to the retina, the cerebrum relies on the continued presence of oxygen and oxidative phosphorylation (OXPHOS) to meet its energy demands.[19]

It is clear that underlying experimental hyperglycemia has contrasting effects on neuronal survival in different regions of the CNS subjected to *acute* injury paradigms. For these findings to have greater clinical relevance, however, it is necessary that they be replicated in *chronic* models of neuronal degeneration. The demonstration of retinal protection against more prolonged periods of ischemia would suggest that retinal energy production may be able to continue indefinitely as long as glucose is abundant, and would support the concept of further bioenergetics-based nerve cell protection for retinal degenerative diseases where energy depletion may be a causative factor (such as glaucoma, ischaemic retinopathies and age-related macular degeneration). The overarching aim of the work described in this thesis, then, is to determine the effect of experimental hyperglycemia on the retina following a prolonged hypoperfusion insult.

Chronic, ischemic-like injury to the retina can be achieved via permanent, bilateral occlusion of the common carotid arteries (2 vessel occlusion; 2VO) in rats.[20] The rat vascular

system is similar to that of the primate with blood supply to the head originating from the common carotid and ventral arteries, which feed into the circle of Willis. The inner retina is then supplied by the central retinal arteries, which branch from the ophthalmic arteries,but the outer retina is supplied indirectly from the choriocapillaris. Due to the redundancy in the circle of Willis blood supply, some cerebral and retinal perfusion can be maintained in the event of occlusion of one or two of the common carotid arteries by supply from the ventral arteries. Unilateral common carotid artery occlusion typically elicits only subtle changes, but bilateral common carotid occlusion has been shown to cause ischemic-like sequelae in the retina and brain. Interestingly, 2VO appears to precipitate a more severe injury in the retina than the brain at any given time point.[20] In the brain, gliosis appears at early time points, but neuronal loss is very gradual developing over many weeks.[20] In the retina, however, studies have reported extensive death of ganglion cells by 7 days.[21-25]Nevertheless, there is some disagreement in the literature concerning the extent of retinal pathology after 2VO with other studies reporting that 2VO-induced neuronal injury in the retina is of a similar nature to the brain, comprising glial cell activation, but minimal neurodegeneration.[26-28]

In order to examine whether hyperglycemia is neuroprotective to the retina during chronic retinal hypoperfusion induced by 2VO, it was first necessary to characterize the model in order to establish the rate of neuronal degeneration and hence determine the optimal endpoint for analysing neuroprotection. Animals were subjected to 2VO and killed at time points from 6 hours to 2 weeks. The results (supplementary table 1) indicated that there was pronounced death of ganglion cells after one week, a pathologic phenotype in complete agreement with the former set of studies.[21-25]However, relatively little has been published about the sequence of events in the optic nerve during 2VO. Thus, the opportunity was taken during the characterization phase of the project to explore the effect of 2VO on axonal, glial and extracellular matrix elements of the optic nerve head (ONH) and ON using histology, qPCR, immunohistochemistry and Western blotting. These results, which comprise published paper one, serve to facilitate a greater understanding of the 2VO model of injury and more generally of the effect of vascular hypoperfusion on these important structures.

Following the completion of the characterization of the model, the principal objective of the thesis could be investigated. To reiterate, the aim of the project was test the hypothesis that elevated vitreal glucose, induced by short-term diabetes, attenuates the effects of chronic

retinal ischemia induced by 2VO in the rat. A benefit of using this model is that the insult is common to both the retina and the brain. Because of the differences in energy metabolism between the retina and brain, it was particularly interesting to determine whether our data would indicate clear, opposing effects in the two tissues, namely protection against retinal but not brain injury. As indicated above, the model precipitates a severe retinal injury, making neuroprotection more difficult to achieve than in the pressure-induced ischemia model, yet causes a relatively mild injury in the brain, thereby providing an interesting comparison with results from acute stroke models.

The body of this thesis contains two published manuscripts in international peer-reviewed ophthalmic journals. Together, they outline the study philosophy, design, methods and results, together with the deductions that can be drawn. The final conclusions chapter summarises these findings and recommends areas of further research.

References

1. Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res.* 2004;23:91-147.

2. Gilmore RM, Stead LG. The role of hyperglycemia in acute ischemic stroke. *Neurocrit Care.* 2006;5:153-158.

3. Kagansky N, Levy S, Knobler H. The role of hyperglycemia in acute stroke. *Arch Neurol.* 2001;58:1209-1212.

4. Helgason CM. Blood glucose and stroke. *Stroke.* 1988;19:1049-1053.

5. Heuschmann PU, Kolominsky-Rabas PL, Misselwitz B, Hermanek P, Leffmann C, Janzen RW, Rother J, Buecker-Nott HJ, Berger K. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: the German Stroke Registers Study Group. *Arch Intern Med.* 2004;164:1761-1768.

