TARGETING METABOLIC VULNERABILITIES FOR NEUROPROTECTION IN GLAUCOMA


B_3 or not B_3? That is the question.

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Glaucoma remains a major cause of vision loss globally. Advancing age, genetics, and high IOP are all considerable risk factors. With an increasingly aged population, the number of patients with glaucoma is set to increase, and thus glaucoma will continue to be a significant health and economic burden.

Current treatment strategies for glaucoma target only IOP, the principal treatable risk factor. Patient adherence is variable, and many individuals are subject to surgical interventions due to progressive damage. In addition, many patients do not respond to pressure-lowering treatments or progress to blindness despite low IOP.

To date, the search for a treatment that targets retinal ganglion cells (RGCs) and arrests disease progression has been unsuccessful. Neuroprotective treatments for glaucoma are of great therapeutic need, as are new clinical trial paradigms that facilitate translation of candidate treatments from bench to bedside in an expedited fashion.

THE KEY TO RGC DEGENERATION: METABOLISM?

The progressive dysfunction and loss of RGCs (and their axons that make up the optic nerve) are the hallmark features of glaucoma. RGCs are highly sensitive to metabolic fluctuations and sit on a metabolic knife-edge during times of stress that may be exacerbated by aging, genetic impairment, or increased IOP (Figure). During these periods of metabolic stress, the viability of RGCs is reliant on mitochondria and supportive glial cells to maintain cellular homeostasis and bioenergetic needs.

Disease-causing mutations in mitochondrial protein-coding genes are prevalent in the human population

AT A GLANCE

▶ KEY POINTS

- Metabolic and mitochondrial dysfunction is present in human glaucoma patients and animal models of glaucoma.
- Declining systemic levels of nicotinamide adenine dinucleotide or nicotinamide (NAM) may be risk factors and biomarkers for glaucoma.
- NAM treatment is robustly protective in animal models of glaucoma.
- Ongoing NAM clinical trials show promise.
- New paradigms for clinical trial execution will allow the identification of potential neuroprotective treatments for glaucoma in the short to medium term.

▶ CRUCIAL NEXT STEPS

- Establish meaningful parameters for neuroprotective clinical trials.
- Continue to identify systemic and retinal ganglion cell–specific risk factors.
- Focus on protecting the vulnerable retinal ganglion cell rather than regenerating the dead retinal ganglion cell.

and are present throughout the majority of cell types in the body. Yet, interestingly, abnormalities in these genes predominantly affect RGCs and present in the form of blinding disorders that, in most cases, have little or no overt extra-ophthalmic pathology (eg, autosomal dominant optic atrophy or Leber hereditary optic neuropathy).

Emerging research suggests that a systemic vulnerability to mitochondrial abnormalities exists in glaucoma patients. Genomic analysis has demonstrated altered mitochondrial DNA content and a spectrum of mitochondrial DNA mutations in individuals with glaucoma. These abnormalities are also present systemically in leukocytes, suggesting a systemic susceptibility to metabolic defects. Such systemic susceptibility conspires with elevated IOP to increase glaucoma susceptibility with age, this research suggests (Table 1). Metabolic decline may thus be a critical, and targetable, pathogenic component of glaucoma.

"EMERGING RESEARCH SUGGESTS THAT A SYSTEMIC VULNERABILITY TO MITOCHONDRIAL ABNORMALITIES EXISTS IN GLAUCOMA PATIENTS."

TARGETING MITOCHONDRIA AND METABOLISM

The mechanisms by which mitochondrial defects influence neuronal metabolism and lead to neurodegeneration are a topic of active research and interest. Current research has discovered metabolic dysfunction and mitochondrial abnormalities occurring prior to neurodegeneration in multiple experimental models of glaucoma. Targeting mitochondria and metabolism has shown promise in animal models of glaucoma (Table 2). Importantly, many of the changes discovered in animal models sensitize RGCs, leaving them vulnerable to the insults of elevated IOP.

One such molecule is the essential redox cofactor and metabolite nicotinamide adenine dinucleotide (NAD), which declines in the retina in an age-dependent manner. NAD is well established to be a potent mediator of axonal and neuronal survival following damaging disease-related insults. A key pathway to NAD synthesis in neurons is through the salvage pathway whose input is nicotinamide (NAM), the amide form of vitamin B_3.

