THE ROLE OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE)– AMYLOID BETA AXIS IN RETINAL GANGLION CELL DEATH IN GLAUCOMA

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Declaration

I declare that:

This thesis presents work carried out by me and does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university.

To the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, is clearly acknowledged.

Nafiseh A. S. Hosseini Fin

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Dedication

To My Beloveds

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Overview of Thesis

The current work is structured as a "thesis by compilation" and is presented in the following format. Chapter 1 provides a comprehensive review of the literature. This chapter discusses the role of RAGE and its ligand amyloid beta in Alzheimer's disease, the known epidemiological and pathological similarities between Alzheimer's disease and glaucoma, and the current evidence of the involvement of RAGE in retinal pathology and its potential role in glaucoma. A summarised version of this chapter will be submitted as an invited review paper to the Journal of Experimental Eye Research with the title 'Receptor for advanced glycation end product mediates retinal ganglion cell loss in glaucoma'.

Chapter 2 describes how $RAGE^{-/-}$ mice are protected against retinal ganglion cell loss and retinal dysfunction in experimental glaucoma. Chapter 3 reports how $RAGE^{-/-}$ mice are protected against retinal ganglion cell loss after exogenous amyloid beta oligomers injection into the vitreous. Finally, Chapter 4 summarises the findings and discusses how RAGE and its ligand amyloid beta may mediate retinal ganglion cell loss in glaucoma. This thesis also includes two separate supplementary sections of figures and details of the protocols used in the thesis, which have been added to the Appendix.

List of Publications

1. Hosseini N, Sukkar M, Golzan SM, "Receptor for advanced glycation end product (RAGE) mediates retinal ganglion cell loss in experimental glaucoma", Investigative Ophthalmology and Visual Science, 59(9), 3726; 2018 (Conference abstract).

Contribution of authors

1. Nafiseh A.S. Hosseini Fin
Ethics application preparation, experimental setup and implementation, data acquisition and analysis

2. Maria Sukkar
Experiment Design, data analysis, manuscript preparation

3. Mojtaba Golzan
Overview of ethics approval, experiment Design, data acquisition and analysis, manuscript preparation

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List of abbreviations

AD	Alzheimer's Disease
AGE	Advanced Glycation End products
AMD	Age-related Macular Degeneration
AOH	Acute Ocular Hypertension
APP	Amyloid Precursor Protein
Aβ	Amyloid Beta
BBB	Blood-Brain Barrier
BRB	Blood-Retinal Barrier
CAMs	Cell Adhesion Molecules
CNS	Central Nervous System
DMSO	Dimethylsulfoxide
DR	Diabetic Retinopathy
ECM	Extracellular Matrix
ERG	Electroretinogram
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GCL	Ganglion Cell layer
HFIP	1,1,1,3,3,3-Hexafluoro-2-Propanol
HMGB1	High Mobility Group Box 1
IOP	Intra Ocular Pressure
IPL	Inner Plexiform Layer
LBP	Lycium Barbarum Polysaccharides
LGN	Lateral Geniculate Nucleus
MAPK	Mitogen Activated Protein Kinase
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloprotease
NADPH	Nicotinamide Adenine Dinucleotide Phosphate-oxidase
NF-κB	Nuclear Factor-kappaB
NFL	Nerve Fiber Layer
OCT	Optical Coherence Tomography
OHT	Ocular Hypertension
ONH	Optic Nerve Head
pSTR	Positive Scotopic Threshold Response
RAGE	Receptor for Advanced Glycation End products
RGC	Retinal Ganglion Cell
ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
SAPK	Stress Activated Protein Kinase
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
TRT	Total Retinal Thickness
WT	Wild Type

ABSTRACT

Glaucoma encompasses a heterogeneous group of neurodegenerative processes associated with progressive damage to the resident neurons within the retina known as retinal ganglion cells (RGCs). The early stages of glaucoma are not associated with any symptoms, pain or change in sight. As a result, up to 40% of RGC loss occurs before a clinical diagnosis is made. Current treatments are effective at reducing intraocular pressure (IOP), the major risk factor for the disease, but a significant proportion of patients still experience vision loss despite treatment. Identifying new treatments that prevent RGC death caused by glaucomatous pathology is a major unmet need.

The receptor for advanced glycation end products (RAGE) is implicated in the pathogenesis of many chronic diseases, particularly neurodegenerative diseases such as Alzheimer's disease (AD) in which RAGE and its ligand, amyloid beta (Aβ), have been shown to mediate neuronal loss. Interestingly, higher RAGE expression and Aβ deposits have also been identified in the RGC layer in glaucoma. Given the current evidence for the involvement of similar underlying pathophysiological mechanisms in AD and glaucoma and that both RAGE and Aβ are linked to cell death pathways, I hypothesised that RAGE–Aβ signalling underlies RGC loss in glaucoma.

To address this hypothesis, RAGE knockout ($RAGE^{-/-}$) mice and wild-type (WT) control mice were exposed to acute IOP elevation. Further, the time-dependent effects of intravitreal injection of Aβ on RGC loss, retinal dysfunction and structural damage in $RAGE^{-/-}$ and WT mice were also investigated. In this study, $RAGE^{-/-}$ mice were protected against RGC loss in experimental glaucoma compared with WT mice. The potent effects of Aβ on RGC loss was significantly diminished in $RAGE^{-/-}$ mice compared with WT mice.