6. Mooradian AD. Central nervous system complications of diabetes mellitus--a perspective from the blood-brain barrier. *Brain Res Brain Res Rev.* 1997;23:210-218.

7. Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. *Stroke.* 2001;32:2426-2432.

8. MacDougall NJ, Muir KW. Hyperglycaemia and infarct size in animal models of middle cerebral artery occlusion: systematic review and meta-analysis. *J Cereb Blood Flow Metab.* 2011;31:807-818.

9. Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. *Eye (Lond).* 2002;16:242-260.

10. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest.* 1998;102:783-791.

11. Kern TS, Barber AJ. Retinal ganglion cells in diabetes. *J Physiol.* 2008;586:4401-4408.

12. Casson RJ, Chidlow G, Wood JP, Osborne NN. The effect of hyperglycemia on experimental retinal ischemia. *Arch Ophthalmol.* 2004;122:361-366.

13. Casson RJ, Wood JP, Osborne NN. Hypoglycaemia exacerbates ischaemic retinal injury in rats. *Br J Ophthalmol.* 2004;88:816-820.

14. Winkler BS, A quantitative assessment of glucose metabolism in the isolated rat retina. In Y. Christen, M. Doly and M.-T. Droy-Lefaix (Eds.), Les Seminaires ophtalmologiques dIPSEN: Vision et Adaptation, Elsevier, Amsterdam, 1995, pp. 78-96.

15. Winkler BS, Sauer MW, Starnes CA. Modulation of the Pasteur effect in retinal cells: implications for understanding compensatory metabolic mechanisms. *Exp Eye Res.* 2003;76:715-723.

16. Wang L, Tornquist P, Bill A. Glucose metabolism in pig outer retina in light and darkness. *Acta Physiol Scand.* 1997;160:75-81.

17. Winkler BS. Glycolytic and oxidative metabolism in relation to retinal function. *J Gen Physiol.* 1981;77:667-692.

18. Racker E. History of the Pasteur effect and its pathobiology. *Mol Cell Biochem.* 1974;5:17-23.

19. Verweij BH, Amelink GJ, Muizelaar JP. Current concepts of cerebral oxygen transport and energy metabolism after severe traumatic brain injury. *Prog Brain Res.* 2007;161:111-124.

20. Farkas E, Luiten PG, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev.* 2007;54:162-180.

21. Kobayashi M, Kuroiwa T, Shimokawa R, Okeda R, Tokoro T. Nitric oxide synthase expression in ischemic rat retinas. *Jpn J Ophthalmol.* 2000;44:235-244.

22. Stevens WD, Fortin T, Pappas BA. Retinal and optic nerve degeneration after chronic carotid ligation: time course and role of light exposure. *Stroke.* 2002;33:1107-1112.

23. Lavinsky D, Arterni NS, Achaval M, Netto CA. Chronic bilateral common carotid artery occlusion: a model for ocular ischemic syndrome in the rat. *Graefes Arch Clin Exp Ophthalmol.* 2006;244:199-204.

24. Yamamoto H, Schmidt-Kastner R, Hamasaki DI, Parel JM. Complex neurodegeneration in retina following moderate ischemia induced by bilateral common carotid artery occlusion in Wistar rats. *Exp Eye Res.* 2006;82:767-779.

25. Kalesnykas G, Tuulos T, Uusitalo H, Jolkkonen J. Neurodegeneration and cellular stress in the retina and optic nerve in rat cerebral ischemia and hypoperfusion models. *Neuroscience.* 2008;155:937-947.

26. Osborne NN, Block F, Sontag KH. Reduction of ocular blood flow results in glial fibrillary acidic protein (GFAP) expression in rat retinal Muller cells. *Vis Neurosci* 1991;7:637-639.

27. Barnett NL, Osborne NN. Prolonged bilateral carotid artery occlusion induces electrophysiological and immunohistochemical changes to the rat retina without causing histological damage. *Exp Eye Res.* 1995;61:83-90.

28. Osborne NN, Safa R, Nash MS. Photoreceptors are preferentially affected in the rat retina following permanent occlusion of the carotid arteries. *Vision Res* 1999;39:3995-4002.

Chapter2 – 2VO AS A MODEL OF OCULAR ISCHEMIA

Spatio-Temporal Characterisation of Optic Nerve Degeneration after Chronic Hypoperfusion in the Rat

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Chapter 3 – THE EFFECT OF HYPERGLYCEMIA

The Effect of Hyperglycemia on Hypoperfusion-Induced Injury

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Chapter 4 - CONCLUSION

Introduction

The conclusion provides an overriding discussion of the work presented in the thesis. It includes comments on the significance of the work, problems encountered and future research. It is not aimed to be a detailed reworking of the discussion of each paper published.