Recently, NAM has been demonstrated to be low in the sera of patients with primary open-angle glaucoma. In mouse models of glaucoma, dietary supplementation with NAM or intravitreal introduction of gene therapy (*Nmnat1*, a terminal enzyme for NAD biosynthesis) robustly protects against neuronal metabolic decline and prevents glaucoma. NAM has a long clinical history and a robust safety profile, even at megadoses (up to 12 g/day long-term); therefore, it is an ideal target for neuroprotection in glaucoma, and clinical trials of NAM in glaucoma are under way.

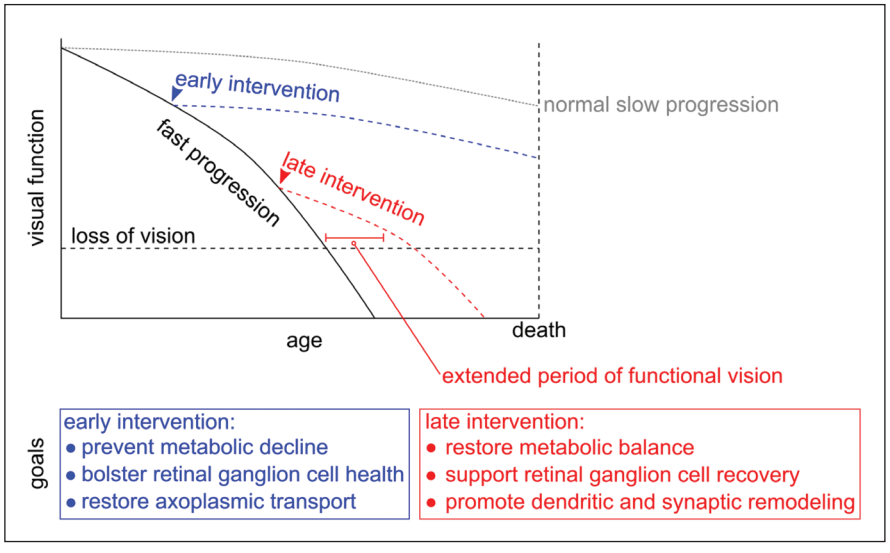

Figure. There is potential for functional vision recovery with neuroprotective treatments at multiple stages during glaucomatous progression. It is becoming increasingly important to understand the early factors that influence retinal ganglion cell health during normal aging and following insults from elevated IOP. Many systemic risk factors predispose retinal ganglion cells to bioenergetic failure, such as advancing age, genetics, and loss of metabolic substrates (Table 1), whereas elevated IOP, neuroinflammation, and hypoxic damage necessitate the initiation of energy-expensive repair processes. A consequence of this process is an induction of compensatory mechanisms that divert energy use from retinal ganglion cell axon potential propagation to repair, initiating retinal ganglion cell degeneration and remodeling. The following degenerative processes, synapse and dendrite pruning, protect injured retinal ganglion cells from excitatory bipolar cell inputs facilitating repair. If compensatory processes permit repair function, then dendrites and synaptic inputs can be restored. If not, then potentially irreversible apoptotic processes are induced. Preventing retinal ganglion cell decline prior to the initiation of apoptosis should be a key factor in designing the next generation of glaucoma neuroprotective treatments. (Figure adapted in part from Caprioli J.[1])

THE PROMISE OF NAM FOR NEUROPROTECTION IN GLAUCOMA

NAM's widespread availability in health food stores, excellent safety profile, good tolerability, and affordability will all facilitate its rapid

TABLE 1. METABOLIC SUSCEPTIBILITIES IN GLAUCOMA PATIENTS

Metabolic Factor	Function	Comments	References
Complex I	Generation of ATP at the mitochondrion (OXPHOS)	mtDNA sequencing, functional metabolic assays	2-6
Complex III	Generation of ATP at the mitochondrion (OXPHOS)	mtDNA sequencing	2
Complex IV	Generation of ATP at the mitochondrion (OXPHOS)	mtDNA sequencing	2,4,6
Complex V	Generation of ATP at the mitochondrion (OXPHOS)	mtDNA sequencing	2,4,6
Increased mtDNA content	mtDNA is a circular DNA consisting of a light and heavy chain encoding 37 genes and is essential for mitochondrial function	mtDNA sequencing	2
Other mtDNA mutations and mtDNA haplogroups	As above	mtDNA sequencing	6-13
Decreased plasma nicotinamide levels	The amide of vitamin B_3 and an essential precursor to NAD	Metabolomics	14
Decreased plasma citrate levels	Citric acid cycle intermediate essential for metabolism	Ion chromatography	15
OPA1	Encodes Optic Atrophy Protein 1, the master regulator of mitochondrial fusion	rtPCR, GWAS, SNP genotyping	16-19
TXNRD2	Encodes Thioredoxin Reductase 2, which plays a key role in redox homeostasis	GWAS	20
ME3	Encodes Malic Enzyme 3, which catalyzes the oxidative decarboxylation of malate to pyruvate	Gene set analysis	21
VPS13C	Encodes Vacuolar Protein Sorting 13 Homolog C, which is essential for mitochondrial transmembrane potential	GWAS	22
GCAT	Encodes Glycine C-Acetyltransferase, which regulates mitochondrial glycine	GWAS	22
PTCD2	Encodes Pentatricopeptide Repeat Domain 2, which is involved in mtRNA maturation and OXPHOS function	GWAS	22