These findings suggest that RAGE–Aβ is involved in RGC loss in an acute model of glaucoma. Similar experiments in other animal models of glaucoma are needed to confirm whether inhibition of RAGE–Aβ binding helps to slow the development of glaucoma.

Introduction

Glaucoma is a neurodegenerative disease with no significant symptoms or signs indicative of disease activity. It is a leading cause of preventable blindness that currently affects 300,000 Australians. Studies suggest that over 50% of people with glaucoma are unaware [1] of their disease and more than 72% of new cases are detected incidentally during examination for another reason unrelated to glaucoma [2], [3]. Despite numerous pharmacological attempts to lower intraocular pressure (IOP), the single most significant risk factor for the disease, glaucoma can continue to progress and eventually lead to retinal ganglion cell (RGC) loss and subsequent vision loss.

During normal vision, light passes through the cornea and lens before reaching the retina, the light-sensitive layer of the eye. The retina is divided into two main structures: the *outer* and *inner* retina. The outer retina houses the photoreceptors, the cells that receive light and transmit it through electrical signals, which are then transmitted to RGCs located within the inner retina. RGCs are responsible for relaying visual signal from the inner retina to the brain. The cell body of RGCs is located within the inner layer of the retina, and their axons extend to the brain via the optic nerve.

In glaucoma, it is believed that elevated IOP induces haemodynamic stress at the optic nerve head, which leads to optical nerve axonal compression and subsequent RGC loss via apoptosis [4]. As a progressive neurodegenerative disease, glaucoma affects peripheral vision in its early stages and can cause central visual dysfunction later in the disease process (Figure 1).

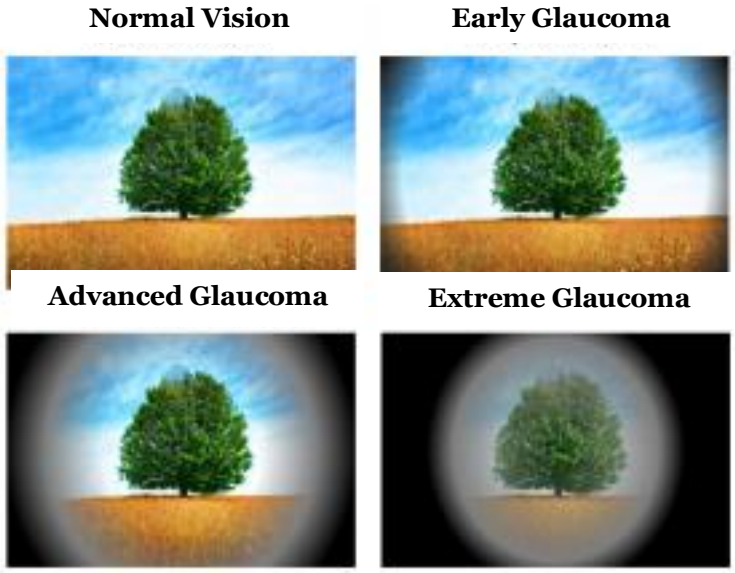

Figure 1. Alterations in normal vision with glaucoma [4]. The loss of sight in glaucoma is generally continuous and peripheral vision might be lost without any symptoms.

Statement of the problem

Elevated IOP is considered to be a prime factor for glaucomatous damage to the optic nerve head, which leads to RGC loss. Management of IOP, the sole modifiable risk factor, is at the centre of most pharmacological interventions targeted at halting disease progression. However, a significant proportion of patients experience some degree of vision loss despite the therapeutic management [5]. As a result, the focus of recent research has shifted from IOP management to devising strategies that delay or halt RGC loss as potentially the most beneficial approach to preserve vision in glaucoma.

A variety of molecular signalling pathways have been investigated to understand better the mechanisms that mediate RGC loss. The receptor for advanced glycation end products (RAGE) is a pattern recognition receptor that binds many molecules involved in tissue injury and is involved in various chronic diseases associated with aging. RAGE binds a number of ligands and is highly expressed in RGCs. Prominent among the various

ligands amyloid beta (Aβ), the central player in Alzheimer's disease (AD), has a potent neurotoxic effect on RGCs [6]–[8]. Although RAGE–Aβ-mediated neuronal cell death has been widely studied in AD, its potential role in RGC death in glaucoma has not been investigated.

Hypothesis

That in glaucoma, 1) RAGE takes part in RGC loss and 2) the RAGE–Aβ axis is involved in RGC loss.

Research aims

To address this hypothesis, IOP was increased in $RAGE^{-/-}$ and wild-type (WT) control mice to replicate a model of retinal ischaemia–reperfusion injury and experimental glaucoma. Following on from the results of the first experiment, in the second experiment, the RAGE ligand, Aβ, was intravitreally injected in to the vitreous of $RAGE^{-/-}$ and WT control mice. The specific aims of the project were as follows.

AIM 1: Determine whether RAGE mediates RGC loss and retinal dysfunction in a model of acute retinal ischaemia.

AIM 2: Determine whether RAGE–Aβ activation exacerbates RGC loss.