Linkage between publications

Chapter 2 outlined in detail the affect of 2VO on the optic nerve and retina of normal rats, and concluded that it was a robust model of ocular ischemia in the rat. Chapter 3 further extended this to include findings of the effect on the brain, and continued on to compare these results to those of hyperglycemic rats. As such the objectives of the study have been achieved.

Problems encountered

Significant problems were encountered throughout the experimental period due to a higher then expected mortality rate amongst the 2VO rats. This resulted in significant concern in regard to the projects animal ethics approval, and numerous evaluations and alterations to the animal care protocol were made in attempt to overcome this with moderate success. However it wasn't until the final data were analyzed that the most enlightening result was found, being a greater than 2 fold higher mortality rate among the hyperglycemic rats when compared to the normoglycemic rats, which when analyzed in isolation was within the limits of previously published mortality rates. Ironically this hurdle which on numerous occasions threatened to end the study resulted in a significant relevant finding.

Future research

This work hypothesizes that a combination of the metabolic factors discussed provides a mechanistic explanation for the neuroprotective affect we have found. This provides a new avenue for further research which could lead to novel treatment strategies for common blinding diseases where energy failure is involved in the

pathogenesis, including ischemic retinopathies, age-related macular degeneration, and glaucoma.

In the intervening period between the experiments conducted and the submission of this thesis, further research has been conducted that supports a protective effect of glucose against RGC loss. Ebneter et al used a rat model of laser-induced glaucoma to conclude that elevated glucose levels attenuated RGC stromal and axonal injury. In addition, research has been published that postulates a possible mechanism for the protective effect of glucose. Han et al used retinal cell cultures treated with the mitochondrial toxin rotenone to induce a metabolic insult. They found that an elevated glucose level attenuated neuronal death. Their evidence suggests that the mechanism by which elevated glucose achieves this effect is via both the pentose phosphate pathway and glycolytic pathway, thereby maintaining cellular ATP and nicotinamide adenine dinucleotide phosphate, and reducing the build up of reactive oxygen species.

Potential clinical applications

The findings of this thesis open possibilities for the development of new treatment options for ocular disease and injury where there is a mechanism of metabolic insult to ocular neural tissue. This includes retinal artery occlusions, retinal detachment and possibly macular degeneration and glaucoma.

Two factors would need to be considered however. Firstly the glucose would need to be delivered effectively to the target tissue, and secondly treatment would need to be ceased before the deleterious effects of long term high glucose levels such as small vessel disease and lens oxidative stress are initiated.

In the setting of acute retinal artery occlusion or retinal detachment it is the loss of blood supply from the retinal arteries that results in the metabolic insult so delivery of glucose via normal vascular pathways is not an option. Something as simple as intravitreal glucose injections could possibly provide metabolic support to the neural tissue before blood supply is re-established, or a retinal detachment is repaired. Such injections could be repeated in the short term.

A role for intravitreal glucose in the treatment of macula degeneration and glaucoma is also possible from a metabolic viewpoint, however as these are not acute conditions presumably the negative effects of long term high levels of intravitreal glucose would negate any benefit. Furthermore intravitreal injections in the setting of glaucoma may be unwise due to transient increase in intra-ocular pressure.

References

Ebneter A, Chidlow G, Wood JP, Casson RJ (2011) Protection of retinal ganglion cells and the optic nerve during short-term hyperglycemia in experimental glaucoma. *Arch Ophthalmol.* 2011;129:1337-1344

Han G, Wood JP, Chidlow G, Mammone T, Casson RJ Mechanisms of neuroprotection by glucose in rat retinal cell cultures subjected to respiratory inhibition. *Invest Ophthalmol Vis Sci.* 2013;54:7567-7577

Summary

The work described in this thesis has added to the body of knowledge of the differences in brain, retinal and optic nerve metabolism and their respective response to ischemic injury. It has shown that short term hyperglycemia is neuroprotective to retinal and optic nerve ischemic injury. This has created possibilities for further research into elucidating the exact mechanism of this protective effect, and its possible manipulation and therapeutic application in the clinical setting.

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Supplementary Table 1. Effect of 2VO on retinal ganglion cell (RGC) survival

	Day 1	**Day 3**	**Day 7**
Thy1 mRNA level [*] (exp as % of sham)	93 ± 16	53 ±13†	23 ±12†
Number of Brn-3 RGCs[**] (exp as % of sham)	98 ± 5	67 ±11†	18 ± 9†

[*]Expression of RGC-specific Thy1 mRNA in 2VOretinas relative to the shams. Data (expressed as mean±SEM) are normalized for *GAPDH* (†P<0.01by Pair-wise Fixed Reallocation Randomization Test).

[**]For each retina, cells were counted to a distance of 2 mm either side of the optic nerve head. Data are expressed as the mean±SEM (†P < 0.01, by Student's unpaired *t*-test).