Abbreviations: ATP (adenosine triphosphate), OXPHOS (oxidative phosphorylation), mtDNA (mitochondrial DNA), nicotinamide adenine dinucleotide (NAD), rtPCR (real-time polymerase chain reaction), GWAS (genome-wide association study), SNP (single-nucleotide polymorphism), redox (reduction-oxidation reaction), mtRNA (mitochondrial RNA)

translation into clinical trials. At least two such trials are currently registered. The first, a crossover study (ACTRN12617000809336) based in Melbourne, Australia, is completed, and the manuscript is under review. The second, a New York–based NAM-pyruvate combination study (NCT03797469) has started recruitment and is underway, with a planned completion date of December 2019. A third Sweden-based study of NAM is planned for 2019 to 2020.

DESIGNING EFFICIENT AND EXPEDITED CLINICAL TRIALS

An ongoing challenge is the time required to conduct clinical trials for neuroprotection in glaucoma. The United Kingdom Glaucoma Treatment Study (UKGTS) demonstrated that, with intensive visual field testing, a change in glaucomatous progression rate could be determined as early as 11 months (when a prostaglandin was compared with placebo).

In a neuroprotective clinical trial, the test agent is assessed with placebo but in the presence of concomitant IOP lowering, a requirement on ethical grounds. Subsequently, longer follow-up periods are required, reducing feasibility and increasing cost. Thus, there is a clear need to develop surrogate clinical markers that provide information on RGC health and accurately predict longer-term progression rates.

We have been exploring whether short-term improvement in inner retinal function as determined by electroretinography or contrast sensitivity is seen after IOP lowering or in the presence of candidate neuroprotective treatment (ie, NAM). One of the aims of our collective research programs is to more accurately match clinical biomarkers with markers of glaucomatous neurodegeneration between human samples and donor tissue and animal models of glaucoma. This is essential to understanding the pathogenesis of RGC degeneration in glaucoma and will therefore aid in the search for relevant markers of RGC health.

TABLE 2. POTENTIAL TRANSLATIONAL NEUROPROTECTIVE TREATMENTS TARGETING MITOCHONDRIA AND METABOLISM

Treatment	Target	Model	References
Nicotinamide	NAD salvage pathway	DBA/2J mouse chronic glaucoma, mouse retinal axotomy model, mouse TNF-alpha inducible neuroinflammatory glaucoma	23-25
Ketogenic diet	Mitochondrial biogenesis, energy availability through ketone body production	DBA/2J mouse chronic glaucoma, mouse bead model of ocular hypertension	26
Coenzyme Q10	Component of mitochondria electron transport chain	DBA/2J mouse chronic glaucoma, rat hypertonic saline episcleral vein injection, rat ischemia/reperfusion	27-29
Resveratrol	Multiple targets of metabolic regulation (AMPK, SIRT1, PGC-1alpha)	Rat hyaluronic acid model, mouse controlled optic nerve crush, pig trabecular meshwork cells	30-32
Citicoline	Likely sirtuins during neuroprotection	Rat kainic acid-induced retinal degeneration, cultured mouse retina, rat partial optic nerve crush	33-37

THE FUTURE OF NEUROPROTECTION IN GLAUCOMA

With improvement in clinical trial testing and available resources for exploring early degenerative events in glaucoma, in addition to a better understanding of the utility of animal models, we are stepping into the future of neuroprotection in glaucoma. Over the coming year, we will see the first results from the NAM clinical trials, which will inform clinicians whether targeting neuronal metabolism is a viable strategy for protecting the vulnerable RGC in glaucoma patients. ■

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